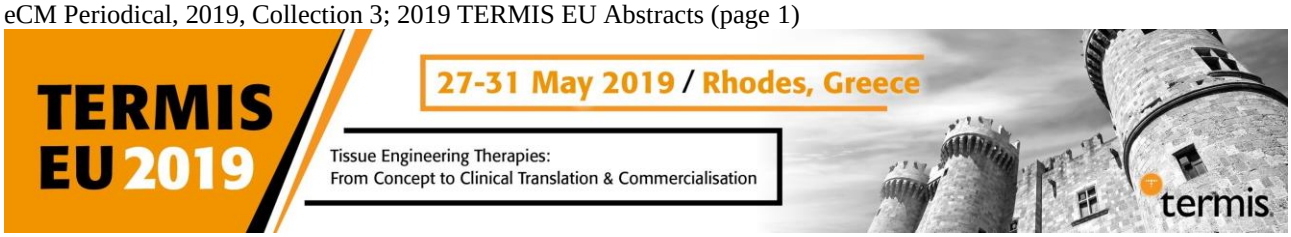


Awardees

In silico regenerative medicine: from living implants to digital patients

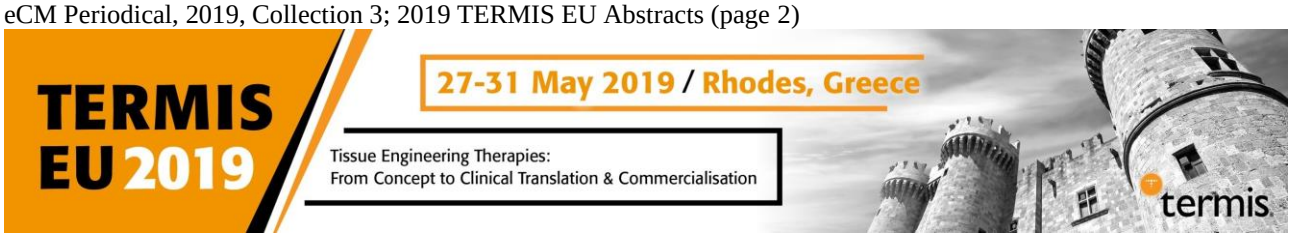
Liesbet Geris
Presenting Author: Liesbet Geris, liesbet.geris@uliege.be
Prometheus, R&D Division for Skeletal Tissue Engineering, KU Leuven, Belgium. GIGA In silico medicine, University of Liège, Belgium. Biomechanics Section, KU Leuven, Belgium

As basic science advances, one of the major challenges in Tissue Engineering (TE) is the translation of the increasing biological knowledge on complex cell and tissue behavior into predictive and robust engineering processes. Mastering this complexity is an essential step towards clinical translation of TE applications. Computational (in silico) modeling allows to study the biological complexity in a more integrative and quantitative way. Specifically, in silico and in vitro tools can help in quantifying and optimizing the TE products and processes but also in assessing the influence of the in vivo environment on the behavior of the TE products after implantation. We develop computational models related to all aspects of the TE product development cycle. Depending on the specific questions that need to be answered and on the available information, model systems can be purely data-driven or more hypothesis-driven in nature. At cell level we study cellular regulation through knowledge-based and multi-omics approaches (transcriptomics & metabolomics). For carriers, we use models that combine effects of geometry, composition and degradation on tissue growth to optimize scaffolds printed in a variety of materials. With respect to culture strategies, we combine the development of in vitro set-ups (e.g. stand-alone perfusion bioreactor) with their digital twins. For a prediction of in vivo processes (clinic), we look at normal and pathophysiological healing cases and design possible treatment strategies that we test in in silico clinical trials. To bring these models to the clinics and/or the market, various collaborations have been set up with clinicians and companies, in Belgium and Europe. An important prerequisite to this translation is the acceptance of digital evidence generated by in silico tools in biomedical R&D activities and in regulatory submissions to EMA and USA-FDA (the latter is a driver of the use of in silico tools). Through our involvement in the Virtual Physiological Human institute and the Avicenna Alliance, we are involved in interactions with a variety of stakeholders, including policy makers and regulators across the world, to establish proper policies, regulations and harmonized guidelines related to the use of in silico tools in medicinal product development & translation.

ACKNOWLEDGEMENTS: A big thank you to all current & former members of the Biomechanics Research Unit (U Liège/KU Leuven) & Prome-theus. Funding gratefully acknowledged from ERC CoG, INTERREG VL-NL, MSCA-ITN, BIOWIN, FNRS, REGMED XB, fond. Francqui & FWO.

REFERENCES

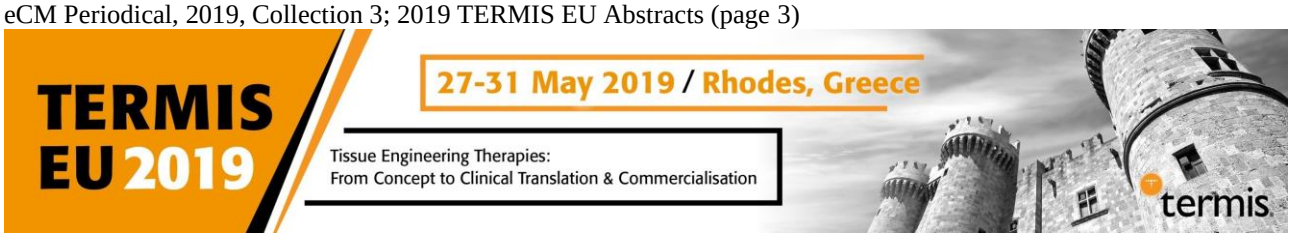
[1] Kerkhofs et al., PLoS ONE, 2016, 11(8).
[2] Manhas et al., BMMB, 2017, 16(2):425-438.
[3] Guyot et al. BMMB, 2016, 15:169-80.
[4] Carlier et al., Sci Rep, 2018, 8(1):2465.



My career: A lifetime of TERM research and innovation & a lifer of TERMIS

Rui L. Reis [1,2,3]
Presenting Author: Rui L. Reis, rgreis@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

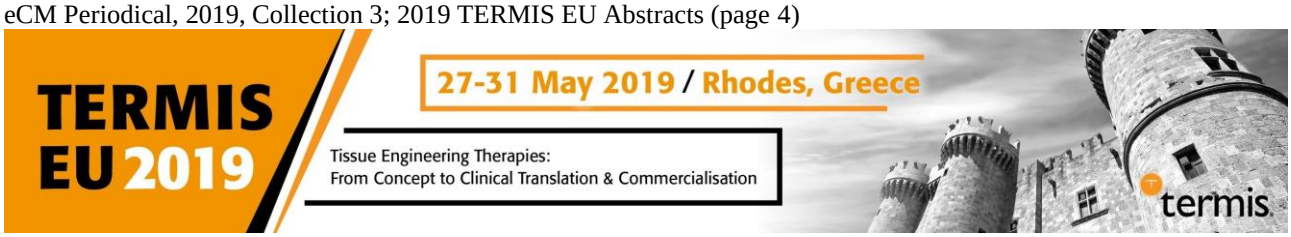
In this talk, as it is typical for such kind of lifetime achievement award, I will describe my career as researcher and innovator in the field of tissue engineering and regenerative medicine (TERM) and my involvement with and contributions to TERMIS and the field as a whole along the years. I will of course present some examples of my own research. The selection of a proper material to be used as a scaffold or as a hydrogel to support, hold or encapsulate cells is both a critical and a difficult choice that will determine the success of failure of any TERM strategy. We believe that the use of natural origin polymers, including a wide range of marine origin materials, is the best option for many different approaches that allow for the regeneration of different tissues. In addition to the selection of appropriate material systems it is of outmost importance the development of processing methodologies that allow for the production of adequate scaffolds/matrices, in many cases incorporating bioactive/differentiation agents in their structures. Furthermore, an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation, and in many cases the selection of a specific sub-population, methodologies should be developed and optimized. The development of dynamic ways to culture the cells and of distinct ways to stimulate their differentiation in 3D environments, as well as the use of nano-based systems to induce their differentiation and internalization into cells, is also a key part of some of the strategies that are being developed in our research group. The potential of each combination materials/cells, to be used to develop novel useful regeneration therapies will be discussed. The use of different cells and their interactions with different natural origin degradable scaffolds and smart hydrogels will be described. Several examples of TERM strategies to regenerate different types of tissues will be presented. It has been my honor to serve TERMIS in different leadership roles since its foundation. I have participated in almost every meeting of all chapters of our Society. I will also describe those roles and my involvement with the society along the years, as well as my present responsibilities in TERMIS. I was and I am a lifer of TERMIS!



'Do not go gentle into that good night' Courtesy of Dylan Thomas, Welsh Poet, 1914-1953

David F Williams
Presenting Author: David F Williams, dfwillia@wakehealth.edu
Wake Forest Institute of Regenerative Medicine, North Carolina, United States of America

Towards the end of a career, some people should be encouraged to fade away gently, having little more constructive to say as the world moves on apace. Others have every right to shout out their views until quietened by death. Dylan Thomas was very upset by the late-life quieting of his father and would have preferred that he stay angry; "Rage, rage, against the dying of the light". Both are valid positions and while I tend to favour the latter, I have much sympathy with the words of Archbishop Desmond Tutu; "Don't raise your voice, improve your argument". For many years I have argued that our understanding of mechanisms of biocompatibility has been based on false assumptions. I have written many papers on this subject, drawing attention to two main consequences of these misconceptions; first the continued belief that biocompatibility is a property of a biomaterial rather than of a biomaterial-host system, and secondly that one of the most important specifications of a tissue-engineering biomaterial is prior approval by the FDA for use in an implantable medical device. These views lead to erroneous descriptions of materials as being 'biocompatible' and to the use of some degradable synthetic polymers as scaffolds when they have no chance of success. I will not raise my voice here but improve my arguments, based on theory, experiment and clinical experience, to show that a major change in our understanding of biocompatibility mechanisms and in our practices of testing the biosafety of biomaterials, should lead to better success in these potentially valuable components of medical technology. To combine Desmond and Dylan, let us "improve our arguments as we go into that good night".

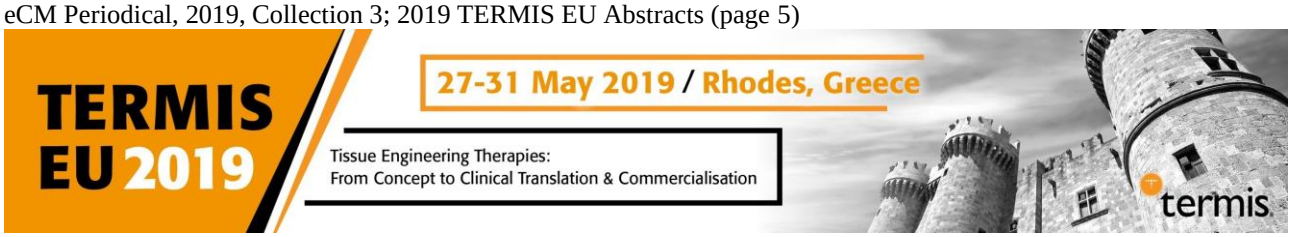


Plenary

Biomaterials for tissue engineering and modeling disease

A.G. Mikos
Presenting Author: Antonios G Mikos, mikos@rice.edu
Department of Bioengineering, Rice University, Houston, Texas, United States of America

Advances in biology, materials science, chemical engineering, and other fields have allowed for the development of tissue engineering, an interdisciplinary convergence science. For the past two and a half decades, our laboratory has focused on the development and characterization of biomaterials-based strategies for the regeneration of human tissues with the goal of improving healthcare outcomes. In a collaborative effort with physicians, surgeons, and other scientists, we have produced new material compositions and three-dimensional scaffolds, and investigated combinations of biomaterials with cell populations and bioactive agents for their ability to induce tissue formation and regeneration. We have examined the effects of material characteristics, such as mechanical properties, topographical features, and functional groups, on cell behavior and tissue guidance, and leveraged biomaterials as drug delivery vehicles to release growth factors and other signals with spatial and temporal specificity. This presentation will review recent examples of biomaterials-based approaches for regenerative medicine applications and highlight future areas of growth, such as the use of tissue engineering to model tumor microenvironments for validation of cancer therapeutic discovery.

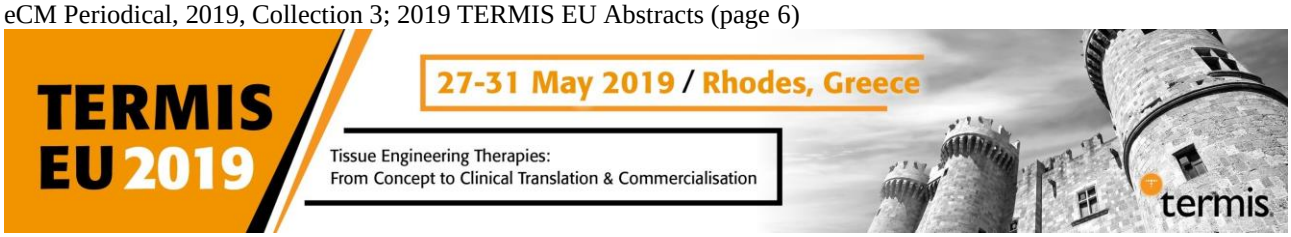


Magnetically assisted tissue engineering technologies for tendon regeneration

Manuela E. Gomes[1,2,3]
Presenting Author: Manuela E. Gomes, megomes@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark-Parque de Ciência e Tecnologia, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

The poor healing ability of tendons, which play a critical role in the musculoskeletal system, as well as the limitations of currently used therapies have motivated tissue engineering (TE) strategies to develop living tendon substitutes. However, the limited knowledge on tendon development and healing processes has hindered the design of TE procedures that more closely recapitulate tendon morphogenesis. Therefore, we've been exploring conventional and innovative tools to design magnetic responsive systems mimicking specific aspects of tendon tissue architecture, composition and biomechanical properties, which, combined with adequate stem cells, are expected to render appropriate behavioral instructions to stimulate the regeneration of tendon tissue. Stem cell bioengineering approaches based on superparamagnetic nanoparticles (SPMNs), are being used to unveil the cellular signaling pathways that trigger the tenogenic differentiation of the widely and easily obtained human adipose derived stem cells. Simultaneously, efforts have also focused on understanding possible uses of magnetic stimulus, in combination with magnetic responsive materials, to modulate inflammation in tendon/tendon cells, which can provide an additional valuable tool to trigger tissue regeneration instead of simple repair.

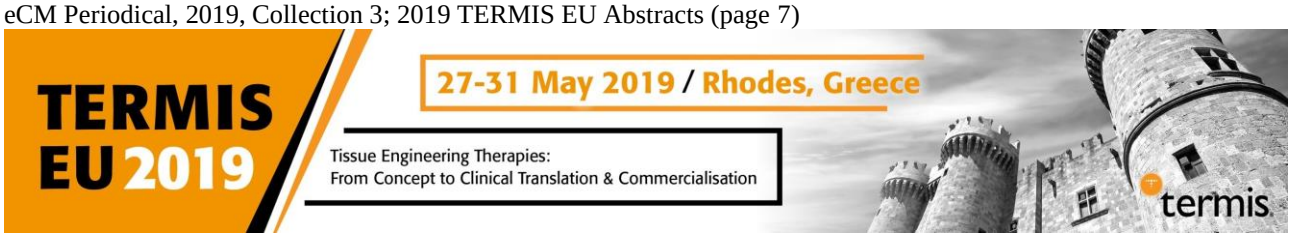
ACKNOWLEDGEMENTS: Project "Accelerating tissue engineering and personalized medicine discoveries by the integration of key enabling nanotechonologies, marine-derived biomaterials and stem cells", supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). HORIZON 2020 (TEAMING Grant agreement No 739572) - The Discoveries CTR and ERC CoG MagTendon nr 772817.



Regulation and roles of autophagy in the brain

Nektarios Tavernarakis
Presenting Author: Nektarios Tavernarakis, tavernarakis@imbb.forth.gr
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas
Medical School, University of Crete, Heraklion, Crete, Greece

Autophagy is crucial for neuronal integrity. Loss of key autophagic components leads to progressive neurodegeneration and structural defects in neuronal synapses. However, the molecular mechanisms regulating autophagy in the brain remain elusive. Similarly, while it is widely accepted that protein turnover is required for synaptic plasticity, the contribution of autophagy to the degradation of synaptic proteins is unknown. We find that BDNF signaling via the tropomyosin receptor kinase B (TrkB) and the phosphatidylinositol-3 kinase (PI3K)/Akt pathway suppresses autophagy in vivo. Autophagy is differentially regulated by fasting in different brain regions. Suppression of autophagy is required for BDNF-induced synaptic plasticity and for memory enhancement, under conditions of nutritional stress. BDNF signaling suppresses autophagy in the forebrain of adult mice. Indeed, BDNF ablation in the neural lineage causes uncontrolled increase in autophagy. In turn, increased autophagy mediates the synaptic defects caused by BDNF deficiency. Thus, fasting suppresses autophagy in regions of the mouse forebrain, thereby promoting synaptic remodeling and memory through a BDNF-regulated mechanism. We identify three key remodelers of postsynaptic densities as cargo of autophagy. Our results establish autophagy as a pivotal component of BDNF signaling, which is essential for BDNF-induced synaptic plasticity. This molecular mechanism underlies behavioral adaptations that increase fitness in times of scarcity.



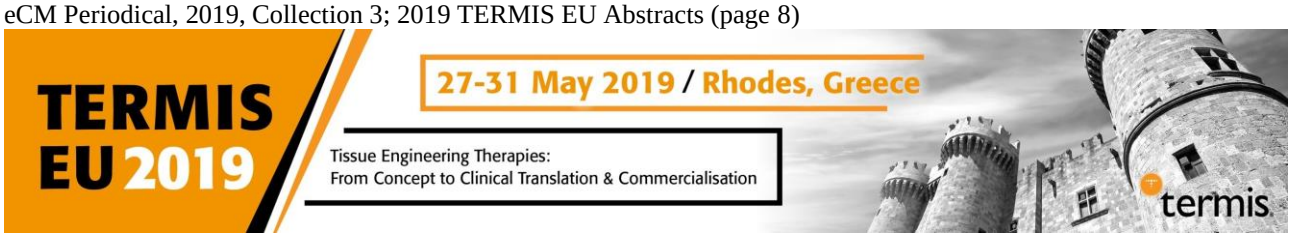
Vascular endothelial growth factors. From basic science to clinical trials

Seppo Yla-Herttuala[1,2]
Presenting Author: Seppo Yla-Herttuala, seppo.ylaherttuala@uef.fi
[1]A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland [2]Gene Therapy Unit and Science Service Center, Kuopio University Hospital, Kuopio, Finland

Therapeutic vascular growth is a potentially useful strategy for ischemic heart disease and peripheral arterial occlusive disease. It involves generation of new capillaries, collateral vessels and lymphatic vessels in ischaemic muscles using either recombinant growth factors or their genes. Arteriogenesis is a process caused by increased sheer stress at the arteriolar level resulting in the formation of large conduit vessels from preexisting small vessels whereas angiogenesis and lymphangiogenesis refers to generation of new vascular structures in vivo. Most commonly used growth factors for therapeutic angiogenesis are members of the vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) families. Some other cytokines and growth factors can also have angiogenic effects. Improved perfusion and functional parameters can be achieved by angiogenesis and arteriogenesis in large animal chronic ischemia models and in man. Safety of the clinical gene therapy of cardiovascular diseases has been excellent with long-term follow-up to 10 yrs after the therapy. Small non-coding RNAs can also be used for angiogenic gene therapy. Most promising results have so far been obtained with direct catheter-based intramyocardial injections of VEGF-D genes with adenovirus and AAV vectors.

REFERENCES

Yla-Herttuala S, Bridges C, Katz MG, Korpisalo P. Angiogenic Gene Therapy in Cardiovascular Diseases: Dream or Vision? Eur Heart J 2017. doi: 10.1093/eurheartj/ehw547.



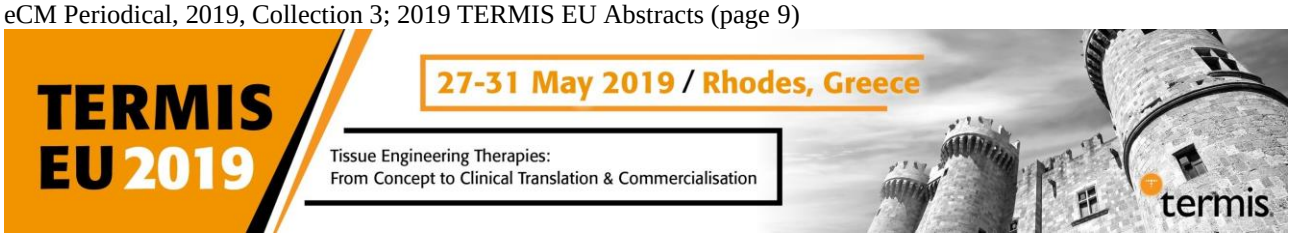
Polymeric nanoparticles for the intracellular delivery of biotherapeutics for cancer treatment

Wim E. Hennink
Presenting Author: Wim E Hennink, W.E.Hennink@uu.nl
Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

An attractive approach to induce a strong immune response against a certain antigen is its targeting to dendritic cells (DCs). Targeting can be established by loading the antigen in a nano-sized carrier and keeping the antigen encapsulated or associated with the particles until they are internalized by DCs. We designed reduction-sensitive cationic dextran nanogels in which an antigen (ovalbumin, OVA) is reversibly immobilized to the hydrogel network via disulfide bonds. These bonds are stable in the extracellular environment but are cleaved in the cytosol of DCs due to the presence of glutathione resulting in triggered released of the loaded antigen after internalization of the nanogels by DCs. These nanogel OVA conjugates showed intracellular release of the antigen when the nanogels were internalized by DCs and boost the MHC class I antigen presentation Besides that nanogels can be used for the intracellular delivery of tumor antigens, these nanoparticles can also be used for the cytosolic delivery of therapeutic proteins. Ribonuclease A (RNase A), as an anti-tumor therapeutic, has its target site (mRNA) inside the cell and has shown poor efficacy in preclinical models as well as in clinical trials due to its insufficient cellular internalization. To this end, anionic dextran nanogels were prepared by an inverse mini emulsion technique followed by post loading at neutral pH of positively charged RNase A. To stabilize the electrostatically loaded protein in the nanogels, Traut's modified RNase A was immobilized covalently in the nanogel network via disulfide bonds that can be cleaved in the reductive cytosolic environment. To enhance the cellular uptake of these anionic nanoparticles, the particle surface charge was reversed by noncovalent attachment of polyethylenimine (PEI). Glutathione triggered release of covalently conjugated RNase A was demonstrated. PEI coating of anionic nanoparticles facilitated their cellular uptake by MDA-MB 231 breast cancer cells. Importantly, PEI coated RNase A loaded nanogels exhibited a concentration-dependent cytotoxic effect by apoptosis after 24 h incubation with cancer cells, whereas the free protein and non-loaded nanogels at equal dose did not show any cytotoxicity. In conclusion, nanogels are attractive vehicles for the intracellular delivery of therapeutic proteins.

REFERENCES

[1] Li D, Sun F, Bourajjaj M, Chen Y, Pieters EH, Chen J, van den Dikkenberg JB, Lou B, Camps MG, Ossendorp F, Hennink WE, Vermonden T, and van Nostrum CF. Strong in vivo antitumor responses induced by an antigen immobilized in nanogels via reducible bonds. Nanoscale 8, 19592-19604, 2016.
[2] Kordalivand N, Li D, Beztsinna N, Sastre Torano J, Mastrobattista E, van Nostrum CF, Hennink WE, and Vermonden T. Polyethyleneimine coated nanogels for the intracellular delivery of RNase A for cancer therapy. Chemical Engineering Journal 340, 32-41, 2018.



Invited

The modern biomaterial paradigm in tissue engineering and regenerative medicine

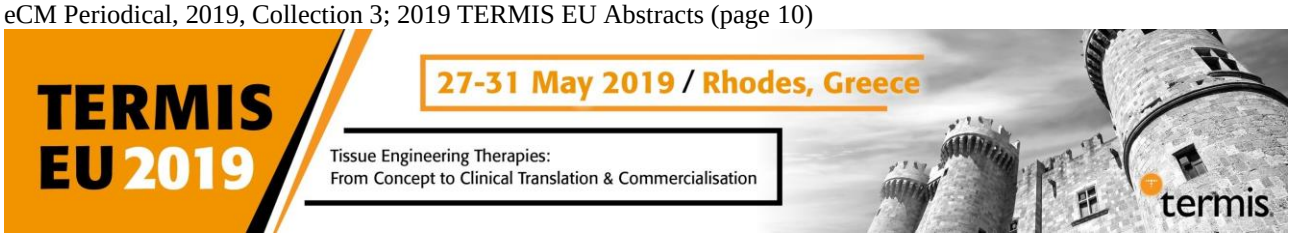
A. Pandit
Presenting Author: Abhay Pandit, abhay.pandit@nuigalway.ie
CÚRAM, Center for Research in Medical Devices, National University of Ireland, Galway, Ireland

Biomaterials are no longer considered innate structures and using functionalisation and biofabrication strategies to modulate a desired response whether it is a host or implant is currently an important focus in current research paradigms. Fundamentally, a thorough understanding the host response will enable us to design proper functionalisation and biofabrication strategies. The input from the host response needs to be weighed in depending on the host disease condition. In addition, biomaterials themselves provide immense therapeutic benefits which needs to be accounted in the design paradigm. Using functionalisation strategies such as enzymatic and hyperbranched linking systems, we have been able to link biomolecules to different structural moieties. The programmed assembly of biomolecules into higher-order self-organized systems is central to innumerable biological processes and development of the next generation of biofabricated scaffolds. Recent design efforts have utilized a developmental biology approach toward both understanding and engineering supramolecular protein assemblies. Structural moieties have taken a variety of different forms such as nanofibers and nanoparticulate. This approach has resulted in functionalisation of micro and nanoparticles with biomolecules that include designed peptide motifs, growth factors and a multitude of gene vector systems. In addition, nature itself has abundant structural complexity that can be biofabricated for harnessing in key targeted clinical applications.

ACKNOWLEDGEMENTS: This publication has also emanated from research conducted with the financial support of Science Foundation Ireland (SFI) and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073.

REFERENCES

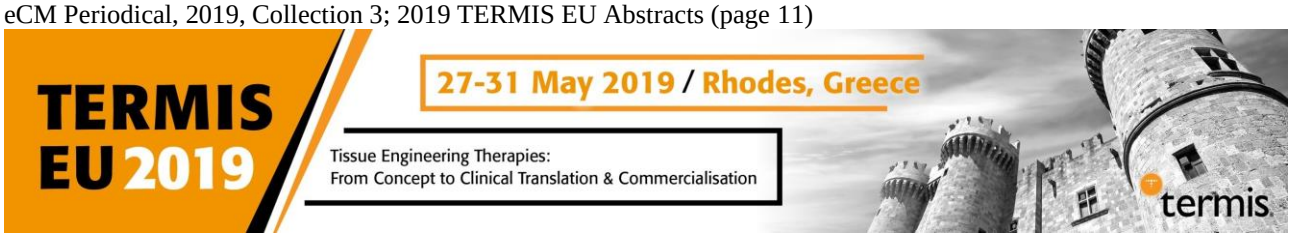
[1] Thomas, D., Fontana, G., Chen, X., Sanz-Nougés, C., Zeugolis, D., Dockery, P., O'Brien, T., Pandit, A. A shape-controlled tuneable microgel platform to modulate angiogenic paracrine responses in stem cells. Biomaterials. 2014: 35(31): 8757-66.
[2] Lang, Y., del Monte, F., Collins, L., Rodriguez, B.J., Thompson, K., Dockery, P., Finn, D.P., Pandit, A. Functionalization of the living diatom Thalassiosira Weissflogii with thiol moieties. Nature Communications. 2013: 4: 3683
[3] Tapeinos, C., Pandit, A. Physical, chemical and biological structures based on ROS-sensitive moieties that are able to respond to oxidative microenvironments.' Advanced Materials. 2016: 28(27): 5553-5585
[4] Mohd Isa IL, Abbah SA, Kilcoyne M, Sakai D, Dockery P, Finn DP, Pandit A. Implantation of hyaluronic acid hydrogel prevents the pain phenotype in a rat model of intervertebral disc injury. Sci Adv. 2018 Apr 4; 4(4):eaaq0597



Xeno-free generation and differentiation of human induced pluripotent stem cells on the surface immobilized with ECM and ECM-derived oligopeptide

A. Higuchi
Presenting Author: Akon Higuchi, higuchi@ncu.edu.tw
Department of Chemical and Materials Engineering, National Central University, Jhongli, Taiwan & Nano Medical Engineering Laboratory, Riken Center for Pioneering Research, Wako, Japan

Stem cells are attractive sources in regenerative medicine. Human pluripotent stem cells (hPSCs), such as embryonic stem cells (hESCs) or induced pluripotent stem cells (hiPSCs), are one of the promising cell source to be used in the future. Therefore, developing appropriate biomaterials for stem cell culture is necessary. In this study, we targeted on three aspects in order to develop an optimal hiPSCs and cultivation method for its applications: (1) Xeno-free extraction and cultivation of human mesenchymal stem cells (hMSCs) from tissue samples as reprogramming cell sources. (2) A safer reprogramming method (Sendai virus vector) was applied to host cells for reprogramming into hiPSCs. (3) Xeno-free conditions for hiPSCs cultivation on biomaterials. We applied PVA-IA hydrogel, poly (vinyl alcohol-co-vinyl acetate-co-itaconic acid), and immobilized extracellular matrices (ECMs) or ECM-derived nanosegments for specific cell cultivation. In PVA-IA-oligopeptide immobilized dish, we can provide both physical and biological properties on the surface by adjusting hydrogel stiffness from 10 to 30 kPa and grafting specific oligopeptides on it. Our results showed high stability of reprogrammed cells grew under PVA-IA grafted substrates having optimal elasticity. PVA-IA grafted substrates performed the highest reprogramming efficiency (0.03-0.21%). Furthermore, hiPSCs were induced to differentiate into cardiomyocyte on the biomaterials. We discuss which biomaterials and nanosegments are preferable to induce differentiation of hiPSCs into cardiomyocytes.



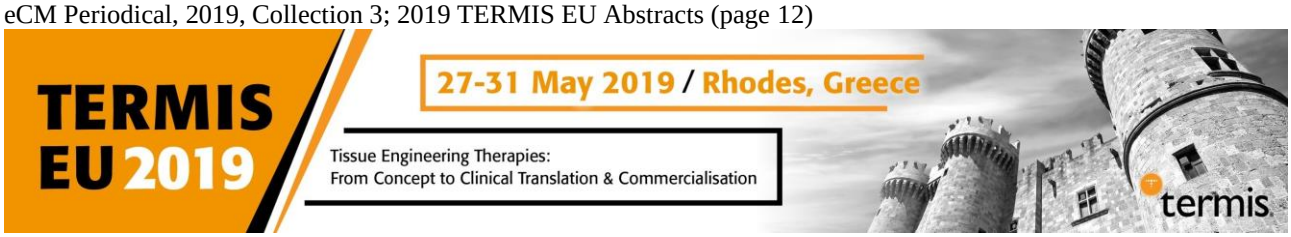
Prospects of tissue engineering in wound management

A. Piaggesi
Presenting Author: Alberto Piaggesi, alberto.piaggesi@med.unipi.it
Diabetic Foot Section, Department of Medicine, University of Pisa, Italy

The European Wound Management Association [EWMA (www.ewma.org)], is a multi-professional umbrella organization which comprehends more than 30 national organization throughout Europe, whose aim is to improve the quality of care for patients affected by chronic and acute wounds. EWMA was founded in 1991 by a group of wound care specialists who believed that focusing on wound management both from scientific, clinical and social point of view would be beneficial for the many patients affected by the various pathologies which are responsible of wounds in Europe. Since then EWMA has continuously worked to sustain and promote the research in wound management, to implement the education of health care professionals in this field, and to advocate for patients' rights and lives, both at European and global level. EWMA has international partnerships worldwide with other society in America, Asia, Africa and Australia, thus covering the whole world for wound care. EWMA developed and maintains a strong and transparent relationship with the most important companies in the field of wound management and one of its recognized roles is to connect the latest research with the potential utilizers, both companies and patients' organizations, both creating occasion of visibility for the researchers, who are encouraged to present their work at the annual scientific meeting of the association, and by publishing the EWMA Journal, which is the official paper of the association, on which the contributions of the scientists and clinicians are welcome. EWMA periodically produces position documents on various aspects of wound management, with a special interest in all the new technologies and strategies for wound management, and recently a document on advanced therapies has been released. The interest of EWMA for basic and translational sciences has been increased in recent years, and a closer relationship with the world of scientists interested in wounds and wound-related issues has been actively searched, in order to secure a most profitable and productive channel for the augmentation and amelioration of the diagnostic and therapeutic options for our patients. The participation of EWMA at TERMIS should be considered as the first bridge that has been set between the basic and translational science and the clinical fields in wound management, where EWMA proposes itself as the place to be for the year to come anyone interested in both aspects.

REFERENCES

Piaggesi A, Låuchli S, Bassetto F, Biedermann T, Marques A, Najafi B, Palla I, Scarpa C, Seimetz D, Triulzi I, Turchetti G, Vaggelas A. Advanced therapies in wound management: cell and tissue based therapies, physical and bio-physical therapies smart and IT based technologies. J Wound Care. 2018 Jun 1;27(Sup6a):S1-S137. doi: 10.12968/jowc.2018.27.Sup6a.S1.



Engineering cell niches for TERM applications exploiting peptide self-assembly

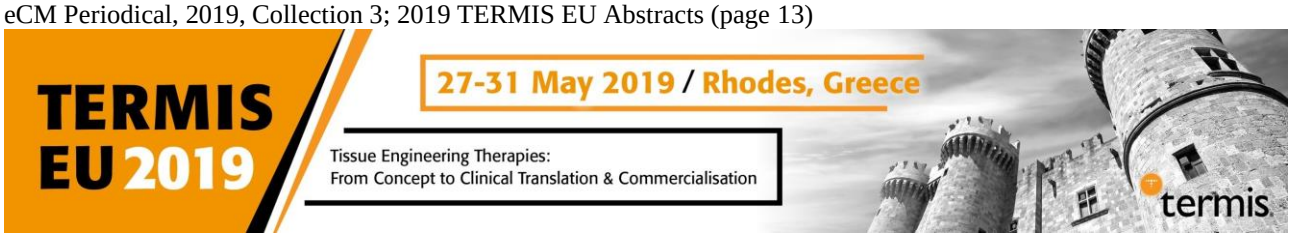
A. Saiani
Presenting Author: Alberto Saiani, a.saiani@manchester.ac.uk
School of Materials and Manchester Institute of Biotechnology, The University of Manchester, M13 9PL Oxford Road, Manchester, UK

The use of non-covalent self-assembly to construct materials has become a prominent strategy in biomaterials science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from tissue engineering to in-vivo cell and drug delivery.[1-2] A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is de-novo designed peptides.[3-4] Peptides offer a number of advantages to the biomaterial scientists. The library of 20 natural amino acids offers the ability to play with the intrinsic properties of the peptide such as structure, hydrophobicity, charge and functionality allowing the design of a wide range of materials. Synthetic peptides are chemically fully defined and being build form natural amino acids they result usually in low toxicity and low immune response when used in-vivo. Our group has focussed on the development of a technological platform for the design of novel bio-functional materials exploiting the self-assembly of β-sheet forming peptides.[5-9] The β-sheet motif is of increasing interest as short peptides can be designed to form β-sheet rich fibres that entangle and consequently form stable hydrogels. These hydrogels can be easily functionalised using specific biological signals and or drugs. Through the fundamental understanding of the self-assembly and gelation of these peptides [5-9] we have been able to design hydrogels with tailored properties for a range of applications including cell culture[9-17], tissue engineering[12-17], cell and drug delivery and 3D-bioprinting. Here I will present our group's journey from molecular self-assembly to bio-functional biomaterials and their commercialisation and the challenges encountered along the way.

ACKNOWLEDGEMENTS: Financial support was received from EPSRC, EU-FP7 (BIOSCENT), Manchester BIOGEL.

REFERENCES

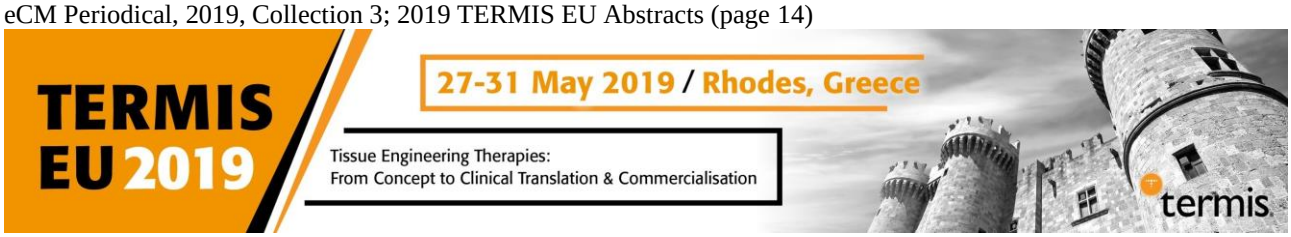
1. Zhang, S. G., Nature Biotech. 2003, 21, 1171.
2. Langer, R. et al, Nature 2004, 428, 487-492.
3. Zelzer, M. et al. Chem Soc Rev 2010, 39, 3351.
4. Caplan, M. R. et al., J. of Biomaterials Science-Polymer Edition 2002, 13, 225-236.
5. Saiani, A. et al., Soft Matter 2009, 5, 193.
6. Elsawy, M. A. et al., Langmuir 2016, 32, 4917.
7. Gao, J. et al., Biomacromolecules 2017, 18, 826
8. J Wychowaniec et al. Biomacromolecules 2018, 19, 2731
9. Burgess K. al. BioTechniques 2017, 63, 253
10. Burgess K. al. PLOS ONE 2018, 13(6), e0197517
11. Markey A. J of Pep Science 2017, 23, 148
12.-13. Castillo Diaz, L. A. et al., Journal of Tissue Engineering 2016, 7, 2041731416649789 & 2014, 5, 2041731414539344.
14. Mujeeb, A. et al., Acta Biomat 2013, 9, 4609.
15. Wan, S. et al., Acta Biomat 2016, 46, 29
16. Kumar D. et al. Adv Func Materials 2017, 27, 1702424
17. Morris O. et al. J. of Labelled Comp and Radiopharm 2017, 60, 481



High definition bioprinting – An enabling technology for TERM

A. Ovsianikov
Presenting Author: Aleksandr Ovsianikov, Aleksandr.Ovsianikov@tuwien.ac.at
Additive Manufacturing Technologies Group (https:/amt.tuwien.ac.at), Institute of Materials Science and Technology, TU Wien, Vienna, Austria, Austrian Cluster for Tissue Regeneration (www.tissue-regeneration.at)

3D bioprinting and biofabrication are already providing disruptive solutions for tissue engineering and regenerative medicine (TERM). However, the most widespread technologies are based on computer-controlled deposition of cells or assembly of cellular units, and thus cannot achieve spatial resolution better than few tens of micrometres. Lithography-based methods approach the problem from a different direction, by producing 3D structures within cell-containing materials and can therefore overcome this limitation. Among these methods, multiphoton lithography (MPL) is an outstanding one as it can produce features even smaller than a single mammalian cell (down to around 100 nm). Our recent breakthroughs on the material development side enabled the use of MPL for direct fabrication of cell-containing constructs, giving rise to High-Definition Bioprinting. HD Bioprinting is able to recreate fine tissue structure with an unprecedented accuracy using biomimetic hydrogels closely resembling extra-cellular matrix (ECM). The further development of HD Bioprinting will facilitate the realization of elegant biological experiments, helping to elucidate biomimetic aspects of cell interaction with the surrounding environment. Our results demonstrate a wide range of exciting applications, from controlling morphology of stems cells in 3D to modelling barrier tissues in vitro and patterning of hierarchical vascular networks using co-culture of endothelial and stem cells. In this contribution the principles of HD Bioprinting, its recent progress of as well as its perspectives for further TERM applications, will be discussed. The presentation is supported by numerous examples.



Bone marrow niche and blood cell formation

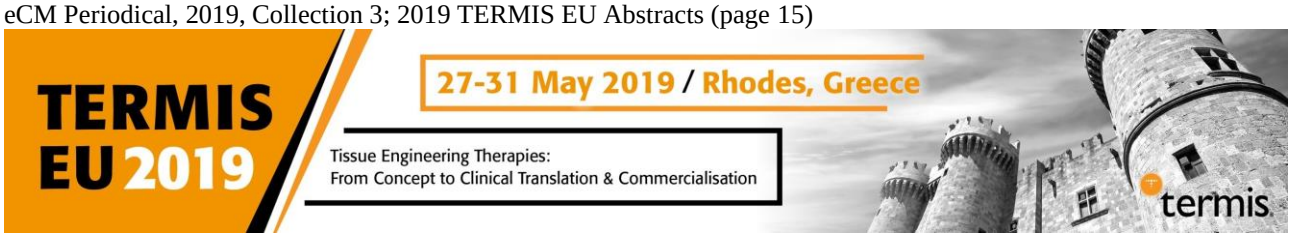
A. Balduini [1,2]
Presenting Author: Alessandra Balduini, alessandra.balduini@unipv.it
[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy [2]Department of Biomedical Engineering, Tufts University, Medford, MA, USA

Bone marrow failure is the result of diseases, trauma, or cancer treatments, leading to a decreased production of blood cells and consequently, the necessity of blood transfusions. A number of studies point to the bone marrow niche as the core of blood cell production but with many interesting complex environmental factors for consideration. Thus, future advancement in the study of blood cell production will depend on the evolution of bioengineering techniques for reproducing physiologically relevant conditions in the bone marrow niche environment. To achieve this goal, the field is moving towards the reproduction of the characteristic features of the physiologic bone marrow microenvironment ex vivo by the use of relevant biomaterials and bioreactors, along with appropriate human cell sources. These models are expected to provide better mechanistic understanding and control of blood component production as well as insight towards the development of systems for the generation of functional blood cells necessary in transfusion and regenerative medicine to replace blood-donor supply. We have successfully developed different bioreactor systems, using silk fibroin, mimicking the bone marrow environment and thereby supporting haematopoiesis and megakaryopoiesis to generate significant numbers of human platelets ex vivo [1-2]. Silk fibroin, derived from Bombyx mori silkworm cocoons, is a promising biomaterial for bone marrow tissue engineering because of its tunable architecture and mechanical properties, the capacity of incorporating labile compounds without loss of bioactivity and demonstrated ability to support platelet production without premature activation. Herein, our experience with bone marrow niche structure and composition, in combination with ex vivo models, in physiological and pathological conditions, will be discussed.

ACKNOWLEDGEMENTS: Cariplo Foundation (2013-0717), US National Institutes of Health (R01 EB016041-02), H2020-FETOPEN-1-2016-2017-SilkFUSION.

REFERENCES

[1] Di Buduo CAet al. Blood. 2015 125(14): 2254-64.
[2] Di Buduo CAet al. Biomaterials, 2017. 146:60-71

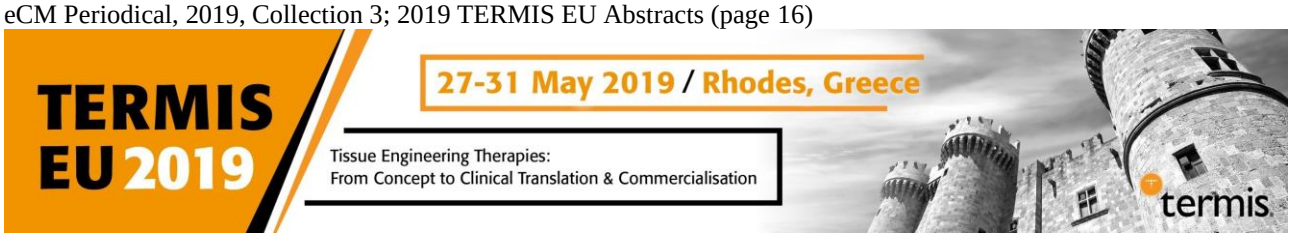


Prospects of tissue engineering in wound management

A.P. Marques[1,2,3]
Presenting Author: Alexandra P. Marques, apmarques@i3bs.uminho.pt
[1]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Avepark 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark 4805-017 Barco, Guimarães, Portugal

Tissue Engineering is currently one of the most promising areas of research aiming developing substitutes to restore, maintain or improve the function of a specific tissue. While the first tissue engineering product was a skin substitute, Apligraf®, and skin wound care has the longest history of clinical application and the highest record of marketing, current options proved to be replacement strategies rather than promoting tissue regeneration. In the case of chronic wounds, delayed healing is also associated to a very high recurrence rate to which current wound management approaches are not sufficiently effective to respond to. Healed tissue of higher quality, which is the outcome of an adequate healing progression and is critical to reduce wound recurrence rate, is therefore the outcome to pursue. Tissue engineering will certainly play a key role in targeting this issue by developing skin substitutes with improved functionality capable of directing the healing mechanisms into the regenerative pathway. On the other side, tissue engineering also allows, by taking advantage of the tailoring of biomaterial features, to advance wound dressings. Both perspectives require the need to better understand the pathophysiology of each wound as well its progression under specific conditions to be able to control their therapeutic action. Gene therapy or gene editing approaches are examples of how a better knowledge of the healing factors or of a skin disease is a demand for a successful outcome. However, as one solution does not fit all wounds, future therapies must be adapted to the healing stages. For example new methods have been employed to create innovative 3D matrices with intrinsic features capable of respond in situ to micro-environmental wound stimuli. In these cases, the temporal changes can determine the release, at a specific time point, of a mediator that was missing for the adequate progression of the healing. Interestingly, this is also in line with 4D printing, an emergent area of the field of tissue engineering that integrates time as the fourth dimension. In fact, in addition to a requirement for an automated process, the complexity of skin tissue and consequently of its healing, is one of the reasons for the use of 3D printing technologies to develop improved and more realistic tissue engineered skin grafts. In conclusion, being a multidisciplinary area in which different expertise stakeholder actively collaborate, tissue engineering reunites the ideal setting to improve wound healing therapies holding therefore great prospects for this area.

ACKNOWLEDGEMENTS: Authors acknowledge the financial support from FCT/MCTES under the scope of IF/00945/2014, H2020-TWINN-2015 Gene2Skin, M-ERA-NET2/0013/2016 Dressing4Scars and 726061 ERC-2016-CoG ECM_INK grants.



Engineering the stem cell niche – Translation to the clinic

A. El Haj
Presenting Author: Alicia El Haj, a.elhaj@bham.ac.uk
Healthcare Technology Institute, Institute of Translational Medicine University of Birmingham UK

Engineering complex tissues creates major challenges for tissue engineering and regenerative medicine strategies. Complex form and function in tissues comprise multiple musculoskeletal tissues with cross-tissue integration and junctions. Our lab has been involved in understanding the ways we can engineer a stem cell niche which can go on to regenerate tissues. Our aim is to deliver donor progenitor cells which can build and define replacement tissues in vivo. The challenge lies in engineering control solutions for stem cells which can re-engineer the surrounding environment in dynamic and static conditions with clinical relevance. We have developed multiple control systems which can be aligned with cell based therapies and are currently translating these approaches towards first in man. Using experimental and theoretical modelling, we have designed tissue models which allow us to test control strategies in vitro. New strategies for labelling and defining tissue turnover and dynamics have been designed which can be used for validating our tissue models and defining success[1]. In this presentation, I will describe the development of our novel stem cell bandage which can be used for directing and maintaining stem cell communities in bone and cartilage[2]. I will present our work in nanomedicine with remote magnetic particle control strategies for stem cell activation and optimised differentiation which is now in preclinical trial[3]. Finally, I will align these strategies to potential treatments for conditions such as in Orthopaedics and Craniofacial repair. Our aim is to translate these approaches towards a clinical solution for patients.

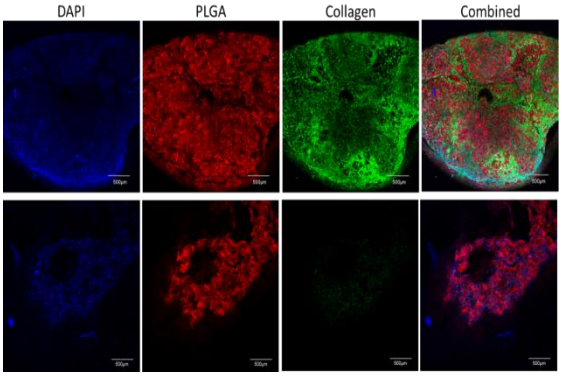
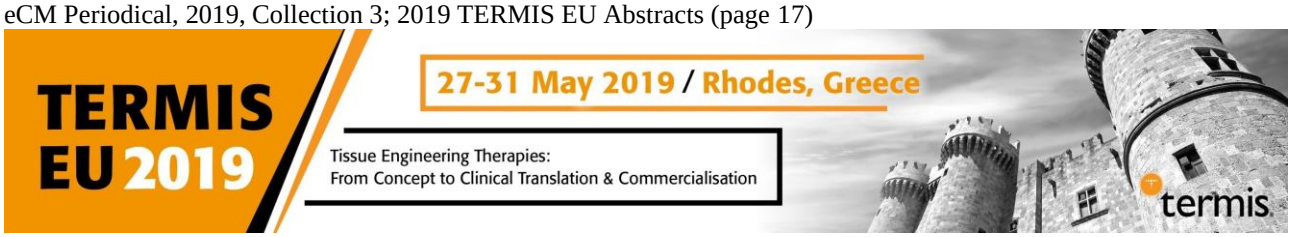


Figure 1: Fluorescent monitoring of collagen matrix production and biomaterial degradation using azide-L-proline chemistry;Scale bars=500mm. Color images available online at www.liebertpub.com/tec (1)

REFERENCES

[1] Bardsley K, Yang Y, El Haj Aj. (2017) Fluorescent Labeling of Collagen Production by Cells for Noninvasive Imaging of Extracellular Matrix Deposition. Tissue Eng Part C Methods. 23(4):228-236.
[2] Loundes, M, Rotherham, M., El Haj AJ, Habib S (2016) Immobilised Wnt proteins act as a stem cell niche for tissue engineering Stem Cell Reports Jul 12;7(1):126-37
[3] Markides H, Mclaren J, Telling, N, Alom N, Al-Mutheffer E, Oreffo R, Zannettino, A, Scammell B, White L, El Haj A.J. (2018) Translation of remote control regenerative technologies for bone repair. NPJ Regen Med. 2018 Apr 17;3:9.



Self-assembling peptide hydrogels for 3D cell culture; From molecular self-assembly to commercial product

A.F. Miller[1,2]
Presenting Author: Aline F. Miller, a.miller@manchesterbiogel.com
[1]Manchester BIOGEL, 19F4 Mereside, Alderley Park, Alderley Edge, Cheshire, SK10 4TG, United Kingdom [2]School of Chemical Engineering and Analytical Science, Manchester Institute of Biotechnology, 131 Princess Street, University of Manchester, Manchester, M1 6NF, United Kingdom

Over the past two decades, significant effort has been made to develop soft materials, i.e.: hydrogels, for the design of 3D cell niches. Taking inspiration from the many millennia proteins have had to perfect their structure-property-function relationships, self-assembling peptides have emerged as a class of promising material building blocks as has they allow the design of very stable, highly versatile hydrogels for use in a variety of biomedical applications (see Figure 1). We have developed a platform for the design of hydrogels exploiting the self-assembly of short (8-9 amino acids) β-sheet forming peptides, and the design is based on the alternation of hydrophilic and hydrophobic residues. The properties of these materials (e.g.: functional and mechanical) can easily be tailored to accommodate different cells' needs. We have used these novel materials for the culture of a variety of cells including: chondrocytes, osteoblasts, nucleus pulposus as well as mesenchymal stem cells. By varying the properties of the hydrogels (e.g.: stiffness, charge) we were able to design 3D niches capable of directing cell behaviour. We are currently using these novel materials in a variety of tissue engineering and regenerative medicine applications (e.g. cardiac regeneration, Barrett's oesophagus disease). Moreover we are using the scaffolds to build 3D vascularized tumour models and physiologically relevant gut models. Our results clearly demonstrate that our materials offer great promise for the design of specific 3D cell niches due to their low immunogenicity, high biocompatibility and the ability we have to control and tailor their properties. We are currently commercializing a range of these materials to offer researchers and clinicians an easy to use and ready to use matrix for 3D cell culture, 3D bioprinting and drug delivery applications. These materials are fully synthetic and have no batch to batch variation hence can be clinically translated.

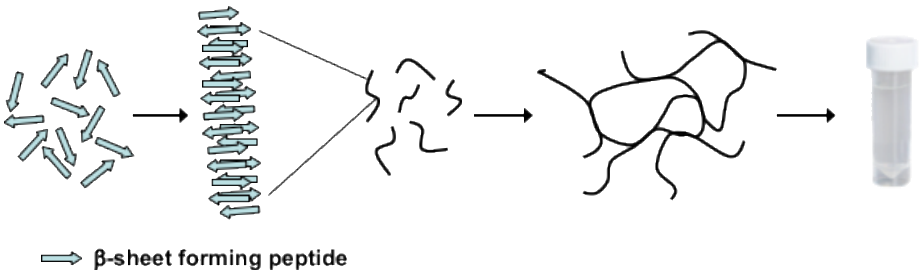
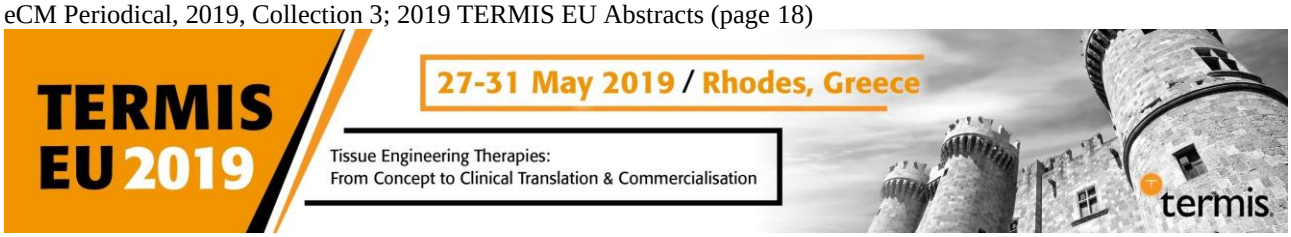


Figure 1: Schematic of the self-assembly of alternating amphiphilic oligo-peptides into beta-sheet rich fibres which entangle to form a 3D hydrogel with structure and properties reminiscent of natural extracellular matrix.

REFERENCES

[1] Kumar D et al. Adv. Func. Mater. 2017; 27:1702424
[2] Raphael B et al. Materials Letters 2017; 190:103-106
[3] Wan S et al. Acta Biomaterialia 2016; 46:29-40



Transforming proteins into functional biomaterials for complex tissue engineering

A. Mata[1]
Presenting Author: Alvaro Mata, a.mata@qmul.ac.uk
[1]School of Engineering & Materials Science, Queen Mary University of London, London, UK

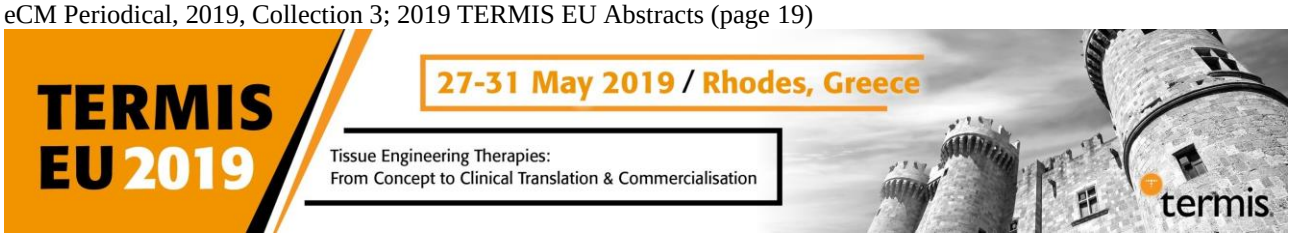
There is great interest to develop new materials with properties that resemble those of biological systems such as hierarchical organization, the capacity to grow or self-heal, and the ability to guide complex biological processes. These kinds of materials would open opportunities to engineer tissues with a much-needed higher level of complexity and functionality. To this end, supramolecular chemistry offers an exciting opportunity to grow such materials from the bottom-up using molecules and processes found in nature. However, the ability to transform molecular and nano-scale design into functional devices with practical utility at the macroscale remains a challenge. The talk will describe new strategies that integrate supramolecular chemistry with engineering principles to develop practical materials with tuneable and advanced properties such as hierarchical organization, the capacity to grow, tuneable mechanical properties, and specific bioactivity [1-3]. These materials are being used towards new regenerative therapies of tissues such as enamel, bone, and blood vessels as well as more biologically relevant in vitro models for applications such as cancer and neurological disorders.

Figure 1: Sample images of functional materials and devices made by supramolecular biofabrication.

ACKNOWLEDGEMENTS: Financial support was received from the ERC Starting Grant (STROFUNSCAFF), The Marie Curie Integration Grant FP7-PEOPLE-2013-CIG (BIOMORPH), and the AO Foundation.

REFERENCES

[1] Hedegaard et al (2018). Advanced Functional Materials 10.1002/adfm.201703716.
[2] Elsharkawy et al (2018). Nature Communications 10.1038/s41467-018-04319-0.
[3] Inostroza-Brito et al (2015). Nature Chemistry 7(11), 897-904. 10.1038/nchem.2349.



Dual alginate crosslinking and two degradation modes for local patterning of biophysical and biochemical properties

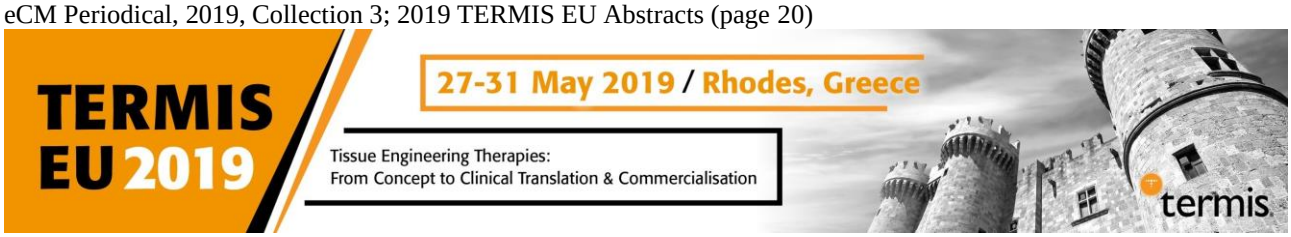
A. Cipitria[1]
Presenting Author: Amaia Cipitria, amaia.cipitria@mpikg.mpg.de
[1]Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany

Native microenvironments are dynamic and exhibit biophysical and biochemical cues orchestrated in space and time. Biomaterials with tunable mechanical and degradation properties, and spatial control of polymer-bound biomolecules can be used to mimic the dynamic cellular microenvironment that cells are exposed to and thereby recapitulate the reciprocal interplay between the cells and extracellular matrix. We have developed an alginate-based material system that relies on two crosslinking types and degradation modes: (i) spontaneous Diels-Alder click chemistry of norbornene and tetrazine functional groups, with the potential to impart passive, hydrolytic degradation via oxidation [1] and (ii) UV-initiated thiol-ene click chemistry with the potential to impart enzymatic degradation via incorporation of MMP-sensitive peptide crosslinkers. Enzymatically degradable alginate hydrogels were fabricated by combining norbornene-modified polymer and MMP-sensitive peptides. The degree of crosslinking and stiffness were controlled by the polymer molecular weight and crosslinker concentration. The degradation kinetics were characterized by changes in wet weight upon incubation in collagenase. Mouse embryonic fibroblasts were encapsulated into degradable and non-degradable controls and cell viability and morphology were tracked over 14 days. Cell viability remained high for all conditions, spread morphology was observed in degradable gels with a significant difference in the cell longest axis, while cells remained round in non-degradable gels. Ongoing work includes tracking of in-vivo degradation upon subcutaneous implantation in mice via fluorescence imaging and ex-vivo characterization of cell and tissue infiltration. Diels-Alder and thiol-ene click crosslinking were combined orthogonally for dual crosslinked materials. Patterns in stiffness were generated by spatially controlling the location of UV-mediated thiol-ene crosslinking via photomasks with stripes of 500 um thickness, and confirmed by micro-indentation surface mapping. Fibroblasts seeded on these materials exhibited a different morphology, with spread cells aligned along the channel axis on stiff regions, and round cells on soft regions. Patterns in biomolecules for cell attachment were achieved by first crosslinking the gel with Diels-Alder chemistry and then coupling a thiol-RGD peptide on regions exposed to UV through photomasks. Ongoing work explores the effect of 3D patterned hydrogels and polymer-bound bone morphogenetic protein-2 and peptide mimetics to guide the osteogenic differentiation of primary human mesenchymal stem cells. This biomaterial-based strategy allowing spatial patterning of biophysical and biochemical cues could direct cell behavior and support guided tissue regeneration.

ACKNOWLEDGEMENTS: Financial support was received from the German Research Foundation (DFG grant CI 203/1-2 and grant CI 203/2-1).

REFERENCES

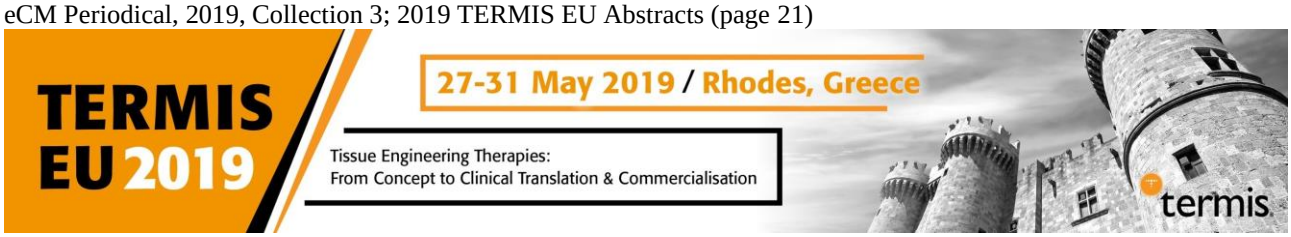
[1] Lueckgen A et al. Biomaterials. 2018; 181:189-198.



Bioengineering materials for orthopedic regenerative medicine

A.M. Ferreira Duarte
Presenting author: Ana M. Ferreira Duarte, ana.ferreira-duarte@ncl.ac.uk
School of Engineering, Newcastle University, Newcastle-Upon-Tyne, UK

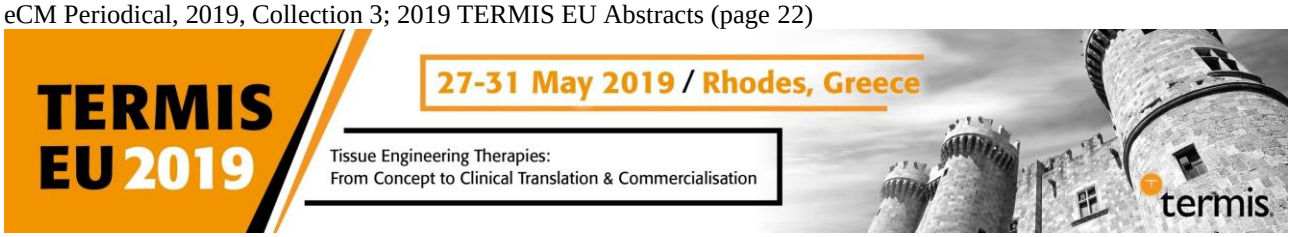
Although only few cell therapies are currently available in clinics that allow the regeneration of a damaged tissue, cell therapies have the potential to treat a broad range of diseases and change the way on how medicine is practiced. Among the main challenges that limit the widespread clinical use of cell therapies in orthopaedics are the maintenance of cell functions, reproducible cell expansion for transplantation, protection and control of cells condition pre- and post- transplantation in patients. Biomaterials offer the possibility to tune the bio-chemical and mechanical properties to overcome these difficulties by facilitating transplanted cell survival, therefore speeding-up the drug discovery and screening to treat diseases, and accelerate the clinical application of cell-based therapies. Synthetic and natural polymers are often used to recruit specific cells to support or guide their function for therapy. Moreover, biomaterials can be modified to regulate specific cellular functions such as migration, attachment, proliferation and differentiation by incorporating mechanical and/or chemical cues. The combination of advanced biomaterials and bio-fabrication technologies (e.g. bioprinting) can facilitate the control and reliability of cell manipulation procedures and protocols for developing and scaling up effective therapies. In our recent works, we demonstrated the importance of cell density in the development of bone microtissues derived from human bone marrow stem cells and its effect in the tissue maturation rates, as a result of bioprinting high cell densities (e.g. 40 million cells/ml) in low-viscosity and natural-based bioinks. Herein, the combination of materials and use of advanced additive manufacturing technologies for the development of orthopaedic cell therapies will be discussed.



Novel biodegradable dendritic block copolymers as nucleic acid vectors to the central nervous system

A.P. Pêgo
Presenting author: Ana P. Pêgo, apego@ineb.up.pt
INEB – Instituto de Engenharia Biomédica / i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto; Faculdade de Engenharia da Universidade do Porto (FEUP), Porto, Portugal; Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal

Dendrimers are attractive carriers for several bioactives due to their unique structural features: globular, well-defined, very branched and controllable nanostructure, low polydispersity and multivalency. Among such bioactives nucleic acids (NAs) are readily compacted into nanostructures named “dendriplexes”, when complexed with positively charged dendrimers that are able to protect them from enzymatic degradation and rapid renal clearance after i.v. administration. However, one important disadvantage of the most commonly used dendrimers is their non-degradability under physiological conditions, that can lead to toxicity by bioaccumulation. Moreover, in the gene therapy field, vector stability can further hinder the intracellular release of the NA, consequently leading to low transfection efficiencies (TE). Therefore, recent interest has focused on the development of biodegradable dendrimers, but only few works report their biomedical applications. Here, we present a new family of fully biodegradable and biocompatible PEG-dendritic block copolymers, as well as their function as nucleic acid vectors. Interestingly, the fully degradable character was crucial for a better nucleic acid release from the dendriplexes, contributing to an amazing improvement of the TE compared to their hybrid biodegradable counterparts. The ultimate application we are seeking for the proposed dendrimer-based vectors is the nucleic acid delivery to neuronal cells in the brain. Consequently, neuronal targeting is being explored.



Therapeutic vascularization of skeletal muscle

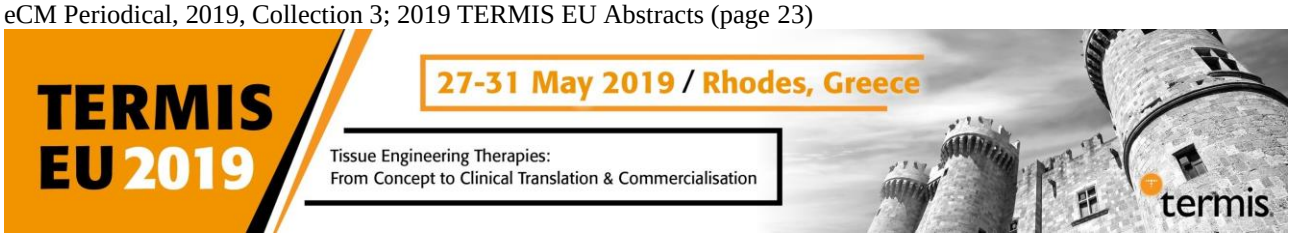
A. Banfi[1,2]
Presenting Author: Andrea Banfi, andrea.banfi@usb.ch
[1]Cell and Gene Therapy, Department of Biomedicine, Basel University, Basel, Switzerland, [2]Department of Surgery, Basel University Hospital, Basel, Switzerland

Therapeutic angiogenesis in skeletal muscle, i.e. the generation of new vessels by delivery of specific factors, is required both for rapid vascularization of engineered grafts and to treat ischemic conditions. In particular, muscle tissue presents some specific features, like a high density of capillary networks and a paucity of extracellular matrix between myofibers, which require consideration when promoting muscle angiogenesis. Vascular Endothelial Growth Factor (VEGF) is the master regulator of angiogenesis. However, uncontrolled expression leads to vascular tumors (angiomas). Major challenges to fully exploit VEGF potency for therapy include the need to precisely control the in vivo distribution of growth factor dose and its duration of expression. We previously found that the therapeutic window of VEGF delivery depends on the VEGF amount in the microenvironment around each producing cell rather than on the total dose, since VEGF remains tightly bound to extracellular matrix and a few "hotspots" of high expression are sufficient to cause angioma growth even if the total dose is rather low. On the other hand, short-term expression of less than about 4 weeks leads to unstable vessels, which promptly regress following cessation of the angiogenic stimulus. Here I will present recent work aimed at: 1) translating fundamental principles of VEGF function into clinically applicable approaches to induce controlled angiogenesis, through the use of recombinant factors-decorated fibrin matrices [1]; and 2) investigating the mechanisms that regulate vascular stabilization and the switch between normal and aberrant angiogenesis in skeletal muscle in vivo, to identify novel molecular targets to improve both the safety and efficacy of VEGF [2-3].

ACKNOWLEDGEMENTS: Financial support was received from the Swiss National Science Foundation (163202 and 182357); and H2020 Research and Innovation Action ELASTISLET (646075).

REFERENCES

[1] V. Sacchi et al. (2014) Proc. Natl. Acad. Sci. U.S.A. 111:6952-6957.
[2] E. Groppa et al. (2015) EMBO Mol. Med. 7:1366-1384.
[3] E. Groppa, S. Brkic et al. (2018) EMBO Rep. e45054



Response of mesenchymal stem cells to flow in the Nichoid 3D microstructured substrate

A. Remuzzi[1]
Presenting Author: Andrea Remuzzi, andrea.remuzzi@unibg.it
[1]Department of Management, Information and Production Engineering, University of Bergamo

INTRODUCTION: The communication between stem cells and the microenvironment affects cell fate and behavior. Physical and mechanical factors have a crucial role in triggering mesenchymal stem cells (MSCs) response. Here, we assess the effect of flow exposure on MSCs cultured in 2D conditions and in the Nichoid nano-engineered 3D substrate [1].

METHODS: We designed and constructed a perfusion chamber and a closed circuit. Rat bone marrow MSCs were seeded on 2D or 3D culture supports. Substrates were then maintained in static conditions or exposed to a wall shear stress of 3 dyn/cm^2. Morphological analysis, SEM analysis, and immunocytochemistry (ICC) were performed at 48 hours.

RESULTS & DISCUSSION: After flow exposure 2D-cultured cells displayed a more elongated morphology, while Nichoid-cultured cells were strongly anchored to the 3D structure. ICC showed that MSCs cultured in cell monolayer have large and flattened cytoplasm and nuclei, while on the Nichoid they have long actin filaments and small but spherical nuclei. Dynamic conditions induced F-actin fibers thickening on both substrates. The 3D Nichoid substrate, as well as flow exposure, induced a different intracellular localization of the mechanotransducer YAP, consisting in an increased cytoplasmic retention of this transcriptional co-activator, confirming that mechanical cues deriving from cell anchorage to the structure and from shear stress are able to influence stem cell response.

CONCLUSIONS: Our versatile and optically accessible system can be used to reproduce physiological flow conditions, able to influence cell response, both on 2D and 3D culture substrates.

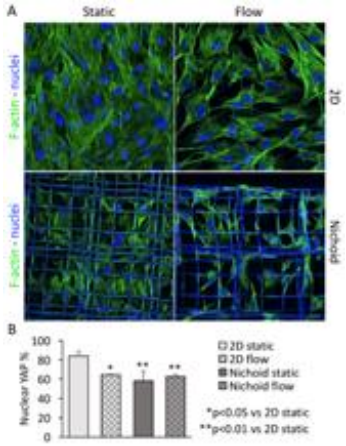
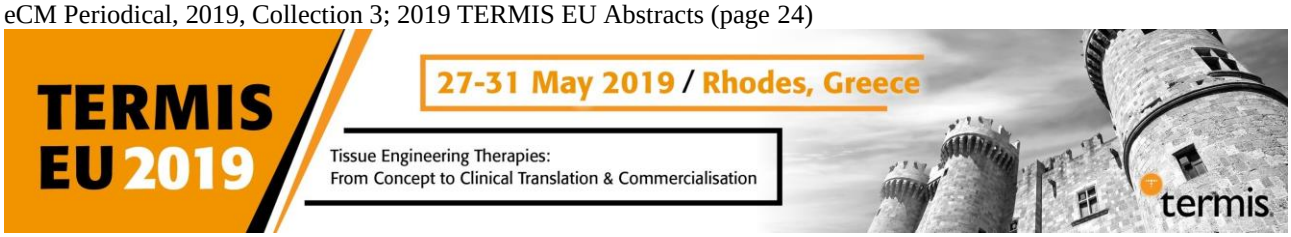


Figure 1: (A) Cytoskeletal organization of MSCs on 2D or 3D substrates in static or dynamic culture. (B) 3D culture conditions and flow exposure induced a decrease in YAP nuclear localization.

ACKNOWLEDGEMENTS: European Research Council, grant no. 825159 - MOAB.

REFERENCES

[1] Nava MM et al. Stem Cell Res Ther. 2016;7(1):132



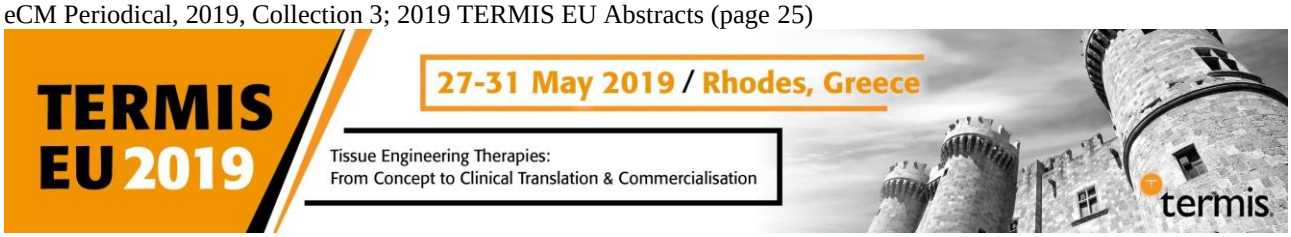
On the convergence of the sciences and why veterinary science can take center stage in tissue engineering innovation

A. Kaasi[1,2]
Presenting Author: Andreas Kaasi, ak@evascientific.com
[1]Eva Scientific Ltd., São Paulo, SP, Brazil [2]Santa Casa de São Paulo, Institute for Research, Technology Innovation and Education, São Paulo, SP, Brazil

Veterinary science is embedded, though often inconspicuously, into many of the other sciences and facets of human culture. The involvement may take place in different phases of each specialty, ranging from research, development, production, sales and marketing and support, here connoting the versatility of veterinary science in the different stages of the traditional innovation life cycle. As an example of an involvement in research and development, the one most familiar to us engaged in Tissue Engineering and Regenerative Medicine (TERM), veterinarians run vivariums for biological and medical R&D. In production, vets are required by law to be involved in the safety and efficacy of tissues such as meat and dairy from normal animals for human consumption, and also of tissues from intentionally modified animals, such as transgenic animals seeking an increased yield or enhanced properties, such as humanized cow milk [1]. As for the sales, marketing and support activities, vets play a role in the commerce, beautification (grooming) and training (sports) of small and large animals. Multidisciplinarity is, thus, a central trait of veterinary science and of the individual vet, yet their potential of interdisciplinarity has perhaps not been exploited optimally. Tissue engineering, a branch of science that is both multidisciplinary and interdisciplinary at the same time, would benefit greatly of a more intimate involvement of veterinary scientists and clinicians. Speaking from a personal perspective, our work with decellularized tissues such as heart valves [2], or decellularized whole organs, such as the kidney or pancreas [3], insights of veterinary anatomy nature that the aortic valve right coronary cusp is muscular[4], that the kidney is similar albeit with some differences [5] and the pancreas vastly different [6], aid in the process of engineering these tissues and organs. The concepts of “One Medicine” [7], and “One Health”[8] have both been around for centuries, forming useful links between veterinary and human medicine and health, respectively. The more recent shift from a pure medical (diagnosis, treatment and prevention of disease in humans and animals) to a health (well-being in humans and animals) focused collaborative initiative, signals a direction of increasingly transdisciplinary and holistic approaches. The next shift involves the impact of tissue engineering methods on the health or state of animals, the impact this has on the tissues and organs of these animals which we, humans, consume for food, and the impact this has on our psychology and culture. Besides these examples, this talk will explore additional situations or opportunities wherein veterinary professionals already are or would be indispensable for TERM.

REFERENCES

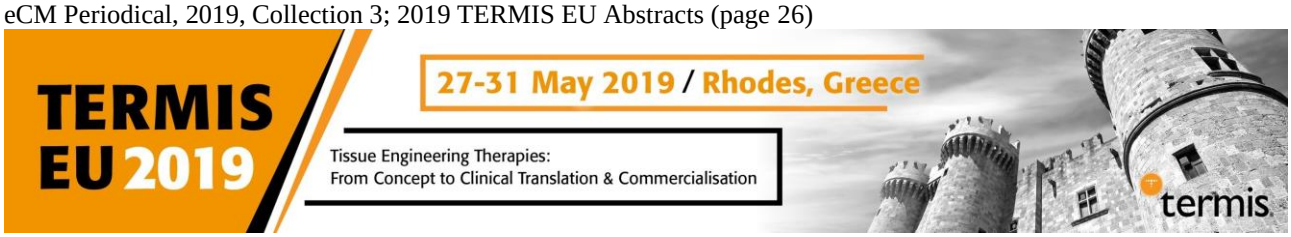
[1] Kaiser G et al. J Dairy Sci. 2017;100(3):1605–17.
[2] Kaasi A et al. J Tissue Eng Regen Med. 2011;5(4):292–300.
[3] de Souza F et al. J Biomimetics, Biomaterials and Biomedical Engineering. 2018; 38:67-74.
[4] D’Cruz I. Echocardiographic anatomy: understanding normal and abnormal echocardiograms. 1996.
[5] Mohammed-Ali Z et al. In: Conn PM, editor. Animal Models for the Study of Human Disease. 2017.
[6] Tsuchitani et al. J Toxicol Pathol. 2016;29(3):147–54.7.
[7] Schwabe C. Veterinary Medicine and Human Health. 1984.
[8] Mahr R. Vet Rec. 2014;174(5):124–124.



SPARC-mediated mechanotransduction in tendon homeostasis and tissue interface maturation

A. Traweger
Presenting author: A. Traweger, andreas.traweger@pmu.ac.at
Paracelsus Medical University, Institute of Tendon and Bone Regeneration, Spinal Cord Injury & Tissue Regeneration Center Salzburg, Austria

Tendons and tendon interfaces display a poor regenerative capacity and injuries remain clinically challenging. Tendons sense mechanical load, however our knowledge on how external loading affects tendon homeostasis and degeneration remains fragmentary. Here we provide evidence that the matricellular protein SPARC is critically involved in the mechanobiology of tendons and is required for homeostasis and tissue interface development. We show that loading tendons at the early postnatal stage resulted in tissue hypotrophy and the fibrocartilaginous transition zone at the Achilles tendon enthesis was impaired in mice lacking SPARC. Further, tendon insertion length at the myotendinous junction was significantly shorter and was characterized by fatty infiltrations. In vivo, treadmill training led to a higher prevalence of spontaneous tendon ruptures and a net catabolic adaptation in SPARC-/- mice. Interestingly, tendon hypoplasia was attenuated in SPARC-/- mice in response to muscle unloading with botulinum toxin A injection. In vitro culture of tendon organoids showed that, while the constructs from both WT and SPARC-/- mice responded to uniaxial loading by increased AKT phosphorylation, the downstream activation of S6K was only impaired in organoids derived from SPARC-/- mice, resulting in the reduction of type I collagen synthesis. To underscore the clinical relevance of our findings, we have further revealed that a novel missense mutation in SPARC is associated with a full-thickness tear of the rotator cuff in patients. Together we provide evidence that SPARC is an essential matrix protein necessary for mechanotransduction in load-induced tendon and tissue interface maintenance.



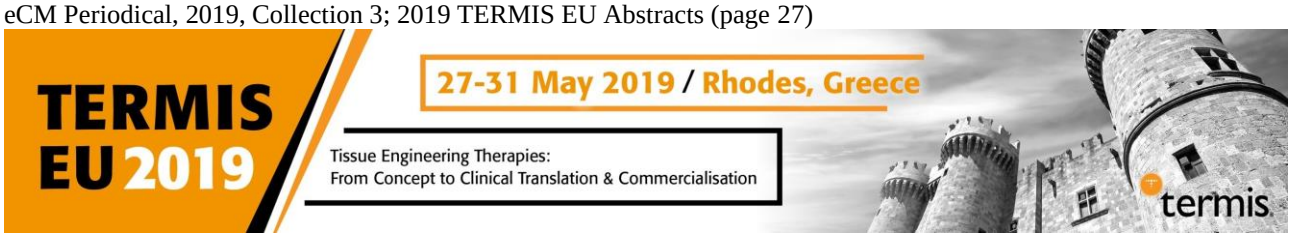
Seeing is believing: Application of radiopaque biomedical polymers in interventional medicine

A. L. Lewis
Presenting Author: Andrew L. Lewis, andrew.lewis@btgplc.com
VP R&D Innovation, Biocompatibles UK Ltd, Lakeview, Riverside Way, Watchmoor Park, Camberley, Surrey, GU15 3YL, UK

X-Ray Radiopacity: A growing number of therapeutic procedures are being conducted using minimally-invasive approaches, usually under the guidance of some type of imaging modality in order to navigate the therapy to the target site, and in some cases to subsequently assess the effectiveness of the treatment. X-ray based imaging techniques such as fluoroscopy and computed tomography (CT) are commonly employed for real time intra- and post-procedural imaging. Certain materials, such as most metals, can absorb the X-ray energy (a property known as radiodensity or radiopacity) and can therefore be clearly seen using X-ray imaging, whereas conventional biomedical polymers are generally radiolucent and must be modified in order to render them specifically radiopaque. **Radiopaque Embolisation Devices:** Embolisation devices are used to divert blood flow or completely occlude the lumen of vessels for a variety of purposes, including prevention of internal bleeding, treatment of organ dysfunction or blocking of vessels that feed benign or malignant tumours. The devices can range from metallic coils to particulate and liquid-based systems usually composed of polymeric materials. Liquid systems are normally mixed with radiopaque components at the point of use to introduce radiopacity, whilst particulate embolics have been traditionally radiolucent. Recently there have been significant advances in facile chemical routes to the introduction of radiopaque groups onto the polymer structures to confer inherent radiopacity [1]. We investigated many different methods for adding radiodense atoms such as iodine, barium, and bismuth into embolic particles, to create a Radiopaque Bead (ROB). Covalent attachment to the preformed beads using an aromatic iodinated species similar to that used as the basis of soluble contrast agents offered the best balance between radiopacity and ability to handle and deliver the ROB. Optimisation of iodine content was established in vitro by imaging assessment using a variety of X-ray based methods using ROB phantoms. In vitro analyses of mechanical properties, flow penetration, suspension times, microcatheter delivery and robustness and drug loading/elution were undertaken to fully characterise the device. ROB distribution, imaging and long-term biocompatibility was established with rabbit and sheep renal embolisation studies, a rabbit VX2 tumour model and a long-term swine hepatic artery embolisation study using micro-CT, fluoroscopy and CBCT and MDCT, prior to moving to First in Man clinical evaluation. The ability to see where the ROB are going is enabling the physician to more precisely target the area of treatment, identify areas of potential under-treatment and improve patient safety by detecting any off-target embolization – together with the benefits of the sustained localised release of chemotherapeutic agent.

REFERENCES

[1] Lewis AL, et al, Future Oncology, 2018 in press.



Secretome from placental progenitor cells and its role in large animal reproductive regenerative medicine

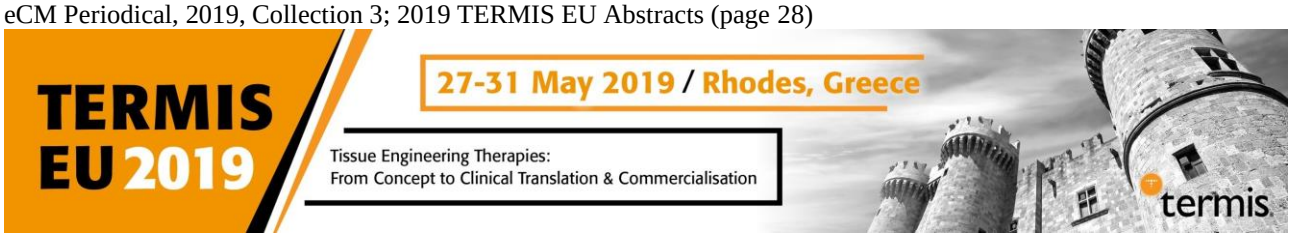
A. Lange-Consiglio[1,2]
Presenting Author: Anna Lange-Consiglio, anna.langeconsiglio@unimi.it
[1]Department of Veterinary Medicine (DIMEVET) Università degli Studi di Milano, Milano, Italy
[2]Centro Clinico-Veterinario e Zootecnico-Sperimentale di Ateneo, Università degli Studi di Milano, Lodi, Italy

Since the engraftment of the transplanted mesenchymal stem cells (MSCs) has been only partially documented, it is suggested that paracrine-mediated signals originating from MSCs must be implicated in tissue regeneration. It is possible that microvesicles (MVs) or soluble factors contained in conditioned medium secreted by MSCs, are mediators of cell-to-cell communications that ultimately lead to tissue repair [1]. It has been suggested that a large number of molecular mediators and MVs are involved in the early embryo-maternal interaction, too. Indeed, equine embryos at day 8 are thought to secrete MVs, which can modulate the functions of the oviductal epithelium through transferring early pregnancy factor and micro-RNAs [2]. From the maternal side, endometrium-derived MVs revealed to have potential targets in biological pathways highly relevant for embryo implantation [3]. In this context, infertility could occur as a malfunction of the endometrium-embryo 'dialogue' when the endometrial production of these mediators could be impaired by persistent endometritis. In view of restoring the fully functional embryo-maternal communications, and to treat endometritis that reduce conception rate and increase delivery-to-conception intervals in both bovine and equine species, regenerative medicine using cell free products from MSCs is proposed as a new approach. In addition, considering that MVs are involved in the regulation of gene decisive in biological processes such as gametogenesis, fertilization, implantation and embryo development, amniotic derived MVs are suggested during bovine in vitro embryo culture and cryopreservation to evaluate survival rate after cryopreservation and on pregnancy rate after embryo transfer of fresh or cryopreserved MV-exposed embryos. Basing on our data, these MVs up-regulate the expression of GPX1 gene and down-regulate the expression of BAX gene improving the survival rate following cryopreservation and the rate of pregnancy, probably for limited lipid peroxidation and apoptosis damages. Least but not last, to date the conditioned medium (CM) obtained from in vitro MSCs has been proven to be sufficient to stimulate the structural and functional regeneration of cardiac, renal, spinal cord, and tendon tissues [4] by a paracrine action due to the action of bioactive factors. In this perspective, we examined whether administration of CM generated from amniotic mesenchymal cells could be useful as mastitis therapy in cattle.

ACKNOWLEDGEMENTS: Grants from Università degli Studi di Milano, Italy is acknowledged.

REFERENCES

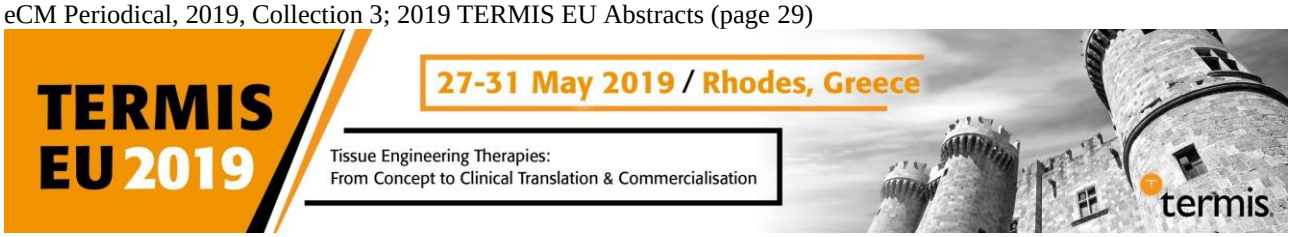
[1] Camussi G. et al. Kidney Int. 2010; 78:838-48.
[2] Bemis L.T. et al. Journal of Equine Veterinary Science. 2012; 32(7):398.
[3] Ng Y.H. et al. PLoS One. 2013; 8(3):e58502.
[4] Lange-Consiglio et al. Stem Cell and Development. 2013; 22:3015-3024.



Towards engineering of bio-instructive materials to guide tissue regeneration processes

A. M. Blocki
Presenting Author: Anna Blocki, anna.blocki@cuhk.edu.hk
Institute for Tissue Engineering and Regenerative Medicine (iTERM) & School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR

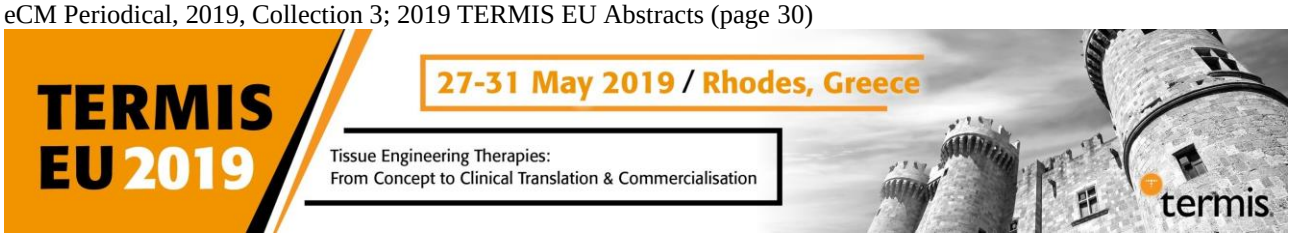
The extracellular matrix (ECM) is a dynamic scaffold full of biochemical, biomechanical and topographical signals that instruct cells in tissue homeostasis, healing and regeneration. Although tissue-derived ECM belongs to the most successful biomaterials in clinical application, these clinical products face many limitations. Besides immunogenicity, risk of disease transmission and shortage of donor organs, these also include a large material variability and a fixed composition thus bioactivity. This is the reason why we set-off to engineer extracellular matrices with customized bioactivities in vitro, which upon processing and implantation will guide regeneration processes. The utilization of the biophysical principle of macromolecular crowding (MMC) during in vitro ECM assembly hereby ensures relevant amounts of ECM to be deposited. As a result, these in vitro matrices exhibit a strong and stable bioactivity and can be tailored to be anti-inflammatory, pro-angiogenic and/or anti-fibrotic. Additionally, this in vitro ECM platform allows us to study and decipher the ECM's mechanism of action. In particular, we were able to shed light on the role of hyaluronic acid in cell-guided fibronectin fibrillogenesis. This novel class of biomaterials based in vitro engineered ECM allows to utilize the whole complexity of signalling employed by the ECM, while being customized in its bioactivity.



Design and study of self-assembling peptides as scaffolds for tissue engineering

A. Mitraki
Presenting author: Anna Mitraki, mitraki@materials.uoc.gr
Department of Materials Science and Technology, University of Crete, Heraklion, Greece, and Institute of Electronic Structure and Laser (IESL) FORTH, Heraklion, Greece

Self-assembled peptides gain increasing interest as biocompatible and biodegradable scaffolds for tissue engineering. Rationally designed self-assembling building blocks that carry cell attachment motifs such as Arg-Gly-Asp (RGD) are especially attractive. We have been using a combination of theoretical and experimental approaches towards such rational designs. We have been especially focusing on modular designs that consist of a central ultrashort amphiphilic motif derived from the adenovirus fiber shaft. This central amphiphilic motif can be further modified with amino acids targeted for various functionalities. We have been studying peptides comprising this central motif, further combined with the RGD motif and cysteine residues that allow further functionalization possibilities, such as conjugation of growth factors or attachment to surfaces. The designer peptides self-assemble into fibrils that are structurally characterized with Transmission Electron Microscopy, Scanning Electron Microscopy and X-ray fiber diffraction. Furthermore, they support cell attachment and proliferation of model cell lines. We further extended this approach to computational design of bifunctional self-assembling peptides bearing calcium and strontium binding residues as well as the cell adhesive motif RGD. Such short self-assembling peptides that are amenable to computational design offer open-ended possibilities towards multifunctional tissue engineering scaffolds of the future.



Intervertebral disc: A journey from embryonic development to cell-based regenerative strategy

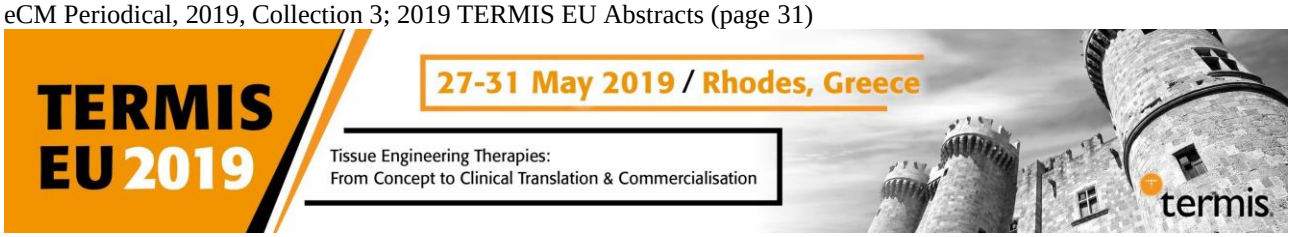
A. Camus[1,2]
Presenting Author: Anne Camus, anne.camus@univ-nantes.fr
[1]Inserm, UMR 1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS, Nantes, F-44042, France [2]Université de Nantes, UFR Odontologie, Nantes, F-44042, France

Low back pain is one of the most common musculoskeletal disorder and the most common causes of disability in industrialized countries. Although the aetiology of low back pain is unknown, it is often (40%) associated with degeneration of the intervertebral disc (IVD). There is currently no effective treatment for disc degeneration [1]. This is largely due to a lack of basic knowledge of the molecular and cellular controls of disc development, growth, differentiation, and homeostasis, during embryogenesis and at different stages of life. The nucleus pulposus (NP) is the highly hydrated central part of the IVD that plays a key role in spine kinematic and where degenerative changes are thought to initiate. Lineage tracing experiments in the mouse demonstrate that notochord cells (NTCs) are the precursor cells that give rise to the NP [2]. There is also considerable evidence that NTCs have a significant influence on the homeostasis of the IVD and their loss has been correlated with aging and degeneration in humans [3]. The generation of NTCs is therefore a promising approach to regenerate IVD. Our research aims to identify key molecules associated with cell fate decisions, morphogenesis and maturation of the NTCs required for disc formation that may also regulates the maintenance of the mature NTCs for functional and healthy adult disc tissue. A thorough knowledge in developmental biology is relevant to elucidate IVD repair in humans and also to the production of NTCs from pluripotent stem cells for regenerative strategies.

ACKNOWLEDGEMENTS: Financial support was received from ANR generiques 2014 (“REMEDIV”), Region des Pays de la Loire, BIOREGATE Scientific Challenge grant (“Caveodisc”); Arthritis Foundation research grant (“NOTOCH”).

REFERENCES

[1] Colombier P et al. Trends Biotechnol. 2014;32,433–435
[2] McCann M and Seguin C. JDB 2016;4,3–18
[3] Purmessur D et al. Global Spine J. 2013;3,201–218



Do dental stem cells of the apical papilla have the teeth for spinal cord repair?

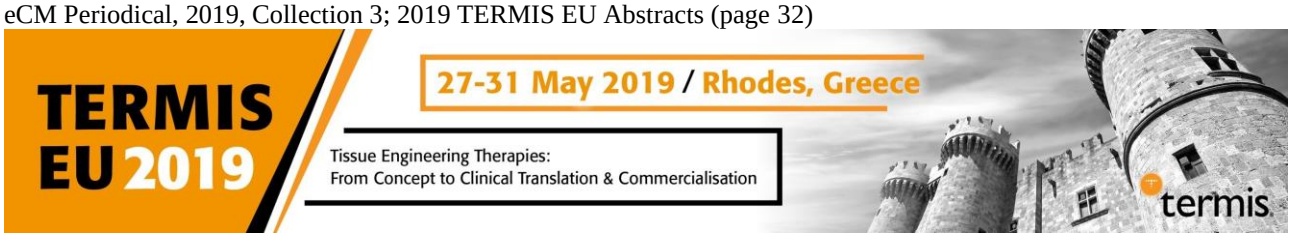
A. des Rieux[1]
Presenting Author: Anne des Rieux, anne.desrieux@uclouvain.be
[1]Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Université catholique de Louvain, UCLouvain, Avenue Mounier, 73, B1 73.12, 1200 Brussels, Belgium

Spinal cord injury (SCI) is the major cause of long-term paralysis via traumatic event. Strategies for spinal cord repair after injury are extensively studied over the world. While cell-based therapy holds great hope for recovery after SCI, this area remains challenging for scientists and clinicians. Transplantation of different type of mesenchymal stem cells (MSC) has been investigated as potential therapies for SCI but, despite this advancement no definitive treatment exists and SCI continues to pose clinical and socio-economic problems. The aim of this work was to provide an innovative strategy for spinal cord repair based on a new MSC source: dental stem cells from apical papilla (SCAP). Dental stem cells (DSC) have been largely investigated in dental therapies, especially in regenerative endodontics. DSC hold a not yet exploited potential in regenerative medicine and are particularly becoming attractive for nervous system repair strategies thanks to their neural crest origin. Up to now, no one investigated SCAP potential for spinal cord repair so far. Stem cells from the apical papilla (SCAP) derive from the neural crest and express numerous neurogenic markers. The goal of the present work was to investigate their therapeutic potential regarding the treatment of SCI. We observed that implantation of a whole human apical papilla at the lesion site improved gait of spinally injured rats(1). We demonstrated that SCAP have immunomodulatory properties and can stimulate oligodendrocyte progenitor cell differentiation(2). And finally, we showed that implantation of the apical papilla exerted an anti-inflammatory and neuroprotector effect. This work underlines the potential therapeutic benefits of SCAP for spinal cord repair.

ACKNOWLEDGEMENTS: Anne des Rieux is a Research Associate at the FRS-FNRS (Fonds de la Recherche Scientifique). We are also grateful to Université Catholique de Louvain (FSR) and IRP for the financial support.

REFERENCES

[1] De Berdt P et al. 2015, J Dent Res.; 94(11):1575-81.
[2] De Berdt P et al. 2018, Cellular and molecular life sciences : CMLS;75(15):2843-56.



Modulating macrophages to drive in situ cardiovascular tissue engineering

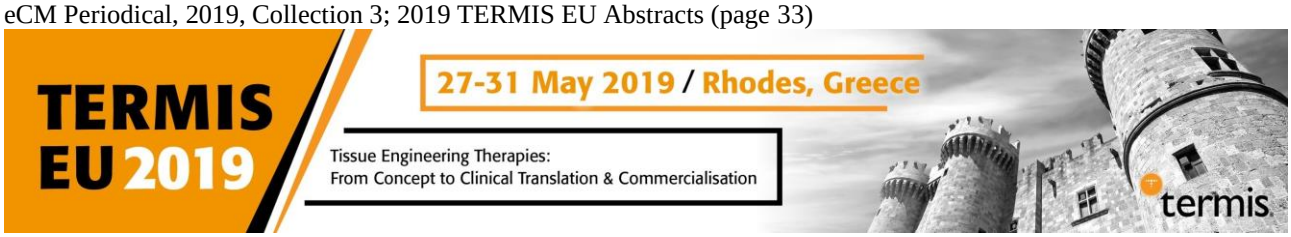
A.I.P.M. Smits[1,2]
Presenting Author: Anthal IPM Smits, a.i.p.m.smits@tue.nl
[1]Soft Tissue Engineering and Mechanobiology (STEM) group, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands [2]Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven, The Netherlands

Materials-driven tissue regeneration using acellular, resorbable synthetic scaffolds is an attractive strategy to obtain living, adaptive cardiovascular replacement tissues, such as heart valves and blood vessels. Upon implantation, such scaffolds induce the recruitment and infiltration of endogenous cells, which degrade the temporary scaffold material and replace that with functional new tissue directly in situ. By omitting lengthy and costly in vitro culturing protocols, the use of such a relatively simple acellular synthetic device offers several intrinsic translational advantages, such as off-the-shelf availability and reduced costs. Various recent studies have demonstrated the proof-of-concept for in situ cardiovascular tissue engineering (TE) using resorbable synthetic scaffolds. One example is the development of a resorbable heart valve scaffold, which demonstrated sustained functionality for 12 months when implanted as pulmonary valve replacement in sheep [1]. Although the in situ TE approach has shown great potential, the in vivo regenerative processes are difficult to control and not well-understood. The host inflammatory response to the implanted scaffold is postulated to play a hinging role during in situ TE [2]. Macrophages specifically display diverse commanding functions in the processes of tissue formation and remodeling, by signaling to tissue-forming cells (e.g. myofibroblasts) -similar to wound healing-, as well as the degradation of the scaffold material. These dynamic cellular functions are dependent on the macrophage polarization state, which can be modulated via the scaffold design parameters (e.g. microstructure), as well as the local biochemical and biomechanical environment, the latter being particularly relevant in cardiovascular applications. In addition to material-dependent parameters, patient-specific, material-independent parameters, such as patient age, sex, and potential systemic comorbidities (e.g. diabetes) are likely to have a profound effect on the efficacy of in situ TE strategies due to their influence on the patient's intrinsic inflammatory state and regenerative capacity [3]. Here, we will elaborate on the current mechanistic know-how of the in situ TE process, with a specific focus on cardiovascular applications (i.e. small-diameter blood vessels, heart valve replacements). Therein, the influence of scaffold design parameters, as well as scaffold-independent patient characteristics will be discussed in order to provide a prospective on the development of robust clinical treatments and the potential for personalized in situ cardiovascular TE strategies.

ACKNOWLEDGEMENTS: We gratefully acknowledge the financial support by the Ministry of Education, Culture and Science (Gravitation Program 024.003.013).

REFERENCES

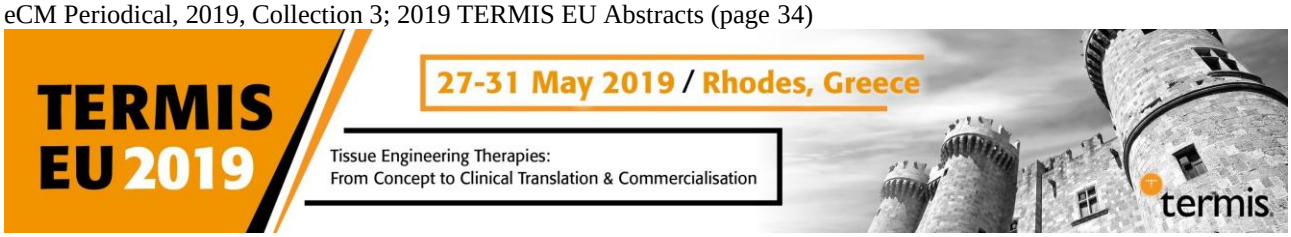
[1] Kluin et al. Biomaterials 2017;125:101
[2] Wissing and Bonito et al. NPJ Regen. Med. 2017;2:18
[3] Smits and Bouten. Curr. Opin. Biomed. Eng. 2018;6:17



Tropoelastin and enhanced wound repair

A.S. Weiss
Presenting author: Anthony S. Weiss, anthony.weiss@sydney.edu.au
Charles Perkins Centre; School of Life and Environmental Sciences; Bosch Institute, University of Sydney, NSW, 2006, Australia

Tropoelastin is crosslinked as an essential step in the formation of elastin where it contributes to elasticity and biological interactions. An emerging model for tropoelastin is that it delivers this potency by emulating extracellular matrix interactions including those through development and repair. This paradigm for enhanced tissue repair encompasses a novel, pure, synthetic material that promotes the repair and fixation of soft tissues. The active component is tropoelastin and leverages its ability to promote new blood vessel formation and its cell recruiting properties to accelerate healing on applied tissues. Key to the technology is a stabilized form of human tropoelastin which allows for tuneable resorption. This implantable material is shaped at will where it hydrates to form a conformable protein hydrogel. Significant benefits in the extent of wound healing, dermal repair and regeneration of mature epithelium in preclinical animal studies are demonstrated superior to current methods of sterile bandaging, commercial hydrogel, and dermal regeneration. These developments will be discussed.

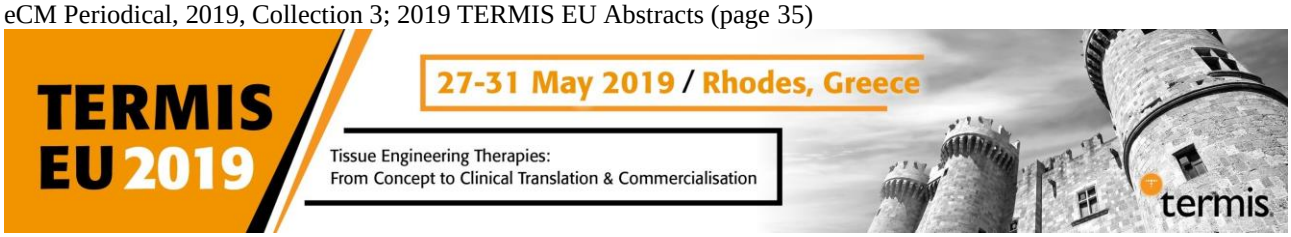


Presentation of the COST Action International network for translating research on perinatal derivatives into therapeutic approaches – SPRINT

A.R. Silini
Presenting Author: Antonietta Rosa Silini, antonietta.silini@poliambulanza.it
Centro di Ricerca E. Menni, Fondazione Poliambulanza-Istituto Ospedaliero, Brescia, Italy

Stem cells hold great promise in the evolving field of regenerative medicine. Over the past two decades, different perinatal tissues have been shown to harbor a vast array of stem cells with therapeutic potential. Perinatal tissues are birth-related tissues and include the amniotic fluid and placenta-derived tissues such as the umbilical cord, the amniotic membrane, the chorion, and the maternal decidua. The cells isolated from perinatal tissues and the factors released from these cells are collectively referred to as perinatal derivatives (PnD). Research on the therapeutic applications of PnD is exponentially growing as documented by the 30-fold increase in scientific publications in the past 10 years, and the number of currently ongoing clinical trials. The multidisciplinary knowledge obtained by the main actors of the scientific, clinical, and industrial research has significantly advanced our understanding of PnD. There are however several issues that need to be addressed to ensure optimal research outcome and safe and efficient clinical applications. These issues range from the need to arrive to consensus on the nomenclature and optimal techniques for cell isolation, characterization, and to more advanced issues such as collating data toward an understanding of the mechanisms of action and therapeutic actions of perinatal derivatives. In 2018 the COST Action entitled “International Network for Translating Research on Perinatal Derivatives into Therapeutic Approaches-SPRINT” was approved. The aim of this Action is to unite a currently fragment critical mass to enhance both the basic understanding and translational potential of perinatal derivatives. This network has an interdisciplinary plan of action which will address open questions (e.g. consensual terms for classification, comparison, standardized operative procedures for harvesting, processing, cryopreservation), monitor and build upon ongoing studies (e.g. from comparison of preclinical studies and identification of mechanisms of action, up to the translation into European clinical practice by addressing regulatory issues and stakeholders’ view), and thus ultimately promote more efficient, safer, and faster advancement of their therapeutic applications and attract industrial investments.

ACKNOWLEDGEMENTS: This abstract is based upon work from COST Action SPRINT, supported by COST (European Cooperation in Science and Technology).COST description: COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. COST Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation. www.cost.eu. COST is supported by the Horizon 2020 EU Framework Programme.



Stem cell secretome as a modulator of CNS regeneration

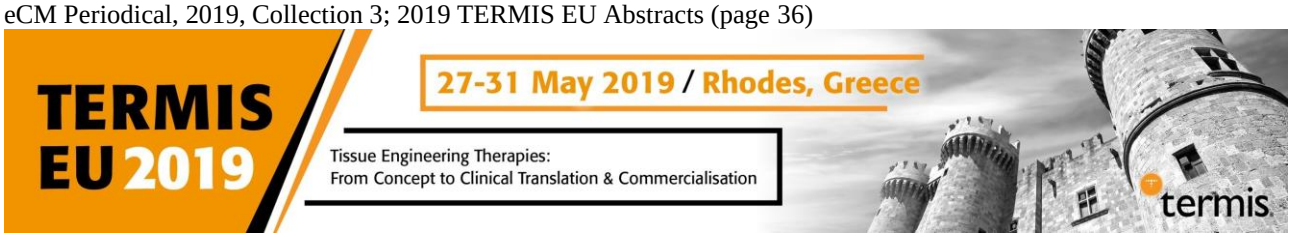
A.J. Salgado[1,2]
Presenting Author: António J. Salgado, asalgado@med.uminho.pt
[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal [2]ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal

The low regeneration potential of the central nervous system (CNS) represents a challenge for the development of new therapeutic strategies. Mesenchymal stem cells (MSCs) have been proposed as a possible therapeutic tool for CNS disorders, namely due to the beneficial actions of their secretome. Indeed, the latter possesses a broad range of neuroregulatory factors that promote an increase in neurogenesis, inhibition of apoptosis/glial scar, immunomodulation, angiogenesis, neuronal and glial cell survival, as well as relevant neuroprotective actions into different pathophysiological contexts. Considering their protective action in lesioned sites, MSCs, and their secretome, might also improve the integration of local progenitor cells in neuroregeneration processes. In this sense their use could represent an important vehicle for the establishment of future CNS regenerative therapies. In the present talk the role of MSCs secretome, on phenomena such as in vitro and in vivo neuronal/glial survival, as well as its characterization will be addressed. Moreover its use as a therapeutic agent for injury and neuro-degeneration of the CNS, namely Spinal Cord Injury and Parkinson's, Diseases and Spinal Cord Injury regenerative medicine will also be presented and discussed. Finally, new trends on how to modulate the secretome MSCs will also be presented.

ACKNOWLEDGMENTS: Foundation Calouste de Gulbenkian to funds attributed to A. J. Salgado under the scope of the The Gulbenkian Programme to Support Cutting Edge Research in Life Sciences; Portuguese Foundation for Science and Technology: FCT Investigator and Grant PTDC(PTDC/SAU-BMA/114059/2009); EU-FP7-Health-2011-collaborative project 278612, Biohybrid - Templates for peripheral nerve regeneration; Santa Casa Neurosciences Awards: Prize Melo e Castro for Spinal Cord Injury Research. Projects NORTE-01-0145-FEDER-000013 and NORTE-01-0145-FEDER-000023, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER). This work has been funded by FEDER funds, through the Competitiveness Factors Operational Program (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT), under the scope of the project POCI-01-0145-FEDER-007038.

REFERENCES

[1] F.G. Teixeira et al., Scientific Reports, 2016, 6: 27791
[2] A.O. Pires et al., Stem Cells and Development, 2016, 25(14): 1073
[3] E.D. Gomes et al., Biomaterials, 2016, 105: 38
[4] F.G. Teixeira et al., Stem Cells Translational Medicine, 6(2): 634
[5] E.D. Gomes et al., Stem Cells, 2018, 36(5): 696



Development of biodegradable microcarrier systems for rapid protein conjugation at physiological condition

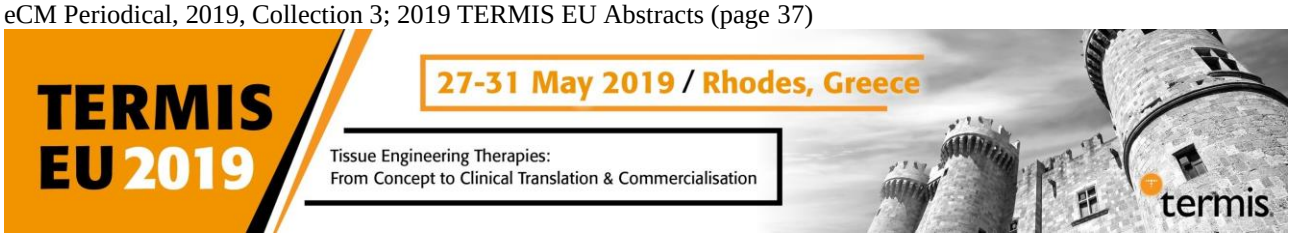
A. Saeed
Presenting Author: Aram Saeed, Aram.Saeed@uea.ac.uk
School of Pharmacy, Norwich Research Park, University of East Anglia, Norwich, Norfolk, UK NR4 7TJ

In the field of tissue engineering and regenerative medicine, there is more demand for biomaterials that are able to provide optimal surface for cell culture and growth, environment for cell expansion and manufacturing, local delivery to enhance new tissue formation. However, for these biomaterials to be transferred from research laboratory toward practical applications where minimal chemical manipulation is required, new simple and efficient synthetic and fabrication tools are required. For examples, synthetic polymers are used as delivery systems need to contain biomimetic adhesion sites for the facile conjugation of bioactive agents. Currently, research towards the synthesis of these types of delivery systems has been met with the challenge of choosing between natural polymers abundant with biologically active interaction sites or mechanically superior synthetic polymers lacking in useful functional groups. Synthetic polymers have advantageous chemical properties, such as defined structure and composition, the versatility of functional groups and synthetic routes, ability to tailor and tune degradation rate and mechanical strength, however, they suffer from poor surface adhesion and an inability to easily conjugate biomolecules such as proteins or peptides that can interact with cellular components. For decades, polyester polymers such poly(lactic-co-glycolic acid) (PLGA), Poly(caprolactone) (PCL) and poly(lactic acid) (PLA) have been the preferred materials for delivery systems. However, these polymers are inherently difficult to work with. They lack functional groups needed to allow for easy conjugation to or control the adhesion of, bioactive agents. This, in turn, has limited their application. Post-functionalisation of these polymers, in which functional groups are substituted or modified using additional reagents, has been attempted to combat this problem. One of the most common techniques employed to functionalise these polymers is aminolysis. This requires physical adsorption on the surface and the use of carbodiimide coupling reagents. However, as these techniques often require multi-step reactions and laborious purification processes, these delivery systems have seen little success in clinical translation. To meet these challenges, herein, we introduce a new way to synthesize polyester polymers with tailored functionals. Choosing a heterobifunctional initiator allowed us to fabricate PCL polymer with clickable terminal groups for conjugation to biomolecules at physiological conditions, in the absence of coupling agents, using copper-free click chemistry. Thus, we simplified and eliminated the need for post-functionalisation process and multistep of conjugation and purification steps. We demonstrated this via the fabrication of microcarrier system, using membrane emulsion technique, and conjugated human serum albumin via a single step. We also showed the conjugation step is highly specific and rapid at physiological conditions. This new platform has the potential to develop a variety of polyester based biomaterials for application in tissue engineering and regenerative medicine.

ACKNOWLEDGEMENTS: Financial support was received Rosetrees Trust Fund, Humane Research Trust and EPSRC ICASE (Grant EP/L505729/1).

REFERENCES

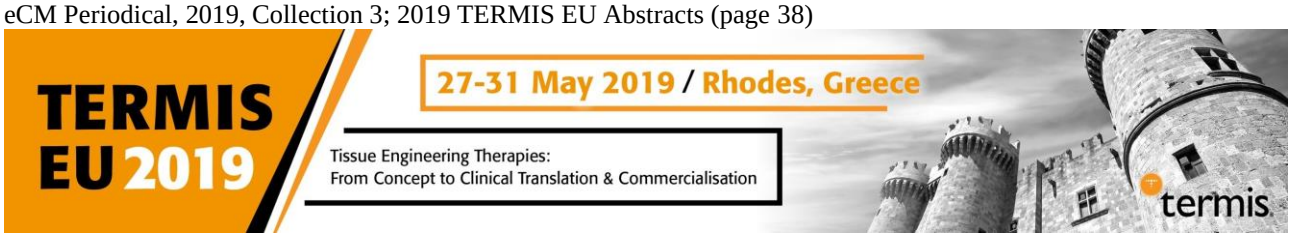
[1] A Saeed et al. Adv Mater. 2014, https://doi.org/10.1002/adma.201403626



Cell therapies - Clinical applications in regenerative dentistry

A. Bakopoulou
Presenting Author: Athena Bakopoulou, abakopoulou@dent.auth.gr
Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, GR-54124, Greece

Oral Mesenchymal Stem Cells (MSCs) have been extensively studied for their biological properties and ability to be applied for the regeneration of hard and soft oral tissues in a wide range of in vitro and in vivo pre-clinical models over the last ten years. These studies have acted as decisive milestones enabling translation towards their clinical application in Regenerative Dentistry, as surrogate therapeutic modules for conventional, biomaterial-based treatment modalities currently used to restore damaged tissues. To this end, implementation of well-designed clinical trials will pave the way for the exploitation of these research achievements in daily dental practice. In this presentation, we will describe the standard operative procedures (SOPs) for the safe and efficient clinical-grade expansion of oral stem cells for application in Dentistry, focusing on our Institutional experience on the development of tissue-engineered constructs tailored to be applied as Advanced Therapy Medicinal Products (ATMPs) for the regeneration of periodontal and peri-implant tissues. Regulatory hurdles in European Union, as opposed to increasing societal needs for applications of cellular therapies in Dentistry, as well as solutions for fostering wider patient access will be also discussed.



Nucleus pulposus progenitor cells positive for receptor angiopoetin-1 alias Tie2 – Multipotent cells of specific capabilities for tissue engineering?

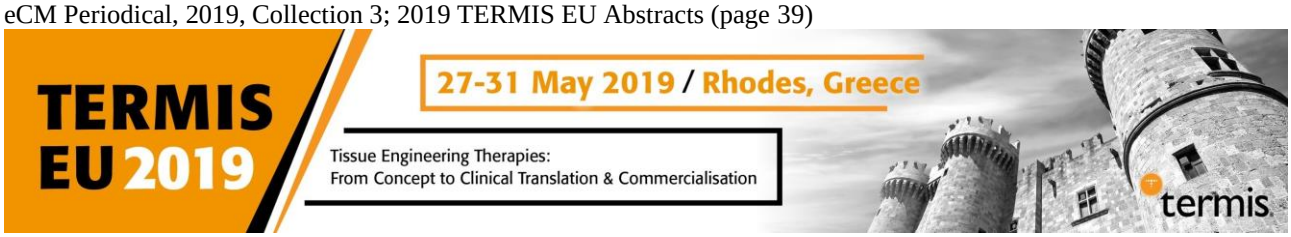
B. Gantenbein[1,2,3]
Presenting Author: Benjamin Gantenbein, bgantenbein@istb.unibe.ch
[1]Institute for Surgical Technology and Biomechanics, University of Bern, Bern, Department for BioMedical Research (DBMR), University of Bern, Switzerland [3]Department for Orthopedics and Traumatology, Insel University Hospital, Bern, Switzerland

Stem cell therapy of the intervertebral disc (IVD) is one of the most warranted but also highly disputed procedures to be applied for treatment of degenerated discs that in many cases cause high low back pain. From previous studies it is known that for instance notochordal cells, a relatively large cell type containing a high number of vesicles, are highly regenerative [1] and may stimulate other differentiated cells, such as nucleus pulposus cells (NPC) to produce more matrix. Lately, a particular tissue-specific progenitor cell population has been identified in the center of the intervertebral disc (IVD), so-called nucleus pulposus progenitor cells (NPPC) [2]. The current hope is that these NPPC could play a particular role for IVD regeneration and/or delay of it. The current knowledge on these cells is obscured and their specific requirements for ex vivo culture are not very clear. Current evidence confirms the presence of these cells in murine, canine, bovine and in the human fetal/surgical samples [3]. Interestingly, Tie2 is a marker for endothelial cells and it is not very clear what their origin and their role might be. Current data using a combination of molecular assays could identify these with about 2-5% in bovine coccygeal IVD samples of one-year age. In human surgical specimens their presence is more obscured depending on the donor's age and the particular condition of the IVD (e.g. based on Pfirrmann grade) [2, 3]. Here, I revisit the recent literature on regenerative cells identified for the IVD in the past decades. Current evidence how these NPPC can be isolated and detected in various species and tissues will be recapitulated. These NPPC are interesting to elaborate more closely from a developmental biology but also from an evolutionary point of you. Future directions will be provided how these progenitor cells could be used for regenerative medicine and tissue engineering.

ACKNOWLEDGEMENTS: Financial support was received from the Horizon2020 Project "iPSpine" # 825925.

REFERENCES

[1] Bach FC Eur Cell Mater 2016; 32:163-80.
[2] Sakai D et al. Nat Commun 2012; 3:1264
[3] Sakai D et al. JOR Spine 2018;2018e:



Open up! Chances and limitations of using open innovation in science

B. Missbach
Presenting Author: Benjamin Missbach, benjamin.missbach@lbg.ac.at
[1]Ludwig Boltzmann Gesellschaft, Open Innovation in Science Center

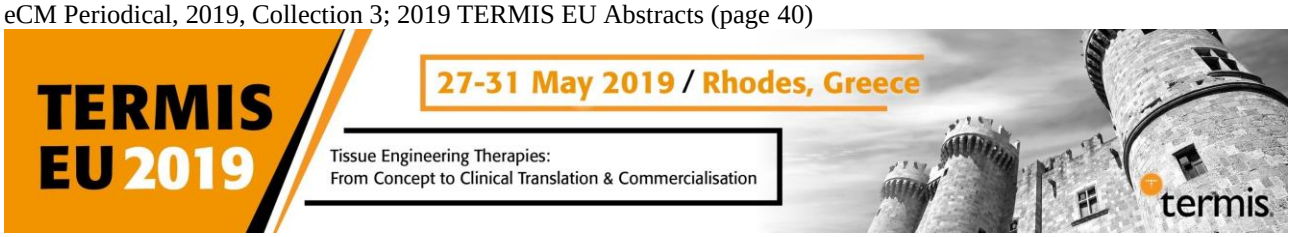
INTRODUCTION: Open research in clinical settings not only refers to open data or open access research artifacts (e.g.: open access publications, open data, open peer-review), but it also includes openness during the entire research flow: From identifying research questions, co-creating study designs with relevant stakeholders, implementing research with patients reported outcomes sets, and utilizing research data for translating scientific knowledge into innovation. This is why the Austrian research organisation Ludwig Boltzmann Gesellschaft has now transformed into an institution that allows for the extensive application of Open Innovation in Science (OIS) methodology. What are the chances and what are the pitfalls of applying these concepts?

METHODS: Open Innovation is a concept formerly used in economic contexts. By making the organizational boundaries more permeable, internal R&D can be stimulated and spark innovation. While this concept is already applied in many businesses, the scientific system is now starting to use open innovation methods to fuel innovation in science.

RRSULTS & DISCUSSION: With the OIS Center, the Ludwig Boltzmann Gesellschaft has created an experimental space for applying OIS and experimenting with different OIS methods. This ranges from opening up the research process at the stage of identifying new research questions (Crowdsourcing) and applying innovative team building methods (Ideas Lab) and finally working with methods and principles that allow to open up research processes. This approach has the potential to change how research organisations can structure their processes in order to create more impact for and with society. The first international crowdsourcing project “Tell us!” is an innovative approach to form research groups based on crowdsourcing research questions on mental health, identifying key issues and topics and finally establishing two strong research groups on the topic “Children of mentally ill parents”. The second crowdsourcing project aimed to identify new research questions in the diagnosis, treatment and rehabilitation of accidental injuries. With this approach, we aimed to identify research questions in a clinical setting by involving patients and experts (Caregivers, therapists, MDs) in order to spark innovative research projects.

CONCLUSIONS: Open Innovation in Science can lead to an increase in societal impact and the relevance to industrial and clinical applications. Open collaboration during one or several of those phases can involve citizen science, multidisciplinary research or strengthen the link between academia, industry and market needs. In order to follow this avenue of opening research, traditional scientific approaches need to be adapted and re-thought.

ACKNOWLEDGEMENTS: No financial interests to disclose.



Using biophysical cues for engineering cell morphology and function

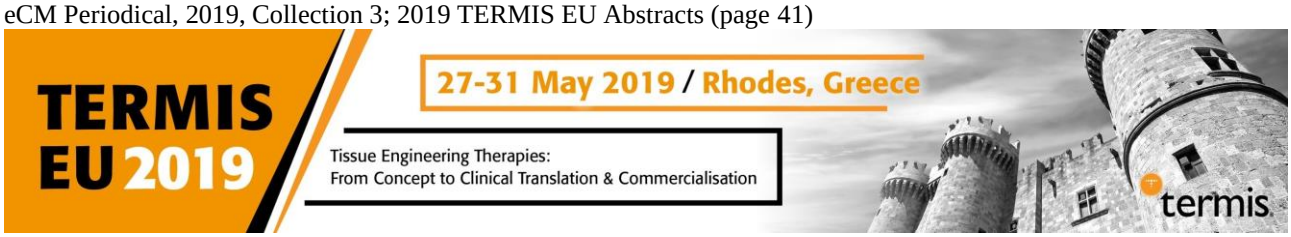
B. Rolauffs[1]
Presenting Author: Bernd Rolauffs, bernd.rolauffs@uniklinik-freiburg.de
[1]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Freiburg University Medical Center, Albert-Ludwigs-University of Freiburg, Freiburg, Germany

Biophysical cues can be as important as biochemical and genetic factors in controlling cell fate. However, compared to growth factors or other biochemical factors, biophysical stimuli are potentially easier to license for clinical use and, thus, harnessing biophysical stimuli for directing cell fate is a powerful and modern strategy for generating therapeutic or preventive interventions. The vision to control cell fate through controlling cell shape is an emerging “hot” topic in regenerative medicine that relies on better understanding, measuring, and controlling cell shape, in order to develop effective, biophysically acting cell-instructive materials. In this context, we have developed an automated, non-destructive, high-throughput quantification method, using images of calcein-stained cells, combined with binary thresholding, to calculate a specific panel of so-called quantitative shape descriptors for efficiently and accurately measuring cell morphological parameters of a large number of cells [1]. Recently, our group demonstrated that a given biomaterial type and its associated nanoscale stiffness are associated with a specific baseline cell morphology of human mesenchymal stromal cells (MSCs), and, thus, that one can intentionally generate desired baseline shapes by choosing biomaterial type and stiffness [1]. We then demonstrated in [2] that defined variations in externally applied biomechanical forces can also prompt a subsequent, defined change in MSC shape. By applying various cyclic tensional forces we engineered significantly different MSC shapes. Interestingly, the key to biomechanically engineering cellular shape was the repetition of a chosen stretch regimen, as cyclic tension had complex time- and amplitude-dependent effects on cell shape. Thus, biomechanically engineering cell shape (and the associated MSC differentiation) relied on complex non-linear processes that were dependent on both active stress input and inactive response time. This was in accordance with [1], which demonstrated that MSCs can be elongated during cyclic stretch, but, after cessation of stretch, the shape reverted back toward the initial baseline shape determined by the biomaterial type and stiffness. It is important to the field that, in the chosen experimental system, dynamic tensile forces were shown to be more significant in defining MSC shape than biomaterial stiffness-related cues – however, the ability of cyclic stretch to override the biomaterial-dictated shape was temporary, as MSC shape ultimately reverted back to the biomaterial-dictated baseline shapes. Together, these studies highlight that MSC shape can be statically engineered through using i. e. micro-engineered adhesion sites, but MSC shape can also be highly dynamic. Under biomechanically dynamic conditions, such as in a patient’s body, MSC shape can be intentionally engineered by utilizing the dynamic forces experienced in vivo.

ACKNOWLEDGEMENTS: Financial support was received from the DFG KFO 273 (RO2511/3-1 to B.R)

REFERENCES

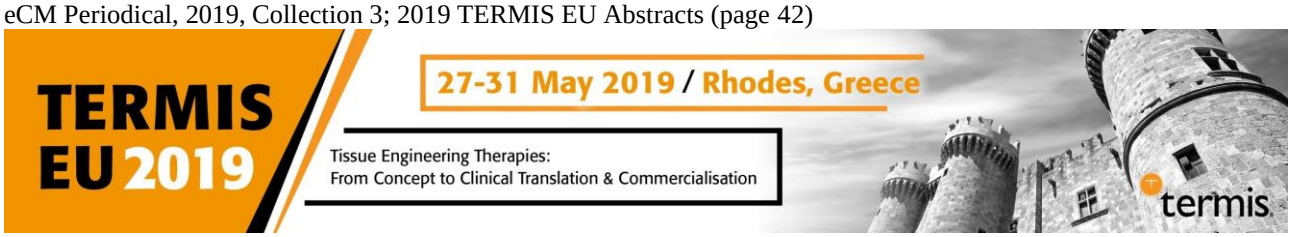
[1] Uynuk-Ool et al. J Tissue Eng Regen Med 2017; doi: 10.1002/term.2263.
[2] Walters et al. Sci Rep 2017; 10.1038/s41598-017-06794-9



Multi-modal imaging of soft and hard tissues in health and disease

B. Müller
Presenting Author: Bert Müller, bert.mueller@unibas.ch
Biomaterials Science Center, University of Basel, Allschwil, Switzerland

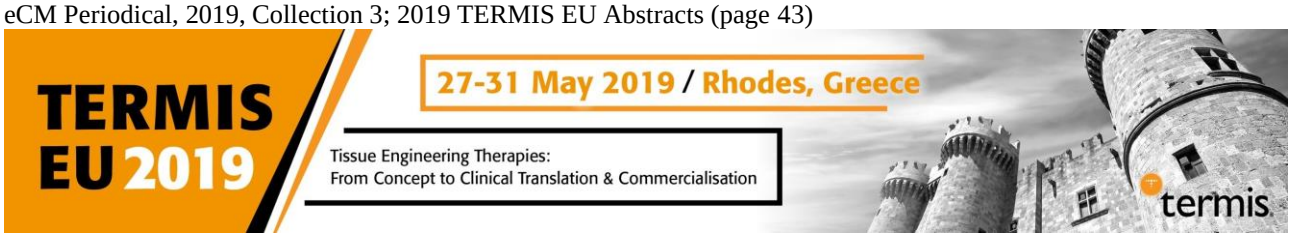
Caries is the most frequent disease worldwide. The currently available treatment options include isotropic fillings, which do not resemble the unique structure of enamel and dentin. X-ray imaging is not only a prerequisite to design bio-inspired dental fillings but also to verify, how far the recently introduced peptide-based remineralization of white spots allows for biomimetic regeneration. The same imaging techniques, i.e. micro computed tomography and spatially resolved small-angle X-ray scattering are used to characterize soft tissues down to the nanometre scale. Using the example of human brain tissue, the author's team has launched nano-holotomography for the isotropic three-dimensional visualization of paraffin-embedded blocks of millimetre size. Nano-holotomography bridges the gap between the optical and electron-based microscopy techniques, while giving access to volumes of reasonable size with a spatial resolution well below 100 nm. This hierarchical imaging approach is going to mediate prospering nano-anatomy of human tissues in health and disease. Even simpler experimental setups enable us to three-dimensionally image entire peripheral nerves after repair and reconstruction. Using a laboratory-based micro computed tomography system, one can obtain three-dimensional data non-destructively, which allow for the characterization of nerves inside state-of-the art conduits. These data also support the selection of cutting planes for the histological sectioning. The combination of the non-destructively obtained tomography data and the subsequently generated histology data gives rise to discoveries, which cannot be received by one technique alone. Furthermore, the information from the more-detailed two-dimensional histology data can be extended into the third dimension through conventional micro computed tomography.



Demineralized bone matrix as a promising carrier for local drug release

B. Wildemann
Presenting Author: Britt Wildemann, britt.wildemann@med.uni-jena.de
Experimental Trauma Surgery, University Hospital Jena, Germany, Julius Wolff Institute & Berlin Brandenburg Center for Regenerative Therapies, Charité-Universitätsmedizin Berlin, Germany

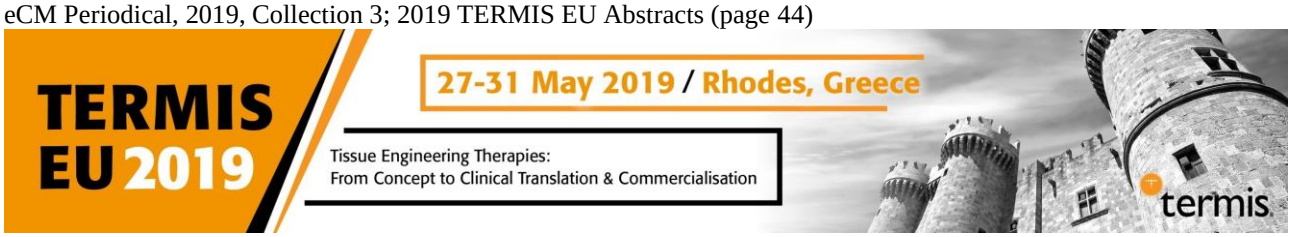
The research and development of materials for bone grafting and local drug release increased in the recent decades. The developed materials are often very complex, making translation into the clinic difficult. A clinically well-established grafting material is allogeneic bone, especially demineralized bone matrix (DBM). We aimed at investigating and optimizing the properties of various demineralized bone matrix products for simple clinical use by quantifying the amount of growth factors and evaluating the osteoinductive properties in vitro and in vivo in critical-size defect models. The quantity and composition of growth factors varied between the donors/lots and different commercial DBM-products. Alkaline phosphatase activity, as an indicator for osteoinductivity, was induced in C2C12 cells when cultured with DBM. In a critical-size rat defect model, bone healing was stimulated without complete bridging. The loading of DBM with bone morphogenetic protein-2 (BMP-2) revealed a burst release within the first 7 days, but also a depot storage of the active growth factor over 56 days. Using this enriched DBM for the filling of drill hole defects in a sheep model showed the regeneration of the defect after 63 days with a reconstitution of the native metaphyseal bone structure. With this in-depth analysis of the DBM properties, limited osteoinduction was observed in vivo, which can be overcome by enrichment with BMP-2. The advantage of this combination is the approval of both components, the ease perioperative loading, and the burst release of BMP-2 in combination with a long-term activity of the factor.



Dynamic biomedical systems based on elastin-like recombinamers

J.C. Rodríguez-Cabello
Presenting Author: J. Carlos Rodríguez-Cabello, roca@bioforge.uva.es
BIOFORGE Laboratory. University of Valladolid CIBEER-BBN. Valladolid, Spain

The use of recombinant technology in the production of macromolecule-based advanced biomaterials has caused a breakthrough increase in achievable degree of complexity and control on the molecular designs and compositions. Those recombinant macromolecules of polypeptide nature are called recombinamers. They are produced from a purely synthetic gene, in which the amino-acid sequence is not restricted to naturally occurring proteins and it is dictated only by engineering design parameters. The high degree of complexity and control of the recombinamer compositions permit to reach unmatched levels of functionality in the materials produced by this way and on the systems based on them. The development of functionality in such systems comes by to different ways. In one hand, these materials can display direct functionality. Such functionality is based on the presence in their composition of functional epitopes, typically inspired by functional epitopes found in natural proteins. The other source of functionality is the holistic functionality that emerges by the precise combination and interactions of direct functions in a precise and well designed macromolecular composition. This holistic function is particularly evident in system with a dynamic nature; systems that rearrange and respond to changes in their environment. Examples of such dynamic systems will be presented. The examples will expand from complex 3D structures for regenerative medicine and tissue engineering that are able to incorporate a designed program of degradation profile and time evolution to more fundamental questions such as the hierarchical spontaneous development of morphology and macroscopical shapes in natural and artificial systems.



Biomaterial scaffolds as 3D tumour models for cancer research

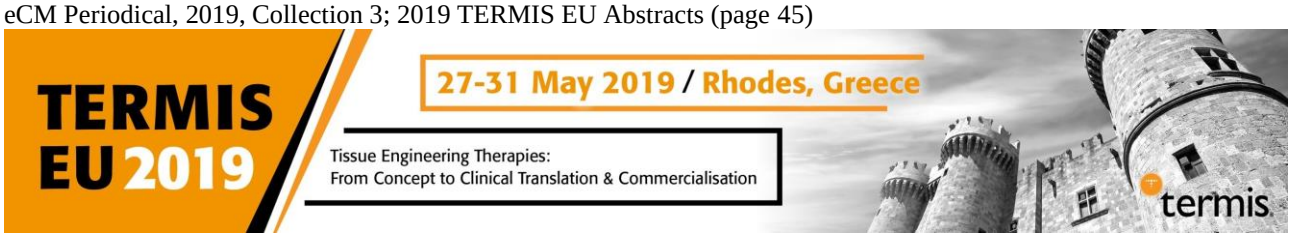
C.M. Curtin [1,2,3]
Presenting author: Caroline M. Curtin, carolinecurtin@rcsi.ie
[1]Tissue Engineering Research Group, Dept. of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Trinity Centre of BioEngineering, Trinity College Dublin (TCD), Ireland [3]Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland

Culturing of cancer cells in 2D has traditionally been used to study complex tumorigenic mechanisms but lacks the structural microenvironment required for cell-cell and cell-extracellular matrix interactions. The alternative involves animal xenograft models but also has various limitations. Recently, 3D cancer cell culturing has been proposed to bridge the gap between conventional 2D culture and in vivo tumours by enabling cells to acquire phenotypes and respond to stimuli similar to in vivo biological systems. Furthermore, gene therapy, encompassing plasmid DNA, microRNA and siRNA delivery, has emerged as a potential therapeutic for cancer treatment as genes can be released locally for a defined timeframe. However, effective gene delivery to abrogate tumour growth remains a crucial barrier to its clinical application. This presentation will describe studies that aimed to develop and characterise 3D in vitro culture models of cancer to simulate the primary tumour and secondary cancer bone metastasis, and to then use the models to determine the efficacy of nanoparticle-mediated gene delivery as competent anti-cancer platforms. Collagen-based scaffolds capable of supporting cell culture have been widely used as gene delivery platforms for tissue engineering and regenerative medicine within our laboratory. Features include excellent biocompatibility and a 3D structure capable of recapitulating the native tumour geometry demonstrating their potential as extracellular matrix models due to their biomimicry. Preliminary work has already demonstrated successful nanoparticle-mediated siRNA delivery in 3D collagen-nanohydroxyapatite scaffolds simulating prostate cancer bone metastasis [1, 2]. Additionally, these scaffolds together with collagen-glycosaminoglycan scaffolds have been used as 3D neuroblastoma models that correlated more closely with in vivo xenograft tumours compared to 2D monolayer cell culture [3]. We hypothesise that collagen scaffolds may be used as 3D in vitro “tumours” that mimic characteristics of in vivo primary tumour progression while collagen-nanohydroxyapatite scaffolds may serve as bone templates for the study of metastasis as the bone component, hydroxyapatite, may be involved in metastasis pathogenesis. Furthermore, these gene delivery scaffold-based models may serve as excellent tools for the development of novel treatment targets.

ACKNOWLEDGEMENTS: Financial support from Science Foundation Ireland, Health Research Board, European Research Council and Neuroblastoma UK.

REFERENCES

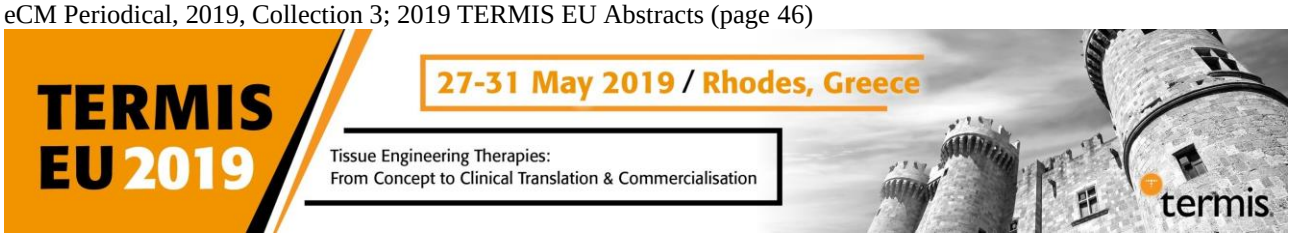
[1] Fitzgerald KA et al. Biomaterials. 2015 Oct;66:53-66.
[2] Evans JC et al. Mol Pharm. 2017 Jan 3;14(1):42-52.
[3] Curtin CM et al. Acta Biomater. 2018 Apr 1;70:84-97.



Multifunctional glycosaminoglycan hydrogels for new therapies and human disease models

C. Werner
Presenting author: Carsten Werner, carsten.werner@tu-dresden.de
Max Bergmann Center of Biomaterials (MBC) and Center of Regenerative Therapies Dresden (CRTD), Leibniz Institute of Polymer Research Dresden (IPF) and TU Dresden, Dresden, Germany

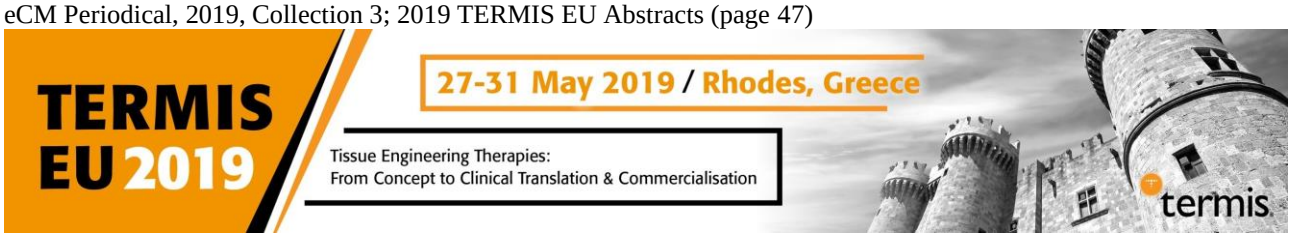
Glycosaminoglycans (GAGs) govern important functional characteristics of the extracellular matrix (ECM) in living tissues and the incorporation of GAGs into biomaterials was shown to open up new routes for the effective modulation of various signaling molecules. For further expanding the related options, we have introduced a rational design strategy for creating biohybrid hydrogels based on GAGs of varied sulfation patterns (heparin and selectively desulfated heparin derivatives), multi-armed poly(ethylene glycol) and a range of functional peptides (to mediate MMP driven reorganization, for covalent in situ assembly of gels from polymeric precursors, and to serve as ligands of cell adhesion receptors). The theoretically predicted decoupling of biochemical and mechanical gel properties was confirmed experimentally and applied for implementing GAG-based biofunctionalization schemes using a broad range of combinations of cytokines. Micro-processing schemes (cyro-gelation, solvent-assisted micro-molding, microfluidic microgel fabrication, multicomponent inkjet bioprinting,) were recently established to allow for the fabrication of GAG-based hydrogels for multiphasic and multifunctional gel materials capable of providing spatiotemporally adjusted signaling characteristics. Applications of the materials platform include 3D culture models to study the role of exogenous cues in fate control of bone marrow derived human stem and progenitor cells, to explore breast and prostate tumor vascularization in vitro as well as scaffolds for exploring new therapeutic approaches to chronic wounds and to neurodegenerative diseases.



Open access publishing: Benefits and challenges

C. Da Re[1]
Presenting Author: Caterina Da Re, caterina.dare@frontiersin.org
[1]Frontiers Media, Lausanne, Switzerland

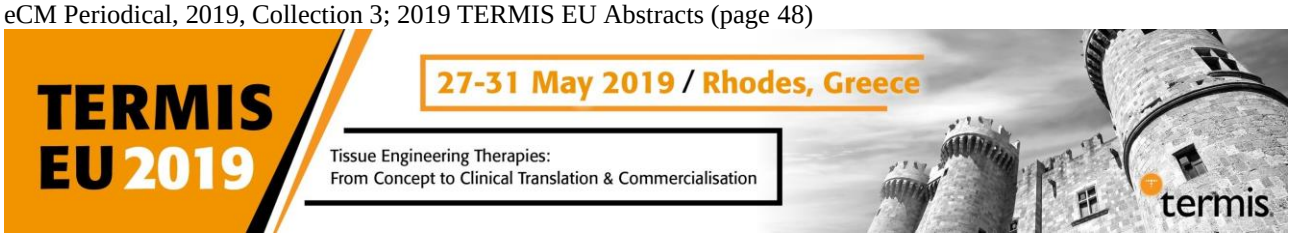
One option for authors who wish to make their work openly accessible is to publish in a journal that makes your work immediately and permanently available online for everyone, worldwide. Open access (OA) is a step towards the democratization of scientific literature. By taking advantage of the digital revolution, OA exploits the interconnected world in order to provide free access and reuse of scientific literature for potentially everyone in the world. The “liberty of re-utilization” drastically promotes the spreading of scientific knowledge and its validation, making OA an efficient system for promoting scientific research. In particular, the Green OA assumes that scientists can publish their achievements in a “closed” journal - the lector should pay to read it - without paying any fees. However, at the same time, these contents are “open”, meaning available, in a sort of archive for everyone ’to read and share. The counterpart of the Green OA is the Gold OA, which implies paid journal publication from the authors but free of charge accessibility to the journal itself. Currently there are major issues about the publishing fees that need to be covered by the authors. The costs of producing an article are somewhat fixed and cover among others manuscript editing, formatting, standardizing, indexing, etc. The introduction of the electronic format has allowed to significantly reduce these expenses, but they still remain a cost that needs to be covered to allow quality publications. There is much room for imagination: some publishing houses only publish OA journals, and some publish a mix of OA and subscription journals, whereas other subscription journals open up or restrict the access after a certain period of time. From another perspective, some journals charge article processing fees (paid by authors or by their grant makers, referred to as the author-pay model) and some receive funding from foundations or institutions, while other journals are based on advertising revenues, priced add-ons, or auxiliary services. Lastly, it is important to point out that OA is compatible with peer review, and all the major OA initiatives for scientific and scholarly literature insist on its importance. Just like subscription-based journals, open access journals must maintain high quality standards of publications in order to be competitive.



Mesenchymal stem cell encapsulation in alginate micro-particles for intra-articular injection in osteoarthritis

C. Le Visage
Presenting Author: Catherine Le Visage, catherine.levisage@inserm.fr
Inserm Regenerative Medicine and Sketeton (RMeS U1229), Université de Nantes, Nantes, France

Osteoarthritis (OA) is a degenerative and inflammatory joint disease that affects the whole joint. Mesenchymal stem cells ability to secrete anti-inflammatory and immuno-modulatory factors represents an attractive tool in the treatment of OA. Considering the risk of cell leakage and the massive cell death upon intra-articular injection, we developed a micromolding method of encapsulation that allows to obtain particles that (i) could be injected with a 26G needle into a mouse joint and (ii) could provide a 3D microenvironment supporting cell biological activity and could. Polydimethylsiloxane (PDMS) chips containing circular micromolds were manufactured and a solution of 2% alginate containing human adipose stem cells (3 M/mL) was deposited on the molds. Cell loading into the micromolds was performed either by sedimentation or by centrifugation. After Ca2+ crosslinking, alginate particles (diameter 150±0.7µm) were obtained. The number of cells per particle was 5 times higher when the micromolds were loaded by centrifugation. Cell number and metabolic activity remained stable for 7 days after encapsulation and injection through a 26G needle had no impact on cell viability. When cells were stimulated with TNF-alpha and INF-gamma, prostaglandin E2 (PGE2) concentration in the supernatant was multiplied by 13 and 7 and indoleamine2,3-dioxygenase (IDO) activity was 2 and 4 times higher when cell loading was performed by sedimentation or centrifugation, respectively. To conclude, we have demonstrated that encapsulated cells were able to sense and respond to an inflammatory stimulus and their potential will now be evaluated in a murine model of osteoarthritis.



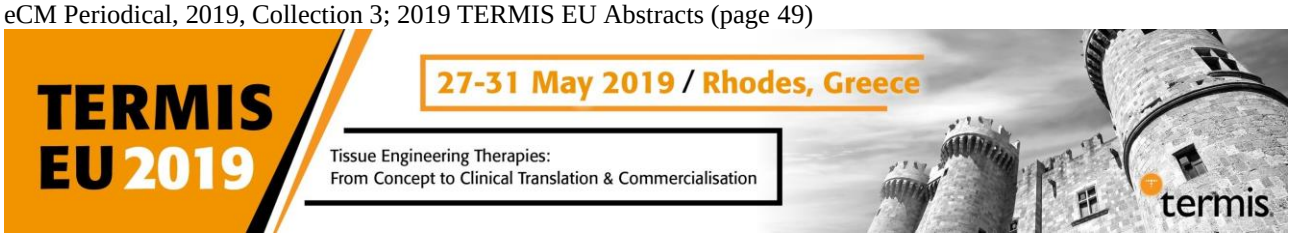
Patient-specific meniscus replacement: Roadmap from artificial over tissue-engineered to smart meniscus

C. Van Der Straeten
Presenting Author: Catherine Van Der Straeten; e-mail: Catherine.vanderstraeten@uzgent.be
[1]Health innovation and Research Institute, Ghent University Hospital, Ghent, Belgium

The fibrocartilaginous menisci between the femoral condyles and the tibial plateau of the knee are essential for the biomechanical and biological homeostasis of the joint and the articular cartilage. They are important for shock absorption, load distribution in weight bearing conditions by increasing the contact area of the opposing articular surfaces, joint stability and lubrication, and articular cartilage nutrition. However, they are often injured and difficult to repair. Meniscal tears are commonly seen in young adults as a result of sports injuries. In the older population, meniscal degeneration, with loss of meniscal tissue and function, plays an essential biological role in the development and progression of osteoarthritis (OA). In vitro pro-inflammatory stimulation of injured meniscal tissue causes production of cytokines, chemokines, matrix degrading enzymes and other catabolic factors by meniscal cells. This effect may be enhanced by obesity and/or age-related dysregulation of anabolic gene expression and changes in cytokine release. Radiographic signs of OA are noted at 8-16 years post-meniscectomy whilst cartilage loss on MRI has been shown at 7 years after meniscectomy. Surgical treatments aim either to repair the menisci in cases of tears or to decrease the loads on the articular cartilage by replacing meniscal tissue or altering joint alignment in severe injuries and/or degeneration. The treatment of meniscus lesions is still the most frequent procedure carried out by knee surgeons (over 1 million annually in the USA). However, clinical results of meniscal implants (allografts and artificial menisci) vary and are difficult to evaluate because surgical and fixation techniques, implant materials, clinical evaluation, scoring systems and criteria for failure differ significantly among publications. In a nutshell, they usually offer symptomatic relief but do not prevent or delay the progression of OA [1]. There is an urgent need to overcome traditional strategies and treatment options. New knowledge and innovative methods are required to develop a patient-specific medically implantable meniscus which will improve the immediate outcome of the surgery, whilst stimulating and expediting regeneration of the injured tissue focusing on innovative and interdisciplinary principles. We present a roadmap to develop a patient-specific 3D printed meniscal implant, using a novel polymer/polymer-composite (PBM patented toolbox) in a fully artificial meniscus and a tissue-engineered implant, being tested for biocompatibility, biodegradability, biotribology and biomechanical properties. We have demonstrated that high-throughput production of uniform micro-tissues is promising for the generation of larger scale tissue engineering constructs [2]. We now focus on micro-aggregates with different cell types and co-cultures of fibro-chondrocytes with mesenchymal stem cells. In parallel we will investigate the best in vivo imaging method for meniscus (CT-MRI), perform statistical shape modelling of menisci and develop an algorithm for fast segmentation and subsequent patient-specific 3D printing. A plan for preclinical testing in an animal model (sheep) and clinical trials will be laid out. Finally, the use of sensor/lab-on-chip technology for the development of a smart meniscus enabling close monitoring and early intervention to prevent failure, will be explored.

REFERENCES

[1] Van Der Straeten C et al, PLoS One, 2016 May 26;11(5)
[2] Berneel E et al, presentation Belgian Symposium on Tissue Engineering 2018



Subchondral bone remodeling concurrent with cartilage degeneration in osteoarthritic joints

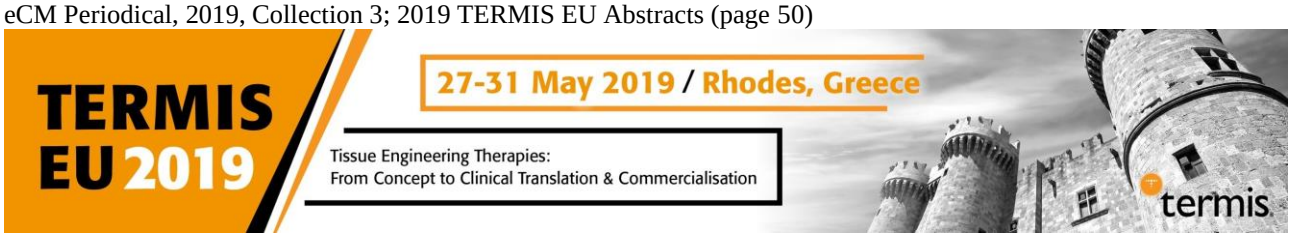
C Liu[1,2]
Presenting Author: Chaozong Liu, Chaozong.Liu@ucl.ac.uk
[1]UCL Orthopaedic Bioengineering Research Group, Division of Surgery and Interventional Science, University College London, Stanmore HA7 4LP, UK [2]Royal National Orthopaedic Hospital, Stanmore HA7 4LP, UK

Osteoarthritis (OA) is a degenerative joint disease that affects both cartilage and subchondral bone [1]. With progression of OA, as results of changes in the loading pattern, bone remodelling and resorption occur in the joint [2]. This weakens the physical environment that supports the overlying cartilage [3]. In this study, we examined the changes in local distribution of volumetric bone mineral density (vBMD) in the subchondral bone, and the biomechanical properties of the overlying cartilage with an aim to understand the effect of subchondral bone remodelling on the overlying cartilage degeneration. Human femoral heads were collected during total hip replacement operation due to OA. Cartilage was graded using ICRS classification, and the mechanical property of cartilage was measure by non-destructive cyclic indentation. To determine the remodelling of the subchondral bone, a peripheral quantitative CT (pQCT) was used to assess the vBMD distribution within the subchondral bone. Non-parametric Kruskal-Wallis method was used for statistical analysis ($p= 0.05$). The examination of retrieved tissues revealed cartilage in different stages of degeneration, from normal to severely abnormal. Subchondral vBMD decreased with cartilage ICRS grade from 576 to 253mg/cm^3 confirming bone remodeling in all samples. Dynamic modulus of cartilage was mapped and showed a weak positive correlation to ICRS grades (3.34±0.93, 2.86±1.11, 4.64±4.37 and 5.56±1.83 N/mm for grade I,II,II and IV respectively), and a moderate positive correlation to subchondral vBMD ($r=0.59$), confirming the concurrence of cartilage biomechanics, degeneration and SCB remodelling. It is believed that that the degeneration of cartilage altered the loading regime in the subchondral bone, leading to bone remodeling, resorption and even cyst formation in the weight-bearing regions of subchondral bone. This changed the physical environment supporting the overlying cartilage, which may lead to further cartilage degeneration

ACKNOWLEDGEMENTS: This work was financially supported by Innovate UK (grant no: 102872), Rosetrees Trust (project no A1184) and European Union through H2020-MSCA-RISE BAMOS programme (grant no 734156).

REFERENCES

[1] Tamaddon, M et al. Bio-Design and Manufacturing, 2018. 1(2): p. 101-114.
[2] Bentley G et al. Injury 2013; 44:S3-S10.
[3] Martin I et al. Journal of Biomechanics 2007; 40: 750-65.

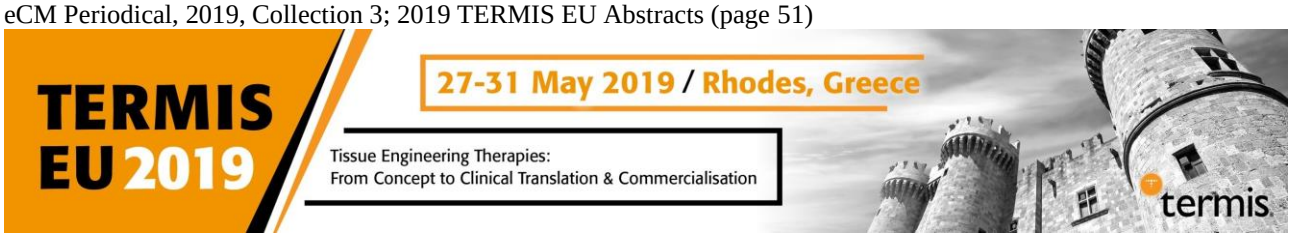


Osteoporosis and 3D bone like scaffolds mimicking the features of human healthy bone

C. Vitale-Brovarone
Presenting author: Chiara Vitale-Brovarone, chiara.vitale@polito.it
Applied Science and Technology Department, Politecnico di Torino, Italy

Osteoporosis is a worldwide spread, bone disease which is continuously increasing due to population ageing; it leads to bone mass loss and increased porosity resulting in fragility fractures. In order to treat osteoporotic fractures and to better understand osteoblast and osteoclast cross-talking, 3D scaffolds mimicking the features of human healthy bone have been developed. Healthy and osteoporotic bones obtained from human bone tissues discarded during surgical interventions have been fully characterised through micro-CT scans, nanoindentations, XRD spectra, Raman spectroscopy and immunohistochemistry. STL files were obtained and used to 3D print a type I collagen solution containing mesoporous bioactive glass/nano-hydroxyapatite particles to mimic both the organic and the inorganic phases of human bone and to stimulate bone regeneration. The rheological properties of the Type I/inorganic particles suspensions were investigated at different collagen concentrations and temperatures as several cross-linking methods have been explored. The incorporation of bone growth factors (GF) in the scaffold struts was carried out using several approaches including ink-jet printing, GF loading into mesoporous glass with enlarged nanoporosity and encapsulation in polymeric nanospheres. Different co-culture of osteoblasts and osteoclasts set-ups were explored and used to assess the influence of the developed 3D scaffolds and their chemical and topographical stimuli on the osteoblast-osteoclast coupling.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 681798-BOOST) and by the Italian Minister of Research, Progetto FARE Ricerca in Italia (GRACE).



Tailoring Laponite® crosslinked hydrogels to control cell function in vitro and in vivo

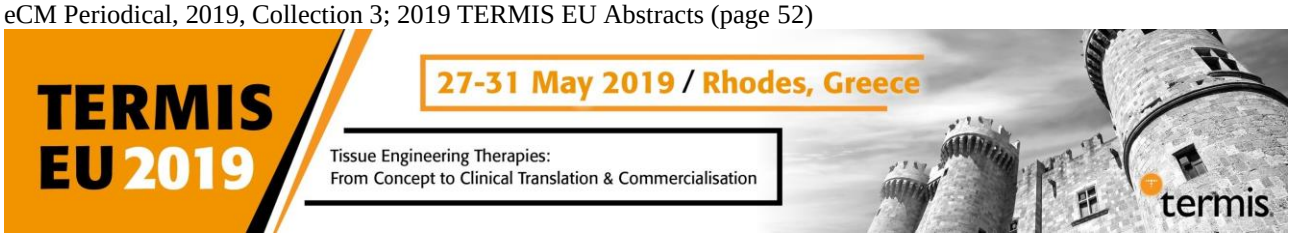
C. Sammon
Presenting Author: Chris Sammon, c.sammon@shu.ac.uk
Materials and Engineering Research Institute, Sheffield Hallam University, Sheffield, UK

Tissue engineering scaffolds which promote regeneration offer potential therapeutic solutions for musculoskeletal tissues and also afford routes to a range of 3D culture matrices. Hydrogels in particular, offer significant potential as a choice of biomaterial for tissue engineering, due to their hydrated nature, matched mechanical properties, biocompatibility and extensive structural framework which can be chemically tailored to mimic the in vivo extracellular matrix (ECM). In this presentation a family of Laponite® crosslinked pNIPAM hydrogels developed in our group will be discussed. These materials have thermal responsive properties close to body temperature, are fully reacted in a liquid state where cells and other factors can be incorporated and require no clean up prior to injection. The presentation will show how it is feasible to fully tailor their mechanical and morphological properties by careful selection of the hydrogel composition and drive the differentiation of human mesenchymal stem cells towards predetermined phenotypes for a range of clinical applications [1,2]. Data will also be presented demonstrating that these materials can be used to generate in vitro 3D culture models of human intestinal epithelium. By careful selection of the culture conditions a range of cell morphologies including villus-like structures can be produced [3].

ACKNOWLEDGEMENTS: Financial support was received from EPSRC (EP/H000275/1, EP/1016473/1), MRC (MR/P026796/1) and ARUK (Project no. 21497).

REFERENCES

[1] Thorpe AA et al Oncotarget 2018; 9(26):18277-95
[2] Thorpe AA et al Acta Biomaterialia 2017; 54:212-226
[3] Dosh RH et al Acta Biomaterialia 2017; 62: 128-143



Injectable hydrogels for musculoskeletal system regeneration: Importance of the disease microenvironment

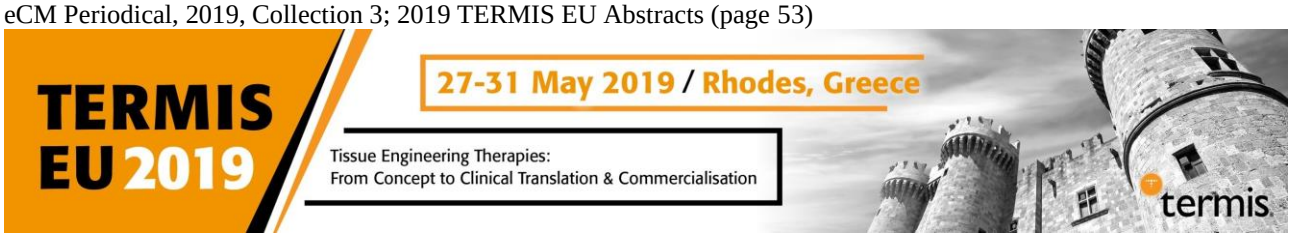
C.L. Le Maitre[1]
Presenting Author: Christine Le Maitre, c.lemaitre@shu.ac.uk
[1]Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, UK

Injectable hydrogel systems have been proposed as an interesting mechanism of delivery of stem cells to promote musculoskeletal tissue regeneration. Our laboratory has developed a platform technology which can be tailored to induce stem cell differentiation to intervertebral disc cells (NPgel)[1] or bone cells (Bgel)[2]. During the design of regenerative approaches for musculoskeletal tissues it is essential to consider the microenvironment and native cell behavior within the site of injection. During degenerative conditions such as intervertebral disc degeneration the intervertebral disc becomes a hostile environment for cells. With a poor nutrient supply, hypoxic environment and low pH the survival of injected cells may be under question. Furthermore the degenerate niche is characterized by high levels of catabolic cytokines which increase matrix degradation and decrease normal matrix synthesis. Thus the effects on any injected cells must be determined otherwise addition of further cells to this hostile environment could further degrade rather than regenerate the native tissue. Here we will discuss the behavior of stem cells within our NPgel system cultured under conditions which mimic the degenerate niche. For the regeneration of any musculoskeletal tissue, consideration of the age of the patient must be considered where acellular approaches which depend on the native cells to induce repair (e.g. in bone repair) or where autologous stem cells are applied. Aged individuals have a reduced ability to heal and regenerate and thus success and limitations of autologous cell sources must be considered. We have demonstrated that our Bgel system could improve bone healing in aged individuals without the need for additional cell sources[3].

ACKNOWLEDGEMENTS: Financial support was received from EPSRC (EP/H000275/1, EP/1016473/1), MRC (MR/P026796/1) and ARUK (Project no. 21497).

REFERENCES

[1] Thorpe AA et al Acta Biomaterialia 2017; 54:212-226.
[2] Thorpe AA et al. Eur Cell Mater. 2016; 32:1-23.
[3] Thorpe AA et al Oncotarget 2018; 9(26):18277-95.



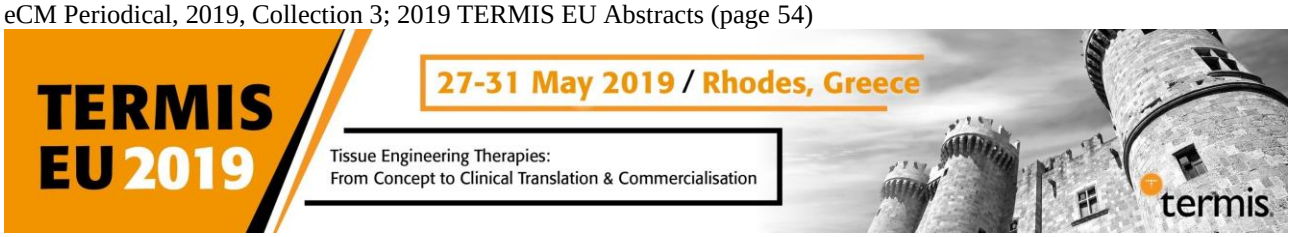
Development of collagen/hyaluronic acid composites for the treatment of nucleus pulposus degeneration: A physico-chemical study

C. Hélary[1]
Presenting Author: Christophe Hélary, christophe.helary@upmc.fr
[1]Sorbonne Université, CNRS, Collège de France, Laboratoire de Chimie de la Matière Condensée de Paris, 4 place Jussieu, 75005 Paris, France

Back pain is considered as the disease of the century because 90% of the world population will be affected during its lifespan. In 50% of cases, chronic back pain is associated with intervertebral disc (IVD) degeneration. The current treatment is based on physiotherapy and administration of drugs. When the pain is too great, a spinal fusion or a discectomy are required. Novel treatments relying on the injection of stem cells embedded in injectable biomaterials have emerged with the aim of regenerating the Nucleus Pulposus (NP) [1]. As the cell behavior depends on the biochemical, mechanical and topological environment, a biomimetic hydrogel would promote IVD regeneration by providing cues to cells to differentiate into a physiological phenotype. Nucleus Pulposus is a hyper hydrated tissue working as a hydraulic shock absorber. Glycosaminoglycans give a high degree of hydration to NP whereas collagen II gives resistance and allows for cell adhesion [2]. With the aim of developing novel biomimetic hydrogels, collagen/hyaluronic acid composites were synthesized to mimic the structure and the mechanical properties of Nucleus Pulposus. For this purpose, different methods to associate hyaluronic acid with collagen were tested and the impact of the formulation on physical properties of composite hydrogels was analyzed. As HA does not spontaneously form gels, it was functionalized with tyramine (Tyr) groups to allow HA-Tyr gelling. This gelling was based on an enzymatic crosslinking by Horse Radish Peroxidase (HRP) and H_2O_2. On the other side, collagen gelling was triggered by pH increase leading to the formation of collagen fibrils populating a physical hydrogel. With a constant collagen concentration (0.4%), HA-Tyr was increased up to 2 % and different HRP and H_2O_2 concentration were used to modulate both kinetic and degree of crosslinking of hydrogels. This physico-chemical study revealed the impact of the different parameters on the physical and biological properties of hydrogels. Two regimes were observed. At low HA-Tyr content (less than 0.4 %), the composite behavior was driven by collagen as HA-Tyr was not able to form a chemical gel at this concentration. Hydrogels exhibited a fibrillary network consisted of cross striated fibrils. The presence of HA-Tyr and a high gelling speed slightly decreased the mechanical properties of composites. From 0.8%, a regime driven by HA-Tyr appeared. The mechanical properties were improved when the HA-Tyr concentration, the gelling speed and the cross-linking density rose. The mechanical properties and the hydration degree were close to those of NP. Unfortunately, this was associated with an inhibition of collagen fibrillogenesis. To evaluate the ability of composites to promote cell adhesion and proliferation, fibroblasts were encapsulated within HA-Tyr/collagen hydrogels. A high HA-Tyr content and the gelling speed negatively impacted cell adhesion and proliferation. This study shows that synthesizing a biomimetic composite hydrogels requires compromises. When the HA-Tyr and gelling speed raise, mechanical properties and hydration are improved but collagen fibrillogenesis and cell adhesion are slightly inhibited. A low gelling speed and a 0.8% HA-Tyr content is the most appropriate formulation to mimic NP.

REFERENCES

[1] Sakai D et al. Biomaterials. 2006; 27: 335-45
[2] Camci-Unal G, et al. Biomacromolecules. 2013; 14: 1085-92



Regulatory considerations for tissue engineered products

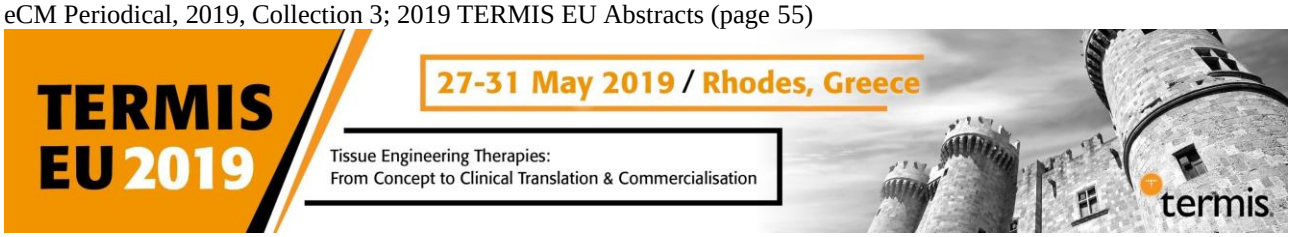
C. J. Mann[1]
Presenting Author: Christopher Mann, cmann@asphalion.com
[1]Asphalion S.L., Carrer de Tarragona, 151-157, planta 10, 08014 Barcelona

Advanced therapy medicinal products (ATMPs) are currently experiencing a boom with several products reaching the market and many others under various stages of nonclinical and clinical development for a broad range of conditions. Tissue-engineered products (TEPs) are a promising subclass of ATMPs for regenerative medicine that can potentially address unmet clinical needs. Here we highlight some of the regulatory considerations and complications of developing TEPs and bringing innovative products to market. To identify several of the most challenging areas of regulatory impact on TEP development we have reviewed guidelines pertinent to both European (EMA), US (FDA) and global (ICH) development of TEPs, the literature and our own experience. The following aspects were identified as important regulatory considerations for the development of TEPs. Classification: most TEPs are borderline products and correct classification can define which regulatory path a product takes and which studies might be required to support clinical development. Free of charge regulatory procedures exist to formally classify ATMPs. Combination: many TEPs are a combination of cells plus a medical device, such as a scaffold or encapsulation material, for example. As medical devices are regulated differently to medicinal products, correct classification, performance and safety testing may be required whereas different documents may also be required at the time of regulatory submission. Manufacturing: there are many potential regulatory issues associated with manufacture of TEPs including scale-up capacity from laboratory to clinical/commercial scale, pharmaceutical acceptability of materials and excipients including traceability and viral safety. New approaches such as 3D printing are not always clearly covered by legislation or guidelines. Characterization: TEPs, especially those combined with a medical device, may be difficult to characterize once combined, for example, cells once encapsulated or integrated in a scaffold, which creates regulatory challenges for assuring safety and mechanism/performance. Relevant species: many TEPs may be considered as personalized medicines as they use autologous cells as an active substance that complicates the design of nonclinical studies, especially toxicity. Allogeneic cells are preferred from a manufacturing point of view, but are not always feasible. Furthermore, human cells are xenogeneic in mice whereas many scaffolds may also be derived from other animal materials such as pig meaning many studies require immunocompromised animals. Finally, recreating the clinical route of administration in animal models can also be challenge and require regulatory input to assure an adequate design. As the number of ATMPs and TEPs under development continues to grow and as new technologies are incorporated into their design, more exceptions and challenges to the current regulatory requirements occurs. TEP development can benefit from continuous regulatory input.

ACKNOWLEDGEMENTS: Financial support was received from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 646075-2 for the Elastislet research project.

REFERENCES

[1] Regulation 1394/2007/EC
[2] EMA/CAT/573420/2009
[3] Directive 93/42/EC



Carbohydrate polymers to modulate macrophage activities for tissue regeneration and immunotherapy

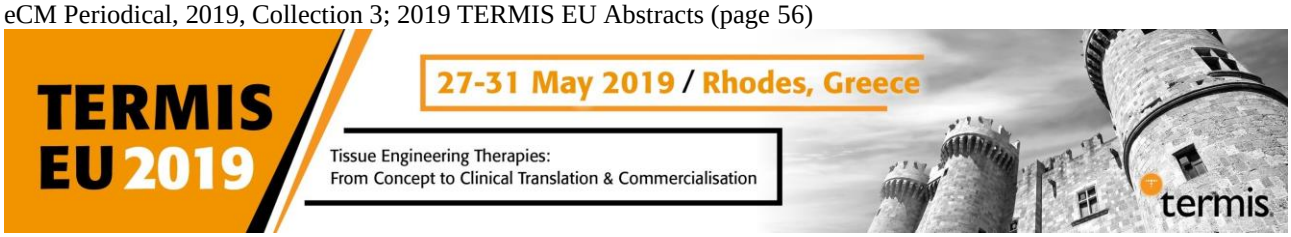
C. Wang[1]
Presenting Author: Chunming Wang, cmwang@umac.mo
[1]Institute of Chinese Medical Sciences, University of Macau, Macau SAR, China

Macrophages are traditionally best known as chief defenders of our immunity. In fact, they play diverse and essential roles in many other physiological and pathological processes, notably including tissue repair and biomaterials integration. They can accumulate at the tissue-biomaterials interface and secrete abundant cytokines to create a microenvironment that benefits or inhibits regeneration. Because the phenotype of these cells is highly plastic in response to varying stimuli, it may be feasible to manipulate their activity at the interface and harness their power to mediate tissue regeneration. Targeting the toll-like receptors (TLRs) on macrophages, our team have devised a series of carbohydrate polymers that can stimulate macrophages to secrete different sets of cytokines [1-3]. We demonstrated that two chemically modified glucomannans could stimulate macrophages to release pro-osteogenic or anti-cancer cytokines, respectively, and devised the first one into the coating of mesenchymal stem cell-laden hydrogels scaffold for osteogenesis. Also, through screening TLR-activating polysaccharides, we modified zymosan/beta-glucan onto titanium implants and assessed their efficacy of integration and potential safety following immune stimulation. Our future research will focus on distinguishing the roles of macrophages of various sub-types and origins in specific regenerative processes.

ACKNOWLEDGEMENTS: We thank the financial support from the Macau Science and Technology Development Fund (FDCT/080/2016/A2 and 126/2016/A3) and University of Macau (MYRG2016-00031-ICMS-QRCM and MYRG2017-00028-ICMS).

REFERENCES

[1] Shi Y et al. Adv Funct Mater. 2018;28: 1804483
[2] Niu Y et al. Biomaterials. 2017;139:39-55
[3] Gan J et al. Biomaterials. 2018;178:95-108.



The application of bioengineering principles to evaluate emerging therapeutics for respiratory disease

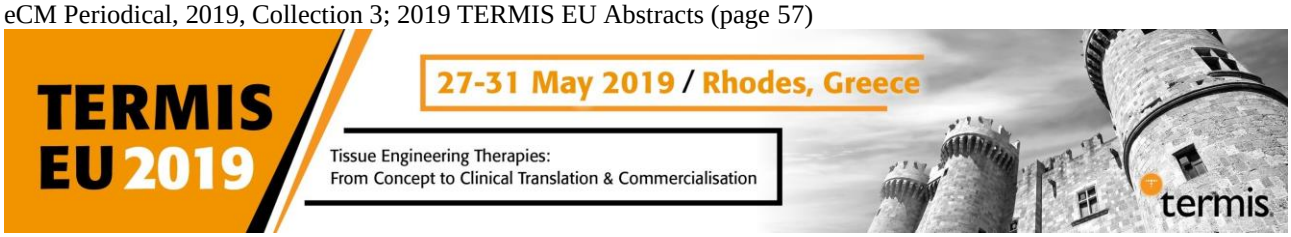
C. O'Leary[1,2,3]
Presenting Author: Cian O'Leary, cianoleary@rcsi.ie
[1]School of Pharmacy, Royal College of Surgeons in Ireland, Ireland [2]Tissue Engineering Research Group, Royal College of Surgeons in Ireland, Ireland [3]Advanced Materials & Bioengineering Research (AMBER) Centre, Royal College of Surgeons in Ireland & Trinity College Dublin (TCD), Ireland.

In tandem with an ageing population and a lack of curative treatments, chronic respiratory disease persists as a leading cause of global mortality, morbidity, and economic burden. The clinical management for conditions such as chronic obstructive pulmonary disease (COPD), cystic fibrosis, and idiopathic pulmonary fibrosis primarily focus on symptomatic therapies, prompting the requirement for further discovery of disease-modifying medicines. Unfortunately, current preclinical models limit the successful translation of new therapeutics in the drug development pipeline, with either in vitro models of single-cell, two-dimensional cultures providing an oversimplification of respiratory tissue, or in vivo animal models lacking key characteristics of the human disease in question. As a result of the disconnect between pre-clinical in vitro models, animal models, and subsequent human trials, most potential drug candidates under investigation fail to exhibit the necessary safety and efficacy to positively influence patient outcomes [1]. Alternatively, bioengineered in vitro respiratory models can enable the exploration of critical features of airway conditions to investigate new mechanisms of disease, drug targets, and overall, bridge the in vitro-in vivo disparity in drug discovery [2]. These models can accurately simulate the complex multicellular environment of the airways, recapitulating the correct three-dimensional anatomical and physiological tissue arrangement that instructs cell behaviour through extracellular matrix (ECM)-ligand interactions and biophysical forces. Moreover, in the case of a particular disease, specific properties of these systems can be modified to parse the important pathophysiological mediators in a manner that is not feasible with current in vitro platforms or animal models. Essentially, by application of the tissue engineering triad, emerging therapeutics can be examined with greater accuracy to provide crucial detail at an earlier stage in the drug development process, facilitating greater use of resources and improving successful translation. Accordingly, the application of bioengineering principles to evaluate novel therapeutics for chronic respiratory disease will be discussed, using examples of tissue-engineered tracheobronchial models and substrates to explore new drug candidates identified in our laboratory.

ACKNOWLEDGEMENTS: The O'Leary Lab receives funding from Science Foundation Ireland as part of the AMBER research centre (17/RC-PhD/3477) and is also supported by research grants from the Fulbright Commission and the State of Kuwait.

REFERENCES

[1] Kola I. Clin Pharmacol Ther. 2008;83:227-30.
[2] O'Leary et al. Tissue Eng Part B Rev. 2015; 21(4):323-44.



Development of synergistic therapeutic biomaterials to manipulate bone metabolism and promote repair

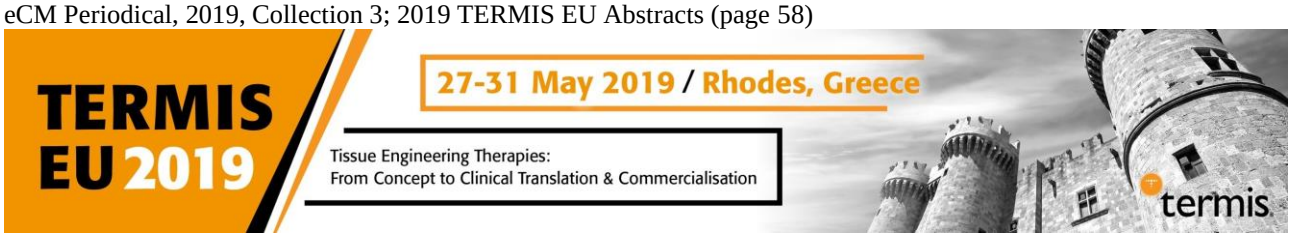
C.M. Murphy[1,2]
Presenting Author: Ciara Murphy, ciaramurphy@rcsi.ie
[1]Tissue Engineering Research Group (TERG), Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Science Foundation Ireland (SFI) Centre for Advanced Materials and BioEngineering Research (AMBER), Trinity College Dublin (TCD), Dublin, Ireland

Healthy bone metabolism is a tightly coupled dynamic process that relies on a balance between osteoclast driven bone resorption (catabolism) and bone formation (anabolism) by osteoblasts. Traditionally, tissue-engineering approaches for non-union fracture repair employ local anabolic therapeutic delivery strategies that target mesenchymal stem cells (MSCs) and osteoblasts to induce bone formation; however, the challenge of healing non-union defects depends on the cause of defect e.g. trauma or disease, and targeting bone formation alone is often insufficient for healing. Our research focuses on targeting both anabolism and catabolism to manipulate bone metabolism and promote bone repair. We have approached this through the synergistic use of both anabolic therapeutics, including recombinant human bone morphogenic protein (rhBMP) -2 and parathyroid hormone $(PTH)_{(1-34)}$, and anti-catabolic bisphosphonates (BPs) in order to manipulate bone metabolism. A major challenge with harnessing a combined dosing regimen is controlling the release of the individual therapeutics to target cells. We have developed a number of polymer-ceramic based biomaterial delivery systems, including both implantable scaffolds and injectable scaffolds, for the controlled release of these therapeutics and demonstrated their efficacy in vivo [1]. A dual therapeutic load of BMP-2 and BPs provided a synergistic enhancement of bone regeneration, demonstrating significantly increased bone formation and remodelling compared to anabolic therapies alone [2]. Incorporating hydroxyapatite in our scaffolds further increased bone formation, demonstrating our polymer-ceramic scaffolds to be osteoconductive in the absence of therapeutics. We have also investigated the manipulation of bone metabolism through a specific dosing regimen of $PTH_{(1-34)}$ to induce bone remodelling and drive healing in BP loaded fractures [3]. Our research to date has shown that optimising both the delivery and regimen of anabolic and anti-catabolic therapeutics to control bone metabolism, augments the bone regenerative potential of these therapeutics in orthopaedic applications. More recently, we have begun to investigate the potential of a number of bioactive ceramics to elicit an anabolic and anti-catabolic bone cell response, and have incorporated them within thermoresponsive collagen-based matrices to develop a range of minimally invasive therapeutic scaffolds that can manipulate metabolism and promote bone repair without the addition of drugs.

ACKNOWLEDGEMENTS: Financial support was received from SFI/AMBER (17/RC-PhD/3477); and H2020, Marie Skłodowska-Curie Actions, Individual Fellowship (705994).

REFERENCES

[1] Yu NY et al. J Biomed Mater Res B Appl Biomater. 2017;105(1):136-144
[2] Murphy CM et al. Acta Biomater. 2014;38(7): 1527-1533
[3] Murphy CM et al. J Bone Miner Res. 2015;30(6):1022-1029



Advanced bioprinting. A surgeon's perspective

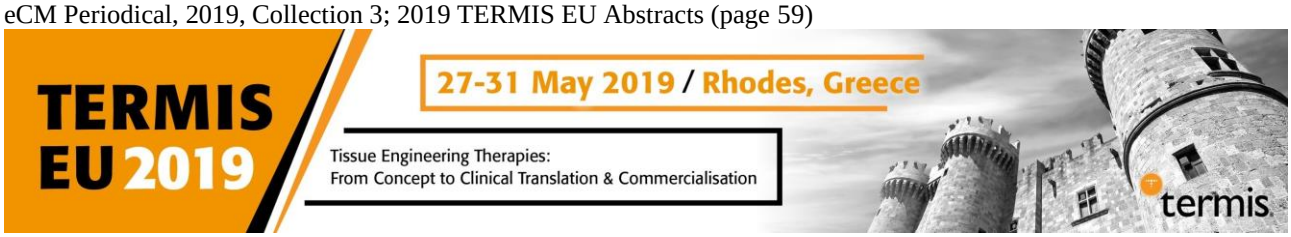
C. Di Bella[1,2,3]
Presenting Author: Claudia Di Bella, claudia.dibella@unimelb.edu.au
[1]Department of Surgery, The University of Melbourne, Australia [2]BioFab3D, Aikenhead Centre for Medical Discovery, Melbourne, Australia [3]Department of Orthopaedic Surgery, St Vincent's Hospital, Melbourne, Australia

Three-dimensional (3D) printing of acellular, non biological material, is already widely used in surgery. In the biomedical field, this technology is in fact increasingly used to create custom-made implants, precise cutting guides, anatomic or models, and can greatly help surgeons in i) identifying specific anatomic variations in complex cases, ii) assisting in the planning of the surgery, or iii) performing precise cuts for bone removal. In recent years, the possibility to print biological materials (Bioprinting), including cells, has gained increasing interest in surgery. Bioprinting has the potential to fabricate living tissues and organs, by delivering biologic elements (not only cells, but also growth factors, drugs, cytokines) within a biomaterial scaffold in a pre-determined way [1]. Bioprinting is typically performed using bench-based sophisticated 3D printers, often in a sterile environment, and allow for the creation of multi-layered scaffolds with cell-to-cell interactions and matrix production [2]. Furthermore, new studies have described the design and applicability of surgical bioprinters, which can deliver, at the time and point of need, a biomaterial that can be deployed during surgery to repair or regenerate human tissues. Ideally handheld directly by the surgeon, this approach can potentially solve some of the difficulties encountered with bench-based constructs [3]. 3D printing and bioprinting now carry huge expectations from the surgical community, because it promises the potential regeneration of entire living organs and tissues. In this talk I will discuss the current state of the art in bioprinting from a surgical prospective, focusing on its application in the musculoskeletal field. I will highlight the current roadblocks for the application of this technique in clinical practice, as well as the expectations and the promises for the future seen with the eyes of the final user.

ACKNOWLEDGEMENTS: Financial support was received from MTP Connect, Royal Australasian College of Surgeon, Australian Orthopaedic Association, Arthritis Australia Project Grant, CASS Grant, The University of Melbourne seeding grant

REFERENCES

[1] Ravnic DJ, Leberfinger AN, Koduru SV, et al. Ann Surg. 266:48–58. 2017
[2] Wu Z, Su X, Xu W, et al. Sci Rep. 6: 24474. 2016
[3] Duchi S, Onofrillo C, O'Connell C, et al. Sci Rep 7: 5837. 2017



Mesenchymal stem/stromal cells - A novel therapeutic tool against infection

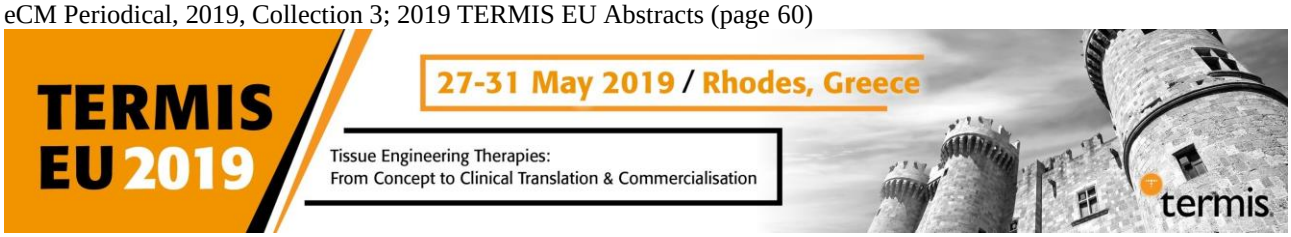
C.L. Esteves[1]
Presenting Author: Cristina Esteves, cristina.esteves@roslin.ed.ac.uk
[1]The Roslin Institute, University of Edinburgh, UK

Increased bacterial resistance to antibiotics is recognized as a major threat to human and animal health. It is a growing problem in the horse, as reflected by a sharp increase over the last 20 years in the number of clinical bacterial isolates that are resistant to a variety of antibiotics. Therefore, addressing antimicrobial resistance (AMR) through more rational antimicrobial use and finding alternative/complementary approaches to antibiotic therapy has become an urgent priority for the management of acute and chronic infection both in humans and companion animals. An added concern with the use of antibiotics is that although effective in combating the cause of disease they do not address tissue damage in infection, which often resolves as fibrosis and scaring, making the tissue prone to re-infection and chronic disease. In humans, new approaches are already being developed that involve not only direct action against bacteria but also stimulation of host immunity and natural tissue healing [1]. Novel strategies harnessing the antimicrobial properties of MSCs are now at the frontline, with early phase clinical trials already underway for multi-drug resistant tuberculosis, Acute Respiratory Distress Syndrome and Chronic Obstructive Pulmonary Disease. Indeed, enormous excitement has been generated recently with the demonstration that MSCs effectively enhance bacterial clearance in preclinical models of infection, both through direct production of antimicrobials and through modulation of host immune cells, namely macrophages and neutrophils. Limited clinical data in domestic animals (dogs) has shown significant benefits in the treatment of drug-resistant skin infections. In the horse, two groups including ours [2,3] have shown that MSCs effectively respond to bacteria by secreting antimicrobials (e.g. Lipocalin-2) and immunomodulatory cytokines (MCP-1, CCL5, IL-6 and -8), significantly reducing growth and biofilm formation by Escherichia coli and Staphylococcus aureus in vitro. We observed these effects with MSCs obtained from different tissue origins, not only from bone marrow and adipose tissue, but also from endometrium, which is naturally exposed to infection. Equine MSCs have been used for musculoskeletal repair for over 15 years and are now the subject of a multimillion dollar business incorporating veterinary clinics and stem cell companies worldwide. However, their clinical potential extends beyond the musculoskeletal system, and their use in the combat of infection could be of great relevance not only in humans but also for treatment of companion animals, namely the horse.

ACKNOWLEDGEMENTS: This work was supported by grants from the Horserace Betting Levy Board (Prj768 and SPrj022), the Petplan Charitable Trust (2017-568-606), Commission for Science and Technology - Government of Chile (CONICYT) and The Roslin Institute Strategic Programme Grant funding from The Biotechnology and Biological Sciences Research Council (BBSRC).

REFERENCES

[1] Alcayaga-Miranda F, et al. (2017) Front Immunol. 8:339
[2] Cortes-Araya Y, et al. (2018) Stem cells and Development. doi: 10.1089/scd.2017.0241.
[3] Harman RM, et al. (2017). Stem Cell Research & Therapy 8:157.



Early simulation of bioengineered Muscle tissue to support volume and function

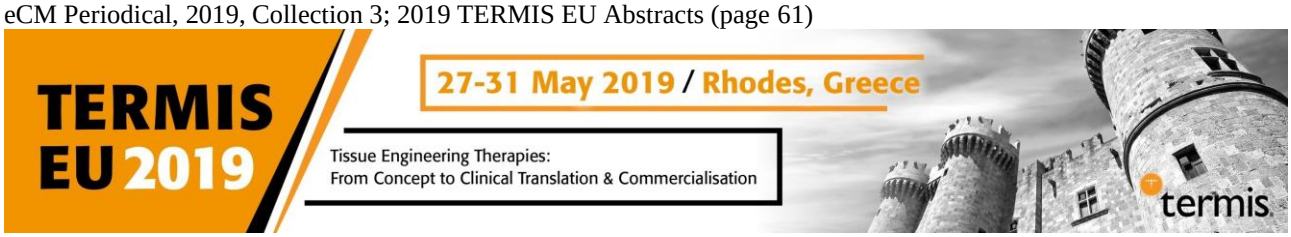
D. Eberli[1, 2]
Presenting Author: Daniel Eberli, daniel.eberli@usz.ch
[1]Urology Department, University Hospital Zurich, Switzerland [2]Urology Department Urologic Tissue Engineering and Stem Cell Therapy, University of Zurich, Switzerland

Disease and traumatic injury can lead to skeletal muscle loss associated with functional impairment. The restoration of lost muscle tissue represents a big clinical challenge and there is urgent clinical need for novel treatment modalities for muscle bioengineering. The use of stem cells constitutes a promising strategy for the generation of autologous, implantable cells to repair damaged skeletal muscle. However, the early quality of the regenerated tissue is of critical importance for its accurate function and thus many efforts have been made to improve the in vivo cell survival and maturation. The muscle tissue development by optimizing the cell population, the nurturing microenvironment and stimulating function are being explored. Current limitations are cellular dedifferentiation, low vascularisation and lack of innervation. Early stimulation using electrical, mechanical and pharmacological stimuli can be used to increase muscle function and performance. Our lab has shown that magnetic stimulation supports muscle and nerve regeneration by activating the neuronal cellular machinery, increasing nerve-muscle cross-talk and inducing the maturation of newly formed neuromuscular junction [2]. In addition, we could show that injected human muscle precursor cells over expressing peroxisome proliferator-activated receptor gamma coactivator 1-alpha (hPGC-1α) enhanced the functional muscle regeneration after trauma [2].

ACKNOWLEDGEMENTS: Swiss Science Foundation Hartmann Müller-Stiftung

REFERENCES

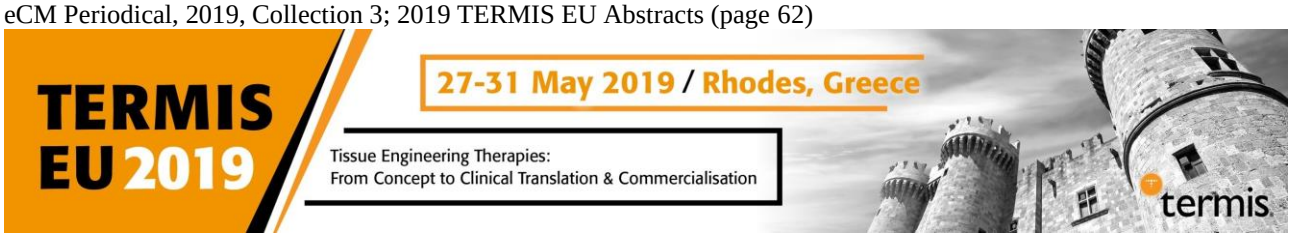
[1] Stolting M.N, et al. Muscle Nerve, 53 (2016) 598-607.
[2] Haralampieva D, et al. Stem Cells international. Stem Cells Int, 2018 (2018) 4658503.



Bioprinting for bone and joint regeneration

D. J. Kelly
Presenting Author: Daniel J Kelly, kellyd9@tcd.ie
Trinity Centre for Bioengineering, School of Engineering, Trinity College Dublin, Ireland

Our musculoskeletal system has a limited capacity for repair. This has led to increased interest in the development of tissue engineering strategies for the regeneration of musculoskeletal tissues such as bone, ligament, tendon, meniscus and articular cartilage. This talk will review our attempts to use biomaterials and mesenchymal stem cells (MSCs) to bioprint functional articular cartilage and bone grafts for use in bone and joint regeneration. It will begin by describing how 3D bioprinting can be used to engineer biological implants mimicking the shape of specific bones, and how these bioprinted tissues mature into functional bone organs upon implantation into the body. Next, it will be demonstrated that different musculoskeletal injuries can be regenerated using 3D bioprinted implants, including large bone defects and osteochondral defects. The talk will conclude by describing how we can integrate biomaterials and MSCs into 3D bioprinting systems to engineer scaled-up tissues that could potentially be used regenerate entire diseased joints.



Mussel-inspired adhesives, coatings and films for biological applications

D. Ruiz-Molina
Presenting Author: Daniel Ruiz-Molina, dani.ruiz@icn2.cat
Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Campus UAB, Bellaterra, 08193 Barcelona, Spain

Inspired by the wet adhesion of mussels to almost any kind of surface, one of the most widespread methodologies used over the last years to control biocompatibility of surfaces has been the melanin-like oxidative polymerization of dopamine into a polydopamine (PDA), followed by its functionalization with appropriate functional groups. Though, despite its versatility and simplicity, PDA modification relies on the existence of a sufficient amount of reactive groups in the primer coating, which cannot be accurately controlled and, because of PDA insolubility, restricted to in situ polymerization methodologies. Recently, we have reported the development of an alternative, simpler approach to fabricate catechol-based materials with interesting adhesive properties. In this novel strategy, a single monomer bearing both a catechol ring and at least one desired functional side chain (i.e., designed to carry a maximum amount of functional groups) is first synthesized and then polymerized by means of a simple and inexpensive procedure consisting of treatment with ammonia in aerobic conditions. Compared to PDA, the main differences would be, first, the nitrogen source - which is endogenous in the case of catecholamines and external in our case (ammonia)- and, more importantly, the fact that this novel approach does not rely on uncontrolled residual reactivity in order to introduce a specific functionality in the coating because it is already built in the catecholic monomer. Following this approach we have fabricated different families of coatings and nanoparticless of applicability as biosurfaces, theransotics carriers and tissue engineering.

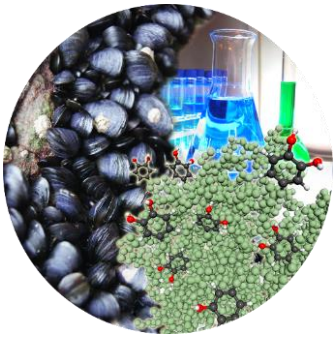
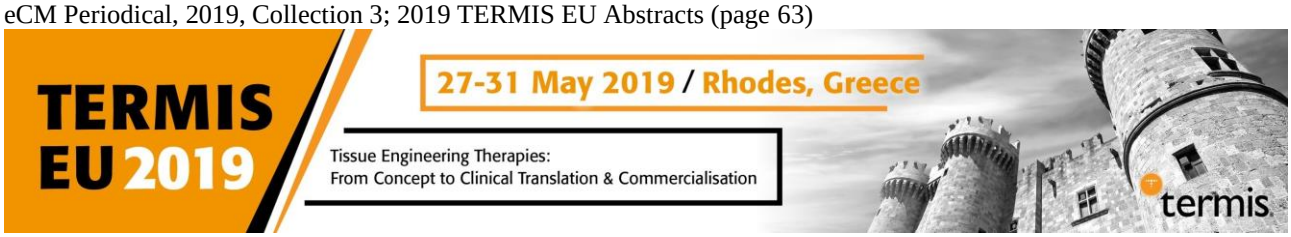


Figure 1: Schematic representation of the bioinspired approach followed for the development of mussel-inspired biomaterials.

ACKNOWLEDGEMENTS: This work was supported by project MAT2015-70615-R from the Spanish Government, the FEDER funds and the COST-Action ENBA CA15216. The ICN2 is funded by the CERCA programme/Generalitat de Catalunya. The ICN2 is supported by the Severo Ochoa program of the Spanish Ministry of Economy, Industry and Competitiveness.

REFERENCES

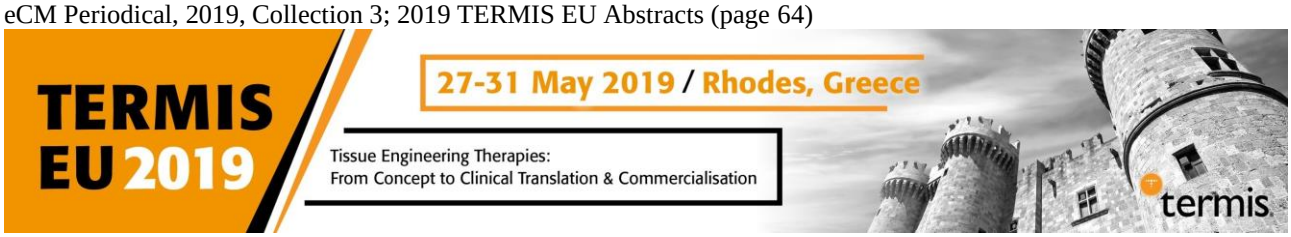
[1] Saiz-Poseu et al. Angew. Chem. Int. Ed.2018,57:2 – 21
[2] Moreno-Villaécija et al. ACS Appl. Mater. Interfaces 2018, 10 (9):7661–7669
[3] Suárez et al. ACS Nano, 2018;submitted
[4] Suárez et al. Biomimetics 2017, 2 (4), 22
[5] Nador et al. Chemistry–A European Journal 2017, 23 (12), 2753-2758



Imaging cardiac regeneration

D. Stuckey
Presenting Author: Daniel Stuckey, d.stuckey@ucl.ac.uk
Centre for Advanced Biomedical Imaging, University College London, London, UK

Imaging has the potential to provide robust tools essential for the smooth translation of promising preclinical studies into effective treatments for heart disease. The possibility of visualising location and extent of therapeutic cell retention and correlating this with local and global measures of cardiac structure, function and viability is tantalising. Yet the majority of preclinical and clinical trials still use the simplest of non-invasive methods for monitoring response to treatment; and despite the development of numerous elegant methods for stem cell tracking, few have been applied clinically, and none in recent years for heart disease. This presentation will discuss the strengths and weakness of current imaging approaches adopted in trials of cardiac cell therapy, and will suggest potential future directions which may allow imaging to save cardiac regeneration before it becomes lost in translation.



Viable and cell-free tissue substitutes from human pluripotent stem cells for bone regeneration during aging

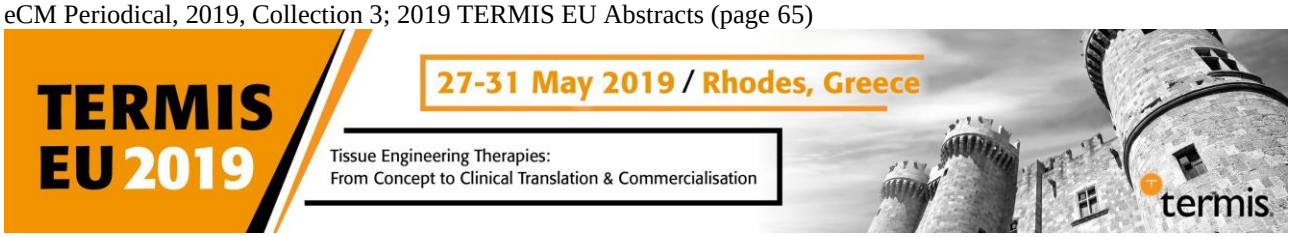
D. Marolt[1,2]
Presenting Author: Darja Marolt, Darja.Marolt@trauma.lbg.ac.at
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology/AUVA Research Center, Vienna, Austria [2]The Austrian Cluster for Tissue Regeneration, Vienna, Austria

Regeneration of bone defects in the elderly patients presents significant clinical and scientific challenges, due to the compromised tissue physiology, decreased function of cells driving tissue regeneration and changes in the systemic environment due to aging and diseases. We have previously reported on engineering of bone tissue substitutes from human induced pluripotent stem cells (hiPSCs) [1], which represent an autologous source of potentially rejuvenated tissue progenitors of any lineage and can be prepared for any patient in unlimited quantities [2]. hiPSC-engineered bone substitutes exhibited stability, continued maturation and vascularization in a subcutaneous transplantation model after 12 weeks, suggesting their potential for bone regeneration. Nevertheless, the development and clinical translation of viable hiPSC-based tissue substitutes present significant challenges, with numerous scientific, safety and regulatory questions to be addressed. On the other hand, hiPSCs and their rejuvenated progeny also represent an unlimited resource for the production of secreted factors, vesicles and extracellular matrix that could be employed in cell-free bone tissue engineering strategies. In our current projects, we prepared and characterized these microenvironment components secreted by the hiPSC-derived mesenchymal progenitors (hiPSC-MPs). We identified major cytokine and growth factor components of the hiPSC-MP conditioned media (CM) and separated the extracellular vesicle fractions. We also developed protocols for in vitro engineering of extracellular matrices (ECM) and evaluated their structure/composition before and after decellularization. Using a library of primary bone marrow stromal cells (BMSCs) from traumatology patients of different ages, exhibiting diverse proliferation and differentiation potentials, we found that hiPSC-MP-derived CM and ECM modulate the primary BMSC osteogenic responses in a patient-, dose- and time- specific manner. In particular, some aged BMSC lines only exhibited significant proliferation and osteogenic differentiation when cultured in the presence of hiPSC-MP secreted components. These results suggest that hiPSC-MP-derived microenvironment components can modulate, i.e. “rejuvenate”, the regenerative potential of adult/aged BMSCs. Further investigation into the mechanisms responsible for patient-specific responses and approaches for the engineering of bone tissue substitutes to enhance the regenerative responses in elderly patients will be discussed.

ACKNOWLEDGEMENTS: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 657716. Financial support was also received from Austrian Research Promotion Agency (FFG) “Industrienahe Dissertation” projects No. 853056 and No. 867803, and “FemTech Praktikum” projects No. 852154 and No 868917.

REFERENCES

[1] de Peppo GM et al. Proc Natl Acad Sci U S A. 2013 110(21):8680-5.
[2] Lapasset L et al. Genes Dev. 25(21):2248-53



Melt electrospun written scaffolds with optimised fibrous and mineral architectures to enhance human MSC osteogenesis for bone regeneration

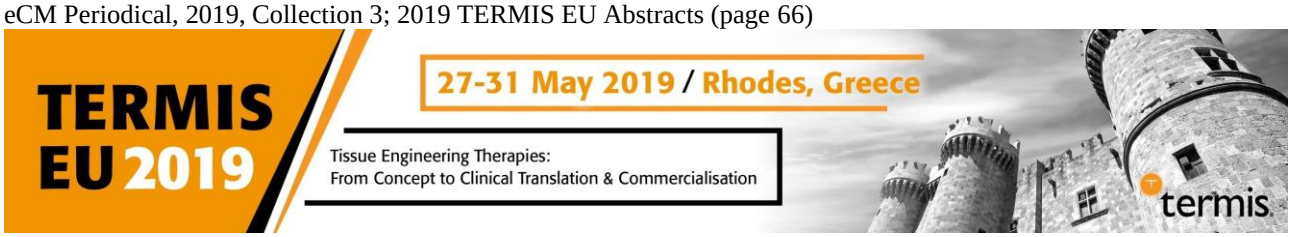
D.A Hoey[1,2,3,4]
Presenting Author: David Hoey, dahoey@tcd.ie
[1]Dept. Mechanical, Aeronautical and Biomedical Engineering, Materials and Surface Science Institute, University of Limerick, Limerick, Ireland [2]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland [3]Dept. of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Ireland [4]Advanced Materials and Bioengineering Research Centre, Trinity College Dublin & RCSI

Material micro-architecture and chemistry play pivotal roles in driving stem cell behaviour. Bone at a cellular level consists of arranged fibres with a cross-fibrillar mineral phase made up of curved nano-sized needle shaped crystals [1]. This nano-structured mineral architecture can bind and stabilise proteins within bone for centuries and thus holds promise as a strategy for therapeutic delivery in regenerative medicine [2]. Herein, we use melt electrowriting (MEW) technology to create fibrous 3D Polycaprolactone (PCL) micro-architectures [3]. These scaffolds were further modified with an extrafibrillar coating of plate shaped micron-sized calcium phosphate crystals (pHA), or with a novel extrafibrillar coating of needle shaped nano-sized crystals (nnHA). A third scaffold was developed whereby nano-sized crystals were placed intrafibrillarly during the MEW process (iHA). Along with vastly differing surface topographies, surface chemistry is altered, with calcium/phosphate ratios differing between groups with values between 1.66 – 1.97, within the range seen in native bone. X-ray diffraction revealed altered crystal structure and crystallinity between groups, with hydroxyapatite (HA) present in all modifications in addition to other minor phases in the extrafibrillar scaffold groups. Water contact angle was investigated revealing increased hydrophilicity with extrafibrillar coatings, while tensile testing revealed enhanced stiffness in scaffolds fabricated with intrafibrillar HA. Biological characterisation demonstrated significantly enhanced human mesenchymal stem cell mineralisation with extrafibrillar coatings, with a 5-fold increase in mineral deposition with plate like structures and a 14-fold increase with a needle topography, demonstrating the importance of bone mimetic architectures. Given the protein stabilising properties of mineral, these materials were further functionalised with BMP2. Extrafibrillar coatings of nano-needles facilitated a controlled release of BMP-2 from the scaffold which further enhanced mineral deposition by stem cells. This study thus outlines a method for fabricating scaffolds with precise fibrous micro-architectures and bone mimetic nano-needle HA extrafibrillar coatings which significantly enhance MSC osteogenesis and therapeutic delivery and thus hold great promise for bone tissue regeneration.

ACKNOWLEDGEMENTS: Financial support was received from European Research Council (ERC) Starting Grant (336882), Science Foundation Ireland (SFI) Support Grant SFI 13/ERC/L2864 and Irish Research Council Postgraduate Scholarship (GOIPG/2014/493).

REFERENCES

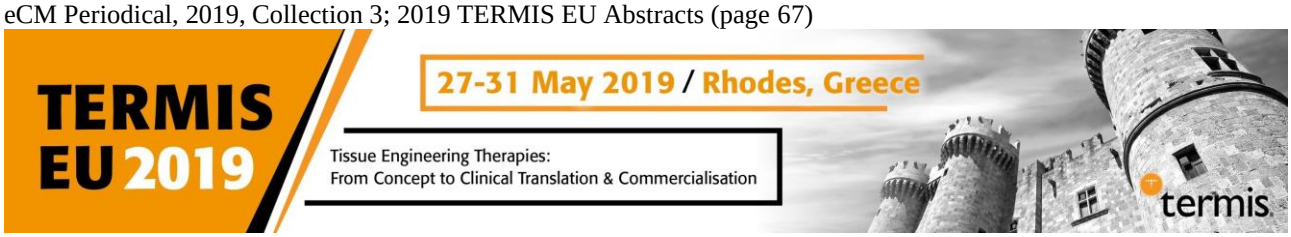
[1] Reznikov, N., et al., Science, 2018. **360**(6388).
[2] Yu, X., et al., Adv Mater, 2017. **29**(33).
[3] Eichholz, K.F., D.A. Hoey, Acta Biomater, 2018. **75**:p.140-151.



Fabrication of stimuli responsive hydrogel microenvironments for cell manipulation

D. Eglin
Presenting Author: D. Eglin, david.eglin@aofoundation.org
AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland

Stimuli responsive hydrogels are being developed for use as two and three-dimensional extracellular microenvironments to mimic the features of the extracellular matrices (ECM), for basic and translational studies. The polymers developed for such application can be biopolymer-derivatives showing responsivity to environmental factors such as temperature, enzyme and light. These external stimuli control the polymeric network formation and in fine the ECM-like environment. However, there is often a lack of understanding on how the external stimuli and crosslinking mechanism of these derivatives can influence the cells behavior. In addition, anisotropy in ECM mimic matrices are seldom achieved in controlled manner and in a cell compatible way. Enhance, we investigated the role of hydrogel enzymatic and light crosslinking and of anisotropy fabrication on the behavior of embedded cells.

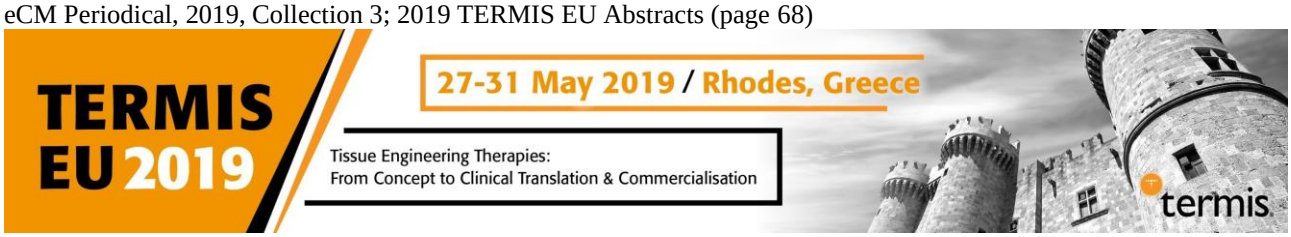


Materials and growth factors for rotator cuff repair

D. Musson[1]
Presenting Author: David Musson, d.mussson@auckland.ac.nz
[1]Bone & Joint Research Group, Department of Medicine, University of Auckland, New Zealand

Surgical repair of rotator cuff tears have high failure rates (20-70%), often due to a lack of biological healing. Augmenting repairs with extracellular matrix-based scaffolds is a common option for surgeons, although to date, no commercially available product has proven to be effective. As such, there has been great interest in developing novel biomaterials and/or growth factor treatments to help solve this debilitating clinical problem. Over the past 7 years we have worked with orthopaedic surgeons, and chemical and material engineers, in an attempt to identify augments that would significantly enhance healing outcomes for patients undergoing rotator cuff surgery. In vitro studies include assessing primary human tenocyte cell growth, matrix production and the expression of genes important in tendon health and disease, as well as in vitro immune response based on pre-macrophage-like cell activation. If an augment appears promising in vitro, we then assess it in a pre-clinical rat rotator cuff defect model. In the search for the ideal augment, we have assessed synthetic and natural materials designed to be used as overlay augments, aimed at providing both mechanical and biological support. While many have fallen at the in vitro stage, the most promising of these have included a decellularised ovine forestomach matrix, and a pure bovine type I collagen matrix. Both these materials enhanced histological outcomes of the tendon itself, but did not enhance the mechanical integrity of the repair to a clinically significant degree. Based on these findings we hypothesised that in order to improve clinical outcomes, an augment needs to address the healing of the tendon, and the tendon-bone junction. Therefore, materials that are designed to be used as overlay augments may not be the best approach to achieve this. Here, we will discuss our assessment of novel rotator cuff augments, and the findings that have led to our recent hypothesis. Finally we will discuss the application of combination therapies which we feel will have minimal impact on the current surgical procedure of orthopaedic surgeons, but have maximum impact on rotator cuff healing outcomes.

ACKNOWLEDGEMENTS: Dr Musson is funded by the Auckland Medical Research Foundation Senior Research Fellowship (3716739). Financial support was also received from Health Research Council of New Zealand programme grant (15/576) and supported by the European Union Seventh Framework Programme skelGEN (FP7/2007-2013) under grant agreement n° [318553].



Skeletal vascularized organoids from patient-derived cells

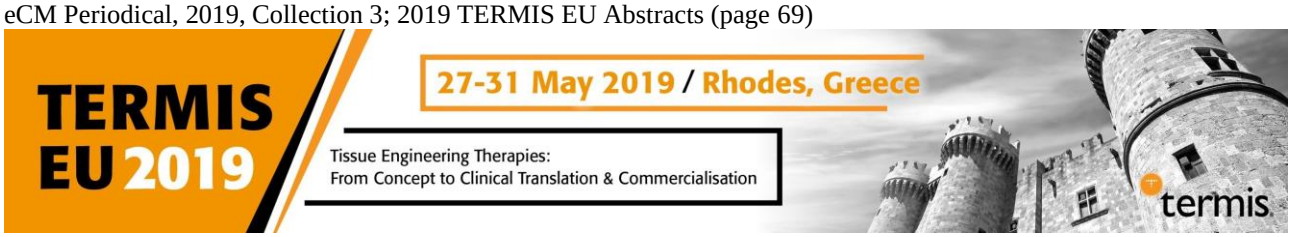
D. Gawlitta[1,2]
Presenting Author: Debby Gawlitta, d.gawlitta@umcutrecht.nl
[1]Dept. of Oral and Maxillofacial Surgery & Special Dental Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands [2]Regenerative Medicine Center Utrecht, Utrecht, the Netherlands

Despite the impressive regenerative capacity of bone tissue, large defects cannot heal without intervention. For example, patients suffering from large maxillofacial bone defects receive autologous vascularized bone flap transplants, harvested from a second surgical site, such as the fibula. Bone regenerative strategies could eliminate the need for autologous bone harvesting. Creation of clinically relevant size bone tissue necessitates the co-engineering of a perfused, intricate vascular network to support cell survival in the construct interior. Such skeletal vascularized organoids can be built with various stem cells and/or biomaterials [e.g. 1]. Typically, co-cultures consist of endothelial or endothelial progenitor cells with fibroblasts, osteoblasts, pericytes or multipotent mesenchymal stromal cells (MSCs). To move to patient-specific tissues, autologous stem/progenitor cell sources can be exploited. Both, for patient-specific disease modeling and for implantation in bone defects of patients, these engineered tissues can be relevant. This talk will highlight our approaches to engineer bone organoids containing capillary-like networks from clinically relevant cell sources. In this, the impact of donor variation on the quality of the in vitro formed networks is discussed. Several biomaterials can support the co-formation of these tissues. Furthermore, the establishment of interconnection of multi-scale vascular networks in engineered bone constructs will be demonstrated [2]. This will pave the way for bringing engineered pre-vascularized bone constructs to cm-scale, with in vitro perfusable endothelial networks.

ACKNOWLEDGEMENTS: This research was partially supported by NWO (Netherlands Organization for Scientific Research) under Graduate Programme Grant #022.005.018 and by the Dutch Technology Foundation (STW) under Veni grant #11208.

REFERENCES

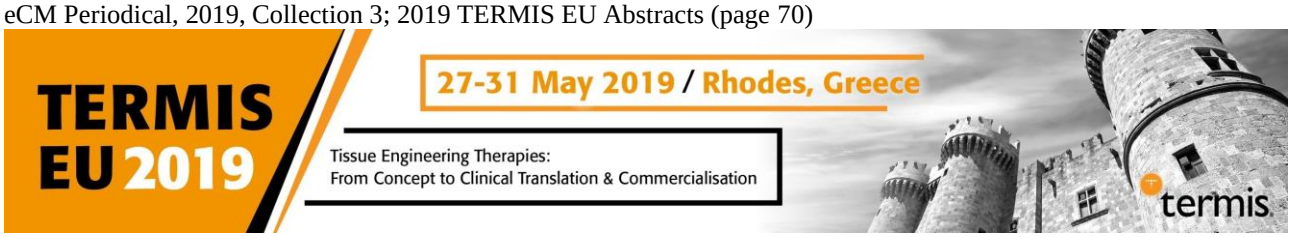
[1] Gawlitta D et al., Tissue Eng Part A. 2012; 18(1-2):208-18.
[2] Klotz BJ et al., Eur Cell Mater. 2018; 30(35):335-348.



Translation of basic tendon science into clinical practice

D. Docheva
Presenting Author: Denitsa Docheva, denitsa.docheva@ukr.de
Laboratory of Experimental Trauma Surgery, Department of Trauma Surgery, University Regensburg Medical Centre, Regensburg, Germany

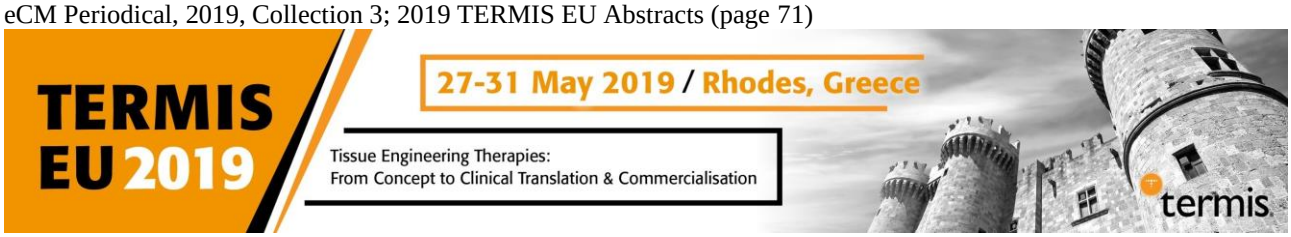
Tendons and ligaments are fascinating in their simplistic appearance of tissue architecture coupled with outstanding biomechanical properties. They are also the biggest compartment of our musculoskeletal system and multicentre research in the last decade has led to improved understanding of how these tissues develop, become diseased and do repair. Resident tendon stem progenitor cells (TSPC) have become a central player in tendon science as they exert important functions in tendon formation, physiology and pathophysiology. Here, I will provide evidence from our basic research on: (i) genes regulating certain TSPC features; (ii) the link between TSPC abnormalities and age-related tendinopathy; and (iii) TSPC therapeutic potential. Lastly, in order to effectively translate the novel findings from basic science to clinical practice, I will discuss what further exploratory efforts are required and how to avoid “lost in translation”.



Product development in an GMP manufacturing environment

D.P. Link[1]
Presenting Author: Dennis Link, dennis.link@bioconnection.eu
[1]BioConnection, Oss, The Netherlands

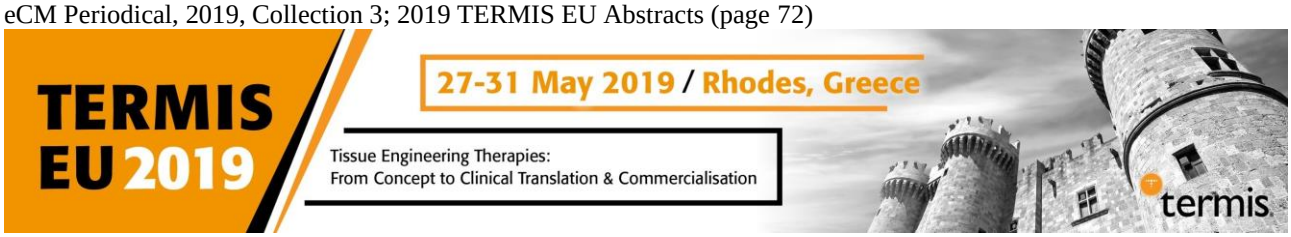
Everybody has great product ideas, but yet only a small percentage of ideas ever get started. The idea of introducing a product into the market seems overwhelming, complicated and very expensive to most people. They don't know where to start or what to do, so eventually nothing will be done. That is quite a shame, so this presentation should encourage scientists to build this bridge to industry and develop their ideas into successful products. Before you can actually use your product in a clinical setting you need to manufacture it in an controlled environment. This production method, called Good Manufacturing Practice, is therefore a requirement for the production of medicinal products for the human and veterinary pharmaceutical industry. The quality of medicinal products can never be completely determined by analyzing the composition. Not all possible impurities can be detected and not every product can be analyzed. The quality can therefore only be guaranteed if the entire production process is also carried out in a precise and controlled manner. Within GMP, it is important that it is accurately recorded how and under which circumstances a product is made. During production, all raw materials, intermediates and end products are checked and the process is precisely kept on the so-called preparation protocol. If afterwards something turns out to be wrong with a certain batch of medicines, one can always find out how it was made, who tested it, where and what raw materials were used. Examples of products being developed from the lab bench leading to eventual clinical productions will be shared and risks in product development in a GMP manufacturing environment will be discussed.



(Bio)engineered organs for blood detoxification

D. Stamatialis
Presenting Author: Dimitrios Stamatialis, e-mail: d.stamatialis@utwente.nl
(Bio)artificial Organs, University of Twente, TechMed Institute of Biomedical Technology and Technical Medicine, Faculty of Science and Technology, P.O Box 217, 7500 AE Enschede, The Netherlands

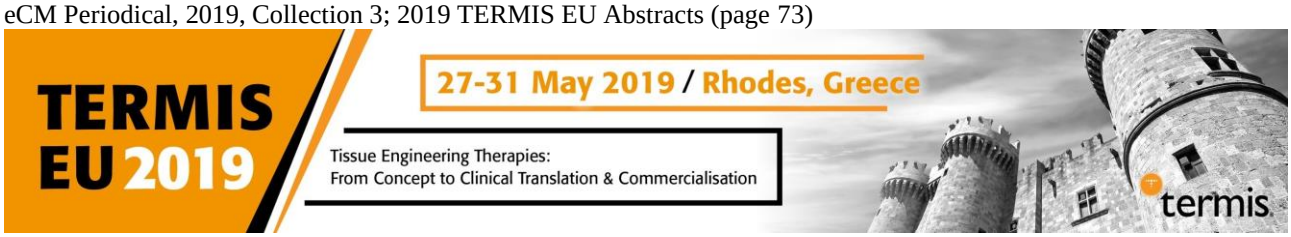
For patients with chronic kidney disease the best solution would be organ transplantation. However, due to shortage in donor organs and the fact that not all patients are eligible for transplantation, most patients are currently treated with therapies using artificial kidney devices. In hemodialysis therapy, a widely available and well-established treatment for the patients with End Stage Renal Disease, the blood of these patients is cleansed 3-4 times a week in dedicated dialysis centres. Major drawbacks of the therapy are the poor removal of middle-sized molecules and protein-bound uremic solutes and the non-continuous treatment, causing large fluctuations in water balance and uremic wastes of the patients. Besides, the need for continuous visits at the dialysis centres is a great social and psychological burden for these patients. In this talk we will discuss bioengineered concepts for achieving better and more continuous removal of a broad range of uremic solutes and for mimicking better the kidney function, including the concept of: Mixed matrix membranes (MMM) which combine the benefits of diffusion and /or convection, provided by the membrane structure, and of adsorption, achieved by sorbent particles dispersed in the membrane; Bioartificial kidney where “living membranes” of tight renal cells monolayer with preserved functional organic ion transporters attached on suitable artificial membranes, can actively remove the uremic solutes mimicking the function of the kidney proximal tubule.



A gel-based model of selective cell motility: Implications for cell sorting, diagnostics and screening

D. Seliktar
Presenting Author: Dror Seliktar, dror@bm.technion.ac.il
Faculty of Biomedical Engineering, Technion – Israel Institute of Technology, Israel

The ability to precisely control cell-loaded material systems is essential for in vitro testing of novel therapeutics poised to advance to clinic. In this report, unique patterns of cell migration by chemotaxis were devised into an in vitro gel-in-gel model for the purpose of obtaining cell response data to potentially therapeutic agonists. The model consisted of co-cultures in a cell-loaded microgel invading an acellular “sorting” gel. Material properties including biophysical and chemical composition of the sorting gel were carefully controlled to guide a desired cell-specific behavior, leading to massive tumor cell invasion by amoeboid migration mechanisms. Optical transparency enabled straightforward and high-throughput measurements of outgrowth response in the presence of either chemical and photo-radiation therapy. Important dosing and drug sensitivity information were obtained with the gel-in-gel model using no more than a light microscope, without further need for arduous genomic or proteomic screening of the tissue samples.



Bioprinting scaffold-free constructs with cell-only bioink

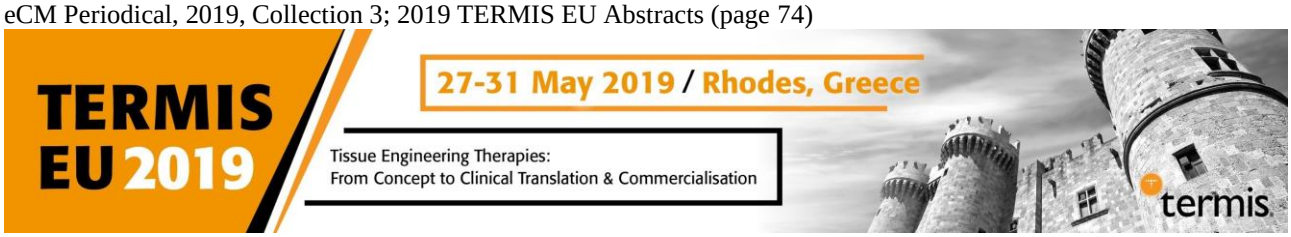
E. Alsberg[1,2]
Presenting Author: Eben Alsberg, ealsberg@uic.edu
[1]Departments of Bioengineering and Orthopaedics, The University of Illinois, Chicago, IL, USA
[2]Departments of Biomedical Engineering and Orthopaedic Surgery, Case Western Reserve University, Cleveland, OH, USA

Three-dimensional (3D) bioprinting has been pursued to build scaffold-free tissue constructs presenting functionally and sophisticated geometry without the presence of an intervening scaffold that can interfere with critical cell-cell interactions. However, to date it has not been possible to print individual cells directly, as preculturing was required to first form cell aggregates or strands prior to printing.[1] Here, the first bioprinting platform using single cells alone, without a macromer solution, as an ink will be reported. A shear-thinning and self-healing support bath permits high-resolution printing of the ink into precise geometries, maintenance of cell viability, cellular condensation formation and long-term culture of constructs for development of engineered tissues. Collectively, the platform may advance cellular condensation-based regenerative medicine strategies, enhance drug-screening capabilities and provide a new tool for addressing questions in developmental biology.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge funding from the National Institutes of Health's National Institute of Arthritis and Musculoskeletal and Skin Diseases and National Institute of Biomedical Imaging and Bioengineering under awards R01EB023907, R01AR063194, and R01AR066193.

REFERENCES

[1] A Ovsianikov et al., Trends in biotechnology 2018;36:348-357.



Cellular brain repair for Parkinson's disease: Is the answer in the (biomaterial) matrix?

E. Dowd[1,2]
Presenting Author: Eilís Dowd eilis.dowd@nuigalway.ie
[1]Pharmacology & Therapeutics and [2]Galway Neuroscience Centre, National University of Ireland Galway (NUI Galway), Galway, Ireland

Cell-based brain repair is a promising option for Parkinson's disease (PD) whereby the nigrostriatal dopaminergic neurons that have degenerated over the course of the disease are replaced by transplantation of healthy neurons into the brain. Given that cell-based brain repair is rapidly accelerating towards the clinic, with the ongoing TRANSEURO trial of fetal tissue and the recently announced Takahashi trial of iPSC-derived dopaminergic neurons in Japan, but that the margin for improvement of such approaches is great, it is critical to continue rigorous preclinical studies to identify potential methods of improving the outcome of cell-based brain repair for patients. In this context, we will be presenting our recent data demonstrating that dopaminergic cell replacement in the Parkinsonian rodent brain, using fetal-derived cells, is dramatically enhanced when the cells were transplanted in a neurotrophin-enriched, immune-shielding collagen hydrogel [1-3]. The hydrogel provided the transplanted neurons with 1) a physical scaffold for cell-matrix adhesion, 2) a neurotrophin reservoir for sustained neurotrophin exposure after transplantation, and 3) shielding from the deleterious effects of the host microglial and astroglial innate immune response (Fig. 1). We will also present data from studies encapsulating human iPSC-derived dopaminergic neurons. Overall, this work suggests that the clinical transplant field should move towards the incorporation of biomaterials, such as neurotrophin-enriched collagen hydrogels, into future clinical trials using primary and/or iPSC derived neurons. Improving the safety and efficacy of such approaches, using this minimally invasive and injectable hydrogel that offers a neuroprotective and immune shielding microenvironment to the transplanted cells, could dramatically improve the reparative capacity of cell therapy for PD, and ultimately lead to an improved therapy for patients.

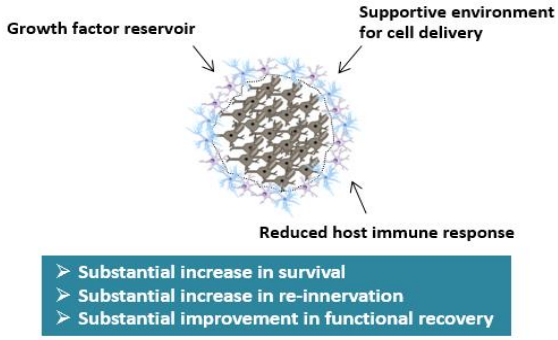
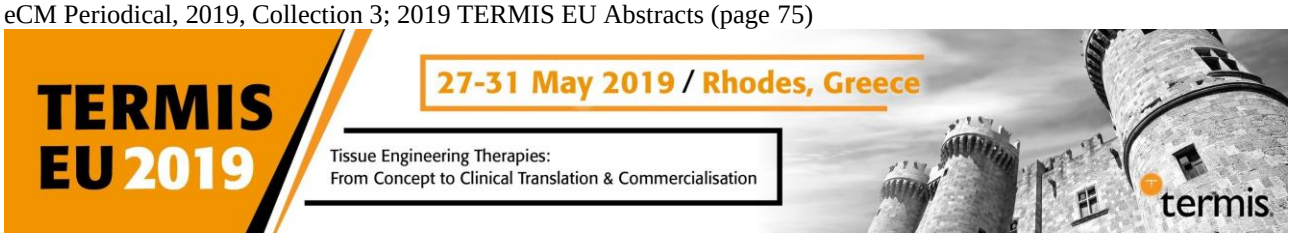


Figure 1: Impact of the neurotrophin-enriched collagen hydrogel on dopaminergic transplants.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland, (Grant/Award Number: 11/RFP/NES/3183) and H2020 Marie Sklodowska-Curie Actions (Grant/Award Number: 676408).

REFERENCES

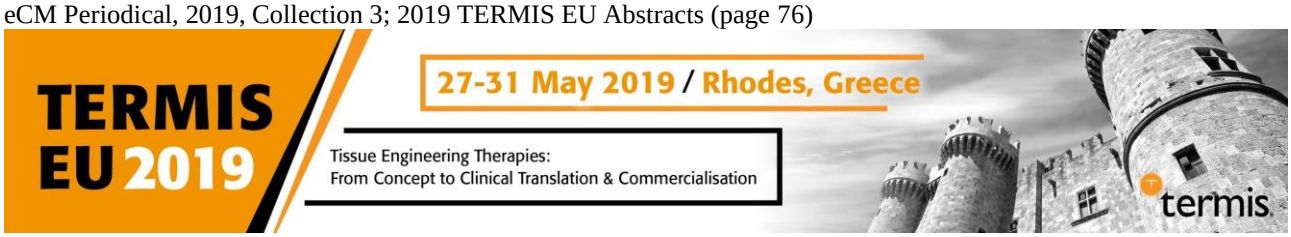
[1] Moriarty et al. Sci Rep, 2017; 7, 16033.
[2] Moriarty N. et al. Eur J Neurosci. 2018; DOI: 10.1111/Ejn.14090. [Epub Ahead Of Print]
[3] Moriarty et al., Eur J Neurosci. 2018; DOI: 10.1111/Ejn.14051. [Epub Ahead Of Print]



A novel integrated low cost tool for accelerating therapies for pancreatic cancer from bench to bed

E.G. Velliou
Presenting Author: Eirini G. Velliou, e.velliou@surrey.ac.uk
Bioprocess and Biochemical Engineering Group (BioProChem), Department of Chemical and Process Engineering, Faculty of Engineering and Physical Sciences, University of Surrey, Guildford, Surrey, UK

Pancreatic Ductal Adenocarcinoma is a deadly disease with 91% of patients dying within five years from diagnosis. This disheartening figure has barely been improved the last 50 years. The low survival rate of patients with pancreatic cancer is partly due to the tumour resistance to currently available treatment, which results from the complex and highly heterogeneous tumour microenvironment (TME). The TME is the biological, biochemical and biomechanical environment that surrounds the tumour and interacts with it in various complex ways, consequently affecting the tumour progression and response to treatment. A key challenge in cancer tissue engineering is ensuring accurate mimicry of the multiple key features of the TME. Our goal is to address this challenge via the development of a high-fidelity animal free pancreatic cancer model, which will enable patient specific treatment optimisation. We use as a basis our developed highly porous polymeric scaffolding system which we advance biochemically and biologically to mimic crucial TME features. More specifically, we create a tri-culture of pancreatic tumour cells, pancreatic stellate cells and endothelial cells. With surface modification of the scaffold we enable coating with various extracellular matrix protein (ECM) compositions and by immersing the scaffold in a 3D printed perfusion bioreactor we achieve mimicry of the blood flow. The versatility of the synthetic scaffold allows to recapitulate and test multiple critical aspects of the pancreatic TME including cell-cell and cell-ECM interactions, stiffness, tissue porosity, diffusion of oxygen, nutrients, metabolites and distribution of the vascularization. Herein, key advancements of our platform will be discussed.



Biomimetic scaffolds for soft tissue engineering via melt electrowriting

E.M. De-Juan-Pardo[1,2]
Presenting Author: Elena M. De-Juan-Pardo, elena.juanpardo@qut.edu.au
[1]Centre in Regenerative Medicine, Institute of Health and Biomedical Innovation (IHBI), Queensland University of Technology (QUT), Brisbane, Australia [2]School of Chemistry, Physics and Mechanical Engineering (CPME), Science and Engineering Faculty (SEF), Queensland University of Technology (QUT), Brisbane, Australia

Melt electrowriting (MEW) is an emerging additive biomanufacturing technique capable of printing fibrous constructs in ultra-high resolution, bringing down the jet diameter to the micrometre-to-nanometre scale. Scaffolds manufactured by MEW are tailorable in terms of fibre architecture, porosity and thickness [1]. Moreover, MEW is a solvent-free process, compatible with the use of medical grade thermoplastic polymers. These exclusive advantages make MEW an ideal manufacturing technique for the production of scaffolds for a wide range of biomedical applications. However, despite the great benefits of MEW, this technique is still at its infancy in a few laboratory set-ups. This work explores the potential of MEW for soft tissue engineering. There is a great need to develop novel biomaterials capable of recapitulating the complex biomechanical features of soft tissues. Native soft tissues are characterised by a strain-stiffening behaviour that makes them very sensitive to load. Herein, the unique capabilities of MEW to produce biomimetic scaffolds with controlled non-linearity, strain-stiffening behaviour, anisotropy and viscoelasticity for soft tissue engineering is presented. This is achieved by applying a biomimetic design approach inspired in the wavy architecture of the collagen fibres present in soft tissues and their load-dependent recruitment. In summary, MEW is an exceptional additive biomanufacturing technique which enables the manufacture of complex biomimetic materials for soft tissue engineering.

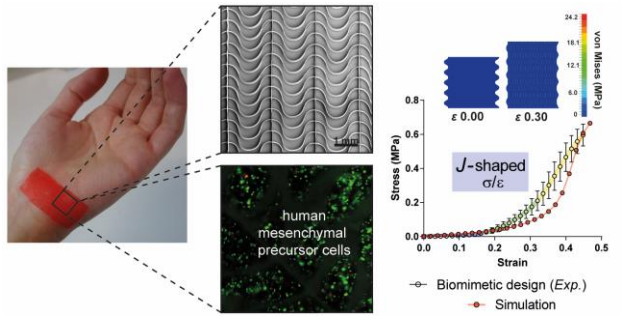
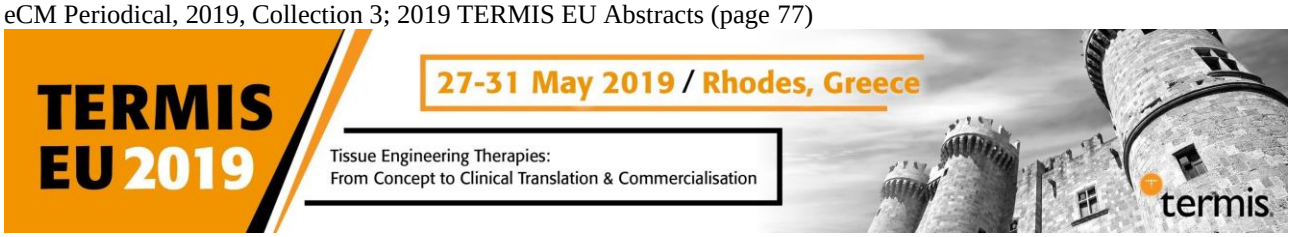


Figure 1: MEW scaffolds present J-shaped stress-strain behavior under tensile load, similar to native soft tissues such as skin. Image reprinted with permission from [3]. ©2017 American Chemical Society.

ACKNOWLEDGEMENTS: Financial support was received from the Centre in Regenerative Medicine (QUT), the CRC for Cell Therapy Manufacturing (CTM 1-03 and 2-06) and the ARC ITTC in Additive Biomanufacturing.

REFERENCES

[1] Wunner F et al. Adv Mater. 2018; 30:1706570
[2] Saidi TN et al. 2018; In review
[3] Bas O et al. ACS Appl Mater Interfaces 2017; 9(35):29430-29437



Label free optical bioimaging for stem cells research

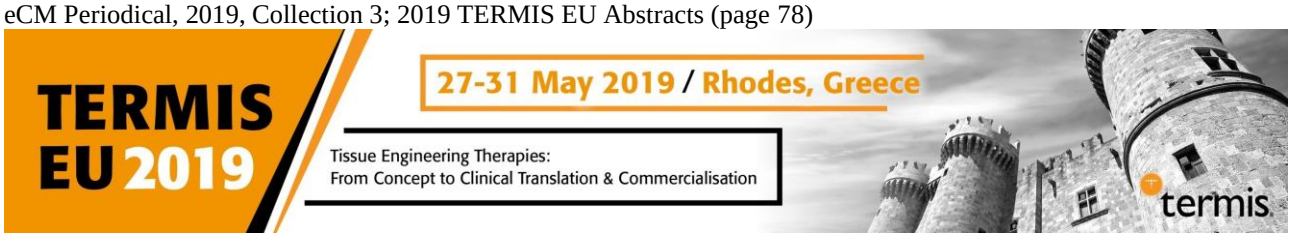
E.V. Zagaynova[1]
Presenting Author: Elena Zagaynova, ezagaynova@gmail.commailto:dimitrios.zeugolis@nuigalway.ie
[1]Institute of Biomedical Technologies Privolzhsky Research Medical University, Nizhny Novgorod, Russia

The promising task in the pre-clinical and clinical testing of the bioengineering constructs and the biomedical cellular products is using multimodal label free imaging. Such unique techniques allow to visualize the individual cells embedded in the constructs, their migration, proliferation, and also to study the cell differentiation processes. The study of the epigenetic mechanisms of stem cell (mesenchymal stromal cells (MSC), induced pluripotent stem cells (iPS)) differentiation is an actual problem. So, using fluorescence lifetime imaging microscopy (FLIM) the metabolic switch from glycolysis to oxidative phosphorylation was shown during MSC and iPS differentiations by the lifetimes changing of NAD(P)H [1]. Also, we studied the involvement of seeded allogeneic MSCs in bone formation using the model of transgenic mice expressing fluorescent protein GFP and genetically labeled cells [2]. Despite the significant progress in developing of skin equivalents (SEs) a problem of non-invasively assessing the quality of the cell components and the collagen structure of living SEs both before and after transplantation remains. Using the methods of optical coherence tomography (OCT), multiphoton tomography (MPT) and FLIM, the structure and quality of dermal SEs before transplantation, and remodeling of collagen matrix and microcirculation in the wound healing after dermal SEs transplantation were studied [3].

ACKNOWLEDGEMENTS: This work has been financially supported by Russian Foundation of Basic Research (grants No. №15-29-04851\17 30.10.2017), and by Russian Science Foundation (grants No.17-75-20178)

REFERENCES

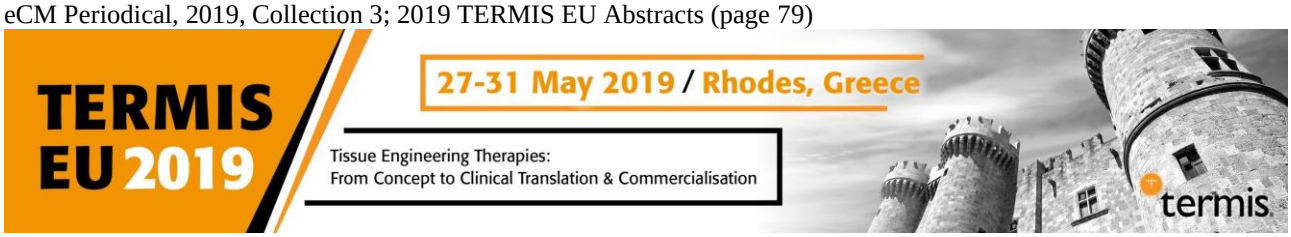
[1] Kuznetsova D et al. Cell Adh Migr. 2017; 11(3): 233–244
[2] Meleshina A et al. Stem cell Res Ther 2018;9:84
[3] Meleshina A et al. Stem cell Res Ther. 2018; 9:84.



Cell-derived microtissues: Design, production and applications

E. Engel
Presenting Author: Elisabeth Engel, eengel@ibecbarcelona.eu
Biomaterials for Regenerative Therapies. Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain, Depart. Materials Science and Metallurgical Engineering, Universitat Politècnica de Catalunya, EEBE, (UPC), Barcelona, Spain. CIBER in Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Madrid, Spain.

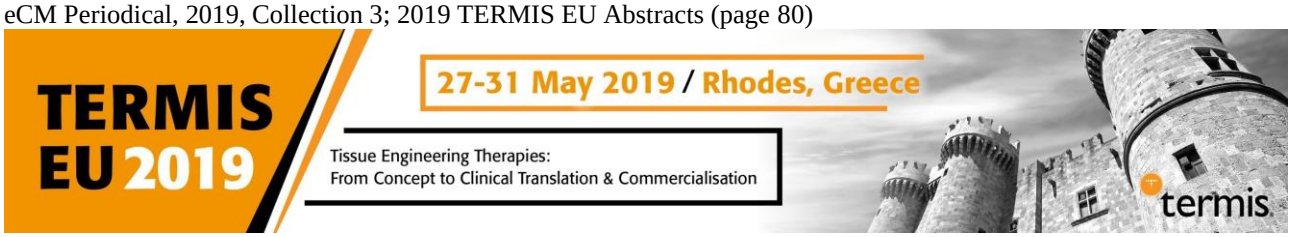
One major approach to regenerative medicine has focused on decellularized tissues and whole organs. Another approach that is getting interest are the cell-derived extracellular matrices (CDMs). These CDMs can be produced using extracellular matrix (ECM) substrates, cell pellets, scaffolds (like hydrogels, microcarriers or 3D printed structures) and scaffold-free living cell sheet culture systems. Alternatively to decellularized tissues, these CDMs can mimic some aspects of the native tissue microenvironment due to its complex composition and the existing possibility to tailor-made them by selecting different production parameters such as cell types and stirred 3D culture among others. This allows to produce patient specific models and disease to understand disease mechanism and test drugs. In this study we will show the production of these CDMs and how they can be used as scaffolds for regenerative therapies.



Laser surface engineering of biomaterials for neural tissue engineering applications

E. I. Stratakis
Presenting Author: Emmanuel I. Stratakis, stratak@iesl.forth.gr
Institute of Electronic Structure and Laser, Foundation for Research & Technology Hellas, Heraklion Greece

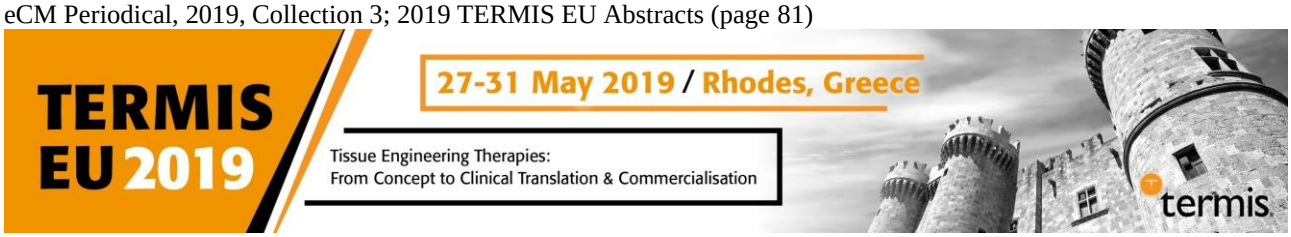
The surface topography of biomaterials can have an important impact on the cellular adhesion, growth and proliferation. Apart from the overall roughness, the detailed morphological features at all length scales, significantly affect the cell-biomaterial interactions in a plethora of applications including structural implants, tissue engineering scaffolds and biosensors. We present a simple, one-step direct laser patterning technique to fabricate various types of micro/nano structured biomaterials platforms. Variation of the laser fluence, alters the surface morphology, leading to a rippled-type, at lower laser fluences, or a conical spiked-type morphology, as the laser fluence increases. Hierarchically-structured cell culture platforms incorporating gold nanoparticles functionalized with specific bio-functional moieties have been additionally developed. Cells with nerve cell phenotype were cultured on the substrates. More specifically, PC12 cells cultured on the developed substrates and treated with nerve growth factor showed a differentiation response that was highly dependent on the surface topography. While, experiments with DRG/SCG nerve cells showed a differential orientation of the cells, depending of the underlying geometry of the laser engineered surface structures. Finally, depending on the laser processing conditions, distinct SW10 cell-philic or cell-repellent patterned areas can be attained with a desired motif, enabling spatial patterning of cells in a controllable manner. Our work provides a versatile method to tune neuron cell responses by proper selection of the surface free energy of the substrate and may be promising for the design of cell culture biomaterial platforms with controlled differentiation environment.



Optimising medical device tissue adhesion and integration through mechanical and surface topographical methods

E.D. O'Cearbhaill
Presenting Author: Eoin D. O'Cearbhaill, eoin.ocearbhaill@ucd.ie
School of Mechanical & Materials Engineering, University College Dublin (UCD) & Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), UCD, Dublin, Ireland

The interface between host and implant is a key predictor of device performance. When seeking to attach or integrate medical devices with host soft tissue, current methods of fixation can lead to suboptimal results. There is a reliance on (1) chemical-based adhesives, which require tissue-specific reactive chemistry and subsequent risk of an inflammatory response, or (2) mechanical methods of fixation (sutures or staples) which can induce significant local tissue damage and associated increased risk of infection. Here, we present two examples of novel interfacing geometries optimised for tissue adhesion and integration respectively. Firstly, a novel two component angled-microneedle patch design is described, which achieves robust and reversible mechanical adhesion to skin (4-5 times the mechanical adhesive strength of cyanoacrylate). This creates a stable platform for 'click-on' drug delivery and biosignal sensing applications, where sensors can be subsequently removed with near-zero force through the reverse mechanical action or designed to bioresorb. Secondly, utilising a novel additive manufacturing approach, we produce soft flexible silicone-based implants with a unique surface topography designed to minimise fibrosis and control the wound-healing response. These geometrical conformations create platforms for optimal tissue fixation and integration for a broad range of medical devices.



Bio-engineering human skin: The long way from basic research to clinical trials

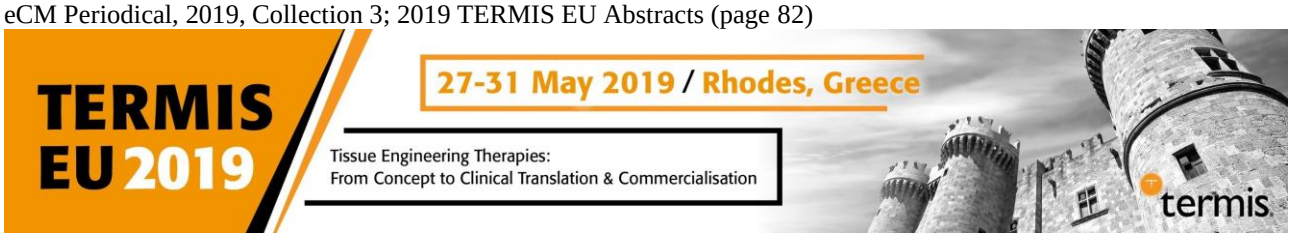
E. Reichmann[1,2]
Presenting Author: Ernst Reichmann, Ernst.Reichmann@kispi.uzh.ch
[1]Tissue Biology Research Unit (TBRU), University-Children's Hospital, Zurich, Switzerland
[2]University of Zurich, Switzerland

New, substantial and clinically applicable achievements in skin replacement and regeneration are rare. The most significant steps forward within the past 45 years have been the development of Cultured Epidermal Autographs (CEA) by the method of Rheinwald and Green (1975) and the invention of the Integra™ Dermal Regeneration Template, by Joannis V. Yannas and John F. Burke in 1981. The Tissue Biology Research Unit (TBRU) in Zurich, Switzerland has more recently come up with several novel bio-engineered skin equivalents, one of which is named DenovoSkin™. DenovoSkin™ is a collagen-hydrogel based, dermo-epidermal skin graft, containing (autologous) fibroblasts in its dermal compartment and (autologous) keratinocytes in its epidermal compartment. To date DenovoSkin™ is presumably the most complex autologous bio-engineered product applied in the clinic. DenovoSkin™ was successfully tested in a Phase I study [1] and is presently used in a prospective, intrapersonally-randomised, interventional, multicentrical Phase II study. In addition DenovoSkin™ is presently applied in compassionate use on a severely injured pediatric burn patient. DenovoSkin™ received the Orphan Drug Designation (ODD) for the treatment of burns from Swissmedic, the EMA and the FDA respectively. On the basic research level the TBRU has developed dermo-epidermal skin equivalents [2-3] exhibiting properties of full thickness human skin, which is a) pre-vascularized by a capillary plexus, and b) pre-pigmented by a functional melanocyte compartment. The TBRU is presently translating this new generation of bio-engineered skin equivalent into GMP-production and clinical application.

ACKNOWLEDGEMENTS: Financial support was received from the Swiss National Science Foundation (SNSF); The Clinical Research Priority Programs (CRPP) of the University of Zurich; The Foundation Gaydoul; The People Programme ‘‘iTERM” (FP7-PEOPLE-2013-ITN) grant agreement no. 607868; The European Union's FP7 Programme for research, technological development and demonstration (FP7/2011-2016 EuroSkin-Graft) grant agreement No. 279024; The People Programme ‘‘MultiTERM” (FP7-PEOPLE-2008-ITN)

REFERENCES

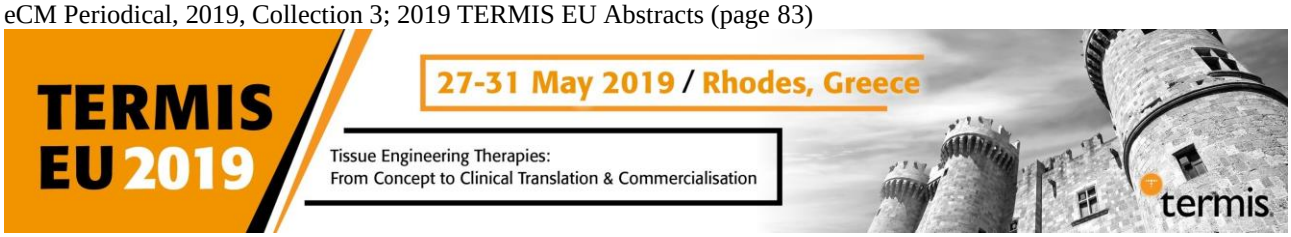
[1] Meuli M. et al. Plastic and Reconstructive Surgery (in press)
[2] Marino D. et al. Sci Transl Med. 2014 6(221):221ra14.
[3] Klar AS, et al. Pediatr Surg Int. 2018 34(2):237-244.



Aging of the skeletal muscle extracellular matrix and its effect on stem cell functionality

F. Ambrosio [1,2]
Presenting Author: Fabrisia Ambrosio, ambrosiof@upmc.edu
[1]Rehabilitation for UPMC International [2]Department of Physical Medicine & Rehabilitation, University of Pittsburgh School of Medicine, U.S.A.

Skeletal muscle trauma resulting from an injury or surgery often results in significant functional declines in older adults. These declines are at least partially attributed to failed muscle healing. Muscle regeneration is predominantly dictated by the action of muscle stem, or “satellite”, cells (MuSCs), a reserve cell population that typically demonstrates considerable dysfunction with increasing age. According to the stem cell “niche” concept, stem cell responses are largely determined by biophysical and biochemical cues that emanate from the surrounding microenvironment. Indeed, expanding recognition of the influence of the microenvironment on stem cell behavior has led to a recent surge in the development of bioinspired and engineered extracellular matrix (ECM) approaches for the treatment of skeletal muscle injuries. Still lacking, however, is an in-depth knowledge of whether and how pathogenic instructional characteristics of the native ECM disrupt MuSC function and skeletal muscle regeneration. While it is evident that MuSC activation, self-renewal, proliferation and differentiation are influenced by physical and dynamic niche interactions, a mechanistic understanding of the direct impact of age-related ECM alterations on skeletal muscle regenerative capacity is unknown. In her presentation, Dr. Fabrisia Ambrosio will share recent in vitro, ex vivo and in vivo data from her laboratory demonstrating that that age-related biophysical alterations in the skeletal muscle ECM promotes a fibrogenic conversion in MuSCs, ultimately driving impaired skeletal muscle regeneration.



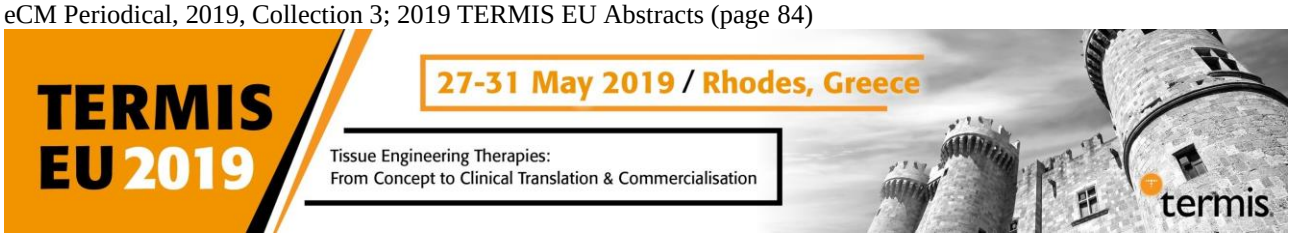
Towards an artificial meniscus: Biomechanical properties as a driver for orthopaedic innovation

F. Barberis[1,2]
Presenting Author: Fabrizio Barberis, fabrizio.barberis@unige.it
[1]Department of Civil, Chemical and Environmental Engineering - (DICCA), University of Genoa, Italy [2]Genova Additive Manufacturing – (GeAM), Genova, Italy

Meniscal tear is still an unsolved problem in regenerative medicine. The almost total inability in self-healing damaged tissue shown by the menisci is due to the extremely reduced amount of vessels for blood supply that does not allow the normal inflammatory response promoter of tissue healing. The use of cadaveric meniscal allografts is subject to several inconveniences as penury of donor tissue, risk of infections and size matching. The challenge is therefore oriented towards the artificial meniscus prostheses. The use of composites based polymeric materials could partially replicate the complex internal structure determined by differently oriented layers operating in a fluid environment. In order to obtain a mechanically efficient prostheses it is indeed fundamental to deeply study the mechanical characteristics of the natural meniscus tissue preserving the features and behaviour of the original structure. A different, non destructive, method able to measure the effective mechanical response of a natural meniscus in its different regions without affecting the results by the sampling inconvenience was set up and described in this work. This technique let to combine a sequent set of classical destructive tests on the same menisci samples, providing an innovative database of mechanical data able to support the projecting phase of a new artificial meniscus.

REFERENCES

[1] A Marrella, A Lagazzo, E Dellacasa, C Pasquini, E Finocchio, F Barberis, L Pastorino **“**3D Porous Gelatin/PVA Hydrogel as Meniscus Substitute Using Alginate Micro-Particles as Porogens**”** Materials Science & Eng C, Polymers 2018, 10(4), 380; doi:10.3390/polym10040380
[2] Marrella A., Lagazzo A., Barberis F., Catelani T., Quarto R., Scaglione S (2017). “Enhanced mechanical performances and bioactivity of cell laden-graphene oxide/alginate hydrogels open new scenario for articular tissue engineering applications”. Carbon, vol. 115, p. 608-616, ISSN: 0008-6223, doi: 10.1016/j.carbon.2017.01.037
[3] Dabiri H, Lagazzo A., Barberis F., Shayganpour A., Finocchio E., Pastorino L. (2017) “New in – situ syntethized hydrogel composite based on alginate and brushite as potential pH sensitive drug delivery system” – Carbohydrate Polymers,Dec 1;177:324-333. doi: 10.1016/j.carbpol.2017.08.046



Advances in mesenchymal stem cell therapy for osteoarthritis: from the cell to its extracellular vesicles

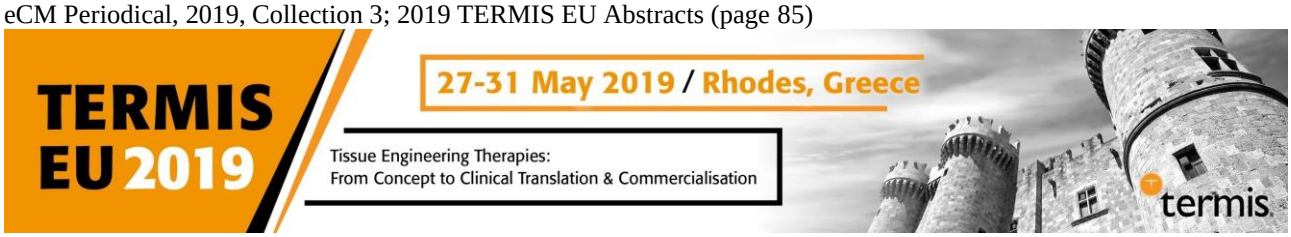
F. Djouad
Presenting Author: Farida Djouad, farida.djouad@inserm.fr
INSERM UMRS 1183, Institute for Regenerative Medicine and Biotherapy (IRMB), Montpellier, France.

Osteoarthritis (OA) is the most common form of inflammatory, degenerative and painful joint disorder affecting the whole joint tissues including the articular cartilage and bone. Despite the increase of OA incidence, there is still no effective therapy able to restore the structure and function of injured articular cartilage. In this context, mesenchymal stem cell (MSC)-based therapies for OA have become thriving areas of research. MSC have been tested in preclinical studies with results highly predictive of clinical success. Thereby, for a decade, MSC have been the subject of many clinical trials in OA demonstrating their safety and efficacy in phase I and II trials and promising results in phase III trials [1, 2]. Tissue repair potential of MSC relies mainly on their paracrine activity mediated through the release of various trophic factors able to enhance tissue repair by regulating the inflammatory response, stimulating endogenous cells and protect tissues from further degradation. More recently, extracellular vesicles (EV), pivotal mediators of cell-to-cell communications, have been shown to be secreted by MSC and to recapitulate their functions. Our laboratory has studied, in vitro, the anti-inflammatory, anti-apoptotic and chondro-protective properties of MSC-derived EV [3]. The data obtained will be discussed as well as the current knowledge on the effects of EV derived from MSC in experimental models of inflammatory or degenerative joint disorders.

ACKNOWLEDGEMENTS: Financial support was received from Inserm Institute and the University of Montpellier; European Union's Horizon 2020 Programme (project ADIPOA2, grant agreement no: 643809) and Agence Nationale pour la Recherche for support of the national infrastructure: "ECELLFRANCE: Development of a national adult mesenchymal stem cell based therapy platform" (ANR-11-INSB-005).

REFERENCES

[1] J.M. Lamo-Espinosa et al. J Transl Med. 2016;14(1):246
[2] H. Iijima et al. NPJ Regen Med. 2018;3:15
[3] S. Cosenza et al. Sci Rep. 2017;7:16214



Scaffold-based delivery of nanomedicines for bone and cartilage repair

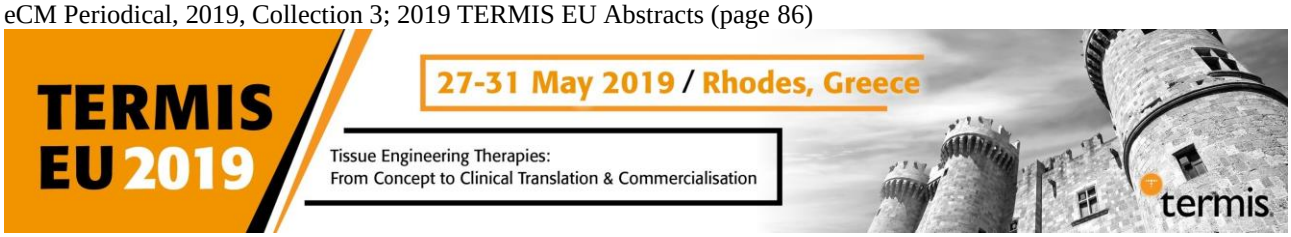
F.J. O'Brien [1,2,3]
Presenting Author: Fergal J. O'Brien, fjobrien@rcsi.ie
[1]Tissue Engineering Research Group, Dept. of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Trinity Centre of BioEngineering, Trinity College Dublin (TCD), Ireland [3]Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland

Developing a biomaterials-based solution for the repair of large scale damage to the articular joint presents a particularly complex challenge due to the complex zonal structure of osteochondral tissue, the issues combining weightbearing with any treatment, and the challenges in directing regenerative stem cells to promote the formation of non-hypertrophic stable hyaline cartilage. Ongoing research in our lab is focussed on the application of multi-layered biomimetic natural polymer-based 3D porous scaffolds for the repair of bone and cartilage in articular joints [1]. Increasingly we are utilising 3D printing to produce such materials with enhanced mechanical properties to facilitate weight bearing. Furthermore, we have been focussing on functionalising these scaffolds for use as delivery systems for biomolecules such as therapeutic nanomedicines enhance their therapeutic potential. These gene activated scaffold platforms thus act as both a template for cell infiltration and tissue formation while also directing autologous host cells to engineer therapeutic proteins in a sustained but eventually transient fashion [2]. Similarly, we have been utilising scaffold-mediated delivery of siRNAs and miRNAs [3] to silence specific genes associated with aberrant effects in bone and cartilage repair. This presentation will highlight some of the particular problems with developing viable clinical treatments to prevent or delay the need for joint replacement and will provide an overview of ongoing research in our lab on gene-activated biomaterials for bone and cartilage repair.

ACKNOWLEDGEMENTS: Financial support from Science Foundation Ireland, Health Research Board and the European Research Council.

REFERENCES

[1] Levingstone TJ et al. Biomaterials. 2016; 87:69-81.
[2] Raftery RM et al. J Control Release. 2018; 10;283:20-31.
[3] Mencía Castaño I et al. Sci Rep. 2016 14;6:27941.



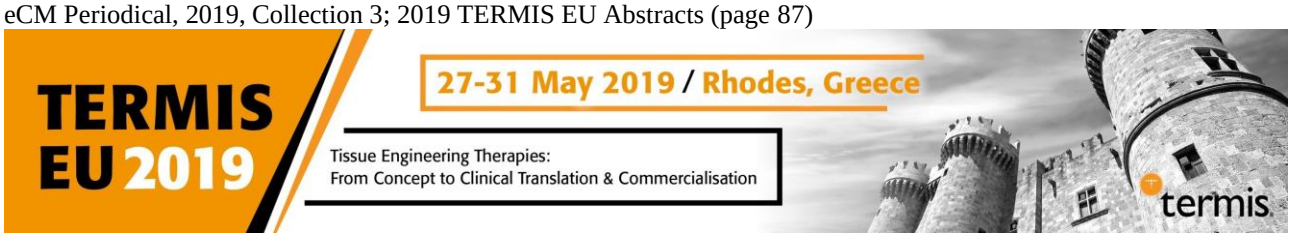
Engineering of extracellular vesicles for drug delivery

F. Gazeau[1]
Presenting Author: Florence Gazeau, florence.gazeau@univ-paris-diderot.fr
[1] Laboratoire MSC, CNRS UMR 7057, Univ. Paris Diderot, USPC, Paris, France

Extracellular vesicles (EVs) are recognized as the nature's own carriers to transport macromolecules throughout the body. Hijacking this endogenous communication system represents an attractive strategy for advanced drug delivery. However, efficient and reproducible loading of EVs with therapeutic or imaging agents as well as high yield cost-effective manufacturing of EVs still represents bottlenecks on the road towards the use of EVs as the next-generation drug delivery system (DDS).[1] To enable clinical translation of EV-based therapy, our group is developing disruptive technologies for large scale, high yield production and engineering of EVs. We will present technological breakthroughs which enable – i) to produce EVs at high yield and high throughput in scalable and GMP-compliant bioreactors based on turbulence stimulation on any producer cells, -(ii) to load EVs with internal or membrane cargoes (inorganic nanoparticles, hydrophilic and hydrophobic drugs…) to endow them with new properties, -(iii) to achieve image-guided photodynamic therapy (PDT) using EVs in a peritoneal carcinomatosis model. **EV production**: Pursuing the analogy with shear-stress induced EV release in blood, we are developing mechanical stress triggering approaches to induce fast EV generation. First proof of concept was done in a microfluidic device allowing fast generation of EVs.[2] In order to upscale shear-stress induced EV production in a controllable manner, we then propose turbulence as a tunable and effective trigger to generate EV into GMP-compliant bioreactors, with a ten-fold gain both in production time and yield. **EV engineering.** We propose versatile technologies to engineer EVs with internal or membrane cargoes, relying either on engineering precursor cells (e.g. nanoparticle cellular uptake [3]), or on post-production EV modification. For example, PEG-facilitated fusion of EVs with functionalized liposomes was used to create smart biosynthetic hybrid vector with improved cellular delivery efficiency of a chemotherapeutic compound as compared to the free drug or the drug-loaded liposomes.[4] **Spatio-temporal control of EV therapeutic activity.** Stem cell or endothelial cell-derived EVs loaded with a photosensitizer show favorable biodistribution, safety and enhanced light-induced therapeutic efficiency in 3D tumoroids [5], subcutaneous tumor xenograft and in a mouse model of peritoneal carcinomatosis. Our unique technologies for EV production and engineering combines decisive assets for clinical translation of EV-based DDS.

REFERENCES

[1] Piffoux et al. Adv Drug Deliv Rev. 2018 Dec [2] Piffoux et al, Adv. Biosys. 2017, 1;1700044
[3] Mulens-Arias et al, Adv. Biosys. 2018, 2, 1800079; Piffoux et al, Nanoscale 2018 10:1234.
[4] A. Silva et al, ACS Nano 2018, 12:9800.
[5] Millard et al, Drug delivery, 2018, 25:1790



Osteoconductive microarchitectures for high performance bone substitutes

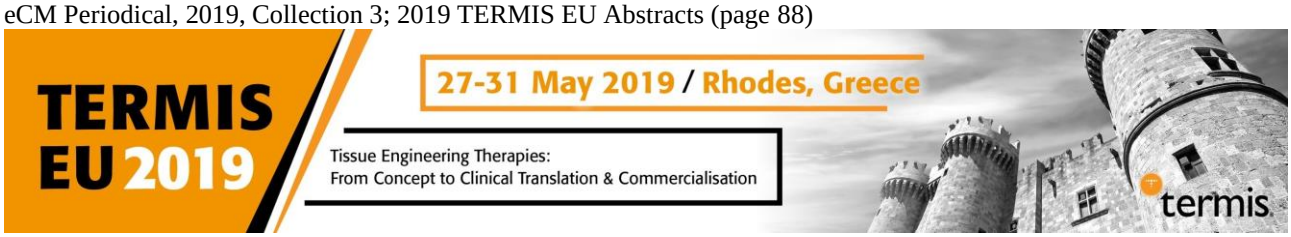
F.E. Weber
Presenting Author: Franz E. Weber, franz.weber@zzm.uzh.ch
Oral Biotechnology & Bioengineering, University of Zurich, Center of Dental Medicine, Plattenstrasse 11, 8032 Zurich, Switzerland

In the 90ties of the last century, the holy grail of pore size for scaffolds in bone tissue engineering was set between 0.3 and 0.5 mm. These values appeared reasonable since they fall in line with the diameter of osteons and influenced the microarchitectural design of bone substitute thereafter. More recently, two papers indicated that pores even bigger than 0.5 mm perform equally well [1-2]. Therefore, the optimal microarchitecture for bone tissue engineering scaffolds in terms of pore size, constrictions, rod thickness, or rod distance is still elusive. Additive manufacturing appears as an ideal tool to study those diverse microarchitecture options since it can generate scaffolds where size and location of pores and connections between pores can be tested. For the production of scaffolds, we applied for titanium-based scaffolds laser sintering and for ceramics lithography-based additive manufacturing. As in vivo test model, we used a calvarial defect model in rabbits. Histomorphometry revealed that all generatively produced structures were well osseointegrated into the surrounding bone. The histomorphometric analysis, based solely on the middle section, showed that bone formation was significantly increased with pores between 0.7-1.2 mm in diameter. Scaffolds with pores of 1.5 and 1.7 mm perform significantly worse. Therefore, pore diameters in osteoconductive bone substitutes should be 1.0-1.2 mm and thus much bigger than previously suggested [3-5]. For lattice microarchitectures we found rods of 0.3 mm in diameter and 0.8 mm distant to each other highly osteoconductive. In essence, additive manufacturing enabled us to generate libraries of microarchitectures to search for the most osteoconductive microarchitecture. Moreover, additive manufacturing appears as a promising tool for the production of personalized bone tissue engineering scaffolds to be used in cranio-maxillofacial surgery, dentistry, and orthopedics.

ACKNOWLEDGEMENTS: Financial support was received from the Synergy program of the SNF (Swiss National Science Foundation) Grant number CR3212.

REFERENCES

[1] von Doernberg, et al. 2006; Biomaterials 27, 5186-5198
[2] de Wild et al 2016; 3D printing + Additve manufacturing, 3(3): 143-151
[3] de Wild et al 2019; 3D printing + Additve manufacturing, in press
[4] Chen et al 2018; Tissue Eng Part A. 19/20:1554-1561
[5] Ghayor & Weber 2018; Front. Physiol. doi: 10.3389/fphys.2018.00960



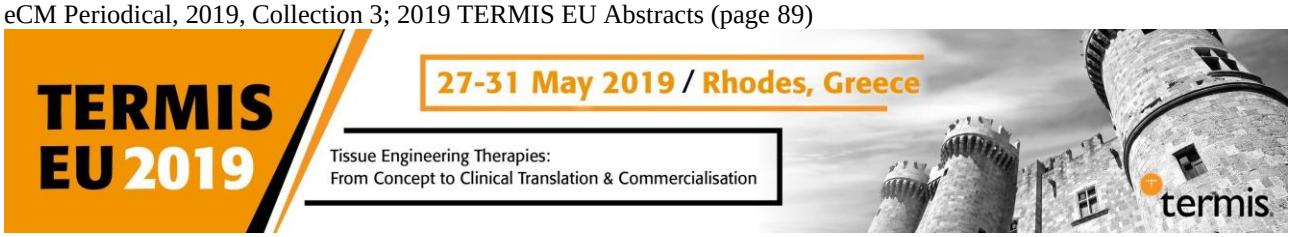
BIOCAPAN: an innovative microcapsule-based advanced therapy medicinal product for the treatment of diabetes mellitus type I

F. Bottausci 1
Presenting Author: Frederic Bottausci, frederic.bottausci@cea.fr
[1]Univ. Grenoble Alpes, CEA, LETI, DTBS, Grenoble, France

Type 1 diabetes (T1D) results from the destruction of insulin-producing Beta-cells by the patient's overactive immune system [1]. The replacement of these lost insulin-producing cells using islet transplantation has proved to be effective for some T1D patients [2]. The main limitation is the need for life-long immunosuppression to prevent rejection of the transplanted cells. Encapsulating the islets appears to be a promising approach [3] since it offers a physical barrier between the cells and the receiver immune system [1] while being porous enough to allow exchange with blood. The technology used in the frame of the BIOCAPAN project relies on microencapsulation. This process has the advantages of producing monodispersed capsules with a controlled diameter that can be adapted to the islets size. The spherical shape and the reduce thickness of the biomaterials between islets and the environment outside the capsule maximizes the exchanges, limiting the necrosis and promoting oxygen, nutrient supply and insulin secretion. We present a method and apparatus for well-controlled microencapsulation of insulin secreting human islets (Langerhans islets) in a high viscous (up to 10 Pa.s) innovative mix of biopolymers and biological elements. The mix aimed at improving the islets viability without altering the insulin secretion as well making the capsule stealthy to the host immune system. The microencapsulation is performed by an advanced equipment we developed that uses disposable encapsulation-oriented microfluidic cartridges. The system, complying with microfluidic standards, is a GMP (Good Manufacturing Practices)-like equipment allowing a fully automated encapsulation process providing ready-to-implant microcapsules. We will finally show encapsulated islets in the mix of biopolymers and biological elements. Encapsulating islets are then ready to be characterized in vivo and in vitro to validate biocompatibility, viability and functionality.
This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 646272 (BIOCAPAN).

REFERENCES

[1] T. Desai and L. D. Shea, 'Advances in islet encapsulation technologies', Nat. Rev. Drug Discov., vol. 16, no. 5, pp. 338–350, May 2017.
[2] E. A. Ryan et al., 'Five-Year Follow-Up After Clinical Islet Transplantation', Diabetes, vol. 54, no. 7, pp. 2060–2069, Jul. 2005.
[3] P. de Vos, M. Spasojevic, and M. M. Faas, 'Treatment of diabetes with encapsulated islets', Adv. Exp. Med. Biol., vol. 670, pp. 38–53, 2010.



The DRIVE consortium - Living implants and delivery devices for the treatment of type 1 diabetes

G.P. Duffy[1,2]
Presenting Author: Garry Duffy, garry.duffy@nuigalway.ie
[1]Regenerative Medicine Institute (REMEDI), School of Medicine, National University of Ireland Galway (NUI Galway), Galway [2]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Islet cell transplantation is a promising treatment for type 1 diabetes mellitus (T1D), but current strategies require immunosuppression and have been plagued by poor islet cell retention and reduced implant viability (1). Technologies to assist the delivery and engraftment of islets in extrahepatic sites are necessary for widespread clinical adoption of islet transplantation. Here we provide an update from Diabetes-Reversing Implants for enhanced Viability and long-term Efficacy (DRIVE), a European Consortium supported by EU Horizon 2020 funding. The objective of DRIVE is to develop a platform for islet cell transplantation that improves cell retention and viability, is delivered in a minimally invasive fashion, and negates the need for systemic immunosuppression.

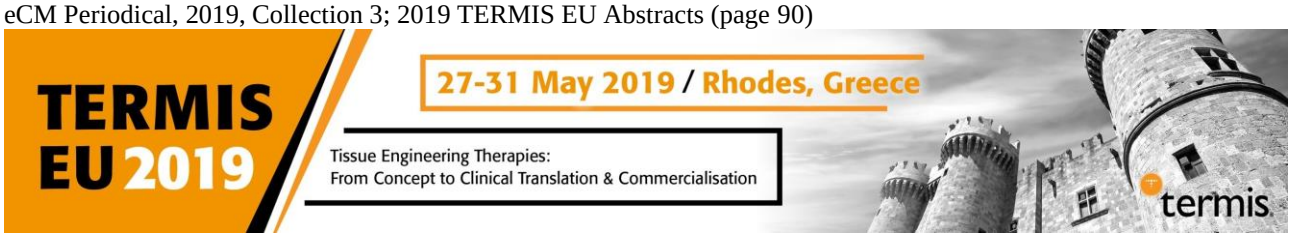
METHODS: DRIVE partners have developed a multicomponent platform to improve islet cell delivery. Technologies under development include β-Gel, a support matrix for islet cell growth, β-Shell, an immunoprotective macroencapsulation device, and TheraPocket, a procedure for delivering islet cells to a target implant site in the anterior abdominal wall.

RESULTS & DISCUSSION: The β-Gel is a pancreo-mimetic hydrogel with pancreatic extracellular matrix proteins and oxygen producing compounds that provides a support matrix for islets. β-Gel has been tested extensively in vitro and supports human islet cell survival and insulin release. A candidate technology and delivery device suite has been developed for a macroencapsulating β-Shell. β-Shell has been tested to deliver a proangiogenic growth factor and β-Gel in a subcutaneous implant model in rats and pigs. Early results demonstrate robust neovascularization around implants and survival of islets and restoration of glycemic control over 4 weeks in a STZ induced rodent model. Exploratory studies in pigs and cadavers have been used to evaluate possible implant sites in the anterior abdominal wall for development of the TheraPocket procedure. Significant progress has been made in developing DRIVE technologies for improved islet cell transplantation. Preclinical studies to evaluate the therapeutic effectiveness of a clinically scaled and translatable DRIVE platform are ongoing.

ACKNOWLEDGEMENTS: Science Foundation Ireland, (13/RC/2073) and DRIVE (EU H2020, 645991), and in part by EDGE (EU H2020 Marie Sklodowski-Curie, 713567)

REFERENCES

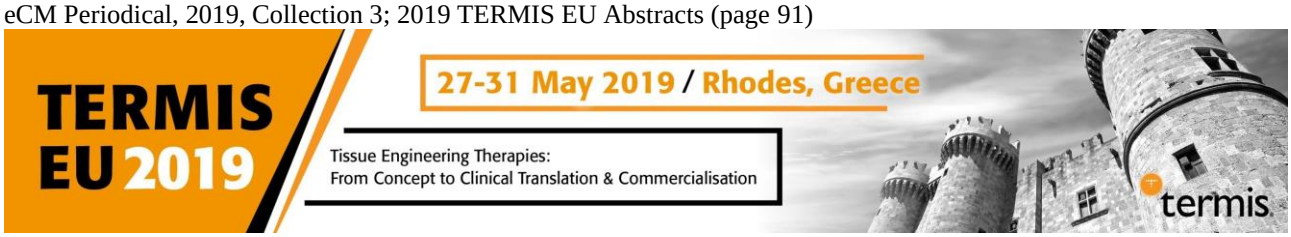
[1] Ryan et al. Curr Opin Pharmacol. 2017; 36:66-71



Academic endeavours and bootstrapped start-up development. How to serve two unforgiving masters at the same time

G. A. Feichtinger[1, 2]
Presenting Author: Georg Feichtinger, g.feichtinger@leeds.ac.uk
[1]Phycosera LIMITED, Shipley, UK [2]Division of Oral Biology, School of Dentistry, Faculty of Medicine and Health, University of Leeds, UK

Almost anyone who embarks upon a career in science shares similar passions, hopes and beliefs culminating in the desire to solve current problems, discover the unknown and develop beneficial innovative products. It was my passion for research coupled with the desire to continuously extend the frontiers of knowledge whilst enthusing the next generation with a love for what current scientific breakthroughs might hold in the future that drove me to become an academic. The world of academia has now however become more challenging as the once sought-after sector of learning now aligns less with the former ideals of independent blue sky thinking where academics could just get on and undertake research. It now has to concede to a more ‘real world’ outside of the ivory tower where academics constantly have to juggle budget management, grant writing, teaching, marking, publishing and research, all whilst living with the constant fear of non-permanent contracts in a relatively slow-moving environment with regards to innovation and rapid new product deployment. The aim of this presentation is to openly discuss alternative career choices for PhDs, to take an objective and honest look at the odds of achieving a fulfilling academic or industrial career in science and the required professional and personal attributes to do so. Most importantly, based on personal experience, this presentation will also cover an often overlooked third career option- starting your own company as an entrepreneur. The third option is particularly interesting if personal motivators include an entrepreneurial streak, a strong desire to apply scientific discoveries in product development and practical problem solving. This presentation will help provide an insight into how it might be possible to balance a demanding academic career whilst also striving to set up a biotech start-up company on a shoestring budget. The talk will furthermore cover trials and tribulations of academic careers, the different avenues available should you want to venture into the exciting world of entrepreneurism and demonstrate that it is possible to synergise both entrepreneurial and academic ambitions across the perceived division of “academia versus industry”.



Bio-inspired bone adhesive proven to be safe in vivo

G. Insley
Presenting Author: Gerard Insley, gerard@gpbio.net
GPBio Ltd. Rathkeale, Ireland

INTRODUCTION: A new combination of α-tricalcium phosphate (αTCP) and calcium silicate cements, modified with phosphoserine, have a proven ability to glue tissues together [1]. The aim of this study was to assess the in vivo safety of this new biomaterial by implantation into subcutaneous pockets, in rats.

METHODS: Precured implants (n=9) with high (45%/28%) and low (25%/2%) concentrations of phosphoserine and calcium silicate, respectively, were implanted subcutaneously in male rats (≈450g) for 6 and 12 weeks. αTCP discs, without silicate and phosphoserine, were used as controls. The implants were analysed via histology (hematoxylin and eosin) and through gene expression, exploring three immune markers and three bone markers (IL1, IL6, TNF1, COL1A, SOX9, RUNX2) to assess any adverse effects or stimulation on the surrounding soft tissue.

RESULTS & DISCUSSION: The histology assessment revealed no signs of adverse effects at the interface between the cured adhesive formulations and the connective tissue. There were no signs of multinucleated cells, nor fibrotic tissue with unaffected connective tissue layers, shown in figure 1. Gene expression analysis confirmed the histology findings, without any measurable regulation of the selected gene markers. The adhesives did not elicit expression of any of the gene markers, and no differences were observed between the test groups and the control.

CONCLUSIONS: This bio-inspired bone adhesive has been proven to be safe with an absence of any side effects on the surrounding soft tissue. These findings encourage further investigation of the full potential of the adhesive biomaterial in tissue repair models.

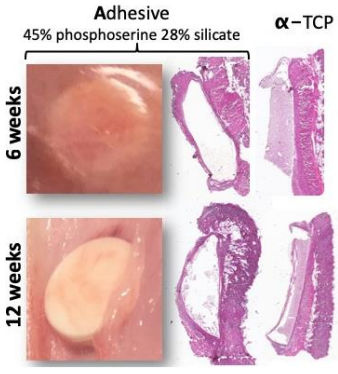
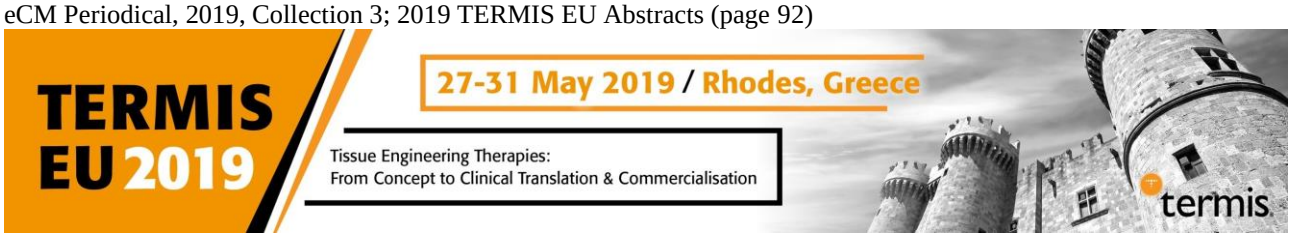


Figure 1: Representative images of the adhesive with 45% phosphoserine and 28% silicate, and control TCP, after 6 and 12 weeks. The explants reveal normal tissue without any adverse reactions. The histology sections depicted thin layers of unaffected connective tissue surrounding the implants.

ACKNOWLEDGEMENTS: This work were partly funded by the Swedish Science Foundation (#RMA15-0110) and EIT Health HeadStart grant (2018 HS PoC 2018-HS-0046).

REFERENCES

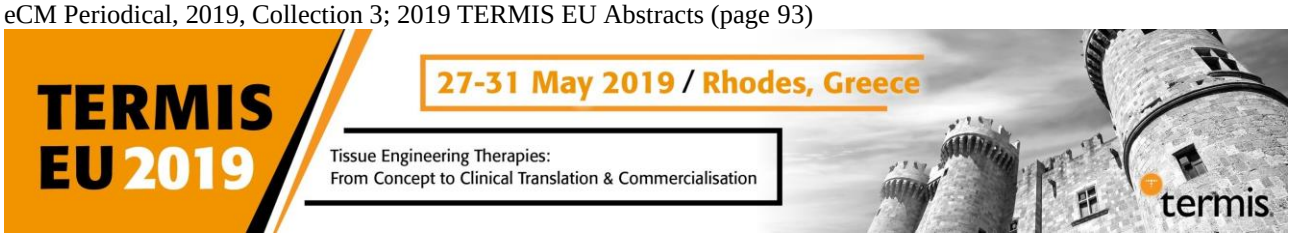
[1] Pujari-Palmer, M. et al. Materials. 2018; 11(12): 2492.



Heterogeneity in adult human mesenchymal stem cells due to origin and culture expansion

G.J.V.M. van Osch
Presenting Author: Gerjo J.V.M. van Osch, g.vanosch@erasmusmc.nl
Erasmus MC, University Medical Center Rotterdam, The Netherlands

Mesenchymal Stem Cells (MSC) are a population of cells that can be obtained from adult tissues and expanded in culture to obtain a large number of cells with multi-lineage differentiation capacity. Thus MSC offer great promise for tissue engineering of many connective tissues. One of the limiting factors of the use of MSC is their heterogeneity. This leads to variable outcomes between patients, labs and experiments. Part of the heterogeneity can be explained by the origin of the MSC. Besides differences caused by harvesting site, different types of MSC are present within one harvest. We have demonstrated differences in chondrogenic capacity between perivascular and lining cells from bone marrow as well as from synovium and have found specific markers to select different populations from the harvested samples. Further heterogeneity is caused by in vitro expansion. Cell expansion is known to cause cellular senescence and loss of differentiation potential. The method of expansion is of large influence and we have found that addition of Wnt3a to the culture medium improves the quality of the MSC. Future research to better understand the heterogeneity of this population of cells and the potential of its subpopulations, will improve the application of MSC in disease model systems and regenerative medicine application and might help us to find methods to stimulate endogenous tissue repair.



Biological polymers as enabling materials for brain tissue model and engineering

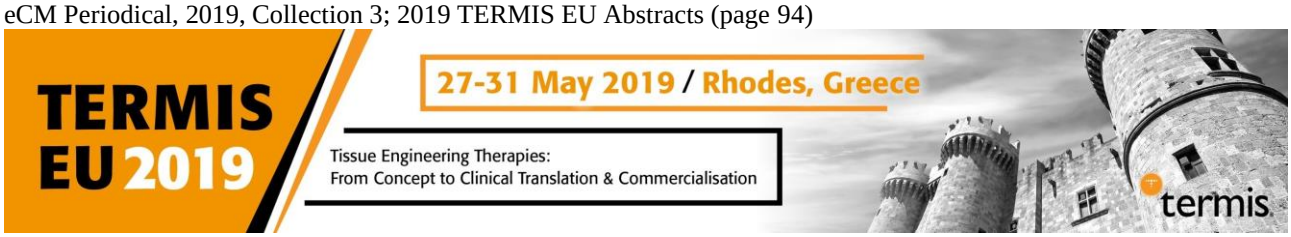
G. Ciardelli [1,2,3]
Presenting Author: Gianluca Ciardelli, gianluca.ciardelli@polito.it
[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy [2]POLITO BIOMedLAB, Politecnico di Torino, Turin, Italy [3]Department for Materials and Devices of the National Research Council, Institute for the Chemical and Physical Processes (CNR-IPCF UOS), Pisa, Italy

Pathologies affecting brain tissue are mainly ascribed to neurodegenerative diseases and central nervous tissue injuries. Neurodegenerative diseases affect more than 100 million individuals worldwide (WHO) causing loss of nerve structure and function and consequently memory loss, change in personality, and dementia. On the other hand, central nervous system injuries, which are mainly related to spinal cord injuries, affect a lower number of patients (approximately 2.5 million people worldwide) but cause enormous functional deficits to patients which lead to disastrous social consequences and huge economic costs. Although intensively studied for over 100 years, no treatment or intervention options are currently available to stop the neurodegenerative pathology progression and to functionally reconnect injured spinal cord. The design of tissue constructs able to recapitulate the complex multicellular environment of brain tissue is a key strategy to model the pathological environment and to progress in treatment efficacy. In this work, biological polymers were applied to engineered an extracellular matrix (ECM)-like environment able to host brain cells of different phenotypes. ECM-like environment can be combined with cells to design advanced therapy medicinal products (ATMPs) to cure pathologies affecting the central nervous system as well as in vitro brain tissue models to study pathologies progression and therapeutic efficacy. Chitosan (CS) was selected as former biomaterial thanks to its well-documented biocompatibility, easy processability, low immunogenicity and anti-inflammatory properties. Several protocols were developed to process CS in form of nanofibers and 3D printable bioinks [1]. Furthermore, gelatin and collagen were combined with CS to achieve a biomimetic ECM-like environment. Additional cues such as antioxidant agents [2], growth factors [3] and cells can be easily loaded within nanofibres and bioinks to better tailor the cell/biological polymer interactions. Physico-chemical properties of the developed nanofibers and bioinks were analysed as well as their ability to allow cell survival of multiple cell populations. By exploiting the biomimetic composition of biological polymers and their tunable properties, the developed 3D ECM-like environment could be applied in the study and treatment of several challenging diseases affecting the central nervous system.

ACKNOWLEDGEMENTS: Financial support was received from Fondazione CRT “Richieste Ordinarie 2014” grant”.

REFERENCES

[1] Tonda-Turo C. et al. Carbohydr Polym. 2017 15(162):82-92;
[2] Marino A. et al. Biochim Biophys Acta Gen Subj. 2017 1861(2):386-395
[3] Tonda-Turo et al. 2017 J Tissue Eng Regen Med. 2017 11(1):197-208



Piezoelectricity applied to tissue engineering: A new approach based on remote cell stimulation

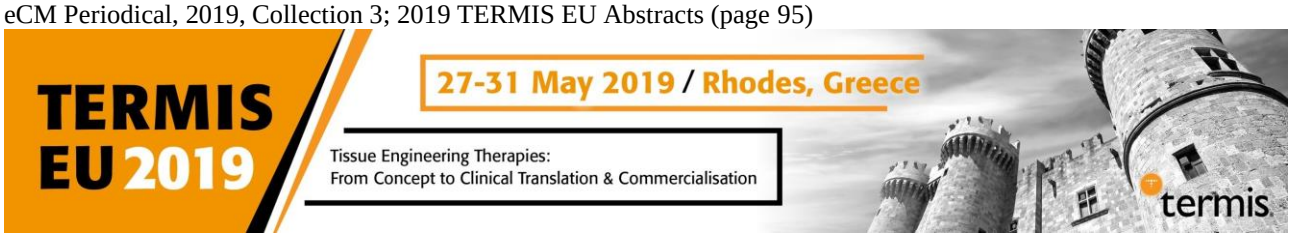
G. Ciofani[1,2]
Presenting Author: Gianni Ciofani, gianni.ciofani@iit.it
[1]Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera, Italy [2]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy

The manipulation of biological activities by non-invasive remote activation is focus of extensive research efforts that, recently, led to the development of different stimulation methodologies based on smart nanomaterials. For instance, magnetic fields, ultrasounds, heating, electric fields, light irradiation can be used in combination with physically-responsive nanomaterials, exploited as real "nanotransducers" [1]. Here, piezoelectric nanostructured materials and their applications in the nanomedicine field will be introduced, with particular attention to tissue engineering and regenerative medicine. Despite their impressive potentials, in fact, this kind of nanomaterials has not yet received significant attention for bio-applications. Our results strongly support the use of piezoelectric nanoparticles in nanomedicine, demonstrating as their exploitation is possible and realistic, and their impressive physical properties can be most useful for several applications, that range from sensors and transducers for the detection of biomolecules, to "reactive" substrates for tissue engineering and cell stimulation [2-3]. After a short introduction to the major classes of innovative piezoelectric nanoparticles that have attracted interest in the latest years, attention will be focused on the research carried out in our laboratories, introducing barium titanate nanoparticles, boron nitride nanotubes, and polymeric composites based on these nanomaterials. In particular, the first example will concern composite poly(vinylidene fluoride-trifluoroethylene), P(VDF-TrFE) / barium titanate nanoparticle (BTNPs) films, prepared and tested as substrates for neuronal stimulation through direct piezoelectric effect. Films were characterized in terms of surface, mechanical, and piezoelectric features before in vitro testing on SH-SY5Y cells. BTNPs significantly improve piezoelectric properties of the films, and support good SH-SY5Y viability and differentiation. Mechanical stimulation elicited by the application of ultrasounds has been proven to promote Ca^{2+} transients and to enhance cell differentiation [4]. Piezoelectric films composed by P(VDF-TrFE) and doped with boron nitride nanotubes (BNNTs) were instead prepared as scaffolds for SaOS-2 osteoblast-like cell culture. After mechanical, surface, and piezoelectric characterization, osteogenic differentiation was evaluated in terms of calcium deposition, collagen I secretion, and transcriptional levels of marker genes (Alpl, Col1a1, Ibsp, and Sparc) in cells either exposed or not to ultrasounds as a source of mechanical stimulation [5].

ACKNOWLEDGEMENTS: This research is partially supported by the Compagnia di San Paolo Starting Grant Number 55_AI16GC01.

REFERENCES

[1] Genchi GG et al. Front Bioeng Biotechnol. 2017; 5:80.
[2] Marino A et al. ACS App Mater Interfaces 2017; 9(21):17663-80.
[3] Marino A et al. Nano Today 2017; 14:9-12.
[4] Genchi GG et al. Adv Healthc Mater. 2016; 5(14):1808-20.
[5] Genchi GG et al. Nanomedicine 2018; 14(7):2421-32.



Development of multimaterial and multiscale approach for fabrication of tissue constructs

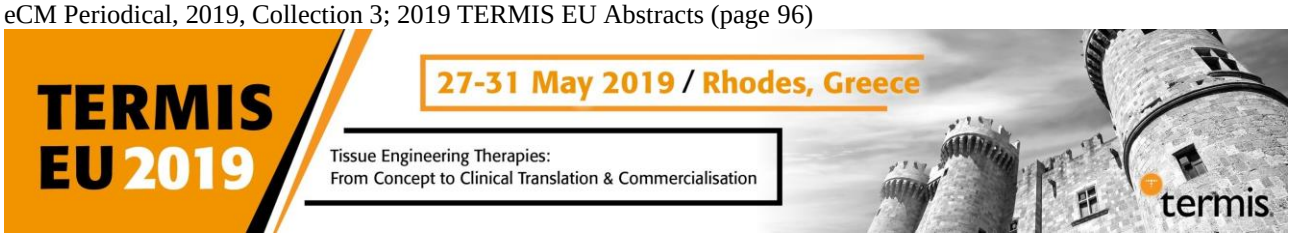
G. Vozzi[1,2]
Presenting Author: Giovanni Vozzi, giovanni.vozzi@unipi.it
[1]Research Center "E. Piaggio", University of Pisa, Italy [2]Dipartimento di Ingegneria dell'Informazione, University of Pisa, Italy

A biological tissue is a composite material with "bottom-up" hierarchical structure that is closely related to its heterogeneous function. The extracellular matrix modulates biochemical and biophysical signalling, and its rigidity is an important micro-environmental parameter that regulates the spatiotemporal dynamics of intercellular signalling. For this reason, many studies are focused on fabricating scaffolds processed at multiple scales with structural and mechanical properties that are optimal for eliciting specific response or mimic those found naturally. These scaffolds have to present large surface areas that have appropriate topology and biochemical cues (e.g, ligands) at the nanoscale for tissue adhesion, while also exhibiting integral porosity to allow for the exchange of molecules that maintain cellular function. In this talk, the use of a multiscale and multimaterial process will be presented to develop 3D in vitro model that can mimic the 3D complexity of natural tissue. These novel 3D in vitro models can be used for the study of physio-pathological condition and for the analysis of effects on cell activities of different biomolecule and/or drugs. For these reasons it is important combine different biomaterials natural and synthetic also in the form of hydrogels that can be processed with several printheads in order to mimic the physiological tissue environment in terms of topology, from nano to milli scale, in terms of biochemical cues and of mechanical properties as it happens in natural tissue. We designed, realized and characterized a multimaterial and multiscale scaffold fabrication approach that combine several micro and nano fabrication techniques such as: a)electrospinning, that is able to process several materials in form of nanofibers[1-3], b) Fused deposition modeling , that allows to realize 3D structure with a millimeters scale made of synthetic polymers[4]; c) piston based extrusion head that is able to process hydrogel materials from micro to milli scale [5]; and finally d) ink-jet head [6], that will allow to decorate the 3D structure with biochemical factors in well defined position in order to better mimic the natural tissue.

ACKNOWLEDGEMENTS: This project has received funding from: a) M-ERA.NET call 2016 network as part of the Horizon 2020 – the Framework Programme for Research and Innovation (2014-2020), project BIOMEMBRANE; b) M.ERA.NET - MANUNET CALL 2017 network as part of the Horizon 2020 – the Framework Programme for Research and Innovation (2014-2020), project KERAPACK; c) the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 681798-BOOST) (www.ercprojectboost.eu).

REFERENCES

[1] Criscenti G et al, Biofabrication. 2018 Aug 20;10(4):045005.
[2] Criscenti G et al, Acta Biomater. 2017 Jun;55:310-322.
[3] Criscenti G et al, Biofabrication. 2016 Jan 29;8(1):015009.
[4] Moroni et al, Trends Biotechnol. 2018 Apr;36(4):384-402.
[5] Vozzi G et al, Methods Mol Biol. 2012;868:57-69.
[6] Tirella A et al, J Biosci Bioeng. 2011 Jul;112(1):79-85.



SmartBone®: The case of an innovative bio-hybrid bone graft, from lab-scale design to clinical translation and commercialization

G. Perale[1]
Presenting Author: Giuseppe Perale, giuseppe@ibi-sa.com
[1]Industrie Biomediche Insubri SA, Via Cantonale 67, 6805 Mezzovico-Vira, Switzerland

INTRODUCTION: Bone is the second most transplanted tissue, just after blood, and early evidences of bone grafts date back even to ancient Egyptians. The last decades saw a constant decline of the use of autografts, the gold standard, and a parallel growth of alternative solutions, being however few and showing their limits [1]. To overcome such limitations, being inspired by nature bone architecture, we have developed a composite bone graft: a mineral matrix obtained by bovine-derived is reinforced with resorbable PLA-CL block copolymer embedding RGD-exposing collagen fragments onto its surface [2], commercially traded as SmartBone® (SB).

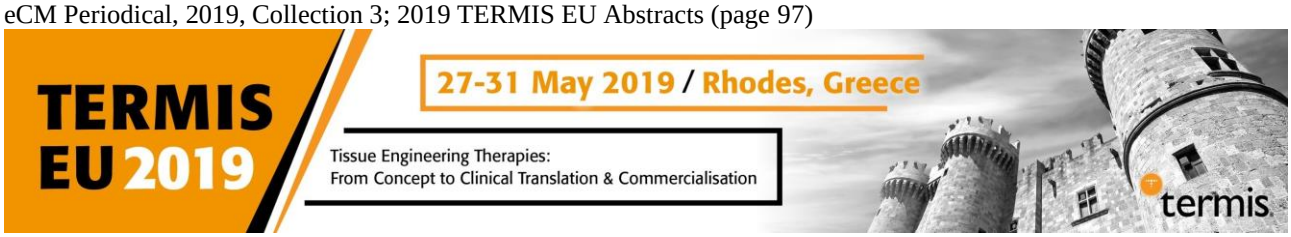
METHODS: SB has been widely investigated, also for regulatory purposes: composition has been assessed by inductively coupled plasma mass spectrometry, microstructure by microCT scan (1172 microCT Skyscan), mechanical performances have been assessed in compression, bending and torsion tests, biocompatibility was proven according to all ISO10993 prescriptions, both in vitro and in vivo animal [3]. Clinical performances were evaluated during investigative clinical trials in bone regeneration applications and mechanism of action was confirmed both during histologic studies [4] and in vitro assessments using human adipose-derived Stromal Vascular Fraction (SVF) [5]. After CE marking, SB has been introduced into the market and monitored via post-marketing surveillance in its clinical applications related to bone regeneration in all skeletal districts.

RESULTS & DISCUSSION: P/Ca ratio resulted 1.9, being the components of >98% of the mineral matrix; open and interconnected porosity was proven, with av. pore size in the 250-600 microns range; compression and torsional breakage stress resulted av. 26MPa, with an av. Young modulus of 1.3GPa. Altogether, clinical investigations in various skeletal districts and indications [3, 6, 7], histological studies and in vitro SVF-based investigations, confirmed the mechanism of action: SB is quickly colonized by host-cells and it is progressively substituted by healthy living bone, thanks to its high performances and its capability to spark the natural remodeling process.

CONCLUSIONS: Overall, with 60'000+ grafts performed in the last years, SB proved to be a safe and performing bone graft, sustaining bone regeneration in a wide spectrum of applications from traumatology to dentistry, from oncology to custom-made cases, and one of the few new biomaterials brought from lab design to market use in the last decade [1].

REFERENCES

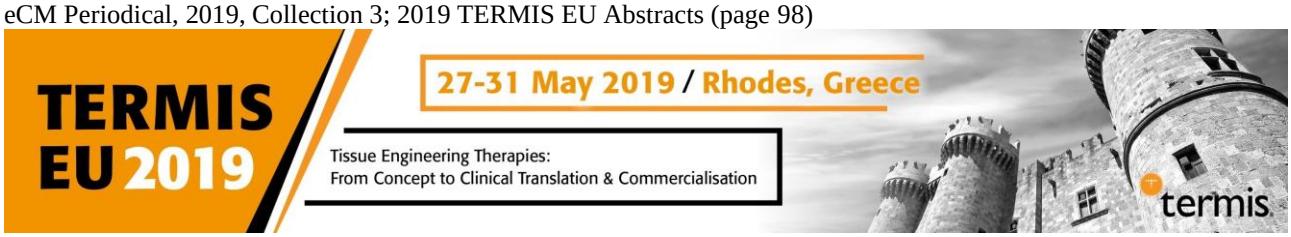
[1] Haugen H., Clin. Periodontol, in press 2018.
[2] Pertici G., Ann. Oral Max. Sur. 2014,14;2:4.
[3] Pertici G., J Biol. Reg. Hom. Ag. 2015,29:136.
[4] D'Alessandro D., Int. J Pharm, 2017.
[5] Roato I., Stem Cells Int, 2018, ID 4126379.
[6] Russo A., Injury, in press 2019.
[7] Piana R., eCM, in press 2018.
[8] Grecchi F., J Biol. Reg. Hom. Ag. 2015,29:42.



Development of tissue scaffolds via 3D biofabrication of organic/inorganic hybrid bioinks

G. Poologasundarampillai
Presenting Author: Gowsihan Poologasundarampillai, g.poologasundarampillai@bham.ac.uk
School of Dentistry, Institute of Clinical Sciences, University of Birmingham, Birmingham, UK

Combination of bottom-up ‘soft-chemistry’ sol-gel processing and top-down manufacturing processes have allowed the synthesis of porous hierarchical structures for biomedical applications. Bioprinting, a layer-by-layer top-down manufacturing process, has the potential to produce biomaterials on demand, customised for individual patient’s needs. One of the potential barriers to future developments in this field is the limited choice of suitable materials. At present, hydrogels from natural biopolymers are used for 3D bioprinting. These have several limitations, such as batch-to-batch variation, low shape fidelity and limited post-printing strength. An alternative are advanced organic-inorganic hybrid materials developed using the sol-gel process. Conventional sol-gel hybrids cannot be used as bioinks due to the release of large amounts of ethanol during the synthesis. In this talk, we will present the development of novel sol-gel derived bioinks for the 3D printing of tissue constructs using novel biocompatible silica precursors. Such precursors can be reacted together at physiological benign condition to result in hybrid hydrogels that show comparable physical and cell-response to hydrogels of biopolymers origin. The hybrid approach presented in this talk offers a number of key advantages for bioprinting over natural polymers: compositional flexibility, improved mechanical properties and controlled degradation.



Cartilage extracellular matrix derived scaffolds for articular cartilage and bone regeneration

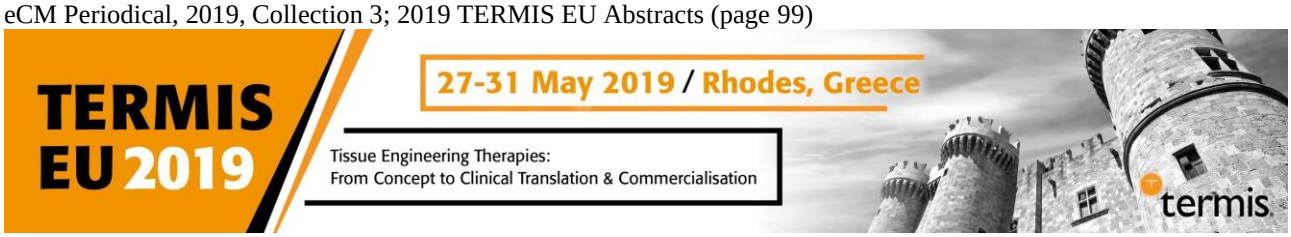
G. Cunniffe[1,2,3,4]
Presenting Author: Gráinne Cunniffe, gcunniff@its.jnj.com
[1]3D Printing Centre of Excellence, Global Supply Chain, Johnson & Johnson, 152-160 Pearse Street, Trinity College Dublin [2]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland [3]Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland [4]Advanced Materials and Bioengineering Research Centre, Trinity College Dublin and Royal College of Surgeons in Ireland, Dublin, Ireland

Extracellular matrix (ECM) derived biomaterials have been used clinically over 1 million times in the surgical repair of different tissues and organs [1,2]. Scaffolds used clinically are typically derived from small intestine submucosa (SIS) or pericardium, and while the ECM of these tissues clearly contain structural and regulatory biomolecules generally supportive of regeneration, it is unlikely that a single tissue source of ECM will be optimal for all clinical targets. This concept is strengthened by recent studies reporting that ECM derived biomaterials can direct the differentiation of mesenchymal stem cells (MSCs) towards the phenotype of the source tissue from which they were derived [3,4]. This motivates the development of tissue-specific ECM derived scaffolds, potentially consisting of different layers or lineage-specific regions, especially when attempting to regenerate complex multi-phasic tissues such as the osteochondral unit of synovial joints. This talk will describe our experience with developing highly porous, cell instructive scaffolds from the decellularized ECM of different musculoskeletal tissues. It will demonstrate how scaffolds derived from growth plate ECM can support large bone defect healing, and that articular cartilage ECM derived scaffolds can support chondrogenesis of adult stem cells and the repair of critically sized cartilage defects. Furthermore, the talk will demonstrate how layered, tissue-specific ECM derived scaffolds can be used for regenerating spatially complex musculoskeletal tissues such as the osteochondral unit.

ACKNOWLEDGEMENTS: Funding for this study was provided to Professor Daniel Kelly by the European Research Council Starter Grant (StemRepair – Project number: 258463), Science Foundation Ireland (SFI/12/RC/2278; 12/IA/1554), Enterprise Ireland (CF/2014/4325) and the Irish Research Council (GOIPG/2015/3186).

REFERENCES

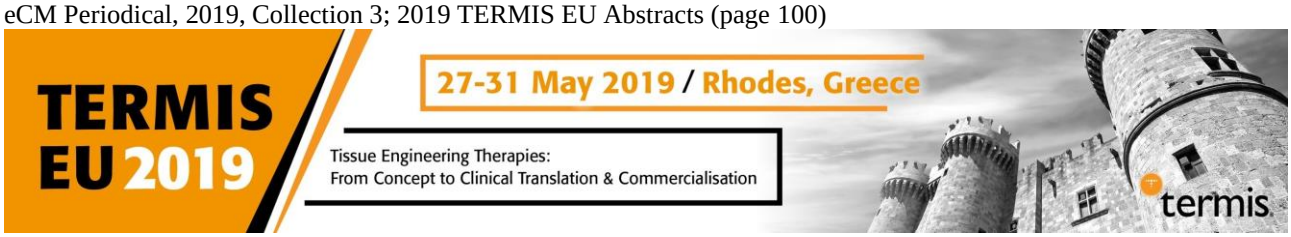
[1] Gilbert TW et al. Biomaterials. 27 (2006) 3675–3683.
[2] Brown BN et al., Transl. Regen. Med. to Clin. 163 (2015) 11–29.
[3] Shimomura K et al Am. J. Sports Med. 45 (2017) 604–611.
[4] Cunniffe GM et al. Eur. Cell. Mater. 33 (2017) 130–142.



Epithelial stem cells: A real tool for effective regenerative medicine treatments

G. Pellegrini
Presenting Author: Graziella Pellegrini, graziella.pellegrini@unimore.it
University of Modena and Reggio Emilia Modena, Center for Regenerative Medicine "Stefano Ferrari", Modena, Italy

Regenerative medicine has generated many efforts to explore new therapeutic potentials of both somatic and pluripotent stem cells with many possibilities envisaged for therapeutic applications. Hematopoietic and epithelial cells are extensively adopted for tissue regeneration, due to their high proliferative capacity and their accessibility. 30 years ago, the method for producing epidermis was discovered by cultivation from a small skin biopsy, allowing life-saving treatment of thousands severely burned patients in the following years. The importance of stem cell content was proven for tissues or organs in different pathologies. For instance, recent developments in cell-based therapy for ocular burns provided support for improvement and standardization of the cure for this disabling disease causing depletion of limbal stem cells. Indeed, biopsies taken from the healthy eye, or other autologous source as oral mucosa in bilateral blindness, can be used for their content of stem cells. Few of these therapies overcame the hurdles related to medicinal product regulation and became available to patients. The combined use of cell and gene therapy represents a further scientific approach for the treatment of congenital diseases. This approach was proven on hematopoietic cells and has recently been established using genetically modified epidermal cells for life-saving treatment on severe genetic diseases, as epidermolysis bullosa.

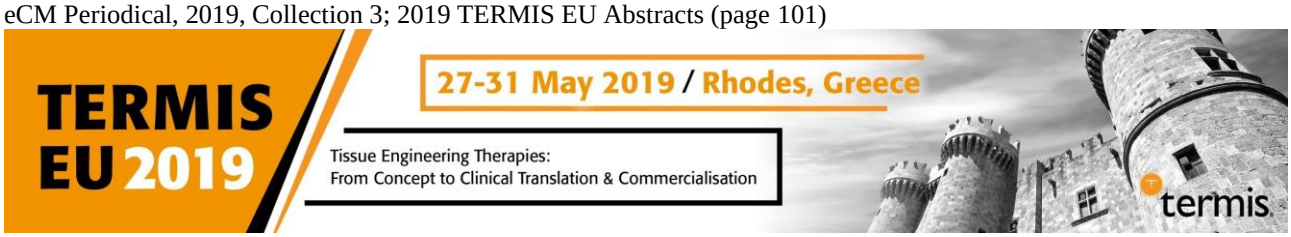


Discrimination of influence of cell adhesion and spreading area on hMSCs differentiation using micropatterns

G. Chen[1,2]
Presenting Author: Guoping Chen, Guoping.CHEN@nims.go.jp
[1]Tissue Regeneration Materials Group, Research Center of Functional Materials, National Institute for Materials Science, Tsukuba, Japan [2]Department of Materials Science and Engineering, Graduate School of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Japan

Cell adhesion and spreading are manipulated by the cell/ECM interactions that play crucial roles in regulating cell functions. Many studies have reported that the physical properties of ECM including geometry, anisotropy, topography and rigidity can influence the mechanosensing of the microenvironment through regulating cell adhesion and spreading. However, it is unclear whether cell adhesion or spreading is the predominant factor to influence cell functions because it has been difficult to separate the two effects by conventional cell culture using uniform surfaces. In this study, the independent influence of adhesion and spreading area on differentiation of human mesenchymal stem cells (MSCs) was investigated by using micropatterning method to precisely control cell adhesion and spreading areas. A series of micropatterns having the same size and different cell adhesion area or having different size and the same cell adhesion area were prepared by UV photolithography for cell culture. The formation of FAs and the cytoskeletal organization in the cells cultured on the micropatterns were investigated to evaluate cell adhesion and spreading state. The mechanical properties of micropatterned cells and the transduction of cytoskeletal force into nucleus were characterized to reveal the mechanism of the influence. The osteogenic and adipogenic differentiation of MSCs were investigated to show how the adhesion and spreading areas independently influenced cell fate determination. The adhesion and spreading of human mesenchymal stem cells (MSCs) were manipulated by the micropatterns due to the regulation of focal adhesion formation. According to the available adhesive and spreading areas, cells assembled their cytoskeletal structure in different manners. However, the binding of myosin to actin fibers was mainly influenced by cell adhesive area. Meanwhile, cell mechanical state and the mechanotransduction were mainly determined by the adhesive area rather than spreading area. Furthermore, large adhesive area facilitated the osteogenic differentiation, while small adhesive area promoted the adipogenic differentiation. Cells with the same adhesive area but different spreading areas had similar potential of differentiation. The results indicated that the adhesive area rather than spreading area played more important roles in regulating cell functions in an isotropically micropatterned cells. The results should inspire the design of biomaterials to process in an effective manner for manipulation of stem cell differentiation.

ACKNOWLEDGEMENTS: This work was supported by JSPS KAKENHI Grant Number 18K19947.



Do we need to better mimic bone microstructure to observe bone cell behavior in vitro?

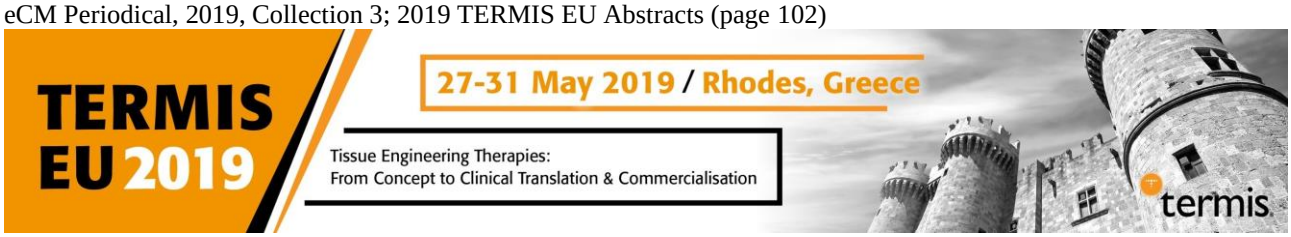
G.C. Reilly[1,2]
Presenting Author: Gwendolen Reilly, g.reilly@sheffield.ac.uk
[1]Insigneo Institute for in silico medicine and [2]Department of Materials Science and Engineering, University of Sheffield, Sheffield, UK

In vitro 3D bone models and 'bone-on-a-chip' style approaches have been advocated as an alternative to in vivo experiments, providing a more physiological environment than monolayer culture, with the opportunity to use patient specific cells for human disease relevance. However, bone is a particularly challenging tissue to create 'on a chip' due to its dense composite structure perfused by blood vessels in osteonal canals, in which ellipsoid cells with multiple processes reside, the osteocytes. The bone extracellular matrix (ECM) is a highly organized structure with arrays of collagen fibres in parallel within one lamella (layer) but changing in orientation between lamellae. Monolayer culture, often described as effectively '2D', does not retain cells' natural morphology, as cells are constrained to flatten onto a highly brittle and stiff substrate, different from the native bone microenrivonment. For example, much previous work in our laboratory used fluid flow over this monolayer to mimic the fluid environment of bone but the cells responses were likely strongly influenced by the non-osteocyte like shape that they are forced to take in monolayer culture. Bone tissue engineering has made huge advances in enabling 3D culture of osteoprogenitor cells in a range of scaffolds and devices. There is much evidence that cells can be easily encouraged to create a 'bone-like' 3D matrix in these environments. In the sense that the matrix is evidenced to contain collagen, other ECM proteins and calcium phosphate/ hydroxyapatite mineral. However in most of these 3D cultures cells reside in clusters or line scaffold surfaces and collagen and mineral are observed in non-organised patches. Recently, our group and others have made advances in better re-creating the bone-like environment and several studies have reported osteocyte-like morphologies in tissue engineered bone. Here, I will mainly focus on two approaches in our laboratory to improve mimicking of bone matrix in vitro. The first involves using emulsion templating (PolyHIPE) techniques to create hierarchical structures with pores of the order of magnitude of osteocyte lacunae and of the order or magnitude of osteonal canals. Such scaffolds are highly tunable in terms of stiffness and in our group we can have cast, printed and created microfluidic devices from such materials. In another approach we use electrospun scaffolds with controlled fibre alignment to guide ECM production by bone cells and re-create lamellar-like structures. It is still not clear from these studies how closely the microstructural morphology of bone needs to be replicated before we can say such models can avoid the need for in vivo studies. However it is clear we are getting much closer to humanized, physiological bone mimics which could be used for better understanding of bone in health and disease.

ACKNOWLEDGEMENTS: Many thanks especially to R. Owen, G. Tetteh, H. Bahmaee and the F. Clayssens research group for input to this work. Financial support was received from the Engineering and Physical Sciences Research Council UK, the British Council and H2020, Marie Skłodowska-Curie Actions: H2020-MSCA-RISE-2017 (777926) and H2020-MSCA-ITN-2017 (766012).

REFERENCES

[1] Owen R. et al. (2016) J. Mechanical Behavior of Biomedical Materials 54:159-172.
[2] Paterson T.E. et al. (2018) APL Bioengineering. 2 026103.
[3] Cigognini D et al. Sci Rep. 2016;6:30746.



Bioprinting of gene-activated scaffolds for musculoskeletal regeneration: Cell-based growth factor delivery

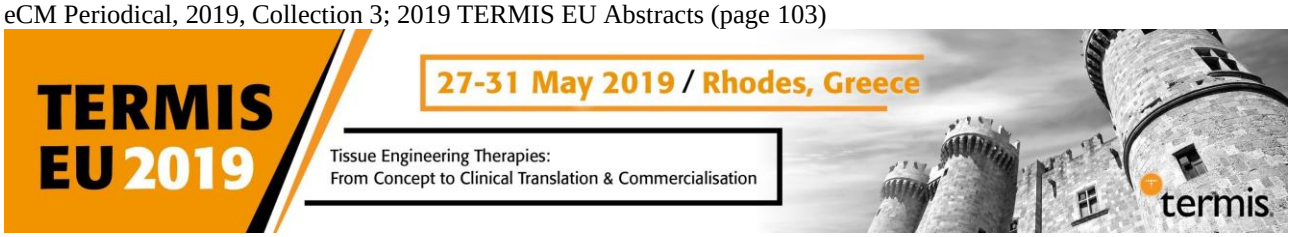
H. Lin[1,2]
Presenting Author: Hang Lin, hal46@pitt.edu
[1]Center for Cellular and Molecular Engineering (CCME), Department of Orthopaedic Surgery
[2]McGowan Institute for Regenerative Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, U.S.A.

Bone morphogenetic protein 2 (BMP-2) is a commonly used inductive factor for osteogenesis of bone marrow mesenchymal stem cells (BMSCs), but sustained delivery of active BMP-2 protein remains challenging. In this study, we first used lentiviral constructs to ex vivo transduce human bone marrow-derived stem cells with human bone morphogenetic protein-2 (BMP-2) gene (BMP-hBMSCs). We then introduced these cells into a hydrogel scaffold using an advanced visible light-based projection stereolithography (VL-PSL) technology, one type of 3D printing technologes that is compatible with concomitant cell encapsulation and amenable to computer-aided architectural design, to fabricate scaffolds fitting local physical and structural variations in different bones and defects. The results showed that the BMP-hBMSCs encapsulated within the scaffolds had high viability with sustained BMP-2 gene expression and differentiated toward an osteogenic lineage without the supplement of additional BMP-2 protein. In vivo bone formation was also observed as early as 14 days post-implantation [1]. We also tested another strategy through gene-activated matrix. We incorporated viral vector encoding BMP-2 gene (Lv-BMP, BMP group) or Green fluorescent protein (GFP) gene (Lv-GFP, GFP group) and human BMSCs into gelatin solution, which were then subjected to VL-PSL. We hypothesized that the Lv-BMP would in situ transduce encapsulated BMSCs, resulting in robust osteogenesis and bony tissue formation without the supplement of extra BMP-2 protein. To test the in vivo bone formation capacity, constructs from GFP or BMP group were implanted intramuscularly in SCID/J mice. Micro–computed tomography (μCT) imaging showed detectable mineralized areas in BMP group, which is restricted within the scaffolds, suggesting a localized bone formation in muscle. Similar areas were not present in the GFP control. Alizarin red staining and immunohistochemistry (IHC) of GFP and osteocalcin indicated that grafted hBMSCs, not host cells, contributed majorly to the bone formation. This novel one-step, gene-activated and live cell encapsulated scaffold fabrication procedure is thus potentially applicable for point-of-care treatment of bone defect.

ACKNOWLEDGEMENTS: Financial support was received from the National Institutes of Health (1UG3TR0021360 and 5R01EB019430).).

REFERENCES

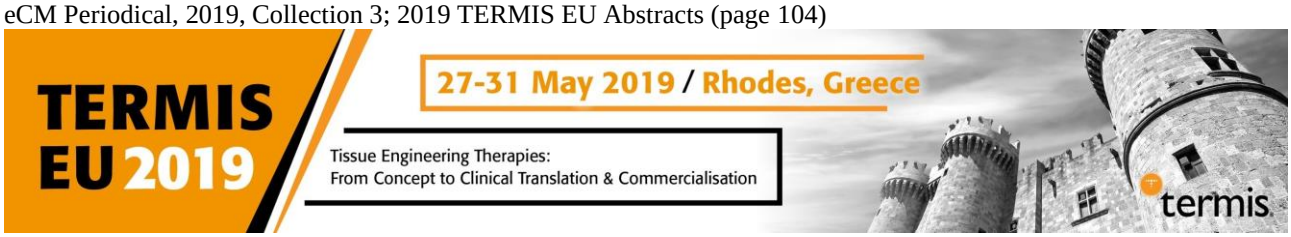
[1] Lin et al. Biomaterials. 2013:331-9.
[2] Lin et al. Sci Rep. 2017;7(1):11327.



Commercialization of tissue engineering products - A user's clinical perspective

H. J. Meisel
Presenting Author: Hans-Jörg Meisel, Hans-Joerg.Meisel@bergmannstrost.de; hj.meisel@googlemail.com
Department of Neurosurgery, BG Klinikum Bergmannstrost Halle, Germany

Official data say that the global tissue engineering market size was valued at around 5 billion US$ in 2016. Growing potential of tissue engineering procedures in the treatment of tissue damages is supporting the market growth. European data show that 1500 procedures using tissue engineered products have been performed and reported annually between 2016 and 2018. (Martin I, et al. The Survey on Cellular and Engineered Tissue Therapies in Europe in 2013.Tissue Eng. 2016;22(1-2):5-16). According to Medicare more than 900,000 surgeries are performed in the US for bone reconstruction or replacement. The segments - Orthopaedics, musculo-skeletal and spine held majority of the market chair in the year 2014 owing to the rising musculo-skeletal disorders. In Europe, most of the start-up companies have failed to participate in this growth. The lack of venture capital and increase in the regulatory requirements have created ahostile climate for start-up companies in Europe for the last 10 years. Companies are now entering into collaboration to gain advantages on research and technology competencies offered by other competitors. Gaining clinical evidence for the use of tissue engineered products is a long lasting process, particularly in the field of musculo-skeletal disorder/research. The increase of minimal invasive procedures due to technical innovation in the field of medical devices are increasing the requirements for tissue engineered products in many medical specialties. The medical unmet need and challenges, in addition, has to be evaluated continuously. The fragmentation of research and development of tissue engineered products and clinical application is requiring an urgent harmonization for the needed translational process.

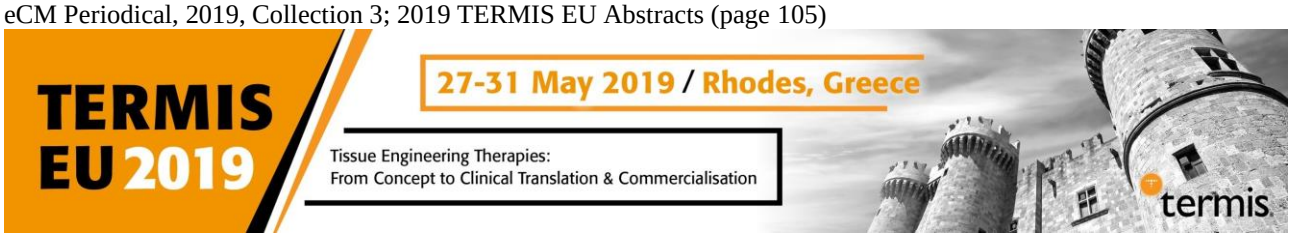


Innovative approach for bone and cartilage tissue engineering

H. Havitcioglu[1,2,3]
Presenting Author: Hasan Havitcioglu, hasan.havitcioglu@deu.edu.tr
[1]Dokuz Eylul University, Faculty of Medicine, Department Of Orthopedics and Traumatology, İzmir, Turkey [2]Dokuz Eylul University, Department of Health Sciences, Biomechanics Department, İzmir, Turkey [3]Dokuz Eylul University, Bone - Cartilage Tissue Biotechnology Application and Research Center (KEKDOB), İzmir, Turkey

Cartilage and bone tissue engineering is highly studied topic for about 20 years. However, it could not be transferred to clinical studies. Therefore, this abstract aims to combine in vitro and in vivo studies for cartilage and bone tissue engineering based on scaffolds with various fabrication techniques. Scaffolds should mechanically and physiologically mimic the cartilage and bone tissue. Porosity, mechanical strength, cell adhesion, biocompatibility, cell proliferation, mineralization and toxicity are important characteristics. In order to improve scaffolds poor mechanical properties, while preserving the porous structure, it is possible to coat the scaffold with synthetic or natural polymers. It is better if you produce scaffolds from natural biomaterials. We produce various bone and cartilage tissue scaffolds with different techniques such as lyophilization, 3D printing and electrospinning. By using Polylactic Acid (PLA), loofah (Luffa Cylindrica), chitosan, collagen, Poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV), fish scale, cellulose acetate, etc. we produced various composite scaffolds. Their chemical, morphological and mechanical properties should be determined by using several tests. Scaffold morphologies were tested by Scanning Electron Microscope (SEM). Also, by using Fourier Transformed Infrared Spectroscopy (FT-IR) chemical compositions were tested. Their mechanical properties were tested by compression or tension tests. Moreover, swelling ratios, thermal gravimetric analysis (TGA) and porosity ratios were completed. Biocompatibility tests could be performed by using mesenchymal stem cells or cell lines. Stem cells are frequently used in regenerative medicine. They can be obtained from many sources such as bone marrow, adipose tissue, periosteum, etc. Viability, cytotoxicity, mineralization, Glucose-Amino-Glycan (GAG) activities were determined by using spectrophotometer. Moreover, some of them were used in Wistar Albino rats and New Zealand rabbits. Analysis indicated, scaffolds are successfully mimicked bone and cartilage tissue. They are suitable for in vivo regeneration. Also, they could be used to increase regeneration. Histological studies showed promising results. Pre-clinical studies were completed. They should be used in clinical studies.

ACKNOWLEDGEMENTS: Financial support was received from Dokuz Eylul University Scientific Research Projects Foundation.



Induction of angiogenesis by placenta derived stem cells and matrix for tissue engineering and regenerative medicine

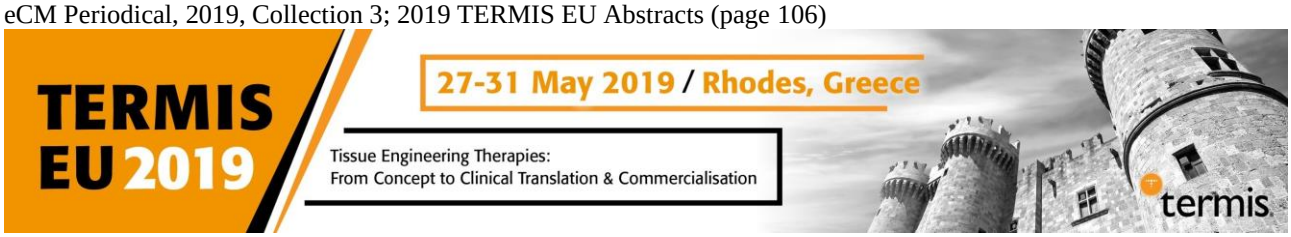
H. Niknejad
Presenting Author: Hassan Niknejad, niknejad@sbmu.ac.ir
Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Despite huge progress in the fields of tissue engineering and regenerative medicine, angiogenesis is still a major challenge. Human placenta including amnion and chorion, cord blood and its matrix have some properties which make them an appropriate source of stem cells and scaffold for tissue engineering. The placenta derived epithelial and mesenchymal cells possess the characteristics of pluripotent/multipotent stem cells which have the capability to differentiate into all three germ layers and can be obtained without ethical problem. Moreover, placenta derived matrix demonstrates properties of an appropriate scaffold tissue substitute via different types of extracellular proteins such as collagens, laminins and fibronectins which serve as an anchor for endothelial cell attachment and proliferation, a substrate for cell delivery and a reservoir of drugs and growth factors involved in angiogenesis. The recent data demonstrate that amniotic epithelial and mesenchymal exosomes have their specific effects on angiogenesis. Moreover, based on in vitro aorta ring assay and microinvital microscopy, both sides of amniochorion membrane can serve as a scaffold for prevascularization of three dimensional tissue engineered constructs. Recently, new insights into developing new methods for surface modification and cross-linking, construction of placenta based hydrogels and micronizing approaches emerged new fields in vascular tissue engineering [1,2].

ACKNOWLEDGEMENTS: This work was supported by National Institute for Medical Research Development (NIMAD), Tehran, Iran under award number [963951].

REFERENCES

[1] Tehrani FA et al. Scientific reports. 2017; 7 (1); 17022.
[2] M Kakavand et al. J tissue eng and reg med. 2017; 11 (6): 1701-1709.



Canine and feline liver organoids as translational models

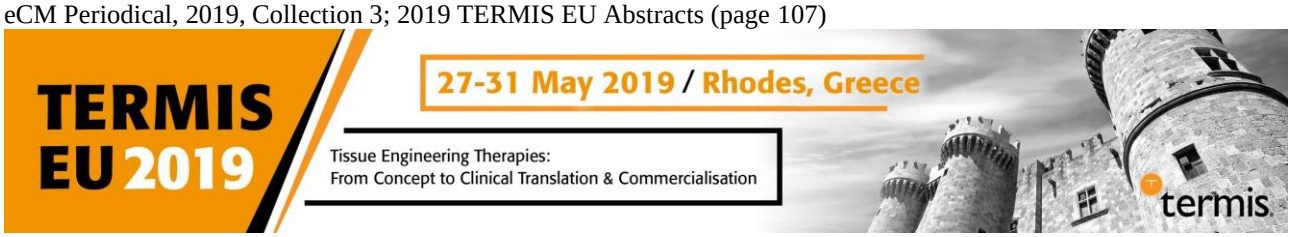
H.S. Kruitwagen[1]
Presenting Author: Hedwig Kruitwagen, H.S.Kruitwagen@uu.nl
[1]Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Organoids are defined as 'structures resembling an organ' and are a 3D adult stem cell culture system. Originally developed from mouse intestinal stem cells, organoid cultures have since then been established from a wide range of tissues and species, including human. Organoid development was a breakthrough in many research areas, as it allowed for highly proliferative robust primary cultures from patient samples from various organs (e.g. stomach, intestine, pancreas and liver). Organoids have already proved to be a valuable tool for disease modeling research (e.g. in human cystic fibrosis) and are also considered for transplantation purposes. Our lab studies canine and feline liver disease and regeneration and established organoid cultures from dog and cat liver [1]. Interestingly, dogs and cats develop spontaneous liver diseases that have clinical counterparts in human hepatology. For example, dogs can suffer from genetic copper toxicosis resulting in chronic hepatitis and cirrhosis similar to human Wilson's disease. Organoids were used to model and genetically correct this disease in vitro [2] and subsequently transplanted in vivo in dogs with copper storage disease. In cats a specific syndrome of liver steatosis is observed upon prolonged periods of anorexia. Feline hepatic steatosis resembles human fatty liver disease, an increasingly prevalent syndrome associated with Western diet and lifestyle. Feline liver organoids were cultured and fed excess fatty acids, which resulted in cellular steatosis [3]. Feline organoids turned out to be more prone to lipid accumulation than human organoids, exemplifying them as an interesting translational research model. Current studies focus on lipid-lowering drug interventions in feline fatty organoid cultures.

ACKNOWLEDGEMENTS: Financial support was received from the Netherlands Organization for Scientific Research NWO ZON/MW (116004121) and the Winn Feline Foundation (W15-037 and W17-015).

REFERENCES

[1] Kruitwagen H et al. BMC Vet Res. 2014; 19;10:137.
[2] Nantasanti S et al. Stem Cell Reports 2015; 5(5):895-907.
[3] Kruitwagen H et al. Stem Cell Reports 2017; 8(4):822-830.



Hybrid bioprinting with vascularized spheroids

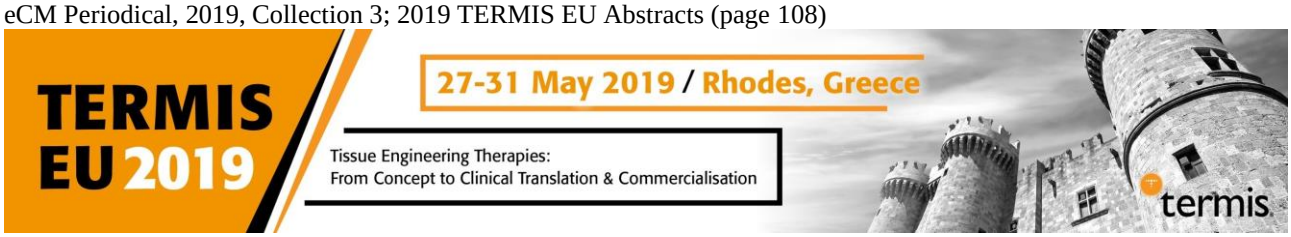
H.A. Declercq[1,2]
Presenting Author: Heidi Declercq, heidi.declercq@ugent.be
[1]Tissue Engineering Group-Bioprint Facility, Department Human Structure and Repair, Faculty of Medicine and Health Sciences, Ghent University, Gent, Belgium [2]GATE (Ghent Alliance for Tissue Engineering), Gent, Belgium

The main challenge in tissue engineering is the creation of a functional engineered vascular system with multiscale vessel networks from capillaries to large vessels within complex 3D structures such as heart, muscle, bone, adipose tissue,… Strategies to engineer these tissues can be categorized in top-down, scaffold-based approaches and bottom-up, developmental biology inspired approaches (scaffold-free). In the bottom-up approach, a 3D tissue is build by assembling modular tissues (spheroids). Although progress was made in both strategies, the inability in fabrication of 3D vascular networks has limited applications in tissue engineering. Now it has become clear that individual approaches will not succeed to the end-goal. In this lecture, we will focus on combining the advantages of both approaches that will have a synergistic effect for future fabrication of 3D tissue analogs. In our approach, cellular building blocks with self-assembling properties and mimicking the tissue of interest will be combined with cell instructive biomaterials. The cellular building blocks are either tissue-specific or vascular. Using a high-throughput non-adhesive agarose microwell system (2865 pores, diameter 200 µm) uniform spheroids with an ideal geometry and diameter (< 200 µm) for bioprinting are formed. High quality homocellular building blocks were already generated that form tissue-specific cellular building blocks ((fibro)cartilage [1], adipose tissue, bone tissue,…) starting from adult cell types or human mesenchymal stem cells derived from adipose tissue or pulp tissue. Dependent on the tissue type, stable spheroid formation was influenced by cell culture medium, cell culture environment and cell types. These tissue-specific spheroids can be combined with vascular spheroids providing the capillary like network. By coculturing endothelial cells with fibroblasts and/or adipose tissue derived mesenchymal stem cells as supporting cells, and applying the favourable coculture ratio, viable vascular spheroids were obtained [2]. Endothelial cells spontaneously organized into a capillary like network and lumina were formed, as shown by immunohistochemistry and transmission electron microscopy. Moreover, the spheroids were able to assemble at random in suspension, creating a macrotissue. The tunable mechanical characteristics of hydrogels (crosslinking efficiency) can influence outgrowth and fusion of the spheroids. 3D bioprinting of vascular spheroids in photocrosslinkable gelatin-methacrylamide was performed with the 3D Discovery (RegenHu) and resulted in high viability and fusion of the vascular spheroids into a vascular network. Reorganization of cells, throughout the entire fused construct and by inoculating with capillaries of adjacent spheroids, creates a branched capillary like network. Combining the advantage of the natural capacity of microtissues to self-assemble and the controlled organization by bioprinting technologies, these vascularized spheroids can be useful as building blocks for the engineering of large vascularized 3D tissues.

ACKNOWLEDGEMENTS: Financial support was received from the Research Foundation Flanders (FWO Research Grant 1510414N); Ghent University (Special Research Fund, BOF, 01B02315); Erasmus Mundus Action 2 Project Basileus V (Scholarship).

REFERENCES

[1] Berneel E et al. Cells Tissues Organs 2015; 202: 369-81
[2] De Moor L et al. Biofabrication. 2018; 10: 035009



Did we run out of ideas? The importance to spark innovation in tissue engineering research and traumatology

H.R. Redl[1,2]
Presenting Author: Heinz Redl, Office@TRAUMA.LBG.AC.AT
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatoloy, AUVA Research Centre, Austria [2]Austrian Cluster for Tissue Regeneration

INTRODUCTION: What if patients and clinical experts could spark and define the direction of multidisciplinary science projects without knowing the scientific literature?
The LBG Open Innovation in Science Center and the LBI for Experimental and Clinical Traumatology set up a project in order to fill the gap between clinical knowledge and scientific research. This novel approach in the field of Traumatology aims to bridge the gap between the conventional bench-to-bedside approach within this discipline.

METHODS: 'Tell us!' — about accidental injuries leverages crowdsourcing methodology to spark new lines of research [1]. Over a period of 4 months, a broad community of international stakeholders and participants was established and motivated to submit research questions in the area of the diagnosis, treatment and rehabilitation of patients with major traumatic accidents.

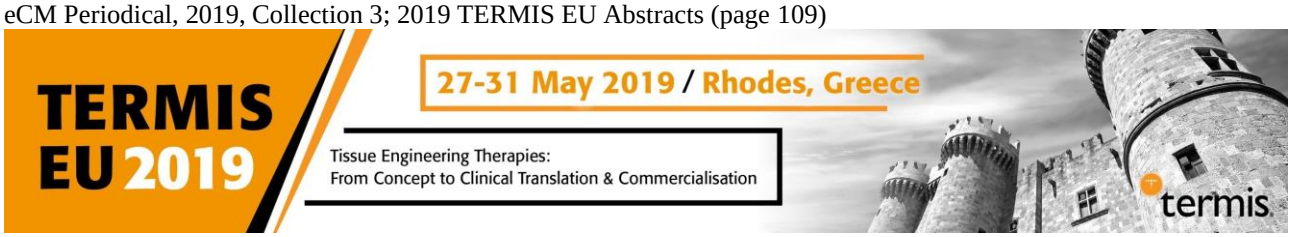
RESULTS & DISCUSSION: 227 participants registered to the tell-us.online platform. In total, 118 users contributed a total of 190 research questions within 4 months of crowdsourcing (Mai — August 2018). Overall, 60% of all contributions came from experts coming from physiotherapy (n = 13), MDs (n = 13) and occupational therapy (n = 11). Most contributions came from the field of nursery with research questions of great detail (e.g.; „What is the effect of irradiated amnion on Chondrogenesis in osteochondral defects in talus or knee?") and more general questions (e.g. „How can a multidiscplinary rehabilitative approach improve the functional outcomes in geriatric clients with cognitive disorders?").

CONCLUSIONS: Applying Open Innovation in Science is a major quest in order to open up scientific workflows. In particular, using crowdsourcing to identify research questions from bedside-to-bench can be a method of great potential to spark innovation in the field of Traumatology.

ACKNOWLEDGEMENTS: No financial interests to disclose.

REFERENCES

[1] Missbach B et al. Orthopaedics Today Europe, April 2018



Towards a better knowledge of cell and tissue responses to strontium-containing bioactive glasses

H. Autefage[1,2]
Presenting Author: Hélène Autefage, helene.autefage@ki.se
[1]Dept of Materials, Dept of Bioengineering, and Institute of Biomedical Engineering, Imperial College London, London, UK [2]Dept of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, SE.

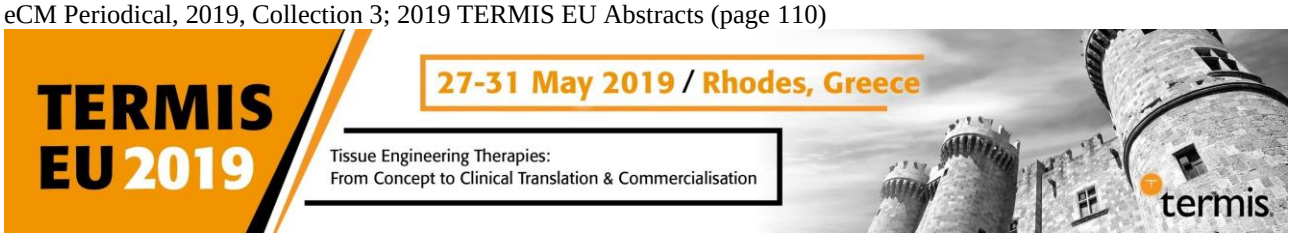
INTRODUCTION: The development of synthetic bone substitutes that are able to repair critical-sized bone defects requires the combination of an optimized composition and architecture, which remains challenging to achieve, partly because of a lack of clear understanding of the mechanisms by which biomaterials drive bone regeneration. Strontium ions have proven anti-osteoporotic properties and can easily be substituted to calcium in inorganic materials. While, strontium incorporation within BG has been shown to increase osteoblastic activity and decrease of osteoclastic activity in vitro, and strontium-containing inorganic bone substitutes increased osteointegration in vivo in small animal models, the mechanisms by which strontium-loaded materials favor bone formation remain unclear. Here, we applied discovery-driven approaches to better identify how strontium incorporation within BG affects osteoprogenitor cell global responses, bone formation and bone/material interaction.

METHODS: A porous SrBG (pSrBG) was created by in situ hybridization, implanted in critical-sized defects in an ovine model, and bone formation was assessed with regards to quantity and quality. To better understand how strontium incorporation into BGs affected human mesenchymal stromal cells (hMSCs) through changes in the ionic microenvironment, a series of SrBGs based on 45S5 composition was produced, and hMSCs were treated with SrBG dissolution extracts before a whole-genome analysis was performed.

RESULTS & DISCUSSION: When implanted in a critical-sized defect in sheep, pSrBG released strontium and outperformed 45S5 control particles with regards to bone-to-material contact and bone architecture. Interestingly, the multiscale analysis of the newly-formed bone quality identified differences between pSrBG and 45S5. In vitro, our objective analysis of hMSC global responses to SrBG dissolution products revealed that Sr^{2+} loading within 45S5 BG composition resulted in strong unexpected changes in the cell metabolism at the gene and cellular levels. Those changes translated to modifications in the cholesterol and lipid raft membranous contents as well as an increased acto-myosin activity.

CONCLUSIONS: This work showed that the simple incorporation of Sr^{2+} in BG can have a strong influence on cell global response. It also illustrated how non-discriminative discovery-driven approaches and in-depth tissue analysis can uncover unexpected cell and tissue responses to specific material properties. By providing a better knowledge of the materials' effects on cells and tissues, such strategies would likely facilitate the development of future biomaterials.

ACKNOWLEDGEMENTS: This work was supported by the Medical Engineering Solutions in Osteoarthritis Centre of Excellence funded by the Wellcome Trust and the EPSRC, the Wellcome Trust Institutional Strategic Support Fund (VIP award 097816/Z/11/B), the Rosetrees Trust, the Technology Strategy Board, UK, and the Swedish Research Council (VR 4-478/2016).

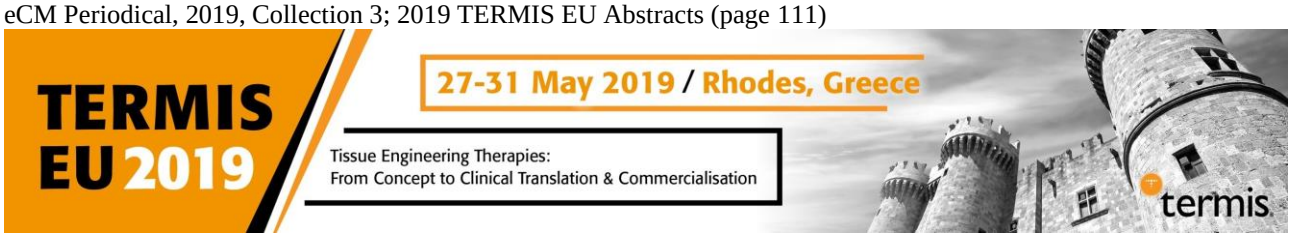


Challenges of manufacturing MSCs for clinical development

H. Roelofs[1]
Presenting Author: Helene Roelofs, H.Roelofs@lumc.nl
Center for Stem Cell Therapy, Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands

Ample experience is available for drug manufacturing involving the culture of cells. Manufacturing processes for drugs where the cells themselves are the active ingredient are relatively new and come with new manufacturing challenges, especially when the cells of interest are adherent cells. MSC products for early phase clinical trials, generated with mainly manual processes - including cell expansion in flasks and multiple open process steps in heavily controlled production environments - have indicated to be safe and efficacious in a number of patient populations. However, the scale-up to increased production volumes has proven to be cumbersome. Simply multiplying the manual process leads to extremely high production costs, driving the price for the cellular drugs to levels that are limiting patient access. Changing the production process itself has its effects on main manufacturing attributes such as identity, purity, safety and potency, hampering clinical development. Here, our experience with MSC production scale-up and automation is discussed as well as alternative options that allow manufacturing scale-up to stay in tune with clinical development.

ACKNOWLEDGEMENTS: Financial support was received from The Netherlands Organization for Health Research and Development, Framework Programme for Research and Technological Development and Horizon 2020.



Modeling of limbal stem cell differentiation using human pluripotent stem cells and tissue engineering

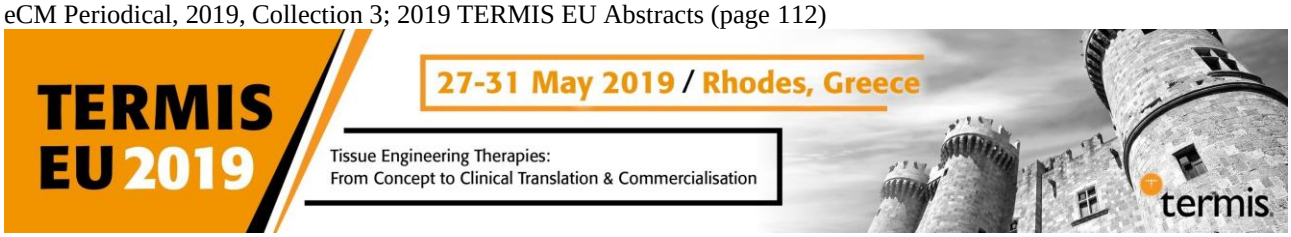
H. Skottman[1]
Presenting Author: Heli Skottman, heli.skottman@uta.fi
[1]Faculty of Medicine and Health Technology, BioMediTech Institute, Tampere University

Corneal blindness affects millions of people worldwide, and there is a constant shortage of high quality donor tissue. Engineering a corneal transplant that could effectively restore functional limbal epithelial stem cells (LESC) and regenerate the corneal epithelium and stroma is an important goal. To achieve this, differentiation of LESCs from human pluripotent stem cells (hPSC-LESC) provides new tools for studying corneal epithelial renewal and providing exhaustless source of cells for regenerative therapy. In addition, human adipose stem cells (hASCs) provide an appealing cell source for corneal stromal regeneration due to their capacity to differentiate towards corneal keratocytes, immunomodulatory properties and good availability from healthy adult donors. We have previously established an efficient and clinically relevant method to produce LESC- like cells from hPSCs [1]. With this method, we are able to produce a cell population highly expressing clinically relevant corneal progenitor markers p63α/p40 in defined and serum-free culture conditions. With detailed analyses of the cell differentiation in several time points, we have gained further knowledge of the differentiation pathway involved. Interestingly, ABCG2, a universally proposed marker of limbal stemness, is only transiently expressed in culture conditions supporting corneal epithelial differentiation. Thus, this cell population at early state of differentiation represented a noticeably different LESC- like phenotype (being ABCG2-positive) as compared to later arising cell population with high level of progenitor marker expression (ABCG2-negative/p63a-positive). Based on our current research hypothesis, these two cell populations may represent functionally different stem/progenitor populations with implications in their regenerative efficacy. For further study and possible utilization of these therapeutic cells, we use novel biomaterials and tissue engineering techniques. In our efforts to develop new constructs for corneal regeneration, we combine the hPSC-LESCs with hASCs into functional biomaterial scaffolds for regeneration of both the corneal epithelium and stroma. To this end, we have recently shown the application of a novel laser-assisted bioprinting (LaBP) method for creating corneal-mimicing structures composed of clinically relevant bioinks, hASC-containing stromal layers and overlying multilayered epithelium bioprinted from hPSC-LESCs [2]. In addition, our new approach for creating layered corneal epithelial and stromal implants with increased transparency takes advantage of our earlier work with hyaluronic acid (HA) based hydrogels for hASCs delivery to the cornea [3]. With incorporation of dopamine moieties to HA hydrogels, we have been able to construct tissue-adhesive implants containing hASCs in the bulk hydrogel and hPSC-LESCs on the surface, and we have demonstrated their proof-of-concept implantation in porcine corneal organ culture model.

ACKNOWLEDGEMENTS: Financial support received from Academy of Finland, Sigrid Juselius Foundation and Business Finland.

REFERENCES

[1] Hongisto, H., et al., Stem Cell Research & Therapy, 2017, 8:291.
[2] Sorkio, A., et al., Biomaterials, 2018. 171:57-71.
[3] Koivusalo, L., et al., Materials Science & Engineering C, 2018, 85: 68–78.



Medical evaluation of mussel protein bioadhesives

H. J. Cha[1]
Presenting Author: Hyung Joon Cha, hjcha@postech.ac.kr
[1]Department of Chemical Engineering, POSTECH, Pohang 37673, Korea

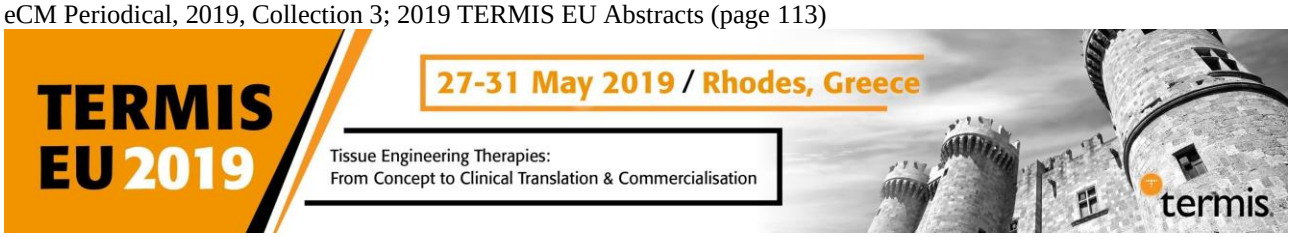
Marine mussel adhesion is known to be mediated by adhesive proteins, which are secreted through the mussel byssus and have great potential as biologically and environmentally friendly adhesive biomaterials due to their biocompatibility and biodegradability. In addition, mussel adhesive proteins (MAPs) have strong adhesion ability even on wet surfaces due to unique amino acid arrangements and composition. However, researches using the natural amino acid composition have been limited due to extreme difficulties in obtaining sufficient quantities of MAPs for practical applications and commercialization. We successfully produced redesigned MAP using a bacterial expression system and this bioengineered MAP showed significant adhesion ability and biological safety. We successfully developed a photo-activated MAP-based hydrogel bioadhesive (LAMBA) inspired by insect dityrosine crosslinking chemistry [1]. The developed LAMBA exhibited substantially stronger bulk wet tissue adhesion than commercially available fibrin glue and good biocompatibility in both in vitro and in vivo studies. Besides, the easily tunable, blue light-activated crosslinking enabled an effective on-demand wound closure and facilitated wound regeneration. Based on these outstanding properties, LAMBA holds great potential as an ideal medical glue for diverse medical applications, including sutureless wound closures of skin and internal organs and effective regeneration of wounded tissues. In addition, we developed a unique water-immiscible MAP-based bioadhesive (WIMBA) exhibiting strong underwater adhesion which was employed by two adhesion strategies of mussels; Dopa-mediated strong adhesion and water-immiscible coacervation [2]. The developed biocompatible WIMBA successfully sealed ex vivo urinary fistulas and provided good durability and high compliance. Thus, the developed WIMBA could be used as a promising sealant for fistula management with further expansion to diverse internal body applications. We developed and evaluated MAP as innovative bioadhesive materials for diverse medical area including scarless wound closure and fistular sealing with proper fabrications and formulations.

Figure 1: Schematic representation of WIMBA employed by the chemistries of DOPA and complex coacervation for urinary fistula sealing [2].

ACKNOWLEDGEMENTS: Financial support was provided by the Ministry of Oceans and Fisheries, Korea.

REFERENCES

[1] Jeon, E.Y. et al Biomaterials, 67, 11 (2015)
[2] Kim, H.J. et al Biomaterials, 72, 104 (2015)



Simplifying corneal regeneration via the use of pliable tissue delivery microfabricated membranes

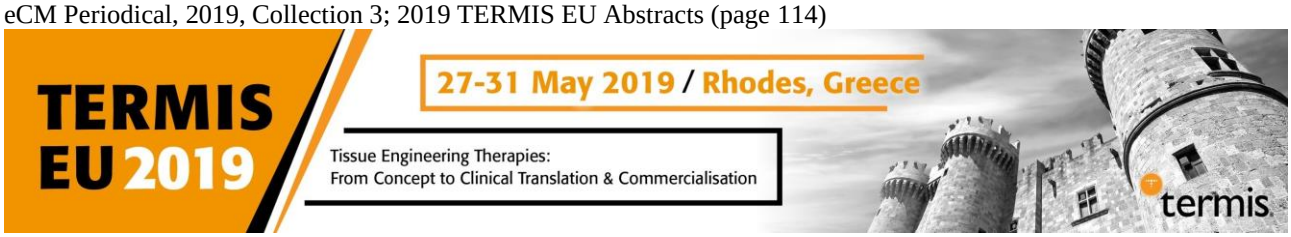
I. Ortega Asencio [1]
Presenting Author: Ilida Ortega Asencio, i.ortega@sheffield.ac.uk
[1]Bioengineering and Health Technologies Group, The School of Clinical Dentistry, The University of Sheffield, Sheffield, UK

Corneal disease affects millions of people worldwide with higher prevalence in developing countries. Corneal transplantation to replace damaged corneas has been used for decades to treat patients with corneal impairment but replacing corneas alone cannot solve corneal scarring if the patient lacks corneal stem cells (1-2). The use of membranes as cell carriers for aiding in corneal disease has been relatively successful, for example, the amniotic membrane (AM) is being used worldwide as a corneal cell carrier (3). Unfortunately, AM carries a risk of viral transmission which can only be reduced by using careful tissue banking protocols. The Sheffield cornea team have been working together with LV Prasad Institute (LVPEI, India) with the aim of delivering new alternatives for simplifying corneal treatments and therefore increasing their accessibility, focusing on (i) the use of tissue explants instead of laboratory expanded cells (Simple Limbal Epithelial Transplantation, SLET which is used now successfully in more than 100 centres worldwide) and (ii) the development of a synthetic AM substitute made of PLGA 50:50 (4). The development of this synthetic PLGA membrane has achieved many of its milestones but frustratingly the membrane that has been developed is stiffer than expected; this occurred as a result of exhaustive solvent removal to reduce solvent concentrations to levels permitted for clinical use (the solvent acts as a plasticizer and when removed the material becomes brittle). Our current challenge is to reduce the stiffness of our previously developed corneal electrospun scaffolds via the utilisation of different solvent-systems and plasticizers while avoiding excessive levels of residual solvent. In parallel to the development of a plain polylactic glycolic acid (PLGA) membrane for corneal disease, Sheffield has been working on the development of a next generation membrane which includes micro features to mimic the limbal niches of the cornea. These niches provide physical support to the stem cells which need to last a lifetime, generating daughter cells to renew the cornea. Specifically, we have developed a corneal PLGA ring in which we have included microfeatures which mimic to a degree the shape and the size of the corneal stem cell niches in the eye. These features are regarded by our clinical collaborators at LVPEI in India as potentially very useful to assist them in stem cell survival as well as providing convenient pockets in which to place tissue explants at the time of surgery. In summary, clinical collaborators in India have been very positive regarding our AM substitutes and they have provided us with very useful feedback which we are now implementing. In essence, we are working towards the improvement of the mechanical performance of current PLGA membranes and we are developing new microfeatured ring scaffolds in which surgeons will able to hold tissue explants in place without the need of using expensive and not easy to access glues (e.g. fibrin).

ACKNOWLEDGEMENTS: Financial support was received from Wellcome Trust, EPSRC E-Term Landscape Fellowship, CONACYT (Chile) and MRC (Confidence in Concept UoS Fund).

REFERENCES

[1] Huang, A.J. and S.C. Tseng. Investigative Ophthalmology & Visual Science, 1991. 32: p. 96-105
[2] Dua, H.S. and A. Azuara-Blanco. British Journal of Ophthalmology, 1999. 83(4): p. 414-419
[3] Ahmad, S., et al. J Cell Physiol, 2010. 225(1): p. 15-9
[4] Deshpande, P., et al, Biomaterials, 2013. 34(21): p. 5088-5106



International standardization and electronic coding of the terminology for regenerated tissue products

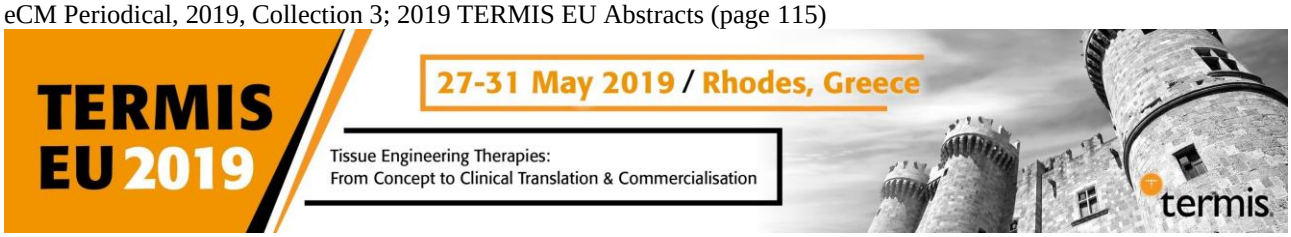
I. Slaper-Cortenbach[1]
Presenting Author: Ineke Slaper-Cortenbach, inekeslaper@gmail.com
[1]Regenerative Medicine Technical Advisory Group (RMTAG), ICCBBA, P.O. Box 11309, San Bernardino, CA 92423-1309, USA

INTRODUCTION: International standardization and electronic coding of terminology for medical products of human origin (MPHO) began in 1994 with the introduction of the ISBT 128 Standard for blood products. In the following decades, the development of the Standard continued and its use expanded steadily to diverse categories of MPHO such as cellular therapy, tissues, organs, and others. The RMTAG was formed to develop a global ISBT 128 terminology for regenerative medicine products. It is composed of technical experts and representatives from professional societies, such as ISCT and TERMIS EU.

METHODS: The ISBT 128 classification ranks: (1) Categories, (2) Classes, and (3) Attributes. Discussions within the RMTAG were primarily accomplished via conference calls and email exchange. Mechanisms for conflict resolution, such as group vote, were used to resolve terminology issues. Comments on proposed terminology were also collected via open public consultation.

RESULTS & DISCUSSION: The product category Regenerated Tissue has been proposed for regenerative medicine products. These products have been categorized into 12 main product groups called Classes (e.g., regenerated tissue, regenerated skin). Attributes that define the specific features of a product have been organized by affinity to eight Attribute Groups (e.g., type of cells, delivery method). Classes and Attributes are combined in the Product Description (CLASS │Attribute │Attribute) to uniquely define a product. To accomplish electronic coding, each Product Description is assigned an internationally unique five-character Product Description Code (PDC) which can be electronically coded into a linear or two-dimensional bar code, or other symbologies.

CONCLUSIONS: The ISBT 128 Standard provides a comprehensive and highly flexible system for describing regenerative medicine products and assigning product codes in an electronically readable format. The international partnership and consensus fostered by RMTAG are crucial to driving advancement of ISBT 128 and keeping pace in the fast emerging field of regenerative medicine.



Cytoprotective single-cell nanoencapsulation

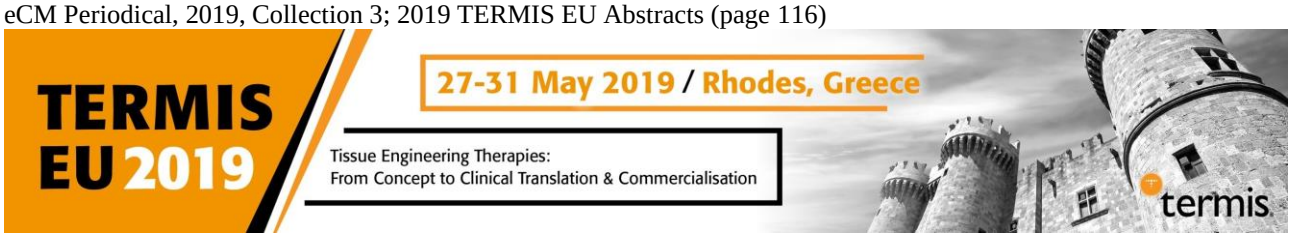
I. S. Choi
Presenting Author: Insung S. Choi, ischoi@kaist.ac.kr
Center for Cell-Encapsulation Research, Department of Chemistry, KAIST, Korea

Nature has developed a fascinating strategy of cryptobiosis for counteracting the stressful, and often lethal, environmental conditions. For example, certain bacteria sporulate to transform from a metabolically active, vegetative state to an ametabolic endospore state. The bacterial endospores, encased within tough biomolecular shells, withstand the extremes of harmful stressors, such as radiation, desiccation, and malnutrition, for extended periods of time and return to a vegetative state by breaking their protective shells apart when their environment becomes hospitable for living. Inspired by cryptobiosis found in nature, researchers have sought to chemically control and tailor the metabolic behaviors of non-spore-forming cells as well as enhancing their viability against adverse environmental conditions, by forming thin (< 100 nm), tough artificial shells [1-3]. These living "cell-in-shell" structures, called artificial spores, enable chemical control of cell division, protection against physical and chemical stresses, and cell-surface functionalizability, armed with exogenous properties that are not innate to the cells but are introduced chemically. The field has further advanced to the stage of chemical sporulation and germination, where cytoprotective shells are formed on living cells and broken apart on demand. The (degradable) cell-in-shell hybrids are anticipated to find their applications in various biomedical and bionanotechnological areas, such as tissue engineering, cytotherapeutics, high-throughput screening, sensors, and biocatalysis, as well as providing a versatile research platform for single-cell biology.

ACKNOWLEDGEMENTS: Financial support was received from the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (MSIP) (2012R1A3A2026403).

REFERENCES

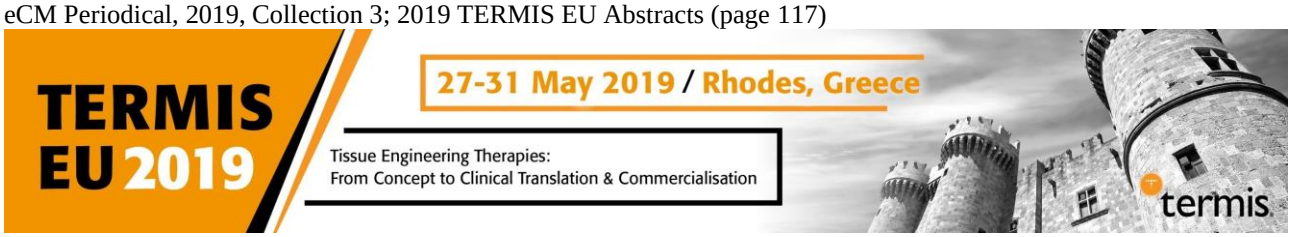
[1] Choi IS et al. Adv Mater. 2018; 30(14):1706063.
[2] Choi IS et al. Acc Chem Res. 2016; 49(5):792-800.
[3] Choi IS et al. Adv Mater. 2014; 26(13):2001-10.



Natural polymers of bacterial origin and their biomedical applications

I. Roy
Presenting Author: Ipsita Roy, royi@wmin.ac.uk
School of Life Sciences, College of Liberal Arts and Sciences, University of Westminster, London, UK

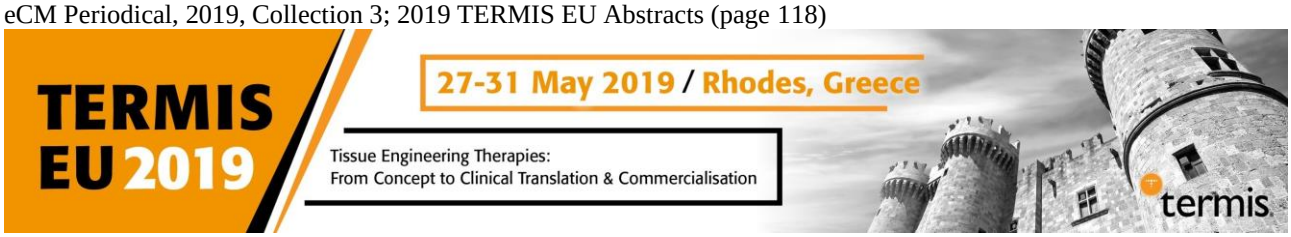
Bacteria produce a large variety of natural polymers including Polyhydroxyalkanoates (PHAs), Bacterial Cellulose, Poly-g-glutamic acid, Alginate and Pullulan. As opposed to other natural polymers that are extracted from natural sources these polymers are produced using bacterial fermentation. Hence, their production is as controlled and regulated as synthetic polymers, a great asset for translational products. PHAs are natural polymers produced by bacteria under nutrient limiting conditions. These polymers are biodegradable and biocompatible in nature and hence can be used in a variety of medical applications such as tissue engineering, wound healing, medical device production and drug delivery. Due to their varied mechanical properties and degradation rates, PHAs can be used to replace a range of tissue types including bone, nerve, cartilage, pancreas, cardiac and skin. In addition, they can be used for short term and long term controlled drug delivery. We have explored various modes of PHA production and their application in hard and soft tissue engineering, biodegradable stent production, nerve conduit production, cardiac patch development, wound healing and controlled drug delivery applications. Bacterial cellulose is another natural polymer produced using static bacterial fermentation. This too is highly biocompatible, has extraordinary mechanical properties and in its natural form is a nanostructured hydrogel highly suitable for the development of wound healing patches. We have initiated the development of antibacterial wound healing patches based on bacterial cellulose. Overall natural polymers have huge potential in biomedical applications and are the next generation sustainable, biocompatible materials, ready to revolutionise the medical world.



Research integrity and publication ethics

I. Bayindir-Buchhalter[1]
Presenting Author: Irem Bayindir-Buchhalter, ibayindirb@wiley.com
[1]Wiley-VCH; Weinheim, Germany

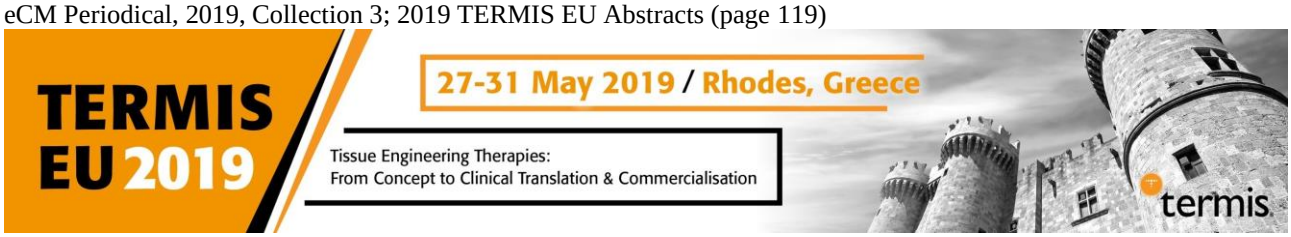
While growing research output is a must to advance a scientific career, reporting research findings should be tied to respecting publication ethics and maintaining integrity. In her talk, Dr. Irem Bayindir-Buchhalter, Associate Editor for the Advanced family of journals, will address several publishing integrity concerns, from plagiarism to authorship rights and conflicts of interest. Come by for a discussion on how integrity will improve the impact of your research.



Horses as large animal models of experimentally induced and naturally occurring musculoskeletal disease

I. Ribitsch
Presenting Author: Iris Ribitsch, iris.ribitsch@vetmeduni.ac.at
Department for Companion Animals and Horses, Veterm, University Equine Hospital, Vetmeduni Vienna, Vienna, Austria.

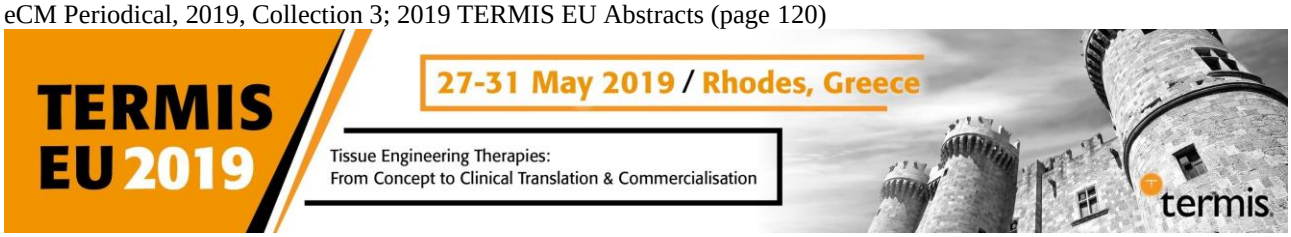
This presentation is following the idea of "One Health" for expanding interdisciplinary collaborations and joining efforts in the development and evaluation of new methods for the prevention and control of disease across species (in this case horses and humans). As one of the few species suffering from naturally occurring musculoskeletal injuries and dysfunction similar to humans, the horse lends itself for this role as it would not only serve as an animal model, but also as a beneficiary of improvements in treatment. As athletic individuals, horses incur idiopathic primary or sports related injuries. An important aspect of clinical research is the precise demonstration of the initial injury, the disease progress, outcome and follow up. The validated applicability of advanced diagnostic methodologies in horses such as arthroscopy and MRI (together with scoring approaches), ultrasound, radiographs, CT and scintigraphy, has made the horse a popular model for which non-terminal studies with thorough evaluation and monitoring are possible. Also second-look arthroscopy and serial sampling are feasible. Moreover, the large size of horses allows for the creation of critical size defects or multiple defects and offers a high amount of material that can be sampled for analysis. Controlled postoperative exercise programs and rehabilitation protocols using e.g. treadmills and horse walkers further support standardization of the results. More recently a broad offer of modern methods to further objectify outcome measures became available including gait kinematics (e.g. lameness locators) and/or kinetics using force plate/ground reaction force analysis. In addition, the horse fulfils the requirements of the European Medicines Agency (EMA), the USA Federal Food and Drug Administration and the International Society for Stem Cell Research (ISSCR) who recommend the use of large animal models to evaluate efficacy, durability, dose response, degradation and safety prior to obtaining market approval of advanced therapeutic medicinal products (ATMPs). Despite obvious anatomic differences between the horse and the human (quadruped versus biped) there are striking similarities when comparing the corresponding anatomical structures. Thus, horses constitute an ideal animal model and studies performed in the horse could serve as preclinical data for human medicine.



How can I contribute to a better scientific community within TERM?

I. Martin
Presenting Author: Ivan Martin, IIvan.Martin@usb.ch
Department of Biomedicine, University Hospital Basel, Switzerland

The field of tissue engineering and regenerative medicine (TERM) has reached a maturity stage, with recognition as a scientific domain of its own and some evidences of promising clinical/commercial fruition. As for any individual coming of age, such progression requires increased awareness of the associated responsibilities. Starting from analysis of the own mistakes and weaknesses, this lecture will offer a personal view on principles of ethics and overall practice of good science that may need to be consolidated in the working routine of TERM affiliates, especially of students and young investigators. Some of the discussed topics will be related to general personal behaviour within a scientific community. Others will have a more specific link to the TERM field, around the challenge of crossing boundaries of several disciplines while addressing fundamental questions of deep scientific relevance. The intent of the lecture, far from the pretension of a moral preach, is to reflect on how TERM can further impact science and society, while receiving growing respect and consideration by other research domains.

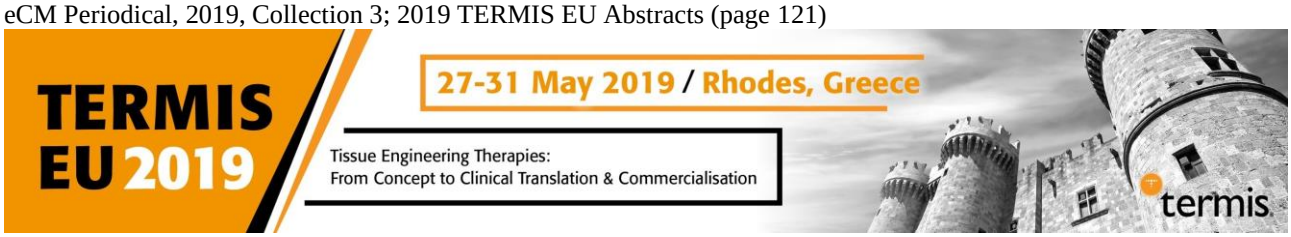


Therapeutic opportunities of dental pulp stem cells

I. Lambrichts
Presenting Author: Ivo Lambrichts, ivo.lambrichts@uhasselt.be
Department of Morphology, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium

Over the past decade, dental tissues have become an attractive source of mesenchymal stem cells (MSCs). Dental stem cells (DSCs) are not only able to differentiate into adipogenic, chondrogenic and osteogenic lineanges, but an increasing amount of research also pointed out their potential applicability in numerous clinical disorders, such as myocardial infarction, neurodegenerative diseases and diabetes. Cell-based therapies are emerging as an alternative treatment option to promote functional recovery in patients suffering from neurological disorders, which are the major cause of death and permanent disability. Together with their multilineage differentiation capacity, their easy availability from extracted third molars makes these stem cells a suitable alternative for bone marrow-derived MSCs. More importantly, DSCs appear to retain their stem cell properties following cryopreservation, a key aspect in their long-term preservation and upscale production. The lecture will address human dental pulp stem cells and their potency to contribute to central and peripheral neuro-regeneration combined with angiogenesis. Human dental pulp stem cells can differentiate into highly differentiated cells including neurons and glial cells. In the peripheral nervous system these glial cells play a crucial role in reinnervation and tissue repair. Application of dental stem cells in stroke models and in arthritis models will be discussed. New data on the effects of L-PRF (Leucocyte Platelet Enriched Plasma) on tissue regeneration, on dental and neural stem cells and on endothelial cells will be presented.

ACKNOWLEDGEMENTS: Financial support was received from FWO Flanders



Tenogenic properties of MSC in inflammatory environment

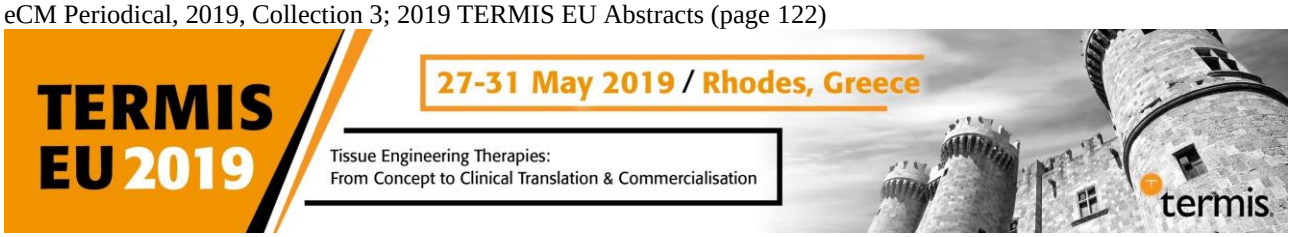
J. Burk[1,2]
Presenting Author: Janina Burk, burk@rz.uni-leipzig.de
[1]Saxon Incubator for Clinical Translation (SIKT), University of Leipzig, Leipzig, Germany [2]Institute of Veterinary Physiology, University of Leipzig, Leipzig, Germany

Tenogenic differentiation of multipotent mesenchymal stromal cells (MSC) occurs in response to physical and biochemical stimulation by tendon extracellular matrix and mechanical loading. However, there is no evidence that tenogenic differentiation contributes to the beneficial effects of MSC observed in tendon healing. The aim of this study was to investigate whether tenogenic properties of MSC are affected by conditions mimicking a pathophysiological tendon lesion environment. A three-dimensional dynamic co-culture model was established, using decellularized tendon matrices seeded with equine adipose-derived MSC, which were subjected to cyclic mechanical stretching and co-culture with peripheral blood leukocytes. In addition, standardized inflammatory conditions were investigated using the pro-inflammatory cytokines IL-1β and TNF-α as cell culture supplements. The attachment and integration of MSC into the tendon matrix was less effective in inflammatory conditions. The expression of the extracellular matrix components collagen I, collagen III, decorin and tenascin-C remained widely unaffected, but inflammatory conditions still impacted on musculoskeletal gene expression. The pro-inflammatory cytokines induced a downregulation of scleraxis, smad8 and osteopontin. These changes were observed in a similar manner in co-culture with leukocytes, possibly due to enhanced IL-1 synthesis by monocytes in the co-cultures. This study demonstrates that inflammation interferes with the tenogenic differentiation potential of MSC. On that basis, it is questionable whether MSC would differentiate into tenocytes when implanted in tendon lesions. Modulatory mechanisms may therefore be more important in this context.

ACKNOWLEDGEMENTS: Financial support was received from the German Research Foundation (BU3110/1-1). Part of the results presented are published in [1].

REFERENCES

[1] Brandt L et al. Int J Mol Sci. 2018; 19(9): pii:E2549.



Cardiosphere-derived cells for allogeneic stem cell therapy in canine dilated cardiomyopathy

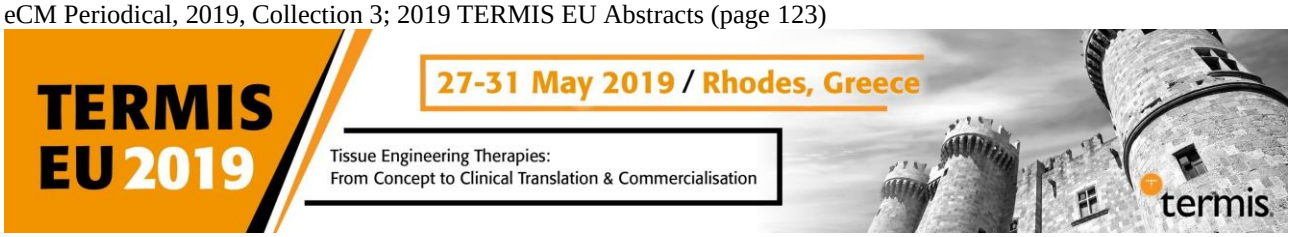
J. Dudhia[1]
Presenting Author: Jay Dudhia, jdudhia@rvc.ac.uk
[1]Department of Clinical Sciences and Services, Royal Veterinary College, University of London, United Kingdom

Non-ischemic dilated cardiomyopathy (DCM) is the second most common cardiac disease of dogs, resulting in congestive heart failure or malignant arrhythmia causing sudden cardiac death. An underlying genetic basis has been proposed in a number of breeds, including the Doberman and Boxer with a cumulative prevalence in European Dobermans >8 years of age of 44% [1]. As treatment options are limited, there is interest in using cardiac stem cells. Cardiosphere-derived cells (CDCs) are an adult cardiac progenitor cell population that can be derived in large numbers from myocardial biopsies. However, for therapeutic use allogeneic CDCs would be a practical solution which also avoid obtaining cells from unhealthy donors. Although mesenchymal stem cells (MSCs) have been shown to induce an immune-tolerant phenotype in recipients from unrelated donors, they are inferior to CDCs in their cardiac regenerative capability and it is currently unknown if canine CDCs possess a similar immune-privileged status. Our aim therefore was to characterise the phenotype of canine CDCs and to assess their immune-regulatory status in in vitro assays. CDCs, MSCs and lymph node cells (LNCs) were obtained from five dogs immediately post-mortem with owners' consent and University ethical approval. These cells were isolated as previously published [2] and characterised for colony formation, self-renewal and multipotency. All dogs in the study were heterozygous for both DLA-88 and DRB-1 and varied in haplotype and cells derived from them were assessed for MHC antigens by flow cytometry. Mixed lymphocyte reactions (MLR) incorporating responder LNCs and allogeneic stimulator CDCs or MSCs were performed to assess proliferation [3]. Canine CDCs demonstrated the ability to self renew, form clonal colonies and commit to multiple lineages (myocardial, endothelial and smooth muscle). In MLR assays, CDCs did not induce significant proliferation in responder LNCs when compared to non-stimulated LNCs. This lack of response was confirmed across multiple donor and responder cells with mismatched MHC I and II haplotypes. Interestingly, allogeneic MSCs stimulated a response in LNCs when compared to non-stimulated cell LNCs. These results demonstrate that CDCs do not produce an immunological response in an in vitro model of transplant immune-reactivity suggesting that CDCs possess immune-privileged status and have the potential for their safe use in allogeneic treatment for canine DCM. Further, exploiting naturally occurring non-ischaemic myocardial diseases in dogs, which exhibit close analogy to an equivalent human condition, can act as an essential bridge between discoveries identified in rodent models and achievable clinical therapies.

ACKNOWLEDGEMENTS: Financial support was received from The PetPlan Charitable Trust.

REFERENCES

[1] Simpson S et al. 2015;204823 Int J Genomics.
[2] Dutton LC et al. Cytometry A. 2018; 93(1):115-124
[3] Dutton LC et al. Sci Rep. 2018; 6;8(1):13351.



Lessons in translation and the immune response to biomaterials

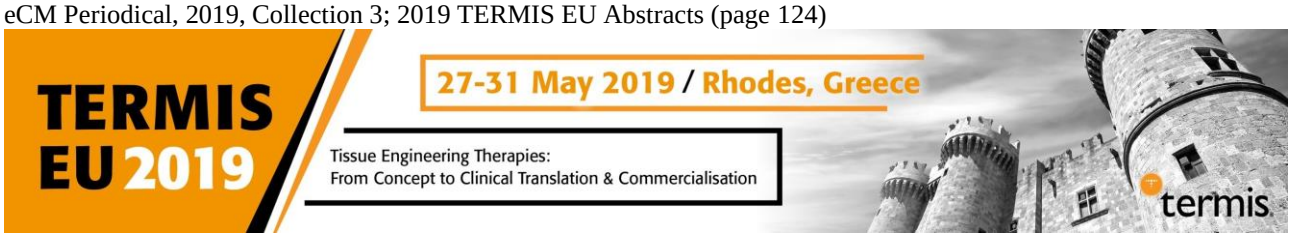
J. H. Elisseeff [1,2]
Presenting Author: Jennifer Elisseeff, jhe@jhu.edu
[1]Translational Tissue Engineering Center, Wilmer Eye Institute and the Department of Biomedical Engineering, Johns Hopkins University [2]Bloomerg~Kimmel Institute for Cancer Immunotherapy, Johns Hopkins Medicine, USA

The immune system is the first responder to trauma and foreign bodies such as biomaterials, yet this response and its capacity to orchestrate tissue repair has not be leveraged in tissue engineering. Biomaterials can be engineered with exquisite control over physical properties and can present an array of spatially controlled biological cues. Until now, these scaffolds have directly targeted stem cells, vascular development, and differentiated cells to stimulate tissue formation or wound healing. Translating tissue engineering technologies to the clinic for multiple clinical applications, we discovered unexpected responses from the adaptive immune system. We profiled in depth the immunological response to the wound environment in combination with biological and synthetic scaffolds. The adaptive immune system, specifically T_H2 T cells, were required for scaffold stimulation of wound repair [1]. We also characterized the tissue immune environment in non-healing wounds and materials that develop a fibrotic capsule and discovered the role of senescent cells [2] and interleukin (IL)-17. Single cell analysis of specific immune populations in the biomaterial response is providing even more resolution of the immune environment around implants and introduces new therapeutic targets. The implications of a pro-regenerative environment on tumor development and the efficacy of immunotherapies will be discussed [3]. Finally, results from clinical trials validating the immune responses associated with tissue repair will be presented. Ultimately, targeting the immune system represents a paradigm shift for the field and will help to realize the promise of regenerative medicine and smart materials.

ACKNOWLEDGEMENTS: Financial support was received from the Morton Goldberg Chair, Department of Defense, National Institutes of Health, Acell Inc, and the Bloomberg~Kimmel Institute for Cancer Immunotherapy.

REFERENCES

[1] Sadtler et al, Science. 2016; 352(6283):366-20
[2] Jeon et al. Nature Medicine. 2017;23(6):775-781
[3] Wolf et al, Science Translational Medicine. 2019; 11(477)



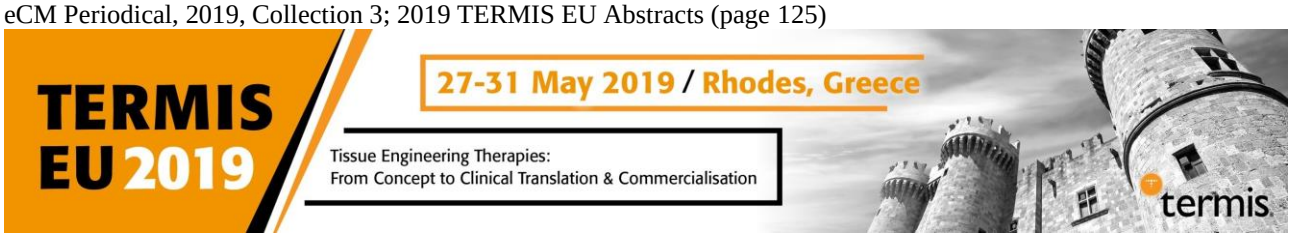
Tissue engineering for drug discovery: Time for implementation

J.M. Kelm[1,2]
Presenting Author: Jens M. Kelm, jens.kelm@precomb.com
[1]PreComb Therapeutics AG, Wädenswil, Switzerland [2]Competence Center Tissue Engineering for Drug Discovery (TEDD), ZHAW, Wädenswil

3D tissue models for efficacy and safety testing have an inherent potential to not only improve in vitro biology but more importantly drug development. However, harmonized validation of these advanced in vitro models throughout the discovery and developmental process is required to unlock the full potential of these high potential technologies and design knowledge-driven novel drug development concepts. Already decades ago 3D cell culture technologies have been postulated to become biology's new dimension changing the way how in vitro research is being accomplished. However, the integration of advanced in vitro models took much longer than anticipated despite the fact that it is agreed within the research community that 3D models reflect more closely the biology of an in vivo tissue or organ. Only over the past 10 years, physiological more relevant models have gained significant momentum in research, especially in drug discovery and development. The expectations are high that by creating more physiological conditions to screening models as well as for safety testing the development of drugs could be performed more efficient than in the past, which basically means an accelerated process with reduced failure rates. However, how realistic is this considering more than 50 years of drug discovery and development incorporating major technological advances on genetic, proteomic and phenotypic level without significant advances on the average success rates of new drug entities with some outstanding years over the past decades? One should think that if the biology of the models are closer to the in vivo biology they should be more translational reducing the gap between in vitro human response and in vivo patient response. However, it has been shown many times that the individual models are much better than current state of the art, but the difficult part is to demonstrate that a process which takes more than ten years can benefit from 3D technologies [1].

REFERENCES

[1] Kelm JM et al. Drug Discovery Today in press



Combining bio-fabrication and guided self-organization of vascular cells to engineer complex vascular networks in engineered tissues

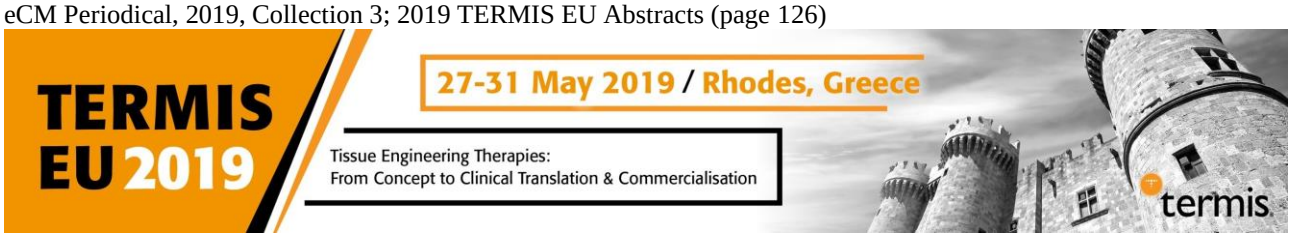
J. Rouwkema[1]
Presenting Author: Jeroen Rouwkema, j.rouwkema@utwente.nl
[1]Department of Biomechanical Engineering, Technical Medical Centre, University of Twente, Enschede, The Netherlands

Optimally engineered tissues will often need to contain a vascular network; either to supply the cells in the tissue with nutrients after implantation, or to ensure a physiological tissue response when the tissue is used as a screening platform [1]. Especially when the tissue is engineered for implantation purposes, this network needs to be properly organized, including macro-vascular structures but also micro-vascular capillaries, to accommodate a functional connection with the vasculature of the patient. Over the past years, multiple approaches have been developed to include vascular networks in engineered tissues. Under the right conditions, the addition of vascular cells within tissue constructs leads to the formation of vascular networks. However, these networks are generally randomly organized and mainly consist of microvascular structures. On the other hand, bio-fabrication approaches can be used to fabricate vascular networks with a tightly controlled and designable organization. However, resolution is often insufficient to include capillary structures and the organization can be lost over time due to tissue remodeling. In order to achieve multiscale organized vascular networks, we are developing a 3D embedded bio-printing approach to spatially control the presence of vascular cells within tissue analogues. This can be seen as a starting situation which will remodel and mature over time. To further guide the organization of the vascular network over multiple scales, both chemical and mechanical cues are included. With this approach, our aim is to control tissue remodeling and maturation, resulting in a vascular network that resembles a vascular tree.

ACKNOWLEDGEMENTS: Financial support was received from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (724469).

REFERENCES

[1] Rouwkema J & Khademhosseini A. TRENDS Biotech. 2016; 34(9): 733-45



The chicken and the egg in bone formation: What cell is the trigger?

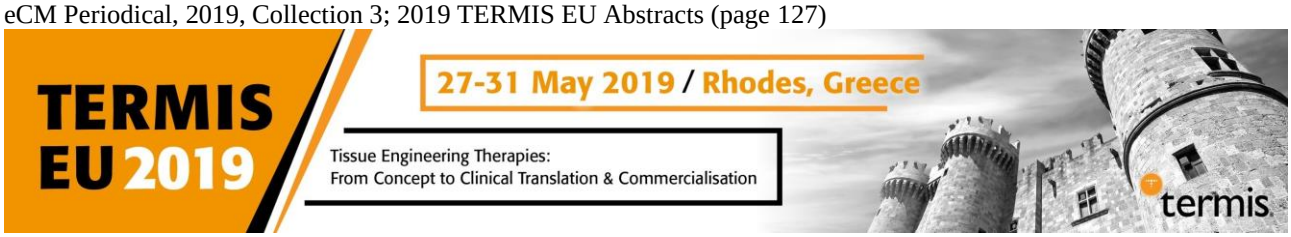
J.J.J.P. van den Beucken[1,2]
Presenting Author: Jeroen van den Beucken, jeroen.vandenbeucken@radboudumc.nl
[1]Regenerative Biomaterials, Radboudumc, Nijmegen, the Netherlands [2]Radoud Institute of Molecular Life Sciences (RIMLS), Radboud University, Nijmegen, the Netherlands

Whereas autologous bone grafts contain all elements for effective bone regeneration (i.e. the appropriate extracellular matrix, cells, and signals), their limited clinical availability and cumbersome harvest urge for (synthetic/allogenic/xenogenic) alternatives with reliable efficacy. Major efforts in the field of biomaterials have resulted in the clinical availability of multiple biomaterials for bone regenerative therapy. Although several of these biomaterials allow direct bone bonding (i.e. bioactive properties) and bone growth over their surface (i.e. osteoconductive properties), the capacity of these biomaterials to induce de novo bone formation is largely lacking. Only few biomaterials have shown such osteoinductive properties [1], but the mechanistic fundamentals of this phenomenon remain elusive. The classic osteoinduction has been described decades ago by Urist, which was caused by bone morphogenetic protein 2 (BMP2). This protein is capable of binding specific receptors on the surface of mesenchymal stromal cells (MSCs), which induces an intracellular signaling cascade resulting in osteogenic differentiation of the uncommitted adult stem cell. Consequently, recombinant DNA technologies has propelled the clinical availability and use of this protein to boost bone formation. However, the cost and supraphysiological amounts of BMP2 required for effective bone formation have tempered its application. Alternatively, de novo bone formation has been shown upon ectopic implantation of calcium phosphate ceramics, for which surface structure appears crucial and related to the formation osteoclastic cells [2]. Strikingly, the application of MSCs for the preparation of cell-based constructs has shown that influx of inflammatory cells and osteoclastogenesis precedes bone formation [3]. Based on these findings and the general knowledge on the interactions between osteoclasts and osteoblasts during bone remodeling, we reasoned that osteoclasts are of pivotal importance in new bone formation. As such, our efforts focused on both the role of biomaterial surface properties on osteoclastogenesis by macrophage precursors, the morphological characteristics of formed osteoclasts, and the functional coupling of these osteoclasts with osteogenic differentiation by MSCs [4]. Further, we challenged the concept of cell-based construct formation by using osteoclasts rather than osteoblasts as the cells of choice in an ectopic implantation model. The data obtained from these studies will revolutionize the traditional concept of osteoinduction and shed light on the elucidation of the mechanism responsible for de novo bone formation.

ACKNOWLEDGEMENTS: Financial support was received from ZonMw (No.40-41400-98-1401), China Scholarship Council (CSC; No.201306990006), and the Erasmus+ Programme.

REFERENCES

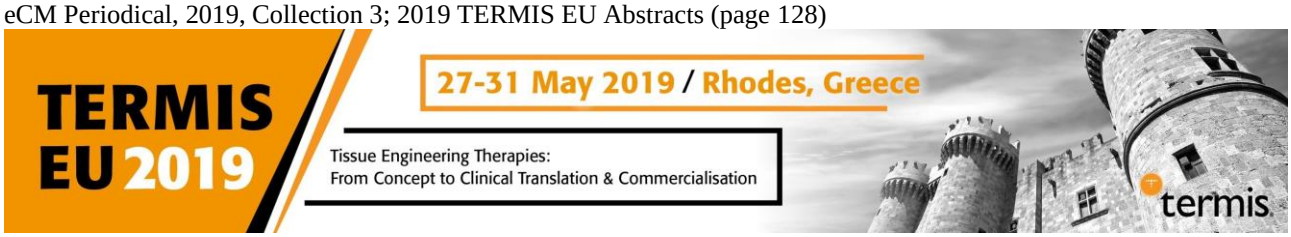
[1] Yuan H et al. Proc Natl Acad Sci U S A. 2010 Aug 3;107(31):13614-9
[2] Davison N et al. Eur Cell Mater. 2015 Jun 20;29:314-29
[3] Gamblin AL et al. Biomaterials. 2014 Dec;35(36):9660-7
[4] Zhang Y et al. ACS Appl Mater Interfaces. 2018 Oct 1 doi: 10.1021/acsami.8b10992. [Epub ahead of print]



Stem cells and biomaterials for the regenerative medicine of intervertebral disc: From clinical data to innovative concepts

J. Guicheux
Presenting Author: Jerome Guicheux, jerome.guicheux@inserm.fr
INSERM UMRS 1229, Regenerative Medicine and Sketeton (RMeS), Université de Nantes & Nantes University Hospital, PHU 4-OTONN, Nantes, France

Intervertebral disc (IVD) is a fibrocartilaginous tissue that grossly consists of a peripheral network of type 1 collagen fibers (namely Annulus fibrosus, AF), which surrounds a highly hydrated gel (namely Nucleus pulposus, NP) rich in type 2 collagen and proteoglycans. IVD degeneration is one of the major causes of low back pain (LBP). Currently, LBP is primarily managed by pharmacological treatments and if unsuccessful by surgical procedures (spine fusion or arthroplasty) that are reserved for severe debilitating LBP. To clinically address LBP earlier in the degenerative cascade of IVD, biology-inspired regenerative strategies could offer less invasive and etiological alternatives to spinal reconstructive surgery. We will first share our view of the mesenchymal stromal cells (MSC)-based therapeutic approaches that have been preclinically developed and, for some of them, clinically transferred in patients with discogenic LBP. Then, we will comment on the recent biomaterial-assisted MSC therapies that recently enter the preclinical and clinical scene of IVD regeneration. Finally, we will share with you our REMEDIV project that aims at developing an injectable NP substitute containing bioactive stem cells-derived IVD cells within a hydrated biomaterial that could be percutaneously transplanted into degenerated IVDs. Finally, we will consider our ability to transplant stem cells-derived IVD cells using a self-setting hydrogel in various animal models. Whether this concept could open new therapeutic windows in the management of discogenic low back pain will finally be discussed.



Injectable thermogels for medical applications

J.D. Ding[1]
Presenting Author: Jiandong Ding, jdding1@fudan.edu.cn
[1]State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, Shanghai 200438, CHINA

Injectable hydrogels are very useful in drug delivery, tissue engineering and regenerative medicine. Compared to chemically crosslinked hydrogels, physical hydrogels formed at body temperature after injection is in particular appropriate for various medical applications. Herein, we report a physical hydrogellable system based on block copolymers composed of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic yet biodegradable polyester such as poly(lactide-co-glycolide) (PLGA).[1-3] We synthesized the block copolymer via ring-opening polymerization of lactide and glycolide initiated by the hydroxyl end groups of PEG. Under appropriate composition, the triblock copolymer PLGA-PEG-PLGA can well be dissolved in water at relatively low temperatures (room temperature or below). The solution is injectable at room temperature, and spontaneously gelled at body temperature. We investigated the underlying mechanism of the physical gelation and revealed that the hydrophobic channel between the semi-bald micelles served as the physical crosslinking points upon heating. We introduced arginine-glycine-aspartate (RGD) peptides into either the PEG block or the PLGA block. While modification of both blocks can enhance cell adhesion of the hydrogel, the immobilizing site in the PEG block was found to be more efficient. Besides potential application of tissue engineering, we have also tried other medical applications of the hydrogels without RGD modification. The hydrogels were confirmed to prevent post-operative adhesion. The mixing with free RGD peptides was found to even further enhance the antiadhesion effect. Such a system with sol-gel transition affords also an excellent platform to encapsulate various drugs.

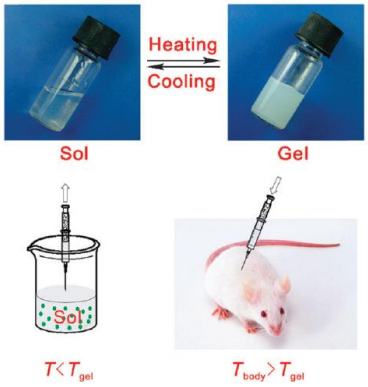
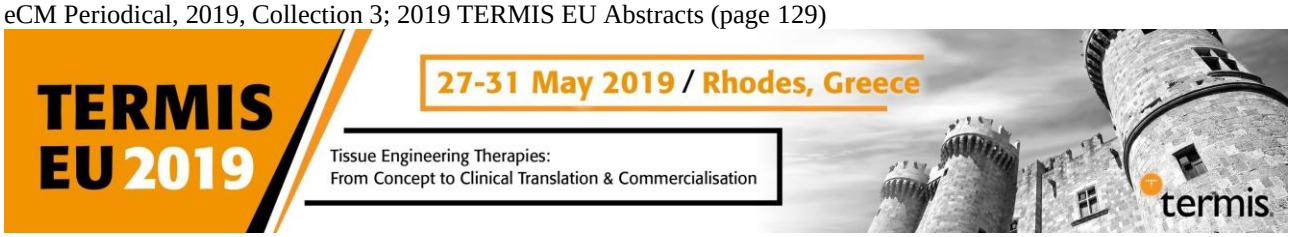


Figure 1: Schematic presentation of a thermogel with sol-gel transition upon heating.[3]

ACKNOWLEDGEMENTS: Financial support was received from National Science Foundation of China (grants no. 21774024 and 51533002), Science and Technology Commission of Shanghai Municipality (grant No. 17JC1400200), and State Key Project of Research and Development (grant No. 2016YFC1100300).

REFERENCES

[1] Shuquan Cui, Lin Yu, Jiandong Ding, Macromolecules, 51, 6405−6420 (2018)
[2] Zheng Zhang, Yuxiao Lai, Lin Yu, and Jiandong Ding, Biomaterials, 31, 7873-7882 (2010)
[3] Lin Yu, Jiandong Ding, Chem. Soc. Rev., 37, 1473-1481 (2008)



Design of biomaterials with micro/nano-structure for regulating cell behavior and tissue engineering

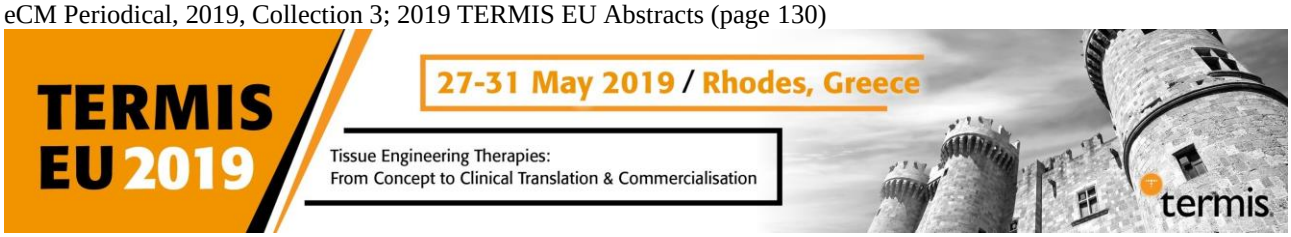
J. Chang[1]
Presenting Author: Jiang Chang, jchang@mail.sic.ac.cn
[1]Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050 China

The micro/nano-surface structure/topography has been found to affect cellular behavior. However, it is still not fully understood the mechanisms of the structural effect on stem cell differentiation and tissue engineering. In particular, the difference of micro and nano structures and the combination of micro and nano structure on tissue regeneration need to be explored. Therefore, we have developed techniques to fabricate biomaterials with micro/nano structure and investigated activity of the surface structure on stimulating stem cell behavior in vitro, and the activity to enhance tissue regeneration in vivo. Our study demonstrates that both micro and nano structure have activity to stimulate cell behavior by activating signaling pathways related to tissue regeneration. The activation mechanisms of micro and nano structures may be different, and the combination of micro and nano structure revealed further enhanced activity as compared to the single structure type. Our results suggest that both micro and nano structure need to be considered in designing biomaterials for tissue regeneration applications.

ACKNOWLEDGEMENTS: Financial support was received from the National Key Research Program of China (Grant No. 2016YFC1100201), the National Natural Science Foundation of China (Grant No. 81430012), and the International S&T Cooperation Project of Shanghai S&T Innovation Action Plan (Grant No.:17540712300).

REFERENCES

[1] Dalby MJ, et al., The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nature Materials, 2007. 6(12): p. 997-1003.



From idea to first-in-human trials: Collagen scaffolds for spinal cord repair

J. Dai[1]
Presenting Author: Jianwu Dai, jwdai@genetics.ac.cn
[1]Center for Regenerative Medicine, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.

Spinal cord injury (SCI) is a devastating injury resulting in changes in the spinal cord's motor, sensory, or autonomic functions. Following SCI, an inhibitory environment develops at the injury site to inhibit neural regeneration. We have developed a functional biomaterial consisting of collagen scaffolds (NeuroRegen scaffolds) and biologically active molecules (neurotrophic factor or the antagonists to myelin-associated inhibitor), and stem cells to build a nerve regeneration microenvironment. Specifically, (1) the linear ordered collagen based NeuroRegen scaffold was developed to guide the neural regeneration along its fibers and decrease the formation of glial scars, (2) collagen binding neurotrophic factors were incorporated into the scaffolds to promote neuronal survival and neural fiber regeneration, (3) antagonists to myelin-associated inhibitors were added to the scaffold to direct the neuronal differentiation of neural stem cells at the injury site, (4) Mesenchymal stem cells (MSCs) were also added to the scaffold to reduce the acute inflammatory response due to SCI (figure 1)[1]. These strategies were found to promote neural regeneration and functional recovery in SCI animals. NeuroRegen scaffolds with stem cells are in the clinical study of spinal cord injury repair for over 3years with over 80 patients enrolled [2][3].

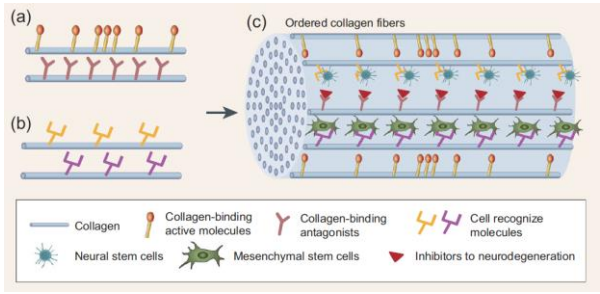
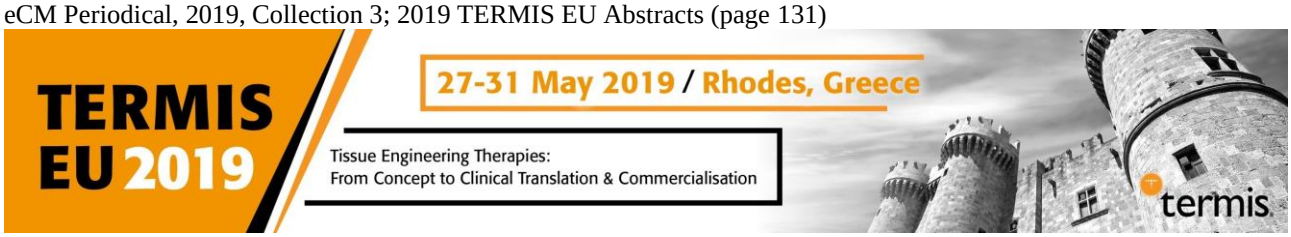


Figure 1: Neuro regenerative microenvironment constructed with functional biomaterials. (a)Collagen-binding biologically active molecules or antagonists were produced, which could be retained at the lesion site in a predetermined spatial arrangement. (b) Collagen-binding cell recognition molecules can be incorporated in biomaterials to capture target cells. (c) A reconstructed neuroregenerative microenvironment produced by multi-functional biomaterials.

REFERENCES

[1] Chen B et al. National Science Review. 2017; 4(4):530-32.
[2] Xiao Z et al. Cell Transplantation. 2018; 27(6):907-15.
[3] Xiao Z et al. Sci China Life Sci. 2016; 59(7):647-55



Bottom-up layer-by-layer assembly technology to process nanostructured multifunctional biomaterials across multiple length scales for tissue engineering and regenerative medicine strategies

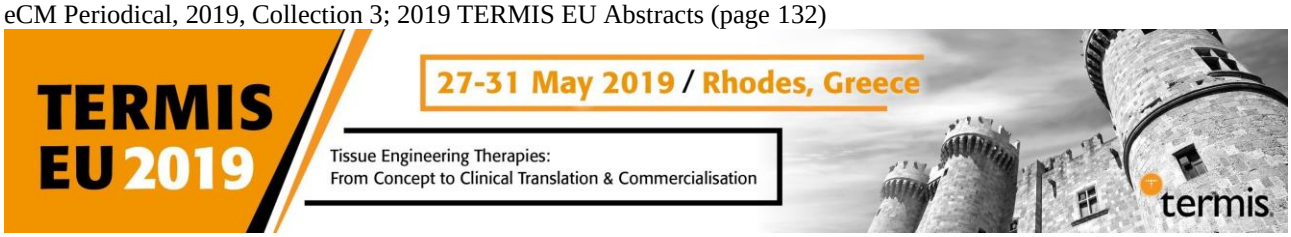
J. Borges
Presenting Author: João Borges, joaoborges@ua.pt
Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Portugal

Over the past decades, scientists have been inspired by fascinating biological systems to develop biomaterial scaffolds aimed at recreating the structural features and functional behavior of native extracellular matrix (ECM) [1]. Natural-origin polymeric materials, including polysaccharides an proteins have shown to be highly promising in emulating ECM features and addressing tissue engineering and regenerative medicine (TERM) strategies owing to their similarities with the ECM, typically good biological performance and high chemical versatility. Despite very promising, natural origin polymers per se often show limited bioactivity, stimuli-responsiveness, and poor mechanical properties, thus extensively limiting their applications, including their capability to support and guide cell fate. Toward this goal, synthetic and biological building blocks have been extensively explored and combined as structural building blocks for the assembly of a wide variety of bioinspired functional architectures with emerging applications in the TERM field. However, most of the developed systems still lack control in thickness and composition, and the functional dynamic nature and structural complexity found in the natural systems. Hence, there is the need for advancing current TERM strategies by resorting to bottom-up assembly methodologies that could finely tune the physicochemical, mechanical, and biological properties of the assembled materials at the molecular level. Among them, the Layer-by-Layer (LbL) assembly technology has emerged as a powerful strategy to achieve surface modification and functionalization, as well as to investigate and regulate the cell functions in physiologically relevant conditions owing to its key enabling features. In particular, it has proved to be a simple, inexpensive, yet robust and highly versatile methodology to fabricate highly organized, robust and functional nanostructured films on virtually any kind of surface, from simple 2D flat platforms to more convoluted 3D surfaces. Such structures are engineered with precisely tailored properties and functions at the nanoscale by resorting to an unprecedented choice of materials, including biological materials assembled through a multitude of complementary intermolecular interactions [2]. In this talk, emphasis will be given to the design of ECM-like biomaterials and advanced biomimetic nanostructured multilayered devices for TERM. In particular, the talk will feature recent work of the group on the use of polysaccharides and peptides, and LbL assembly technology to formulate innovative multifunctional and tunable biomaterials with different shapes and internal structure across multiple length scales, from the nano- to the millimetre-scale. The potential of such devices to act as reservoirs of bioactive molecules and stimulate cell-signaling pathways that are critical in regenerative medicine will be discussed.

ACKNOWLEDGEMENTS: J. Borges acknowledges the financial support by the Portuguese Foundation for Science and Technology (FCT) in the scope of the project "SUPRASORT" (PTDC/QUI-OUT/30658/2017) and postdoctoral grant (SFRH/BPD/103604/2014); Marine Biotechnology ERA-NET project "BLUETEETH" (ERA-MBT/0002/2015); and European Research Council Grant Agreement No. ERC-2014-ADG-669858 for project "ATLAS".

REFERENCES

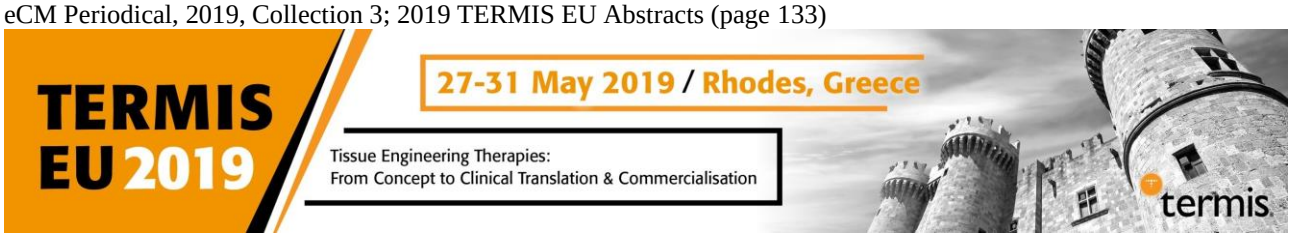
[1] Place ES et al. Nat Mater. 2009;8(6):457-70
[2] Borges J, Mano JF. Chem Rev. 2014;114(18):8883-8942



Compartmentalization of cells and therapeutic molecules into soft polymeric structures for bottom-up tissue engineering strategies

J.F. Mano
Presenting Author: Joao F. Mano, jmano@ua.pt
Department of Chemistry, CICECO — Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

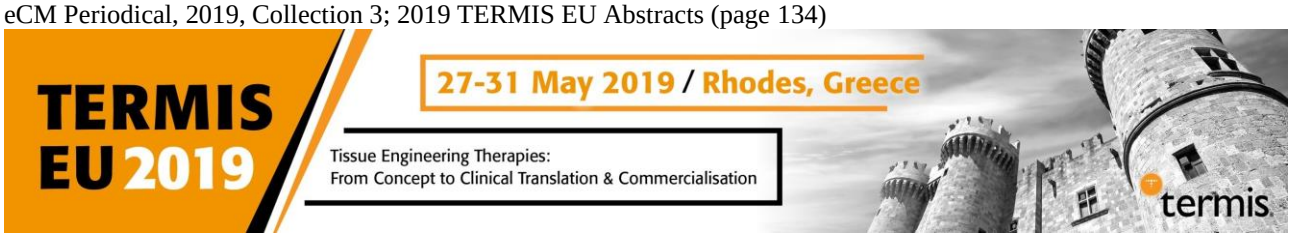
Sophisticated strategies have been employed to encapsulate cells and bioactive molecules in micro-sized matrices using distinct biomaterials, to be explored in cell and tissue engineering. Distinct enabling technologies are overviewed that could be used to encapsulate both molecules and cells in geometrically controlled micro-compartments. Using bioinspired strategies, superhydrophobic surfaces can serve as platforms to process basic units of hydrogels in mild conditions, permitting the encapsulation of living cells and other biological cargo with high efficiency. By using spherical templates containing cells followed by adequate coating procedures it is possible to produce liquefied capsules that may entrap viable cells. The presence of solid microparticles with controlled mechanical properties inside such capsules offers adequate surface area for adherent cell attachment increasing the biological performance of these hierarchical systems, while maintain both permeability and injectability. The liquid environment allows for a free-organization in the space of the cells towards the formation of new microtissues. The basic hybrid elements may be assembled towards larger constructs permitting to engineering tissues in a bottom-up approach with a complete control of the biological cargo.



Novel gram-positive bacteria killing cubosomes with high eukaryotic cytocompatibility – Insights into an interesting lesson from nature

Jochen Salber
Presenting Author: Jochen Salber, jochen.salber@hotmail.com
UMC Knappschaftskrankenhaus Bochum GmbH, Hospital of the Ruhr-University Bochum, Clinic of Surgery, Germany

Since the 1970s, an increasing resistance to antimicrobial agents has become a dangerous threat to the global public health. This requires measures and efforts across all sectors of our society. Effective antibiotics and alternative, complementary antimicrobial strategies also called “drug-free” are necessary for the success of major surgery and interventional therapies or implanted mid- and long-term medical devices. In this context, an international and interdisciplinary team of around 10 scientists and clinicians developed core-shell switchable thermoresponsive colloidal particles based on polyurethane grafted poly(N-isopropylacrylamide)-b-poly(ionic liquid) [NHP407-g-p(NIPAM-b-HPIL)] copolymers. With comprehensive analytics e.g. NMR, dynamic light scattering (DLS), Cryo-TEM etc. the chemical and three-dimensional structure of these new amphiphilic copolymers could be revealed. Cryo-TEM images manifested a cubosome structure. Antimicrobial susceptibility was determined by the broth microdilution method and cytocompatibility evaluation was done on the fibroblast L929 cell line by the Multiplex assay. NHP407-g-p(NIPAM-b-HPIL) cubosomes induced a strong bactericidal effect against non- and antibiotic resistant strains of S. aureus and S. epidermidis. The necessary concentrations were very low. Moreover, the antibacterial effect was induced immediately after the addition of the colloidal dispersion and approximately 99% of S. aureus were killed just within 90 minutes by half of the concentration correspondent to the MBC value. Interestingly, high-resolution SEM-focused ion beam (FIB) experiments showed, that the particles interacted with the bacterial surface only without being incorporated. Biological testing on L929 murine fibroblasts and other eukaryotic cells showed that the system is cytocompatible with concentrations around the MBC values.



Monocyte subsets and macrophage polarization in cell migration and tissue remodeling

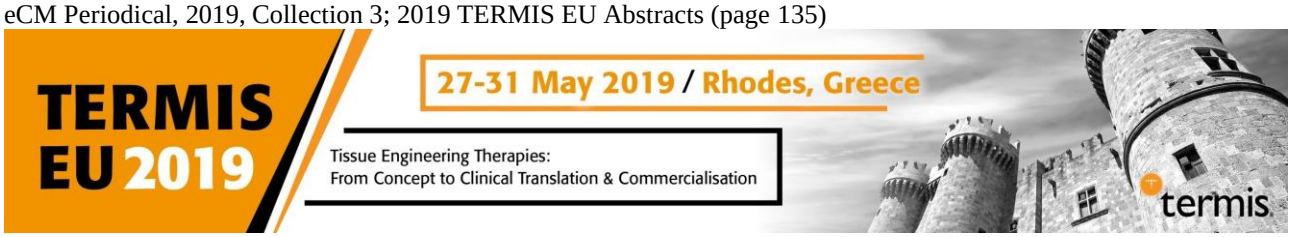
J. Wojta[1,2,3]
Presenting Author: Johann Wojta, johann.wojta@meduniwien.ac.at
[1]Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria [2]Core Facilities, Medical University of Vienna, Vienna, Austria [3]Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, Austria

Human monocytes can be divided into functionally distinct subsets, based on expression of CD14 and CD16, into classical monocytes (CD14++CD16-), intermediate monocytes (CD14++CD16+) and non-classical monocytes (CD14+CD16++) [1]. The CD16+ subsets are associated with pathologies characterized by a chronic inflammatory state including coronary artery disease [2]. We demonstrated recently that in humans with mild inflammation, the CD16+subsets express the highest levels of inflammatory cytokines [3]. Macrophages also exhibit distinct functional heterogeneity and plasticity [4]. Exposure to interferon-γ and lipopolysaccharide primes macrophages towards a proinflammatory phenotype, whereas macrophages exposed to interleukin-4 (IL-4) and IL-13 are linked to tissue repair processes [4]. Recently, we showed that proinflammatory human macrophages exhibit significantly higher matrix degradation activity compared to reparative macrophages and that this reduced ability to degrade matrix by reparative macrophages is due to increased expression of plasminogen activator inhibitor-1 [5]. Implications and pathophysiological roles of these subsets of monocytes and macrophages in processes such as cell migration and tissue remodeling will be discussed.

ACKNOWLEDGEMENTS: Financial support was received from the Austrian Science Funds (SFB 54); from the Ludwig Boltzmann Society; and from the Association for the Promotion of Research in Arteriosclerosis, Thrombosis and Vascular Biology

REFERENCES

[1] Ziegler-Heitbrock, L. et al. Nomenclature of monocytes and dendritic cells in blood. Blood **116,** e74-80 (2010).
[2] Weber, C. et al. Role and analysis of monocyte subsets in cardiovascular disease. Joint consensus document of the European Society of Cardiology (ESC) Working Groups 'Atherosclerosis & Vascular Biology' and 'Thrombosis'. Thromb. Haemost. **116,** 626–637 (2016).
[3] Thaler, B. et al. Differential in vivo activation of monocyte subsets during low-grade inflammation through experimental endotoxemia in humans. Sci. Rep. **6,** 30162 (2016).
[4] Murray, P. J. & Wynn, T. A. Protective and pathogenic functions of macrophage subsets. Nat. Rev. Immunol. **11,** 723–737 (2011).
[5] Hohensinner, P. J. et al. PAI-1 (Plasminogen Activator Inhibitor-1) Expression Renders Alternatively Activated Human Macrophages Proteolytically Quiescent. Arterioscler. Thromb. Vasc. Biol. **37,** 1913–1922 (2017).



Systemic environment, extracellular vesicles and miRNAs in aging: Impact on cell based therapies?

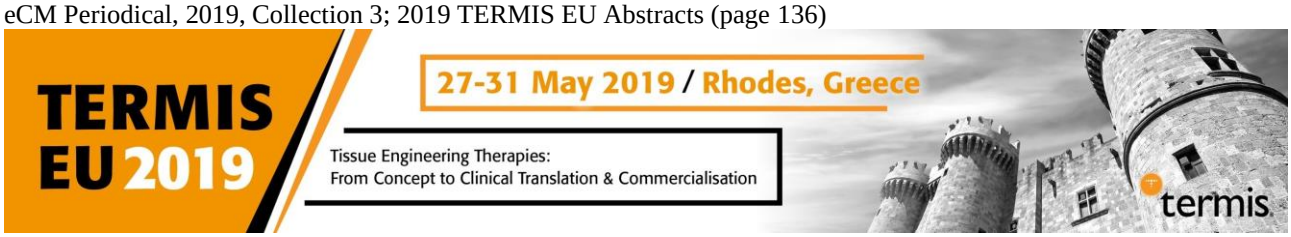
J. Grillari[1,2]
Presenting Author: Johannes Grillari, Johannes.grillari@boku.ac.at
[1]Department of Biotechnology, BOKU – University of Natural Resources and Life Sciences Vienna, Austria [2]LBI for Experimental and Clinical Traumatology, Vienna, Austria

Cellular senescence has evolved from an in vitro model system to study aging to a multifaceted phenomenon of in vivo importance since senescent cells in vivo have been identified and their removal delays the onset of age-associated diseases in a mouse model system [1]. In order to understand how senescent cells that accumulate within organisms with age negatively impact on organ and tissue function, we have started to characterize miRNAs and RNA modifying proteins that are differentially expressed in early passage versus senescent cells and their functional role in the context of cellular and organismal aging. Thereby, we identified circulating miRNAs as bona fide members of the senescence associated secretory phenotype (SASP) that are transferred from senescent cells to their microenvironment or even the systemic environment. These miRNAs are transported via extracellular vesicles and recipient cells taking them up are altered in their cell fate, including altered osteogenic differentiation of mesenchymal stem cells [2,3]. In summary, we present evidence of the importance of specific miRNAs and extracellular vesicles as their carriers, and highlight their potential use as biomarkers of aging and age-associated diseases like especially osteoporosis, or even as therapeutic tools and targets [4].

ACKNOWLEDGEMENTS: This work was supported by the Christian Doppler Society. The financial support by the Austrian Federal Ministry of Economy, Family and Youth; the National Foundation for Research, Technology and Development is also gratefully acknowledged, as is funding by the Austrian Science Fund I2514.

REFERENCES

[1] Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. Nat Med. Springer US; 2018; doi:10.1038/s41591-018-0092-9
[2] Terlecki-Zaniewicz L, Lämmermann I, Latreille J, Bobbili MR, Pils V, Schosserer M, et al. Small extracellular vesicles and their miRNA cargo are anti-apoptotic members of the senescence-associated secretory phenotype. Aging (Albany NY). 2018;10. doi:10.18632/aging.101452
[3] Weilner S, Schraml E, Wieser M, Messner P, Schneider K, Wassermann K, et al. Secreted microvesicular miR-31 inhibits osteogenic differentiation of mesenchymal stem cells. Aging Cell. 2016;15: 1–11. doi:10.1111/acel.12484
[4] Vogt S, Stadlmayr G, Stadlbauer K, Sádio F, Andorfer P, Grillari J, et al. Stabilization of the CD81 Large Extracellular Loop with De Novo Disulfide Bonds Improves Its Amenability for Peptide Grafting. Pharmaceutics. 2018;10: 138. doi:10.3390/pharmaceutics10030138



Strategies for peripheral and central nerve repair

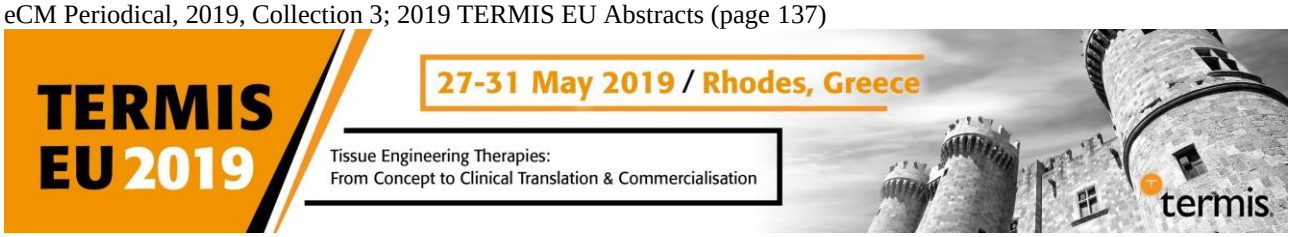
J. C. Knowles[1,2,3]
Presenting Author: Jonathan C Knowles, j.knowles@ucl.ac.uk
[1]Biomaterials and Tissue Engineering Division, Eastman Dental Institute, University College London, London, UK [2]Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 31114, Republic of Korea [3]The Discoveries Centre for Regenerative and Precision Medicine, UCL Campus, London, UK

Peripheral and central nerve damage has severe consequences for mobility and function and general quality of life long-term. The long-term cost of support for patients with these injuries can be extremely high. The current most successful strategy for peripheral nerve repair is the utilisation of autograft to improve function in for example the hand and/or arm. This has obvious limitations and problems due to the need to harvest tissue. Alternatives such as tubing, to maintain space coupled with gels and growth factors are much less successful. Central nervous system repair options are much more limited and the usual procedure is to wait as a small (but unpredictable) percentage of patients will show some functional recovery. More recently some success has been shown with the injection of stem cells, but these procedures were carried out on highly specific injury types. Thus there is a really need for an implant that may give at least some functional recovery. For a paralysed patient, the recovery of even a small amount of function, to allow independent mobility can be transformative. Over the last few years, we have been working on a number of acellular systems for peripheral and central nerve repair. Initial work(1, 2) centred on the utilization of phosphate glass fibres as scaffolds to directionally drive the growth of neurites and this work showed promising results, particularly in the central nerve repair model. More recently it became apparent that maintenance of space was a critical requirement for an implant and so we have developed a new generation of porous implants to drive the repair process. We will present the strategies utilized and the recent in vivo data obtained our work.

ACKNOWLEDGEMENTS: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (2018R1A2B3003446) and Global Research Development Center Program (2018K1A4A3A01064257). This project has received funding from the European Union's Horizon 2020 research and innovation programme,
under Grant agreement No 739572.

REFERENCES

[1] Joo NY, Knowles JC, Lee GS, Kim JW, Kim HW, Son YJ, et al. Effects of phosphate glass fiber-collagen scaffolds on functional recovery of completely transected rat spinal cords. Acta Biomater. 2012;8(5):1802-12.
[2] Kim YP, Lee GS, Kim JW, Kim MS, Ahn HS, Lim JY, et al. Phosphate glass fibres promote neurite outgrowth and early regeneration in a peripheral nerve injury model. J Tissue Eng Regen Med. 2015;9(3):236-46.



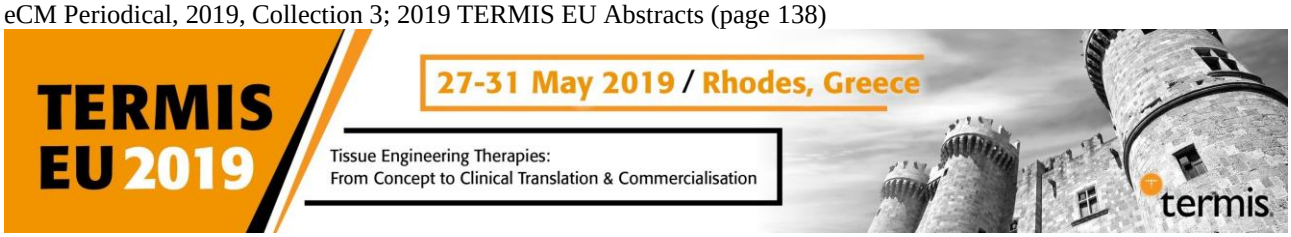
Extracellular matrix derived components as building blocks for tough hydrogels

J. Hilborn[1,2]
Presenting Author: Jons Hilborn, hilborn@kemi.uu.se
[1]Polymer Chemistry, Department of Chemistry- Ångström Laboratory, Box 538, 751 21 Uppsala, Sweden [2]Department of Bioengineering, School of Engineering, The University of Tokyo, 2-11-16, Yayoi, Bunkyo-ku, Tokyo, 113-8656, Japan

In nature, through evolution tissues have uniquely adapted to the demanding mechanical requirements for diverse biological functions. All tissues, with the exception of blood, have to sustain mechanical load. This strength has allowed various medical treatments to be possible where an open wound in the body after trauma can be closed during surgery, to transplant tissues from one site of the body to another and organ transplantation. Repaired tissues can be held together by sutures, staples or screws (mainly for bones) until body´s own repair processes provide healing orchestrated by the controlled release of signalling molecules. To provide suitable scaffolds for tissue growth hydrogels have successfully been used for repair and regeneration in vivo by delivering cells, drugs or signaling biomolecules.[1] The high water content in hydrogels is, however, accompanied by a poor mechanical strength. To address this, extensive efforts have been devoted to synthesizing tough, ductile hydrogels to meet flexibility and load bearing requirements. While they have enhanced mechanical properties, these synthetic polymer-based hydrogels do not have the biodegradability or favorable cell and tissue interactions as given by our body-own extracellular matrix (ECM) components such as proteins and glycosaminoglycans (GAGs). To evaluate if at all it is possible to fabricate a tough transparent biodegradable hydrogel containing collagen, we have recently successfully chemically functionalised collagen molecules to act as reinforcing filler in a hydrogel matrix to make a toughened gel. We have demonstrated in an earlier study that this chemically modified collagen retains its structural and biochemical properties when compared with unmodified collagen.[2] These collagen-based hydrogels have a modulus of 60MPa, can be extended >300% with full recovery upon unloading. This demonstration serves as a proof of concept that designing for toughness is possible using components that individually has gained regulatory accaptance for specific application.

REFERENCES

[1] Yan, H.J. et al. Biomaterials **161**, 190-202 (2018).
[2] Cigognini D et al. Sci Rep. 2016;6:30746. Ravichandran, R. et al. J Mater Chem B **4**, 318-326 (2016).



Potential anticancer therapeutic and antibacterial properties of green synthesized Ag, Au and Ag/Au alloy nanoparticles

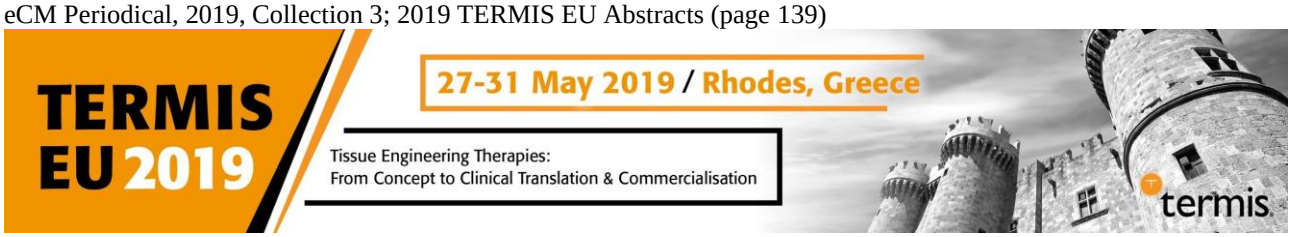
J. L. Cholula-Díaz[1]
Presenting Author: Jorge Cholula, jorgeluis.cholula@itesm.mx
[1]School of Engineering and Sciences, Tecnologico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, 64849 Monterrey, N.L., Mexico

Monometallic silver and gold, and bimetallic silver/gold nanoparticles (Ag-, Au-, and Ag/Au-NPs, respectively) have been object of intense research during the last years, mainly due to their excellent antimicrobial effects, as well as their potential applications in anticancer treatments. The use of bimetallic silver/gold nanosystems are expected to present improved therapeutic efficacy by maintaining the general biocompatibility of gold nanoparticles while decreasing the relatively high toxicity of silver nanoparticles towards healthy cells. Moreover, it is possible to vary systematically their physicochemical properties as a function of the NP composition, especially their optical properties [1]. Nevertheless, with respect to their antibacterial and cytotoxic activity, these do not necessary follow a monotonic NP composition-dependent behavior, but rather, their toxicity depends strongly on the particle size, surface area and surface chemistry of the NP [2,3]. The most common synthesis method is the chemical reduction of silver salt by a reducing agent in the presence of a stabilizing agent. Many reducing agents (hydrazine, N,N-dimethyl-formamide and sodium borohydride) may be associated with potential environmental toxicity as well as biological risks, and they are often difficult to dispose. An alternative to these toxic reagents is the usage of plant extracts and biodegradable natural biopolymers for an eco-friendly synthesis of noble metal NPs. In our research group, we have used starch as reducing and capping agent in the highly reproducible and environmental-friendly synthesis of colloidal mono- and bimetallic Ag/Au alloy NPs. Herein, our experience with the green synthesis and characterization and anticancer, as well as antimicrobial properties of colloidal noble metal nanoparticles will be discussed. Specifically, the determination of the therapeutic potential of colloidal Ag, Au and Ag/Au alloy nanoparticles by evaluating their cytotoxicity on a human breast cancer cell panel and on human melanoma cells will be described. Furthermore, the biocompatibility of the noble metal nanosystems towards human normal cells will be presented. Finally, the antibacterial properties of the Au-containing nanoparticles against antibiotic-resistant bacterial strains will be discussed.

ACKNOWLEDGEMENTS: Financial support was received from the School of Engineering and Sciences from Tecnologico de Monterrey through the Research Group on Optics and Lasers.

REFERENCES

[1] Mallin M P et al. Nano Lett. 2002;2(11):1235-1237.
[2] Padmos J D et al. Phys. Chem. C 2015;119(13): 7472-7482.
[3] Mukha I et al. J. Nanosci. Nanotechnol. 2017;17(12):8987-8994.



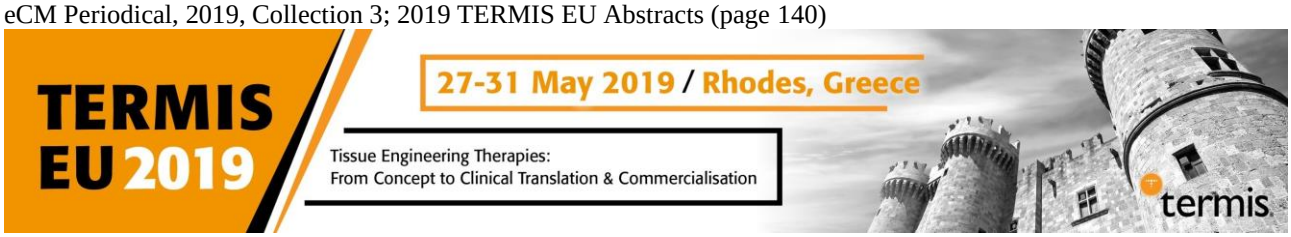
The influence of endotoxin on cellular activities

J. Olijve
Presenting Author: Jos Olijve, jos.olijve@rousselot.com
Rousselot-Gelatin, Ghent, Belgium

Endotoxins are found in the outer membrane of gram-negative bacteria and have profound in vivo responses. They can trigger strong immune responses. They are therefore unwanted contaminants in (bio)materials and their activity must be as low as possible. Hence, FDA defined 2.15-20 EU/medical device or 0.06-0.5 EU/ml as application limits depending on the type of application. Endotoxin received significant less attention during in vitro testing. Effects of endotoxin on various cellular activities have been reported. Levels as low as 0.005 ng/ml selectively silences mesoderm induction during directed differentiation of human embryonic cells [1]. Traces as low as 0.002 ng/ml, significantly alter proliferative response of hematopoietic stem cells [2]. Toll-like receptor 4 (TLR4) is the lipid A inflammatory signal transducer responsible for the TLR4 signal pathway induction [3]. Endotoxin levels in solutions and in use biomaterials must be taken into account when performing in vitro experiments with TRL4 cells. This presentation will give examples of the influence of endotoxin on cellular activities and the critical levels.

REFERENCES

[1] Sivissubramaniyan K, et.al. Regen Med. 2008 Jan; 3(1): 23-31
[2] Rinehart, J.J. and Keville, L. (1997) Cytotechnology (1997) 24, 153-159
[3] Diamond et al. ImmunoTargets and Therapy 2015:4 131–141



Environmentally-friendly coatings with antibacterial properties

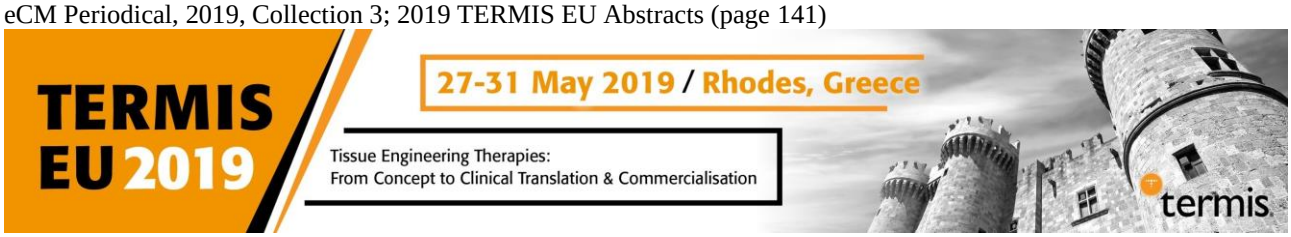
J.M. Garcia-Martin[1]
Presenting Author: Jose Miguel Garcia-Martin, josemiguel.garcia.martin@csic.es
[1]Instituto de Micro y Nanotecnología, IMN-CNM, CSIC (CEI UAM+CSIC), Isaac Newton 8, 28760 Tres Cantos, Madrid, Spain

Orthopedic metal implants improve the quality of life of millions of patients each year. The main problem they present is infection, which depending on the type of device and the patient's health affects 0.5-5% of cases. Our approach to this problem is to use nanostructured coatings to reduce the risk of infection. In short, by magnetron sputtering at oblique incidence [1] we fabricate a coating formed by titanium nanocolumns that develop as the result of atomic shadowing processes in the ballistic deposition regime, see Figure. It is an energy efficient method that can be scaled-up to industry, since it takes place at room temperature in one single step and no chemicals are involved (thus without recycling problems). In a first work, we carried out experiments in vitro with the main pathogen agent in implants, the Gram-positive bacteria Staphylococcus aureus (S.a.): it was demonstrated that the nanocolumns have an antibacterial behavior against S.a. (decreases bacterial adhesion and prevents the formation of the biofilm) without affecting the biocompatibility (cells that promote bone growth, osteoblasts, do proliferate and retain their mitochondrial activity) [2]. Recently, a stay funded by the Fulbright Commission at the Nanomedicine Laboratory of Northeastern University (Boston, USA) has made possible to broaden the scope and impact of this research line. On the one hand, we have verified that the antibacterial activity is maintained in a wide range of manufacturing parameters that control the morphological dimensions of the coating, which allows for validating the robustness of this strategy. In fact, it will be shown that some coatings have been fabricated in a semi-industrial equipment, a first approach proving that with feasible modifications, this strategy can be scaled up to large surfaces. On the other hand, we have verified the effectiveness of these nanostructured coatings also against Gram-negative bacteria such as Escherichia coli, which gives them an added value that extends its potential use: this type of bacteria presents a second lipid membrane external to the cell wall that gives them a special resistance to standard treatments with antibiotics. Finally, we have improved the antibacterial activity of these coatings by combining them with other nanostructures. In particular, the decoration of the nanocolumnar coating of titanium with tellurium nanorods by means of a hydrothermal reaction has exhibited enhanced antibacterial properties. This work will be published shortly.

ACKNOWLEDGEMENTS: Financial support from MINECO (MAT2014-59772-C2-1), MECD (PRX16/00383), Fundación Domingo Martínez and Fulbright Commission are appreciated.

REFERENCES

[1] Alvarez R et al. J. Phys. D: Appl. Phys. 2016; 49:045303
[2] Izquierdo-Barba I et al., Acta Biomater. 2015; 15:20-28



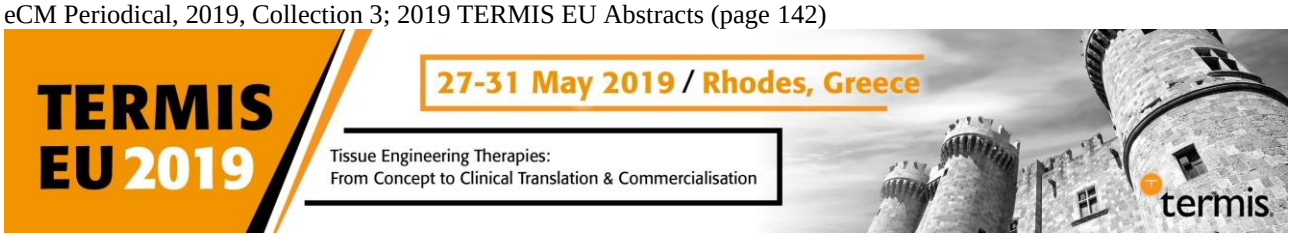
Mimicking the tissue specific extracellular matrix composition by medical electrospinning

J.C. Heikoop[1]
Presenting Author: Judith Heikoop, j.heikoop@ime-electrospinning.com
[1]IME Medical Electrospinning, Van Dijklaaan 6, 5581 WG Waalre, The Netherlands

Fibre-based scaffolds can be designed to form the basis to engineer living tissue replacement by providing mechanical and structural support and to guide the development of well-organized and functional extracellular matrix. In the regenerative medicine field, electrospinning is often the preferred production technique due to its capability to produce 3-dimensional fibrous ECM lookalike scaffolds with similar nanometer to micrometer length scales using an extensive range of natural and synthetic polymers. The process is highly versatile and tunable, allowing to tailor scaffold properties to fit the demands of the various tissue to be engineered and regenerated. Controlling all the parameters, which create the base of this method's versatility, has proven to be a challenge, leading to a lot of inconsistencies and batch to batch variability. It's of outmost importance to tightly control and log all relevant parameters. For example, the humidity affects the structural properties of the scaffold on the macro and micro level. Depending on the extend, these structural changes will have a large effect on the mechanical and biological properties of the scaffolds. Furthermore, during the production of fiber-based scaffolds, it is often assumed that a x-amount of polymer feed will result in a defined y- thickness. The really achieved thickness of fibrous scaffolds is normally measured afterwards at the end of the production line, often even in a destructive manner. In electrospinning especially for 3D shapes this assumption can lead to large batch to batch variation. Contactless thickness measurement technology offers the ability to accurately measure the thickness during production also at high fiber collection speeds and allows producing each batch to the desired scaffold thickness to micrometer level. Using multiple measurement locations, each scaffold obtains critical thickness quality checkpoints. This also makes backwards process alterations after final measurement obsolete. Keeping a tight control of the entire production process, also including electric charges combined with real time thickness monitoring, is the approach to drastically reduce variability of fiber-based scaffolds between and within a batch. Furthermore, it is also minimizing waste during the production process and is therefore a prerequisite for scaling a research process to an economically viable manufacturing process. Tightly controlled Medical Electrospinning will allow for the translation of promising research outcomes into the design and development of scaffolds with well-defined features such as porosity and fiber diameter to promote regeneration of the natural structures of the extracellular matrix in a variety of tissues.

REFERENCES

[1] Putti M, et. al. (2015) Electrospinning poly(ε-caprolactone) under controlled environmental conditions. Polymer 63, 189-195.



3D printable hydrogel-bioinks: Rheological implications and the importance of controlled network formation

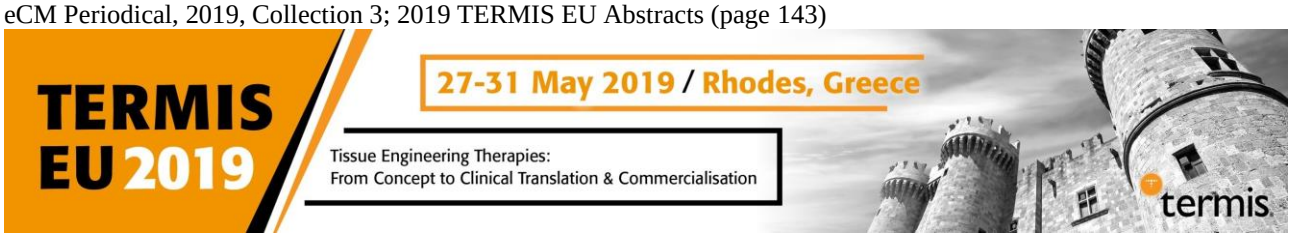
J. Groll
Presenting Author: Jürgen Groll, juergen.groll@fmz.uni-wuerzburg.de
Bavarian Polymer Institute, Julius-Maximilians University of Würzburg, Würzburg, Germany

Biofabrication is a young and dynamically evolving field of research [1]. It aims at the automated generation of hierarchical tissue-like structures from cells and materials through Bioprinting or Bioassembly. This approach has the potential to overcome a number of classical challenges relating to organization, personalized shape and mechanical integrity of generated constructs. Although this has allowed achieving some remarkable successes, it has recently become evident that the lack of variety in printable hydrogel systems is one major drawback for the complete field [2]. In order to be suitable for Biofabrication, hydrogels have to comply with a number of prerequisites with regards to rheological behavior and especially stabilization of the printed structure instantly after printing, while at the same time allowing the cells to proliferate. This contribution will present some of our recent work in this field, starting with a method to assess bioink printability [3]. It will then introduce thiol-ene cross-linking as alternative to the often used free radical polymerization to stabilize printed hydrogel structures with better control over network characteristics [4]. This enables the control over nanoparticle migration in and release from printed hydrogel constructs [5] and can be transferred to gelatin as one of the most widely applied bioink systems [6].

ACKNOWLEDGEMENTS: This work was financially supported by the European Research Council under grant agreement 617989 (project Design2Heal), the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°309962 (project HydroZONES) and the German Research Foundation (DFG; SFB/TRR 225 Projects A06, B02 and B04.

REFERENCES

[1] J. Groll et al. Biofabrication 2016, 8, 013001.
[2] T. Jüngst et al. Chemical Reviews, 2016, 116 (3), 1496.
[3] N. Paxton et al. Biofabrication 2017, 9, 044107.
[4] S. Stichler et al. Annals of Biomedical Engineering, 2017, 45(1), 273.
[5] B. Baumann et al. Angewandte Chemie International Edition 2017, 56, 4623-4628.
[6] S. Bertlein et al. Advanced Materials 2017, 29, 1703404.



Programming of macrophages as the immunomodulatory approach in implantation

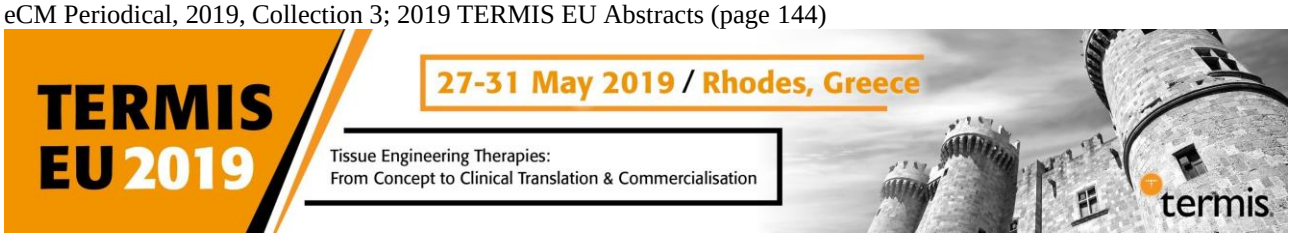
J. Kzhyshkowska[1,2],
Presenting Author: Julia Kzhyshkowska, julia.kzhyshkowska@medma.uni-heidelberg.de
[1]Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University; German Red Cross Blood Service Baden-Württemberg–Hessen, Germany

Implants, transplants, and implantable biomedical devices are mainstream solutions for a wide variety of human pathologies. One of the persistent problems around nondegradable metallic and polymeric implants is failure of macrophages to resolve the inflammation and their tendency to stay in a state, named "frustrated phagocytosis"[1]. During the initial phase, proinflammatory macrophages induce acute reactions to trauma and foreign materials, whereas tolerogenic anti-inflammatory macrophages control resolution of inflammation and induce the subsequent healing stage. However, implanted materials can induce a mixed pro/anti-inflammatory phenotype, supporting chronic inflammatory reactions accompanied by microbial contamination and resulting in implant failure. Titanium and titanium alloys have been successfully used in orthopedics, dentistry, cardiology and otorhinolaryngology. However, up to 20% of implants fail due to the development of various complications. We have established the ex vivo model system for the analysis of human primary macrophage responses to the porous and polished titanium surface. Using systems immunology approach we have identified the detrimental responses of human macrophages that included extensive expression of tissue-destroying matrix metalloproteinases, pro-inflammatory cytokines and chitinase-like proteins. The creation of a long-term anti-inflammatory microenvironment around implants and artificial tissues can facilitate their integration. Macrophages are highly plastic cells that define the tissue reactions on the implanted material. Local control of macrophage phenotype by long-term fixation of their healing activities and suppression of inflammatory reactions are required to improve implant acceptance. In order to develop the therapeutic strategy to overcome the chronic inflammatory reactions to titanium we have develop the have demonstrated proof-of principle for the long-term macrophage programming. We have designed the cytokine cocktail consisting of IL4/IL10/TGFβ1 (M2Ct) that induced long-term anti-inflammatory and pro-healing phenotype in human primary monocyte-derived macrophages. We used the self-standing, transferable gelatin/tyraminated hyaluronic acid based thin films for the controlled release of anti-inflammatory cytokine combinations and demonstrated to maintain the favorable M2-like macrophage phenotype with low responsiveness to pro-inflammatory stimuli. Such self-standing release systems can be used for prolonged local control of macrophage phenotype upon implantation factors.

ACKNOWLEDGEMENTS: This work has received funding from the European Union's Seventh Framework Programme for research and technological development and demonstration (Grant number 606294, IMMODGEL)..

REFERENCES

[1] Kzhyshkowska J et al. J Leukoc Biol. 2015 Dec;98(6):953-62.
[2] Riabov V et al. Acta Biomater. 2017 Apr 15;53:389-398.



3D printed bouncy hybrids for cartilage regeneration

J.R. Jones
Presenting Author: Julian RJones, julian.r.jones@imperial.ac.ukmailto:dimitrios.zeugolis@nuigalway.ie
[1]Department of Materials, Imperial College London, South Kensington Campus, London, UK

Clinicians need bioactive materials that can share load with host tissue (bone and cartilage) and provide a suitable temporary template (scaffold) for tissue regeneration before they biodegrade at a controlled rate. Orthopedic surgeons also need devices that can replace the articular surface of cartilage, then regenerate the cartilage to replace the device, all while recruiting cells from the underlying bone marrow. Such devices do not yet available, but they could be, using our new hybrid biomaterials. Hybrids have nanoscale co-networks of inorganic glass and organic components, e.g. sol-gel silica and biodegradable polymers. We now have hybrids that can "bounce" and self-heal[1]. The hybrids are ideal for 3-D printing inks, which can yield bespoke scaffold architectures. Osteochondral devices can now be 3D printed that stimulate articular cartilage production and also provide the bearing surface with tribology similar to native cartilage, e.g. coefficient of friction tested against living collagen. Mechanical properties and degradation rate are determined by the amount of polymer and degree of cross-linking. Self-healing is due to polymer chain mobility and hydrogen bonds allowing crack bridging[1]. When our hybrids are printed to have 200 μm pore channels, they provoke chondrocytes to produce type II collagen matrix, Sox9 and Aggrecan. Importantly, they do not produce type or type X collagen[1]. Bone marrow stem cells showed similar results. Use of star polymers enables development of hybrids that degrade under action of enzymes[2]. Therapeutic on release from the scaffolds can be achieved by incorporating strontium for enhanced osteogenesis[3,4].

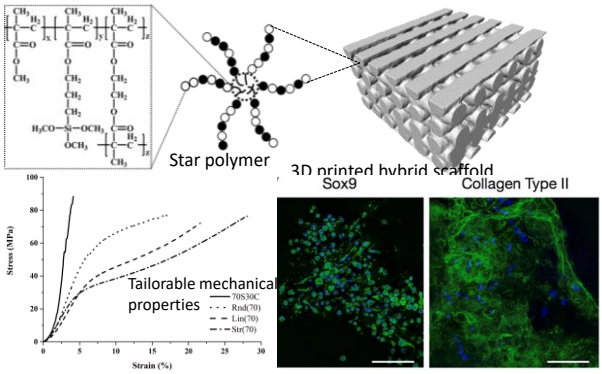
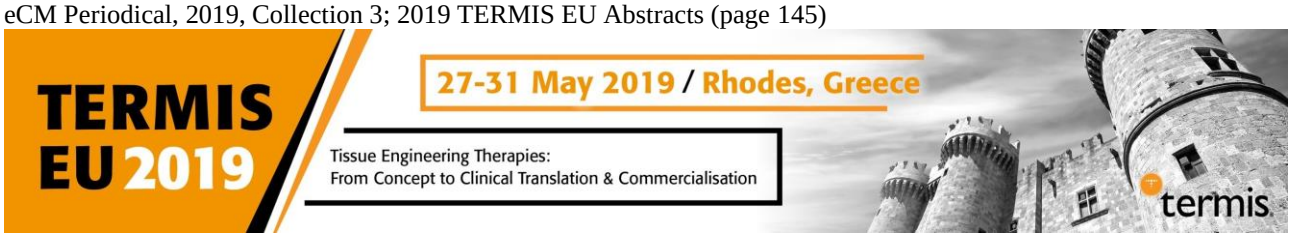


Figure 1: Schematic of a hybrid made with a star polymer with a biodegradable core; a 3D printed hybrid scaffold; stress strain curves comparing star polymer hybrids with polymers of different architectures (random v linear branched v star); Col II and Sox9 production from chondrocytes on 3D printed hybrids of specific architectures.

ACKNOWLEDGEMENTS: European Commission (Marie Curie Initial Training Networks; grant number: 289958) and EPSRC (EP/M019950/1, EP/N025059/1 and EP/I02249X/1).

REFERENCES

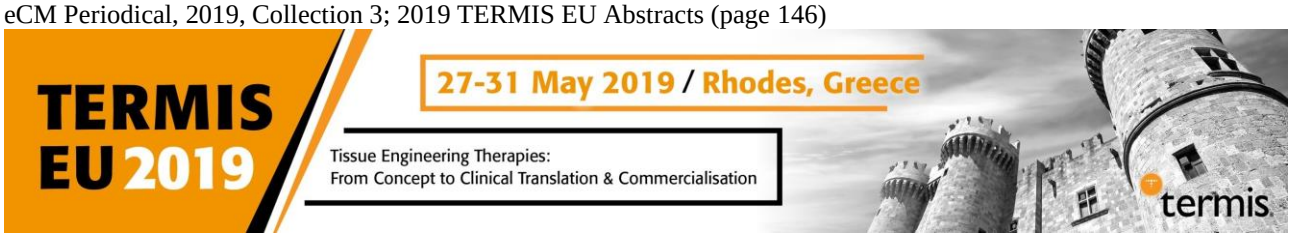
[1] Tallia et al. Materials Horizons, 2018, 5, 849-860
[2] Chung et al. Acta Biomaterialia, 2017: 54: 411-418
[3] Naruphontjirakul et al. Acta Biomaterialia, 2018: 66: 67-80
[4] Nommeots-Nomm et al. Acta Biomaterialia, 2017: 57: 449-461



Exploiting the nexus between biomaterial mechanics, tissue mechanics and gene therapy for enhanced tissue genesis and repair

J. J. Cooper-White[1,2]
Presenting Author: Justin J. Cooper-White, j.cooperwhite@uq.edu.au
[1]Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland, QLD, Australia [2] School of Chemical Engineering, University Of Queensland, QLD, Australia

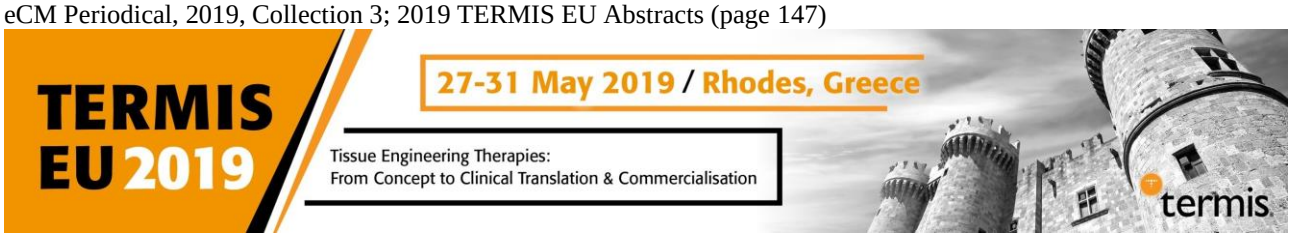
The ability of perivascular-derived mesenchymal stromal cells (MSCs) to sense and respond to variations in nanoscale extracellular matrix (ECM) composition and changes in mechanics of their local microenvironment is crucial to their survival and function as reparative cells within all tissues. Our investigations have confirmed that by mimicking these biophysical and biochemical cues in vitro, variations in matrix mechanics, ligand type and nano-spatial organization of adhesive ligands can not only invoke, but be deterministic of changes in MSC proliferation, migration and differentiation. Most recently, using a range of real-time fluorescent location and FRET-based biosensors, we have probed the dynamics of MSC adhesion to these types of substrates and quantified the subsequent development of tension and regulation of mechano-sensitive signaling pathways, providing new mechanistic insights into the dynamics of MSC mechanosensing and how lateral spacing of adhesive nanodomains drives hMSC fate through biased RhoGTPase signaling. Furthermore, by identifying differentially expressed microRNAs produced by MSCs in response to varying hydrogel stiffness and RhoA activity, or post activation in an injury site, we have most recently shown that modulating these mechanosensitive miRNAs (through targeted delivery) can overcome local ‘soft’ mechanical cues to drive commitment to stiffer tissues (e.g. bone), even when encapsulated in a soft 3D hydrogel, or suppress the creation of ‘stiff’ fibrotic tissue within an injured ‘soft’ tissue. These recent studies provide novel strategies with which to manipulate MSC fate and significantly impact tissue engineering and regenerative medicine applications that focus on their exogenous use or endogenous manipulation.



Natural biomaterials for bone and osteochondral defect repair

K. Alpantaki [1, 2]
Presenting Author: Kalliopi Alpantaki, kalliopialpantaki@gmail.com ; apopaki@yahoo.gr
[1]Department of Orthopaedics, Athens Medical Center, Marousi, Athens, Greece [2]Department of Materials Science and Technology, University of Crete, Heraklion, GR 70013 Greece

As people grow older musculoskeletal disorders are leading causes of morbidity and disability. Degenerative joint disease, mainly osteoarthritis, and intervertebral disc degeneration are considered the most common reason of chronic pain and disability within adult population. A considerable number of sufferers facing severe pain and activity limitation and seek surgical intervention. Apart from degenerative disorders, osteochondral defects following traumatic events, bone defects caused by complex fractures or tumours removal, delayed union or nonunion, and spinal fusion further conduce to the patients' reservoir requiring surgical treatment. The subsequent burden in terms of both quality of life and financial cost is enormous and expected to be further increased. Existing treatment options are vastly invasive; they address acute symptoms while they are inadequate in arresting the process of degeneration. The insufficiency of the current methods to address the problem and the increasing prevalence due to the aging population exacerbates the need of novel treatment modalities. Currently, tissue engineering technology based on the fabrication of natural polymers seeded with cells and enriched with growth factors is aiming to provide effective alternative substitutes for bone and cartilage repair. During this presentation, the main findings gained from our in vitro and in vivo studies will be discussed. We have evaluated the potential of crosslinked chitosan/gelatin (CS:Gel) scaffolds cultured with pre-osteoblastic cells and human bone marrow mesenchymal stem cells (BM-MSCs) to promote osteogenic differentiation. The scaffolds supported the formation of extracellular matrix, while fibroblasts surrounding the porous scaffold produce collagen with minimal inflammatory reaction in vivo. Additionally, we have evaluated the potential of crosslinked chitosan/alginate (Ch/Alg) porous scaffolds to promote the proliferation and fibro/chondrogenic differentiation of human nucleus pulposus cells indicating a potential use in intervertebral disc regeneration. Our findings provide evidence that natural biomaterials represent a promising strategy for tissue engineering for bone and osteochondral defects.

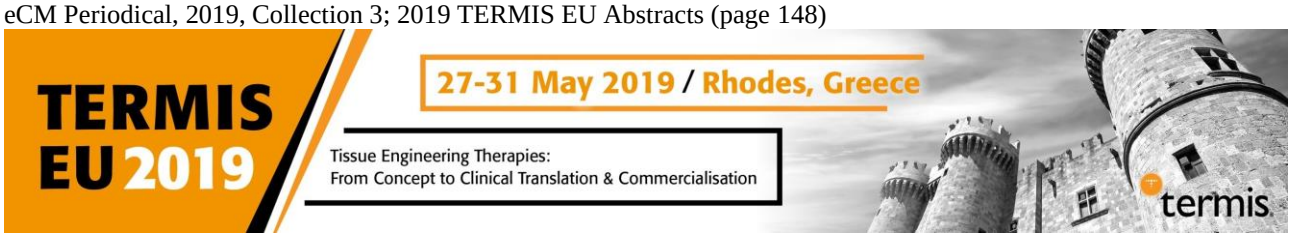


Harnessing the inflammatory response for tissue regeneration

K.L. Spiller[1]
Presenting Author: Kara L. Spiller, PhD, spiller@drexel.edu
[1]Biomaterials and Regenerative Medicine Laboratory, School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, PA, USA

The inflammatory response plays a major role in the body's response to injury, disease, or implantation of a biomaterial. When the inflammatory response functions normally, it can be a powerful force that promotes tissue repair and regeneration, but when it goes awry, disease takes hold and healing is impaired. Our is to understand the mechanisms by which the inflammatory response orchestrates successful tissue regeneration and to develop novel biomaterial strategies that apply these principles to situations in which tissue regeneration is impaired. In particular, we focus on the behavior of the macrophage, which can rapidly change behavior in response to environmental stimuli to promote inflammation, vascularization, tissue deposition, or remodeling. Through their dynamic phenotypic changes, macrophages function as major regulators of healing. In this talk, we will focus on our work to develop novel affinity-based drug delivery strategies that harness macrophage behavior to promote tissue regeneration and healing, with a particular emphasis on angiogenesis.

ACKNOWLEDGEMENTS: Financial support was received from US NIH/NHLBI R01HL130037 and US NSF CAREER awards.

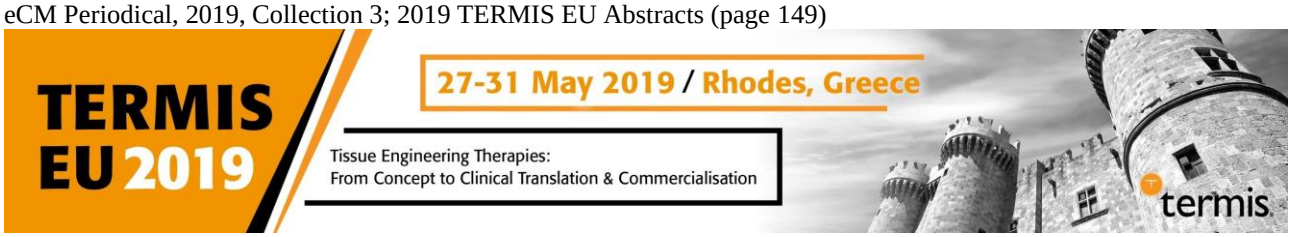


Nidogen-1 improves cardiac function post myocardial infarction via synergistic angiogenesis, neurogenesis, scar modulation and immune response

K. Schenke-Layland [1,2,3]
Presenting Author: Katja Schenke-Layland, katja.schenke-layland@uni-tuebingen.de
[1]Department of Women's Health, Research Institute for Women's Health, Eberhard Karls University Tuebingen, Tuebingen, Germany [2]Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany [3]Department of Medicine, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Myocardial infarction (MI) is a major cause of morbidity and mortality worldwide with the need for safe and scalable cardio-regenerative and reparative therapies. Our study of the extracellular matrix (ECM) during cardiogenesis within differentiating pluripotent stem cells and human fetal cardiac tissues identified a new therapeutic candidate, nidogen-1 (NID1), with a cardioprotective function in the post MI setting. In vitro cytotoxicity, endotoxin and immunological studies of recombinantly produced human NID1 verified its safety for in vivo use and potential for regenerative immune system response. Intra-myocardial injections of a NID1-functionalized hydrogel were tested in a mouse MI and reperfusion model. Echocardiography revealed a significant increase of ejection fraction and fractional shortening in NID1-treated animals when compared with controls. This important functional improvement was attributed to cardioprotection, a significant increase in vascular and neuronal cells in the infarct area, and a significant reduction in scar size. Our study demonstrates the efficacy of NID1 as a potential therapeutic candidate post MI, based on a unique synergy of cardiac protection, angiogenesis, neurogenesis and scar modulation.

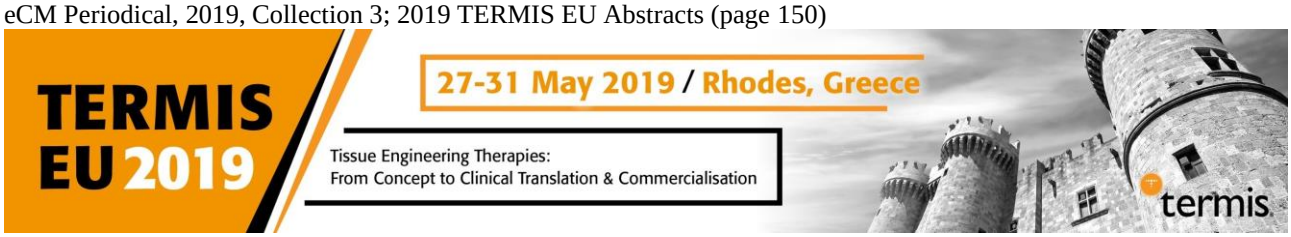
ACKNOWLEDGEMENTS: This work was financially supported by the California Institute for Regenerative Medicine - CIRM (RB3-05086); the Bundesministerium für Bildung und Forschung - BMBF (0316059), the Ministry of Science, Research and the Arts of Baden-Württemberg (33-729.55-3/214 and SI-BW 01222-91), and the Deutsche Forschungsgemeinschaft – DFG (INST 2388/33-1, SCHE701/14-1).



Benchtop grafting of protein-polymer bioconjugates in the absence of external deoxigenation – Synthesis and applications

K. Velonia
Presenting Author: Kelly Velonia, velonia@materials.uoc.gr
Department of Materials Science and Technology, University of Crete, Heraklion, Crete, Greece

Hierarchical architectures are ubiquitous in biological systems where multifunctional materials are produced in situ, under ambient conditions and, through ecologically balanced processes. Synthesized to mimic natural superstructures, amphiphilic protein-polymer conjugates, exhibit enormous potential in nanotechnology. Their synthesis conventionally requires stringent anaerobic conditions and specialized equipment, limiting potential applications. Based on the recent development of oxygen tolerant approaches aiming to simplify polymerization protocols in materials science, herein we report a novel, simple reaction protocol for quantitative synthesis of protein-polymer bioconjugates in the absence of any external additives. We will discuss how, by simply adjusting the headspace of the reaction vessel, we overcame the challenges of conventional synthesis and developed a synthetic approach that offers robustness and versatility for the synthesis of a variety of protein polymer bioconjugates. Different strategies employing several reversible-deactivation radical polymerization (RDRP) approaches leading to multifunctional and responsive polymer-protein conjugates will be presented. The intriguing molecular properties and assembling architectures observed in a variety of such biopolymers together with promising applications of these novel synthetic biomaterials in drug delivery will be presented.



Biomaterial-driven in situ transplantation of mesenchymal stromal cells for myocardial repair

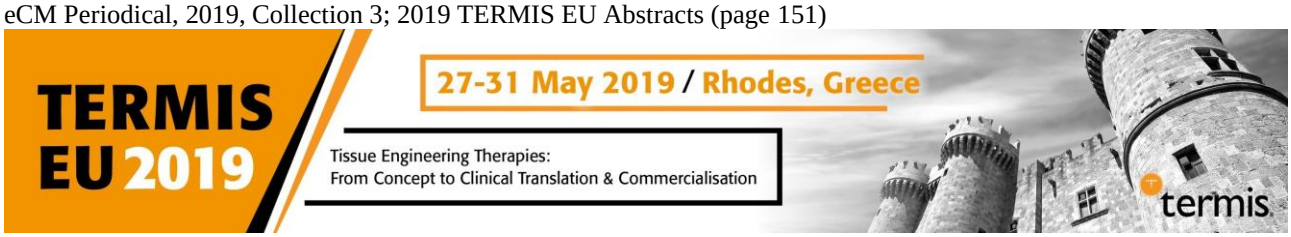
K. Suzuki,
Presenting Author; Ken Suzuki, ken.suzuki@qmul.ac.uk
William Harvey Research Institute, Barts and The London School of Medicine, Queen Mary University of London, United Kingdom

Myocardial infarction (MI) remains a major cause of human death and disability. Although the recent progress in recanalization therapies have significantly improved the mortality of acute MI, this has resulted in an increase of patients suffering post-MI chronic heart failure. The hearts post-MI frequently undergo pathological cellular and molecular remodelling, and the heart function becomes increasingly deteriorated with significant cardiac dilatation. As a result, many patients develop severe congestive heart failure. Thus, development of more effective, widely-available new treatments is urgent requirement. Myocardial repair and/or regeneration by means of transplantation of stem/progenitor cells is a promising new approach for the treatment of MI. Transplantation of adult (tissue-resident) stem cells (i.e. mesenchymal stromal cells, bone marrow stem cells, cardiac resident stem cells) can offer the repair of damaged myocardium by paracrine secretion, whilst transplantation of pluripotent stem cells (i.e. induced pluripotent stem cells, embryonic stem cells) may achieve myocardial regeneration through their cardiomyogenic differentiation. Based on encouraging pre-clinical data, various protocols of this approach have been or are being tested in clinical trials. However, to date, the overall results of the previous clinical trials are rather disappointing; therapeutic benefits observed are not as substantial as expected (though positive absolutely), requiring further refinement of the protocol. One of the main issues associated with this approach is the suboptimal cell-delivery route. Current methods are intracoronary and intramyocardial injection. However, these methods are known to result in limited initial retention, insufficient survival and thereby poor engraftment of donor cells, profoundly limiting the therapeutic efficacy. These injection methods also carry a risk of arrhythmia occurrence and coronary embolism. More recently, biomaterial-driven epicardial placement has been proposed as an alternative, safer and more effective route. We have reported the utility of epicardial placement aided with the cell-sheet technique, fibrin sealants, hydrogels, and films [1-3]. These methods can be completed with little invasiveness to the patinates, particularly when applied to patients undergoing open heart surgeries. This lecture will summarize the advantages and disadvantages of these cell-delivery methods and discuss future strategies.

ACKNOWLEDGEMENTS: Financial supports were received from the British Heart Foundation (Programme Grant RG/15/3/31236), Heart Research UK (Translational Research Grant; RG2618/12/13 and TRP06/15), Kaneka Corporation. This research was also supported by the UK National Institute for Health Research Biomedical Research Centre at Barts.

REFERENCES

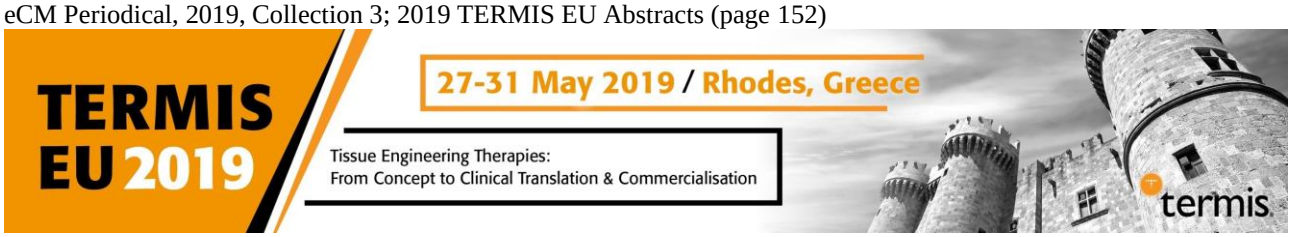
[1] Ichihara Y et al. Biomaterials. 2018;154:12-23.
[2] Kobayashi K et al. Sci Rep. 2018; 8:9448.
[3] Tano N et al. J Am Heart Assoc. 2016;5:e002815.



Bioprinting techniques for high throughput tissue modelling

K.W. Dalgarno
Presenting Author: Kenny W. Dalgarno, kenny.dalgarno@ncl.ac.uk
School of Engineering, Newcastle University, Newcastle, UK

Interest in the generation of multi-cellular tissues for disease modelling has been growing steadily over the past few years, with models proposed in the literature for a wide range of diseases. 3D, multicellular micro-tissue models may be developed in the form of co-culture spheroids, organ-on-a-chip type systems, or engineered tissues. The growth in interest has been fuelled by the continued failure of simpler in vitro models to work effectively as pre-clinical predictors of in vivo performance. However, the use of micro-tissues in the development of therapies or in any clinical context has been very limited as many experiments have low numbers of models, with no dilution series, replications, or positive and negative controls. This makes the models slow to develop and validate and low throughput in terms of their ability to provide information on diseases or therapies. The key to enabling the widespread use of micro-tissue models is throughput, as this will allow models to be developed and validated quicker, and then run as high throughput assays once they have been developed. Bioprinting techniques offer an attractive approach to creating initial co-cultures, with the ability to locate cells, fluids, and biomaterials within a wellplate or microfluidic system, but historically the techniques have not been widely adopted for the creation of micro-tissue models as the technologies have reliability and productivity limitations. This presentation will outline developments in bioprinting techniques and bio-ink formulation which overcome these limitations and will describe how the techniques are being applied to high throughput tissue modelling.



Human organs chips for drug development, disease modeling, and more…

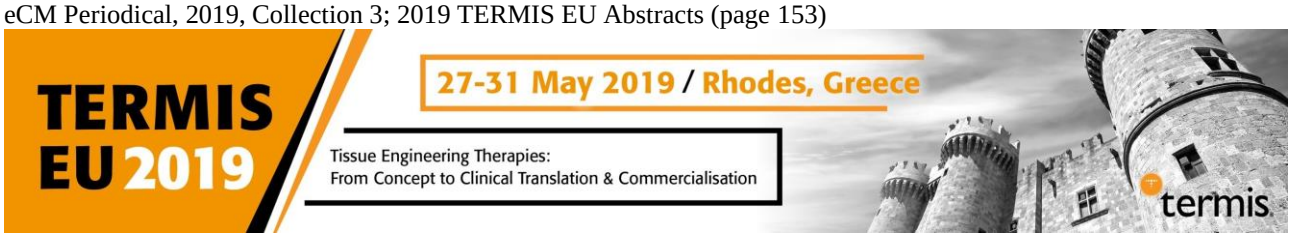
K. E. Healy[1,2]
Presenting Author: Kevin E. Healy, kehealy@berkeley.edu
[1]Department of Bioengineering [2]Department of Materials Science & Engineering, University of California, Berkeley, California, USA

Our work has emphasized creating both healthy and diseased model organ systems, we call microphysiological systems (MPS) or 'organ chips', [1-3] to address the costly and inefficient drug discovery process. The average time to develop and launch a new drug is 10-15 years, and costs ~ €5-3b. The poor efficiency and high failure rates are attributed to the heavy reliance on non-human animal models employed during safety and efficacy testing that poorly reflect human disease states. With the discovery of human induced pluripotent stem cells (hiPSC), we can now develop MPS to be used for high content drug screening, disease modelling, and numerous other applications. By combining the genetic background of hiPSC with microfabrication technologies, we can create MPS with appropriate biophysical tissue architecture and "tissue-like" drug gradients that recapitulate minimal human organoids sufficiently to allow accurate prediction of the toxicity of drugs. This presentation will discuss our progress in developing integrated in vitro models of human cardiac and liver tissue based on populations of normal and patient specific hiPSCs differentiated into cardiomyocytes, hepatocytes, or supporting cells. The benefits of our approach include: 1) robust microengineering platforms that control microtissue organization and function; 2) precise delivery of molecules (e.g., drugs) in a computationally predictable manner; 3) ability to model human disease; 4) cost efficient and high content characterization of an integrated multi-organ drug response; and, 5) reduction in use and refinement of animal experiments. While chips featuring single organs can be of great use for both pharmaceutical testing and basic organ-level studies, the huge potential of organ chip technology is revealed by connecting multiple organs on a single chip to create a scalable integrated human system for mechanistic biological studies and devising therapies for common, rare, and difficult to study diseases. This presentation will also address our cardiac-liver integrated MPS to predict drug-drug interactions. Ultimately, the vision of MPS technology is to reduce or eliminate the use of animals in drug discovery, and conduct 'clinical trials' in patient-specific organ chips that can accommodate variations in genetics, environment, and lifestyle.

ACKNOWLEDGMENTS: This work was funded in part by the US National Institutes of Health (NIH) NCATS UH3TR000487, and the NIH Common Fund for the Microphysiological Systems Initiative, the California Institute for Regenerative Medicine DISC2-10090, and NIH-NHLBI HL130417.

REFERENCES

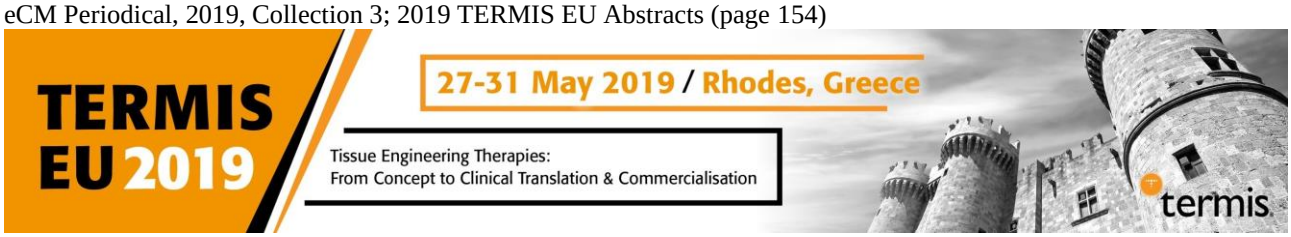
[1] Mathur, A., Loskill, P., et al., Sci.Rep., 9(5), 8883 (2015).
[2] Loskill, P., et al., Lab Chip, **17(9)**, 1645-1654 (2017). DOI: 10.1039/C6LC01590E
[3] Ma, Z., P., Huebsch, N., et al., Nature Biomedical Engineering, 9-10-18: DOI: 10.1038/s41551-018-0280-4



Commercialising university technology

K. Ryan
Presenting Author: Kieran Ryan, kieran.a.ryan@nuigalway.ie
Technology Transfer Office, National University of Ireland Galway, Galway, Ireland

Universities have an obligation to ensure that Intellectual Property (IP) outputs are properly captured and exploited according to various National and European guidelines. There are two main ways which University technology development can take on the road to commercialisation: 1. Licensing the technology to an existing company: A license is permission to do something the granting party (the licensor) has the right to otherwise prohibit. In the context of IP licensing, it is a grant, by the owner of the property, to another (the licensee) of the right to use the IP in question for commercial purposes; 2. Starting a new company: An important university objective is to explore and pursue opportunities for the exploitation of its intellectual property rights. For universities and its inventors, spin out companies often provide an effective means to achieve this objective. A spin out is created when the University creates a new company out of one of its existing departments, institutions or by an inventor. The decision of which path to take is critical and various elements can effect this decision such as the inventors own objectives, the market niche for the technology, the stage of technical development, the potential reward for each option and the types of support structures available. This talk will summarise the main points to consider when deciding on the most appropriate way to commercialise technologies developed in Universities.



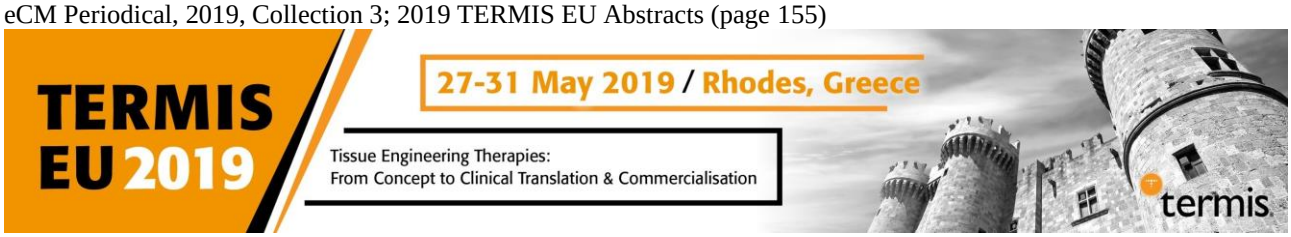
Tailor-made 3D tissue models: What works for industry and what does not

L. Suter-Dick[1]
Presenting Author: Laura Suter-Dick, laura.suterdick@fhnw.ch
[1]Molecular Toxicology, School of Life Sciences, University of Applied Sciences Northwestern Switzerland (FHNW), Muttenz, Switzerland

Tailor-made 3D tissue models, microphysiological systems and organ on a chip are now state of the art devices for the performance of organotypical cell cultures. They can be based on cell lines, primary cells or stem cells (iPS). These systems allow for specific tissues or even organ systems to be construed in vitro. They create a physiologically relevant environment, bridging the gap between in vitro and in vivo models and offering new approaches to research in medicine, biology, and pharmacology [1]. The potential for the implementation of these relatively novel techniques in the industrial context has been widely recognized. On the one-hand side, 3D structures have better physiological properties than planar monolayer cultures. This has been shown to enhance the function and life span of cells in culture. For the liver, for example, hepatocytes grown in 2D quickly de-differentiate and lose their functional characteristics such as metabolic competence. Hepatocytes grown in 3D, however, can be maintained over longer periods of time and still display metabolic functionality. Complex, 3D systems, also allow to better co-culture several cell types in one system or "microtissue". As an example, liver microtissues can contain only hepatocytes or a combination of hepatocytes and non-parenchymal cells. This combination allows the in vitro system to be able to mimic more complex events involving inflammatory responses and the process of fibrosis [2]. Tissues with barrier functions such as epithelia and endothelia, that are usually exposed to flow conditions in vivo profit from growing under microfluidic stress that can support cell polarization and cell function. Classical examples are the proximal tubule of the kidney, a common target for drug induced kidney injury [3]. Multiorgan systems or body on a chip are also slowly gaining acceptance in the pharmaceutical industry as they allow the testing of interactions between two or more tissues. Despite the technical and scientific progress, some major hurdles remain for the adoption of such complex systems in pharmaceutical drug development. On the one hand, complex systems are less amenable to automation and high-throughput screening, which limits their applicability in drug discovery. Inherent to their complexity is also a certain degree of variability, which is not welcomed in the drug screening process. The main contribution these systems can make nowadays is the understanding of mechanisms of toxicity and the partial replacement of animal studies in the early phases of drug development. At this point of the drug development process, fewer chemicals are tested and therefore assays of medium throughput and high complexity can be deemed acceptable. However, high standards need to be met in terms of robustness and standardization. Technical issues related to miniaturization of fluidic systems, compound adsorption to chip-material and tubing, issues inherent to flow, and large dead volumes must be kept to a minimum. In addition, appropriate, measurable endpoints must be optimized in terms of sensitivity to detect cellular responses and lead to scientific and regulatory acceptance of the results.

REFERENCES

[1] Sosa-Hernandez J et al. Micromachines 2018, 9, 536; doi:10.3390/mi9100536
[2] Prestigiacomo V et al. PlosOne, 2017
[3] Wilmer M et al. Trends in Biotechnology, 2016



Angiogenic properties of dental pulp stem cells for tissue engineering

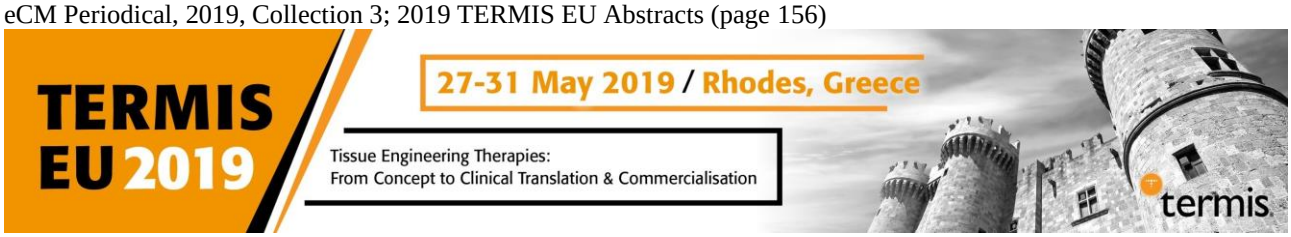
L. Muller
Presenting Author: Laurent Muller, laurent.muller@college-de-france.fr
Center for Interdisciplinary Research in Biology (CIRB), CNRS UMR 7241 – INSERM U1050, Collège de France, Paris, France

A limiting step for the success of cell-based therapies is the requirement for rapid blood perfusion deep in the tissue constructs in order to maintain differentiation and support survival of the implanted stem cells. Dental pulp stem cells (DPSC) have been proposed as optimal candidates for tissue regeneration strategies of lesions and injuries in the craniofacial area, in order to regenerate dental pulp as well as bone. DPSC could participate in construct vascularization either directly through their differentiation into endothelial cells, or through paracrine activation of the host vasculature and promotion of angiogenesis. The latter pathway has been the subject of many investigations in order to identify means of increasing the angiogenic potential of DPSC. In this context, hypoxia has been proposed as a major factor that could be used for pre-conditioning of DPSC through the regulation VEGF release. We have shown that FGF-2 is even more potent through the stimulation of the secretion of both VEGF and HGF by DPSC, since these factors act in synergy for promoting angiogenesis. We indeed demonstrated that a short exposure of DPSC in vitro, prior to implantation, was sufficient for increasing vascularization of the tissue constructs (Gorin et al, 2016). More recently, pre-vascularization strategies based on in vitro generation of micro-vascular networks have been the subject of many studies. We have thus investigated DPSC capacity to promote micro-vascularization of collagen scaffolds and characterized their participation to capillary formation together with their perivascular recruitment and their participation to vessel maturation through the generation of the vascular basement membrane. We also assessed the impact of such in vitro pre-vascularization on perfusion of the tissue construct after implantation in SCID mice.

ACKNOWLEDGEMENTS: Financial support was received from Agence Nationale pour la Recherche (ANR-Pulp Cell 2014-18) and Fondation pour la Recherche Médicale (FRM-DBS20131128438).

REFERENCES

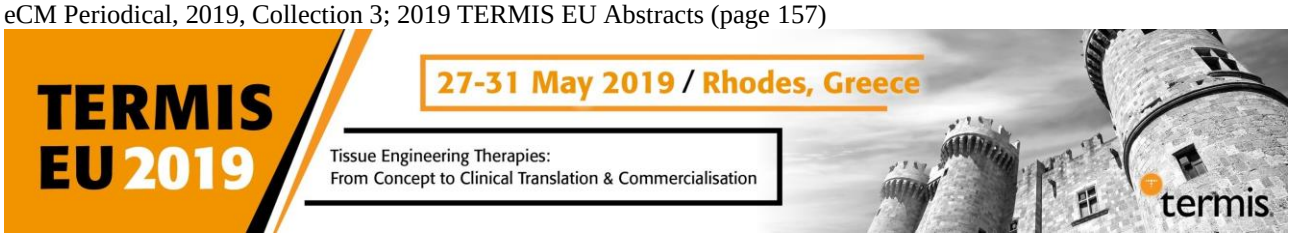
[1] Gorin C et al. Stem Cell Transl Med. 2016; 5(3):392-404



Publishing in a more diverse and connected world

L. Mullen
Presenting Author: Leanne Mullen, l.mullen@elsevier.com
Elsevier Limited, Oxford, United Kingdom

This session will look at key changes in the publishing landscape including open science and the global increase in publications. The analysis of key publishing data and trends will also be discussed in terms of how this information is driving new types of articles, new ways of sharing content and opportunities to increase diversity and global representation within our journal teams.



Transforming the spleen into a functioning liver in vivo

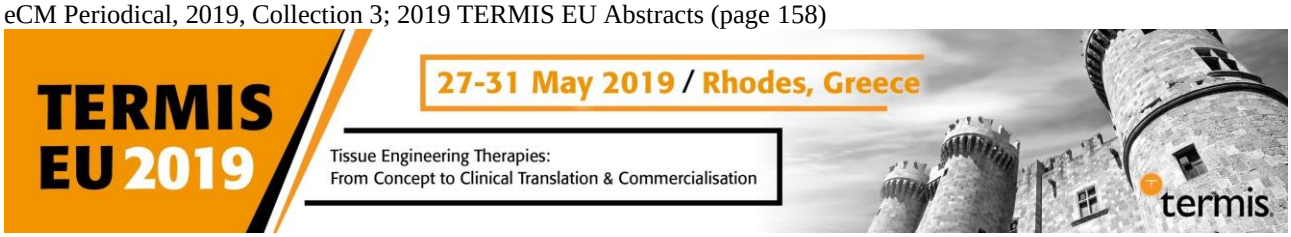
L. Dong[1]
Presenting Author: Lei Dong, leidong@nju.edu.cn
[1]State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University

Regenerating human organs remains an unmet medical challenge. Suitable transplants are scarce, while engineered tissues have a long way to go towards clinical use. In our previous study [1], a tumor homogenate-containing hydrogel (THAG), successfully creates an immunosuppressive enclave after transplantation into immunocompetent mice. Xenogeneic cancer cells of different species and tissue origins seeded into THAG survive well without being eliminated or spreading out of the enclave. It suggested that it was possible to harness the unique features of tumor development for tissue transplantation. Here, we demonstrate a different strategy that successfully transformed an existing, functionally dispensable organ to regenerate another, functionally vital one in the body. Specifically, we injected a tumor extraction into the mouse spleen to remodel its tissue structure into an immunosuppressive and pro-regenerative microenvironment. We implanted autologous, allogeneic or xenogeneic liver cells (either primary or immortalized), which survived and proliferated fast in the remodeled spleen, without exerting adverse responses. Notably, the transplanted liver cells exerted typical hepatic functions to rescue the host mice from severe liver damages including 90% hepatectomy. Our approach shows its competence in overcoming the key challenges in tissue regeneration – including insufficient transplants, immune rejection and poor vascularization. It may be ready for translation into new therapies to regenerate large, complex human tissue/organs.

ACKNOWLEDGEMENTS: We thank the financial support from Nanjing University State Key Laboratory of Pharmaceutical Biotechnology Open Grant (02ZZYJ-201307), the Jiangsu Province Funds for Distinguished Young Scientists (BK20170015), and the National Natural Science Foundation of China (31671031, 81673380).

REFERENCES

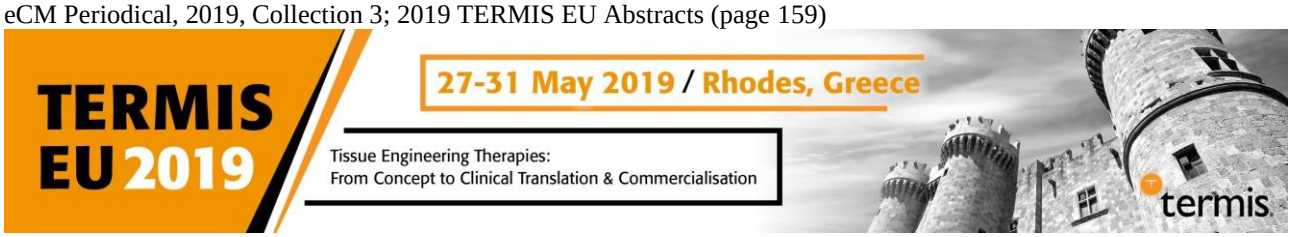
[1] Wang Z et al. Adv Sci. 2018;5: 1700666



No antibiotics biomaterials funtionalization to prevent and fight implants associated infections

L. Rimondini
Presenting Author: Lia Rimondini, lia.rimondini@med.uniupo.it
Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy

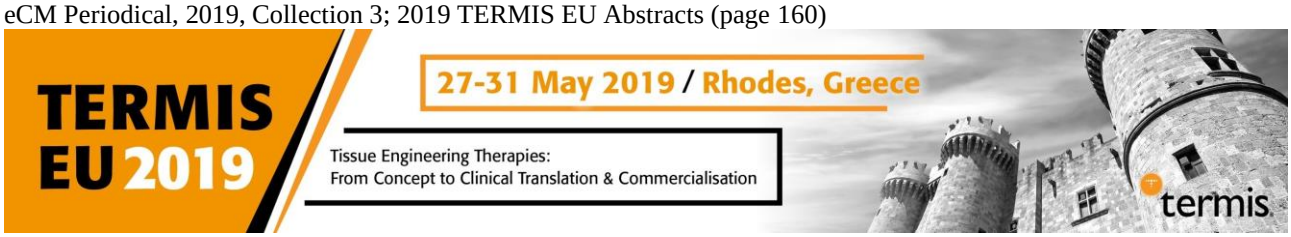
Implant-related infections are one of the most common reasons for surgical failure (14-29% of total failures in orthopaedics), in most cases causing severe disability and leading to a significant reduction in the patient's quality of life. According to the most recent surveys, the mortality rate of patients undergoing primary implant infections ranges from 10 to 18%; moreover, if an infection occurs also in the revised implants, this percentage can double or triple. In most of the cases infections are due to antibiotic resistant biofilm that makes treatment very difficult. Therefore, technologies addressed to prevent biofilm formation onto implants surface, without affecting biocompatible properties, seems to be a promising approach. In the present lecture, not functionalized and functionalized biomaterials with ions, polyphenols, proteins with bacteriostatic properties and "zombies" bacteria will be presented. All these technologies are alternative to antibiotic treatment and especially design to be complaint with tissue regeneration application requirements and to prevent or fight antibiotic resistance.



Creating environments to control biological response

L. Grover
Presenting Author: Liam M Grover, l.m.grover@bham.ac.uk
University of Birmingham, UK

Cells are highly responsive to local physical and chemical stimulus. As such, controlling local environment can be used as a means to modulate pathological processes or could be used to control tissue growth ex-vivo. In this talk, I will discuss how we are using materials to deliver agents that control the inflammatory microenvironment around a wound to control tissue healing and thereby prevent scarring both in the skin and across the surface of the eye. I will also discuss how we are using materials to create environments that can be used to create organotypic cell cultures to screen for novel therapeutics.



Combining in vitro and in silico models in regenerative medicine: A digital twin for perfusion bioreactor processes

L. Geris[1-3]
Presenting Author: Liesbet Geris, liesbet.geris@uliege.be
[1]Prometheus, R&D Division for Skeletal Tissue Engineering, KU Leuven, Belgium. [2]GIGA In silico medicine, University of Liège, Belgium. [3]Biomechanics Section, KU Leuven, Belgium.

INTRODUCTION: One of the major bottlenecks and cost drivers of cell-based therapies at this point is the manufacturing process that all too often is a scaled out version of the cumbersome manual laboratory procedures established during product development. Here, we describe the development of a perfusion bioreactor for cell expansion along with its digital twin, allowing for a precise monitoring and control of the cell culture process.

METHODS: Based on observations made in a suite of experimental results obtained with a pilot version of the perfusion bioreactor set-up, a mechanistic computer model of the neotissue (cells + extracellular matrix) growth was developed [1]. Speed of growth was observed and modeled (in FreeFEM++) to be influenced by local curvature, flow-induced shear stress, oxygen, glucose and lactate concentrations. The different model components were calibrated using in-house experimental data. Predictions on neotissue growth for 2 different triply periodic minimal surfaces were verified in a dedicated experimental set-up. A reduced and reparame-trized version of the model was developed (in MATLAB®) by homogenizing over the scaffold volume, allowing for rigorous single- and multiple objective optimization studies to determine bioreactor settings for maximal neotissue growth. A low footprint standalone version of the perfusion bioreactor set-up was developed, including connections for electronic and optical sensors at the chamber in- and outlet for environmental monitoring and control.

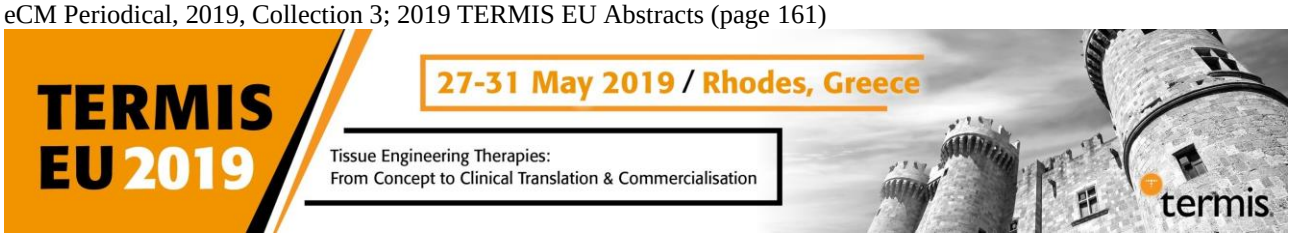
RESULTS & DISCUSSION: Model predictions showed enhanced neotissue formation and lactate production for Gyroid compared to Dcup scaffolds. Experimental results were quantified in 3D by contrast enhanced nanoCT imaging, confirming model predictions. The reduced model showed increased neotissue formation for frequent replacement of the bulk of the medium in the bioreactor. Taking into account the cost of culture shifted the optimal settings towards lower replacement frequencies and medium fractions. Online monitoring of oxygen, glucose and lactate is possible in the standalone set-up and will provide, on the one hand, addi-tional experimental read-outs for model credibility building and, on the other hand, the necessary real-world data to allow real-time model-based monitoring and control of the process.

CONCLUSIONS: The simultaneous development of a standalone perfusion bioreactor and its digital twin ultimately will allow for the precise prediction and follow-up of neotissue formation in a 3D perfusion environment, which is an important step towards automation of cell expansion in the context of TE therapies.

ACKNOWLEDGEMENTS: M. Mehrian, S. de Bournonville, T. Lambrechts, J.M. Aerts, F. Luyten & I. Papantoniou contributed to this work. Funding received from EC (FP7 ERC StG 279100 & H2020 ERC CoG 772418), FWO and VLAIO.

REFERENCES

[1] Guyot et al. BMMB, 2016, 15:169-80.



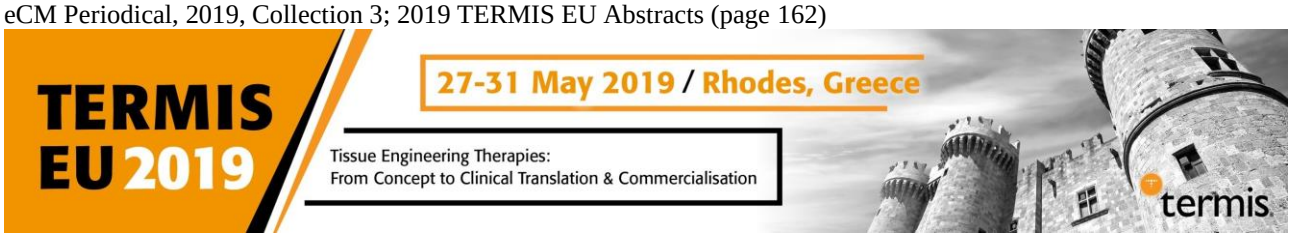
Learning from nature to form new organic materials for tissue regeneration

L. Adler-Abramovich
Presenting Author: Lihi Adler-Abramovich, LihiA@tauex.tau.ac.il
Department of Oral Biology, The Goldschleger School of Dental Medicine, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Molecular self-assembly is a key direction in current nanotechnology based material science fields. In this approach, the physical properties of the formed assemblies are directed by the inherent characteristics of the specific building blocks used. Molecular co-assembly at varied stoichiometry substantially increases the structural and functional diversity of the formed assemblies, thus allowing tuning of both their architecture as well as their physical properties. In particular, building blocks of short peptides and amino acids can form ordered assemblies such as nanotubes, nanospheres and 3D-hydrogels. These assemblies were shown to have unique mechanical, optical, piezoelectric and semiconductive properties. Yet, the control over the physical properties of the structure has remained challenging. For example, controlling nanotube length in solution is difficult, due to the inherent sequential self-assembly mechanism. Another example is the control of 3D-hydrogel scaffold's physical properties, including mechanical strength, degradation profile and injectability, which are important for tissue engineering applications. [1] Here, in line with polymer chemistry paradigms, we applied a supramolecular polymer co-assembly methodology to modulate the physical properties of peptide nanotubes and hydrogel scaffolds. Utilizing this approach with peptide nanotubes, we achieved narrow nanotube length distribution by adjusting the molecular ratio between the two building blocks; the diphenylalanine assembly unit and its end-capped analogue. In addition, applying a co-assembly approach on hydrogel forming peptides resulted in a synergistic modulation of the mechanical properties, forming extraordinary rigid hydrogels. [2] Furthermore, we designed organic-inorganic scaffold for bone tissue regeneration. [3] This work provides a conceptual framework for the utilization of co-assembly strategies to push the limits of nanostructures physical properties obtained through self-assembly.

REFERENCES

[1] Aviv M. et al. ACS Appl. Mater. Interfaces (2018).
[2] Halperin-Sternfeld, M. et al. Chem. Comm. 53, 9586-9589, (2017).
[3] Ghosh, M. et al. Biomacromolecules, 18, 3541–3550, (2017).



3D NephroScreen: High throughput drug-induced nephrotoxicity screening on a proximal tubule-on-a-chip model

L. Gijzen[1]
Presenting Author: Linda Gijzen, l.gijzen@mimetas.com
[1]MIMETAS BV, J.H. Oortweg 19, NL-2333CH Leiden, The Netherlands, info@mimetas.com, www.mimetas.com

INTRODUCTION: Renal toxicity remains a major issue in clinical trials and stresses the need for more predictive models fit for implementation in early drug development [1]. Here, we describe a perfused, leak-tight renal proximal tubule cell (RPTEC) model cultured within a high throughput microfluidic platform (Mimetas' OrganoPlate®), along with recent results from a 12-compound nephrotoxicity screen performed within the "NephroTube" CRACK IT consortium in collaboration with sponsors and the NC3Rs.

METHODS: Human RPTEC (SA7K clone, Sigma) were grown against a collagen I ECM in a 3-channel OrganoPlate®, yielding access to both the apical and basal side. Drug-induced toxicity was assessed by exposing kidney tubules to 4 benchmark and 8 blinded compounds with known clinical effects supplied by the sponsors for 24 and 48h. The tightness of the barrier was evaluated by diffusion of a dextran dye from apical to basal compartment. Parallel to this, cell viability with a WST-8 assay and the presence of LDH in the supernatant were assessed. Finally, kidney tubules were lysed, and RNA was extracted for gene expression analysis of acute kidney injury markers.

RESULTS & DISCUSSION: Upon perfusion flow, RPTEC form leak-tight confluent tubular structures against the collagen I ECM in the OrganoPlate®. The NephroScreen revealed significant decreased barrier tightness and cell viability in 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL was observed in 9, 5 and 7 out of 12 compounds respectively whereas claudin-2 showed a decrease in 6 out the 12. Overall, more effects were observed after 48h in comparison to 24h exposure.

CONCLUSIONS: The kidney-on-a-chip model in the OrganoPlate® provides a promising in vitro renal toxicity tool to answer the desire to provide a better alternative to animal studies in terms of throughput, costs and predictivity and ultimately will be commercialised after further validation.

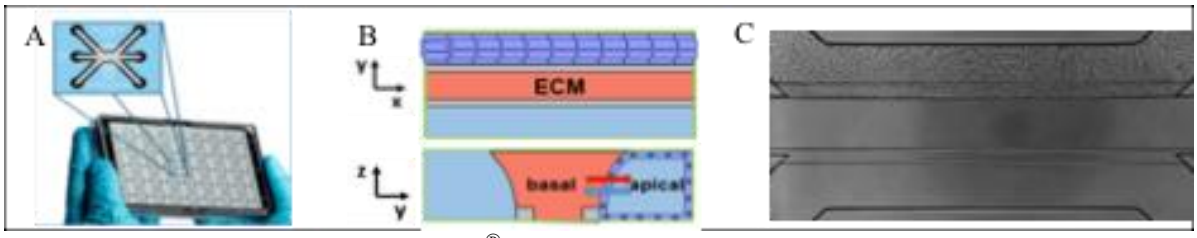
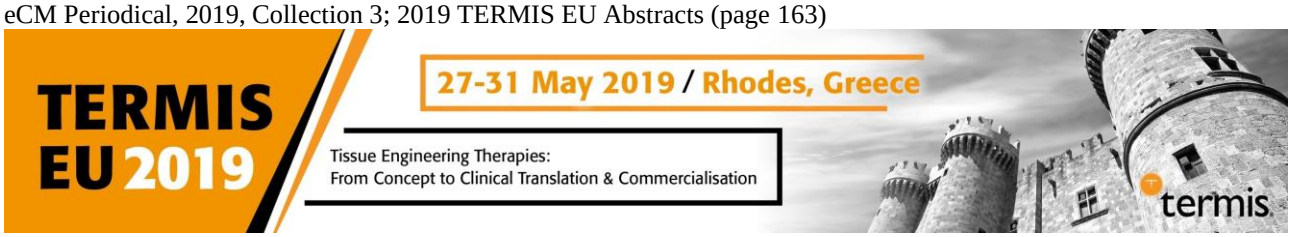


Figure 1: A) The 3-lane OrganoPlate® platform with 40 microfluidic cell culture chips embedded in a standard 384-well microtiter plate. **B)** Schematic overview of RPTC cells cultured against a collagen I ECM in a 3-lane OrganoPlate® yielding access to both apical and basolateral side. **C)** Phase-contrast image of RPTC cultured in the top channel of the OrganoPlate® at day 7.

REFERENCES

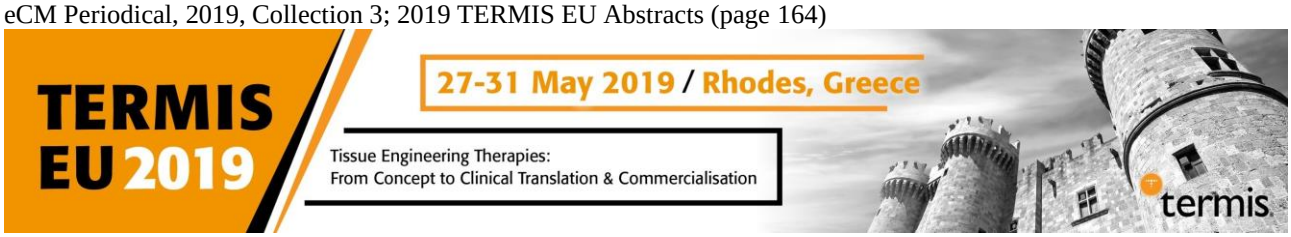
[1] Wilmer, M. J. et al. Trends in Biotechnology. 2016. 34(2):156-170



Biofabrication: A tool set to study biology in 3D with applications in regenerative medicine and in vitro models

L. Moroni
Presenting Author: Lorenzo Moroni, l.moroni@maastrichtuniversity.nl
MERLN Institute for Technology-Inspired Regenerative Medicine, complex tissue regeneration department, Maastricht University, Maastricht, the Netherlands

Organs are complex systems, comprised of different tissues, proteins, and cells, which communicate to coordinate many functions in our bodies. To replicate these structures towards the development of new therapies for tissue and organ repair, as well as for in vitro 3D models to better understand the morphogenetic biological processes that drive organogenesis, more and more advanced technologies are needed. Biofabrication strategies, in particular, are being developed to construct tissues and organs, and impart spatiotemporal control over cell-cell and cell-extracellular matrix communication, often through control over cell and material deposition and placement. Here, we present some of our most recent advancements in biofabrication that enabled the control of cell activity, moving towards enhanced tissue regeneration as well as the possibility to create more complex 3D in vitro models to study biological processes.



Long-term survival of transplanted autologous canine liver organoids in a COMMD1 deficient dog model of metabolic liver disease like Wilson's disease

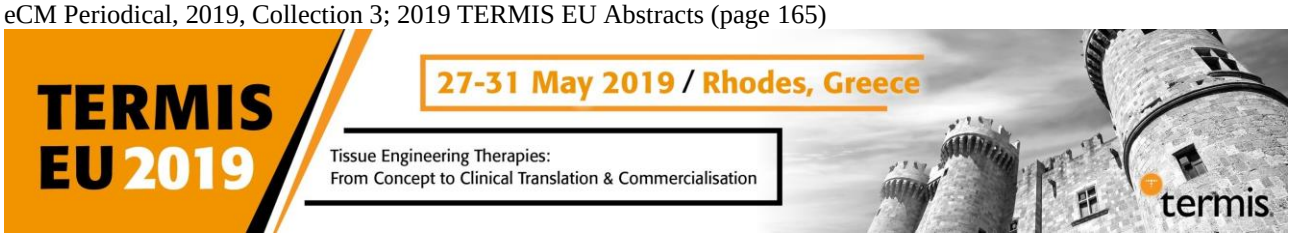
L.C. Penning
Presenting Author: Louis C. Penning, L.C.Penning@uu.nl
Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, 3584 CM, the Netherlands

The low number of patients suffering from rare diseases hampers research investments. Due to inbreeding in dogs these diseases often occur more frequent and are much more prominent in the animals. In view of the molecular, cell-biological, and clinical similarities, and comparable sizes and life-expectancy, dogs are ideally positioned to play a crucial role as models, especially for rare diseases. Here an example is presented how a naturally occurring genetic mutation in dogs is utilized to create a highly relevant animal model to investigate feasibility of liver stem cell transplantations.
For metabolic liver diseases, liver cell transplantation can be a less invasive alternative to liver transplantation. Primary human hepatocytes have been transplanted in patients with various inborn errors of metabolism. However, hepatocytes do not expand in vitro, are obtained from allogenic livers, and do not persist in the recipient. Adult liver stem cells cultured as organoids are a highly expandable cell source, which can be genetically modified and are a potential attractive alternative for cell transplantations [1]. We have transplanted autologous, gene corrected canine liver organoids in a COMMD1 deficient dog model of copper toxicosis, resembling human Wilson's disease [2,3]. Liver progenitor cells were obtained from COMMD1 deficient dogs by liver biopsy, lentivirally transduced with the full length canine COMMD1 gene, and expanded to 4.4-9.3x10^8 cells as organoids within 12 weeks. Cells were transplanted back into the same dog by either intrahepatic injections or intraportal delivery. Four dogs were transplanted, one dog served as vehicle control. Liver was sampled before, short term (1 week) and long term (up to two years) after transplantation. Even though the engraftment and repopulation percentages were low, the transplanted and gene-corrected cells survived in the liver for up to two years after transplantation. Liver organoids can be used for gene correction and subsequent autologous cell transplantation. Intraportal transplantation of high cell numbers is safe and technically this procedure can easily be extrapolated to human transplantation. Further research should focus on optimizing hepatic differentiation of organoids and the effect on repopulation and functional recovery. These preclinical studies in long-living large animals are pivotal for the translation of liver organoid transplantations from an experimental setting to human clinical application.

ACKNOWLEDGEMENTS: This study was funded by the Netherlands Organization for Scientific Research NWO ZON/MW (116004121).

REFERENCES

[1] Huch et al. Cell 2015;160:299-312
[2] Favier et al. PLoS One 2012;7:e42158
[3] Nantasanti et al. Stem Cell Reports, 2015;5:895-907



Functional injectable biomaterials for minimally invasive surgery

L. Ambrosio
Presenting Author: Luigi Ambrosio, luigi.ambrosio@cnr.it
Institute of Polymers, Composites and Biomaterials, National Research Council, Viale J.F. Kennedy, 54, Mostra d'Oltremare, Pad.20, 80125 Naples, Italy

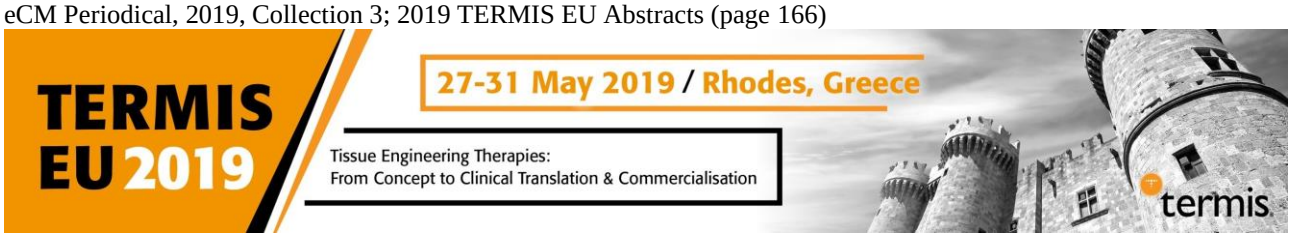
INTRODUCTION: The implementation of a personalised therapy together a less invasive surgery for the restoration of human tissues is becoming an appropriate strategy to mitigate costs of the modern health care system and the maintenance of health and quality of life. The selection of a suitable injectable is often based on material characteristics (including mechanical properties, drug release kinetics and degradation) that serve for the specific treatment function. By a careful selection of materials and processing conditions it is possible to finely control the behaviors from micro to sub-micrometric scale to develop active platforms to support the repair/regeneration of different tissues such bone and intervertebral disc (IVD).

METHODS: Calcium phosphate obtained by sol-gel synthesis combines hydroxyapatite (HA) with other calcium phosphate phases such as dicalcium phosphate (DCP), a precursor of natural HA in bone. Here, a bioactive and osteo-inductive injectable calcium phosphates is prepared by modification of hydroxyapatite with Strontium (Sr) due to its dual mode of action, simultaneously increasing bone formation while decreasing bone resorption. Furthermore, Hyaluronan gels as nucleous substitute were studied. In particular, specific formulation of hyaluronan (HA) polymeric substitute materials HYAFF 120 (an ester of HA), HYADD3 (an amide of HA), and collagen-low molecular weight hyaluronic acid semi-interpenetrating network loaded with gelatin microspheres as a potential material for tissue engineering of the nucleus pulposus (NP) were studied. For in vitro studies BMSCs were seeded on HYADD3. Meanwhile, for in vivo study the gels were injected into the NP of the lumbar spine mini-pigs in which a nucleotomy had been performed.

RESULTS & DISCUSSION: The injectable Sr-containing calcium phosphate bone cement (CPC) presented appropriate radio-opacity and injectability needed for bone filler applications. Moreover, the systems based on strontium modified hydroxyapatite (Sr-HA) at different composition demonstrated also a tunable proliferation and osteogenic differentiation of hMSC. The proposed gels for tissue engineering of the nucleus pulposus (NP) displayed a gel-like behavior, it was easily injectable as demonstrated by suitable tests. Importantly, it supported the growth and chondrogenic differentiation potential of mesenchymal stem cells (MSC) in vitro and in vivo. The properties of the semi-IPN hydrogel were successfully combined with TGF-β3 delivery by gelatin microspheres, which promoted the chondrogenic phenotype. The in vivo study, after 6 weeks of implantation, demonstrated that the two hyaluronan derived polymeric substitute materials, HYAFF120 and cell-loaded HYADD3 had a central NP-like region which had a close similarity to the normal biconvex structure and contained viable chondrocytes forming matrix like that of normal disc.

CONCLUSIONS: The strontium modified hydroxyapatite (Sr-HA) system presented appropriate osteogenic properties. Hyaluronic acid-based gels possess the unique NP extracellular matrix analogue behaviours. Both injectable systems demonstrated their easily injectability and highly bioactivity in both bone and NP repair/regeneration.

ACKNOWLEDGEMENTS: Open Lab project, MIUR progetti premiali 2012. PNR Aging program 2012-2018 from the MIUR and the EU FP7 Grant No. NMP3-LA-2008-213904.



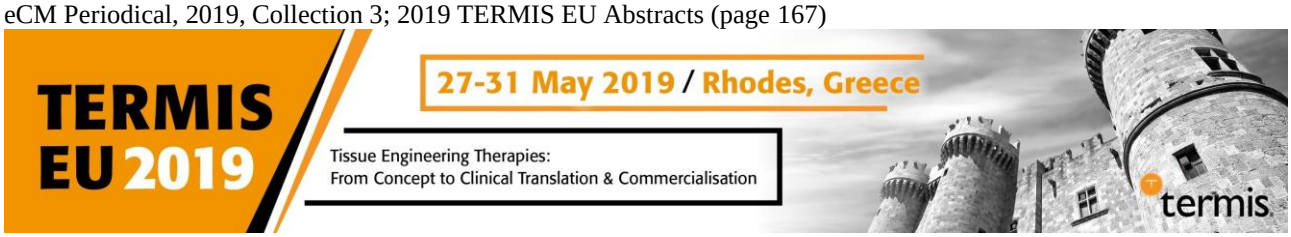
The impact of ancillary materials on your translational journey

L. Csontos[1]
Presenting Author: Lynn Csontos, lynn.csontos@stemcell.com
[1]STEMCELL Technologies Ltd., Cambridge, United Kingdom

As the field of regenerative medicine surges forward with aggressive growth in clinical trials, the regulatory landscape for all aspects of the market continues to evolve. This continued growth has led to increased awareness around the need for specialized and complex materials utilized in their manufacture. Ancillary materials (AMs) are components or reagents used during the manufacture of tissue engineered products but are not intended to be part of the final product(s). Commonly, there are limitations in the availability of clinical-grade reagents used as AMs. Furthermore, AMs may affect the efficacy of the clinical product and subsequent safety of the therapy for the patient. As such, AMs must be carefully selected and appropriately qualified during the tissue engineered product development process. The global regulatory landscape surrounding AMs will be discussed complete with highlighting regional differences [1,2]. Case studies will be reviewed for specific AMs including cell culture media used in clinical applications along with how to identify a good versus poor supplier of Ancillary Materials.

REFERENCES

[1] J Solomon et al. (2016) Current perspectives on the use of ancillary materials for the manufacture of cellular therapies. Cytotherapy 18(1): 1-12
[2] ISO/TS 20399-1:2018 Biotechnology -- Ancillary materials present during the production of cellular therapeutic products -- Part 1: General requirements, ISO/TS 20399-2:2018 Biotechnology -- Ancillary materials present during the production of cellular therapeutic products -- Part 2: Best practice guidance for ancillary material suppliers, ISO/TS 20399-3:2018 Biotechnology -- Ancillary materials present during the production of cellular therapeutic products -- Part 3: Best practice guidance for ancillary material users



Promises and future of bioprinting technic in cosmetic evaluation

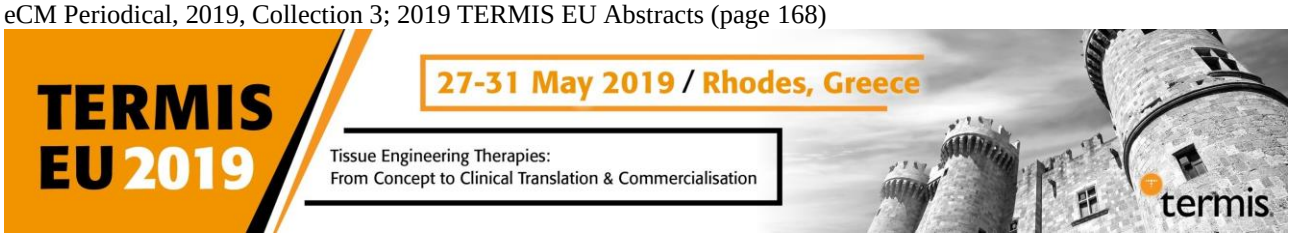
M. Rielland
Presenting Author: Maïté Rielland, maite.rielland@rd.loreal.com
Cellular and Tissue Engineering Platform, Knowledge and Performance Department, Advanced Research, Research & Innovation, L'Oréal, Aulnay-sous-Bois, France

INTRODUCTION: Since last 80', a long time before 2013 European Union ban on animal testing for cosmetic products, L'Oréal has placed itself as a pioneer for reconstructed human skin. It became one of the first cosmetic companies testing its raw materials/actives/formulations on in-house reconstructed human skin. Many different skin models are currently used in-house for efficacy and safety evaluation tests and knowledge of human skin physiology and pathology. However, one of the most challenge with in vitro models is to develop more relevant and predictive models according to cosmetic target (anti-aging, skin imbalance…). Bioprinting is a great alternative to create new models of skin with a complexity that cannot be achieved only by human hands. One of the biggest potential advantages of this technology is the ability to place cells or biological material where it needs to be placed, opening a few doors for tissue engineering. Taking advantages of our long expertize in skin tissue engineering, and thanks to new technologies (bioprinting, gene editing…), L'Oréal R&I aims to develop more predictive and pertinent skin models.

METHODS: Normal human fibroblasts or keratinocytes from skin plastic surgery wastes have been printed using inkjet or micro-extrusion printing methods or manually seeded. To reproduce the dermis part an internal Bioink has been designed.

RESULTS & DISCUSSION: Human normal keratinocytes, fibroblasts and melanocytes can be printed with good viability and can differentiate to form a correct epidermis or full thickness model. Histology and immunofluorescence on the main markers show that each cell types are functional. Taking advantages of those results, we have developed a patterned epidermal model were two type of keratinocytes are printed with different designs (figure 1). Interestingly they keep the design along the culture and form an epidermis with all differentiation layers and express correct epidermal main markers.

CONCLUSIONS: Skin models can be printed with correct viability, differentiation and expression of the main epidermal and dermal markers. We have developed a new epidermal patterned model with resolution that cannot be achieved only by human hands. This model could be used to study skin disease where the pathological and the healthy areas of the skin would be in the same sample leading to a more physiological in vitro model, improve the robustness and reduce the number of samples in evaluation tests.



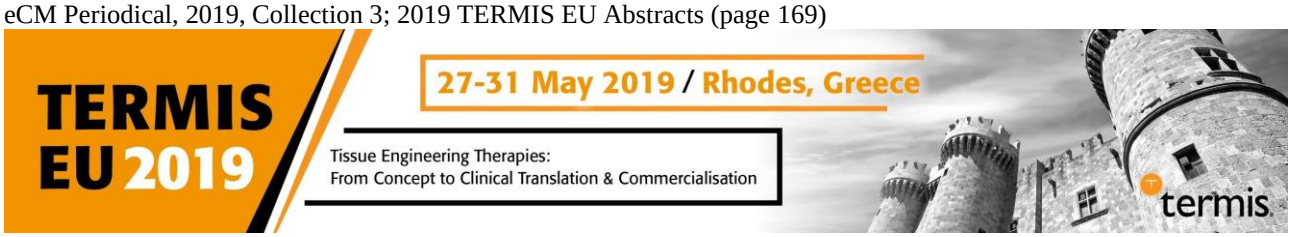
Engineered microenvironments for stem cell engineering

M. Salmeron-Sanchez
Presenting Author: Manuel Salmeron-Sanchez, manuel.salmeron-sanchez@glasgow.ac.uk
Centre for the Cellular Microenvironment — University of Glasgow, Glasgow, UK

It is now well known that stem cells differentiation can be controlled by using the properties of the cell microenvironment. Much work has been done to understand the role of the elastic properties of the substrate and, more recently, viscoelasticity in stem cell behaviour. Stem cell response to these properties relies on integrin mechanotransduction and the activation of signalling pathways in response to the actual force performed by cells on synthetic extracellular matrices. Here we show that other less know properties of the substrate (e.g. pure viscosity without any elastic component) are relevant to control the dynamic properties – mobility- of integrins.[1] We show control of the mobility of integrin ligands by using RGD functionalised lipid bilayers and, unconventionally, introduce functionalities on the bilayer to promote binding of other receptors, such as cadherins (HAVDI) and growth factor peptidomimetics (e.g. BMP-2). We show that the influence of targeting simultaneous integrin/cadherin receotors on substrates of controlled viscosity. In addition, we show the powerful effect of targeting simultaneously integrins and growth factor receptors. Based on this, we engineer 3D systems that promote bone regeneration by using ultra-low doses of BMP-2, and the potential to translate this receptor-targetting technology into clinical settings.

REFERENCES

[1] Bennet M, PNAS 2018;115:1192



Technological challenges for the clinical translation and commercialization of TERM therapies

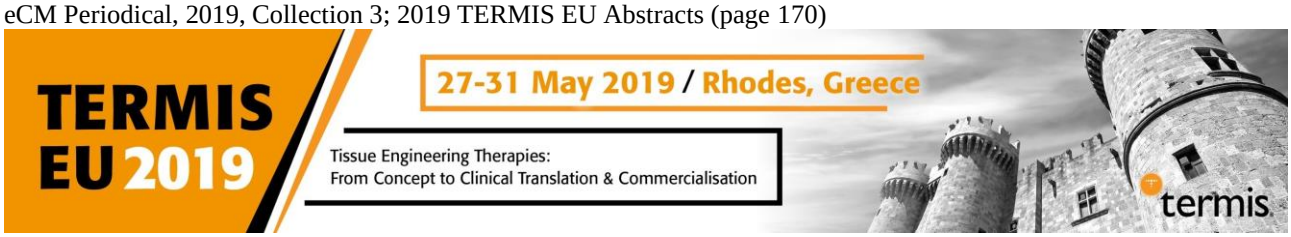
M.T. Raimondi[1]
Presenting Author: Manuela T. Raimondi, manuela.raimondi@polimi.itmailto:dimitrios.zeugolis@nuigalway.ie
[1]Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy

Mesenchymal stem cells (MSCs) raise a great interest for regenerative medicine for many clinical applications such as orthopedic, plastic and reconstructive surgery, within autoimmune or degenerative disease treatment and as immune-suppressive agents in organ transplantation. In stem cell therapy, cells are injected in the patient after an extensive in vitro manipulation aimed at obtaining a sufficient cell number able to guarantee the therapeutic effect. Currently, stem cell "manufacturing" implies the use of additives also from animal sources, hampering their clinical use mainly for safety reasons. I introduced a novel ground-breaking concept for an easy to use, repeatable, cost-effective, and safe substrate for stem cell expansion, called the "nichoid" substrate, capable of maintaining cell stemness through architectural cues, avoiding feeder cells and dangerous additives. The substrate is polymerized with a laser in a biocompatible resin using a frontier nano-fabrication technology called "two-photon laser polymerization" (2PP). The nichoid could drastically reduce down to few years the time to market of stem cell products that is currently 20 years. Another frontier technology developed by my group to accelerate the discovery of new drugs, including nanoparticle-based drugs and stem cell-based drugs, is the MOAB miniaturized optically-accessible perfusion bioreactor. The MOAB allows to culture 3D organoids of few millimeters in size, under continuous perfusion of the culture medium, infusion of the therapeutic agent to be tested and diagnostics of cell response both in real time and also post-cultivation. These two original platforms (the nichoid and the MOAB) are currently being integrated into a single device for the discovery and testing in vitro of new stem cell-based therapies. Another platform developed by my group to accelerate the clinical translation and commercialization of TERM therapies is the MICROATLAS miniaturized imaging window. This miniaturized implantable device explores the possibility to visualize and quantify in vivo the immune response to a biomaterial or a drug, including a stem cell-based biodrug, in the aim to reduce the ex vivo assessment required by current regulation. The use of a three-dimensional microstructured chip will allow repositioning the observation field of view of a two-photon microscope for intravital inspections for repeated and quantitative measurements of cell recruitment, neo-angiogenesis and fibrotic reaction, at different time-points. At the same time, it will act as an efficient auto-fluorescent micro-beacon, providing volume reference to implement adaptive optics methods, which will improve intravital image resolution sub-cute.

ACKNOWLEDGEMENTS: This research is funded by the European Research Council under the Horizon 2020 Research and Innovation program (ERC-CoG-2014 project NICHOID, G.A. n. 646990; ERC-PoC-2016 project NICHOIDS, G.A. n. 754467; ERC-PoC-2018 project MOAB, G.A. n. 82515). Funding was also granted by the Italian Ministry of University and Research (MIUR-FARE-2016 project BEYOND, G.A. n. R16ZNN2R9K).

REFERENCES

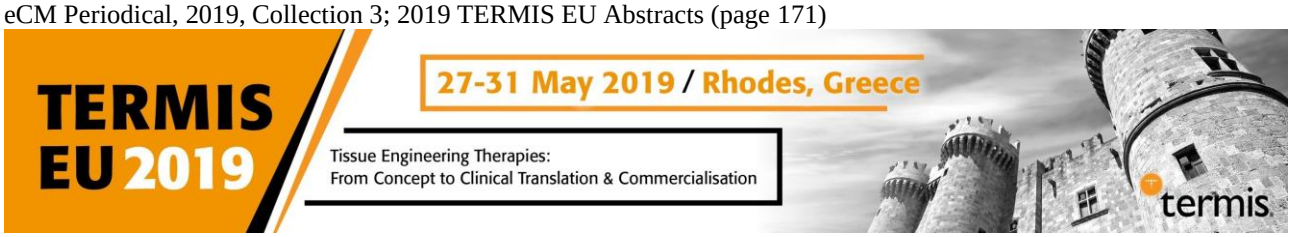
[1] Nava MM et al. J Tissue Eng Regen Med. 2017;11(10):2836-2845.
[2] Nava MM et al. Stem Cell Res Ther. 2016;7(1):132.
[3] Marturano-Kruik A et al. Proc Natl Acad Sci USA. 2018;115(6):1256-1261.



Glyco-functionalised neural interface coatings through heparan sulphate mimetics

M.J. Biggs
Presenting Author: Manus J. Biggs, manus.biggs@nuigalway.ie
Dept. of Biomedical Engineering & Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

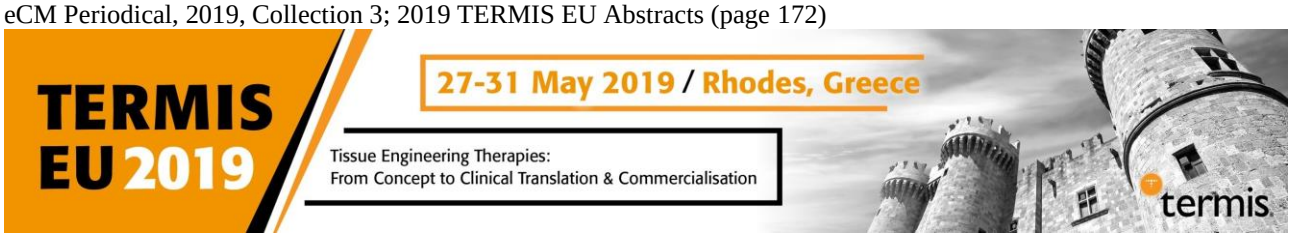
Current strategies in developing functional brain-machine interfaces are focused on reducing electrochemical impedance while presenting neurotropic moieties to enhance device integration. The use of biochemical functionalization of conducting polymer surfaces is being increasingly utilized to promote or inhibit specific biological interaction and promote chronic neuroelectrode functionality. In this study the physical, electrical and biological properties of a conventional conducting polymer - PEDOT are investigated following functionalisation with a heparan sulphate mimetic termed F6. Experimentally, PEDOT/F6 coatings were polymerized galvanostatically on platinum/iridium probe microelectrodes. The cytocompatibility of the functionalized PEDOT coatings was evaluated by culturing isolated rat ventral mesencephalic (VM) cells on the polymeric coatings and performing quantitative immunostaining of relevant neural biomarkers such as β tubulin for neural outgrowth and GFAP for astrocytes. The synthesis of pro-inflammatory cytokines, chemokine factors and reactive proteins were also assessed in vitro. F6 functionalisation was shown to enhance neuroelectrode biofunctionality, as assessed by the promotion of VM cell presence and neurite outgrowth for up to ten days in culture. A neurotrophic effect imparted by F6 was observed through binding to multiple neurotropic growth factors. Further, the modulation of cytokine and chemokine activity in VM populations indicated a protective effect of F6 which may translate to an enhanced availability of neurotropic growth factors. These results may be particularly important for neuroelectrode coating designs with biological dopants as they show an overall reduction in inflammation profile while promoting neural growth. The study further promotes the exploration of other glycan mimetics to potentiate matrix-therapeutics strategies at the neural-tissue interface.



Engineering complex bio architectures to biomimic nature: A step closer to the development of artificial organs

M. Thurner[1]
Presenting Author: Marc Thurner, Marc.Thurner@regenhu.com
[1]regenHU Ltd. Switzerland

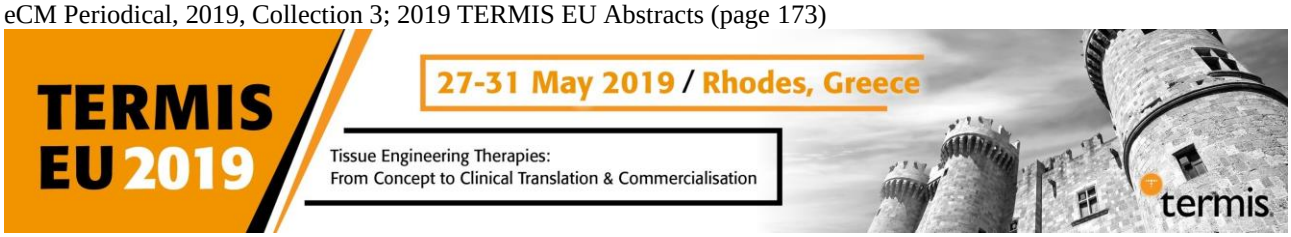
regenHU cutting edge biofabrication process creates infinite possibilities in the manufacture of artificial tissues and organs. By engineering Macro & Nano architectures in a single process unit, we have opened the door to a whole new world of possibilities allowing us to mimic biological systems identical to those found in nature. regenHU — a three dimensional biotechnology company, exploiting the potential of 3D bioprinting & cell based therapies — is a creative innovator in the development of biomedical products for regenerative medicine & drug discovery. Based in Fribourg, Switzerland, regenHU is a pioneer and global leader in its field, converging digital manufacturing (3D printing), biomaterials and biotechnology to create transformational innovations in Healthcare.



Electrospinning as a method for biomaterial manufacturing: An industry perspective

M. Thio
Presenting Author: Marco Thio, marco.thio@electrospinning.co.uk
[1]The Electrospinning Company Ltd., Harwell Campus, Didcot, Oxfordshire, United Kingdom

Restoring damaged tissue through regenerative medicine strategies has gained significant momentum over the last decade, with broad portfolios of biomaterials available to the end user. However, the vast majority of commercially available regenerative medicine products are based on processed collagen, decellularized donor tissue and ceramic-associated processes. Reconstruction of hard tissue such as bone is well established using decellularised bone granules/grafts from either human (allograft) or animal (xenograft) origin or synthetic substitutes such as TCP/HA. In most cases, these bone substitutes contain the natural bone building blocks such as calcium phosphates. In contrast to hard tissue however, soft tissue regeneration relies heavily on decellularised tissues such as skin, intestine, amniotic membrane and pericardium which not always mimic the tissue extracellular matrix they are meant to regenerate, offering the surgeon a compromise rather than an indication-specific solution. Electrospinning is widely acknowledged amongst the academic community as a method to produce scaffolds for cell culture & in vivo experiments. Features such as superior strength, controllable fibre diameter and fibre alignment and variable degradability allow the generation of indication-specific biomaterials that could outperform currently available medical devices. In addition, numerous publications describe the possibility of incorporating active pharmaceutical ingredients (API) within or surrounding the fibres providing a platform for drug delivery with potential use in major sectors such as wound care, orthopaedics and cell therapies. In this brief review, we provide an overview of the status and position of electrospinning as a method for clinical-grade biomaterial manufacturing and share our insights on the challenges and opportunities observed as a centre of excellence in biomedical electrospinning.



Pre-clinical testing of bioprinted cartilage: What has it brought us?

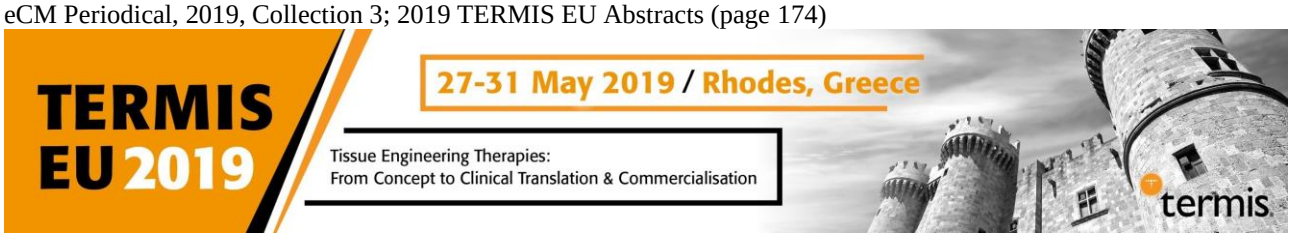
M. Zenobi-Wong[1]
Presenting Author: Marcy Zenobi-Wong, zmarcy@ethz.ch
[1]Tissue Engineering + Biofabrication Laboratory, ETH Zürich, Switzerland

Articular cartilage is composed of distinct layers, each characterized by a unique cell morphology, biosynthetic profile, biochemical composition and ultrastructural organization. These zone-specific properties give each layer its specific functional role, which then allows the whole multilayered organ to provide its well-known mechanical and lubrication properties. There have been many tissue engineering studies performed to recreate aspects of the zonal architecture of articular cartilage in the hope of obtaining a more realistic chondral or osteochondral graft. 3D bioprinting has been proposed as an optimal technology to biofabricate articular cartilage due to its inherent layer-by-layer additive nature. Although 3D bioprinting is in its infancy, with the development of more advanced biomaterials and printing technologies, it would offer the potential to recreate spatial distribution of growth factors, drugs and morphogens necessary for cartilage regeneration. It could also be used to reproduce gradients in stiffness, surface lubricity, oxygen impermeability of the calcified cartilage layer which may be critical in clinical usage. In this presentation, we discuss material, hardware and software developments from our lab towards bioprinting more realistic cartilage grafts. To fabricate osteochondral grafts, a commercial bone bioink together with a custom enzymatically crosslinkable cartilage bioink [1] are used and interlocking mechanisms, mechanical and biological performance studied. Using an ear shape as a model system, we further compare the properties of casted, reinforced hydrogels to that obtained with 3D bioprinting [2] in terms of cell viability, extracellular matrix deposition and shape retention during different stages of the manufacturing. To conclude, as the tissue engineering field strives towards increasing complexity of cartilage models, studies are needed to identify which of these features are essential for in vivo success and which naturally develop as the graft matures. The role of bioprinting in providing these key properties of cartilage is being actively explored and if successful, would allow for major advances in treatment of osteoarthritis, reconstructive surgery and screening for disease-modifying anti-rheumatic drugs (DMARDs) and disease modifying osteoarthritis drugs (DMOADs).

ACKNOWLEDGEMENTS: Funding for this project was received from the Swiss National Science Foundation and Fifa.

REFERENCES

[1] Broguiere et al. Tissue Eng Part C Methods. 2015;21(7):660-70
[2] Kesti et al. Adv Funct Mater. 2015; 25:7406-7417



2D substrates as innovative tools with dual therapeutic/regenerative behaviors

M. Grazia Raucci
Presenting Author: Maria Grazia Raucci, mariagrazia.raucci@cnr.it
Institute of Polymers, Composites and Biomaterials, National Research Council, Naples, Italy

INTRODUCTION: 2D materials such as graphene oxide (GO) and exfoliated black phosphorus (2D bP) can be used as alternative tools for bone metastasis treatment. Recent studies have shown the effectiveness of 2D bP and GO as photodynamic therapy (PDT) agents for cancer treatment [1]. This activity has been ascribed to their capability of generating singlet oxygen and acting as photosensitizers that, in presence of reactive oxygen species (ROS) and infrared light irradiation, constitute an essential component of PDT therapy. The idea was to use GO and 2D bP in the first step as anti-cancer agents and then as osteoinductive compounds. Indeed, 2D_bP is readily biodegradable inside the human body and produces nontoxic intermediates, such as phosphate, upon exposure to water and oxygen thus resulting a safe material able to induce osteogenic differentiation of cells and new tissue formation. Furthermore, the good mechanical properties obtained in a reduced state, high hydrophilicity and good biocompatibility of GO sheets suggest that it could also be an ideal nanoscale reinforcing filler for biocomposites for bone regeneration. Hence, we propose the in vitro use of 2D substrates (GO and 2D bP) as a strategy to inhibit the progression of osteosarcoma after surgery. At the same time, we have investigated the osteogenic effect of these nanosheets on in vitro model of new forming bone tissue.

METHODS: The biological studies were performed to evaluate the effect of 2D substrates on cell proliferation and osteogenic differentiation of human healthy (hMSC and HOb) and cancer cells (SAOS-2) with and without infrared irradiation treatment (IR). The cell morphology was evaluated by scanning electron microscopy, confocal microscopy and hematoxylin-eosin staining. The biocompatibility and osteogenic differentiation were checked in vitro by Alamar blue assay and ALP activity, respectively. The reactive oxygen species (ROS) production was evaluated with and w/o IR treatment by in vitro model inflammation. The effect of 2D bP on inflammatory response through pro and anti-inflammatory cytokine investigations on a co-culture model was also investigated.

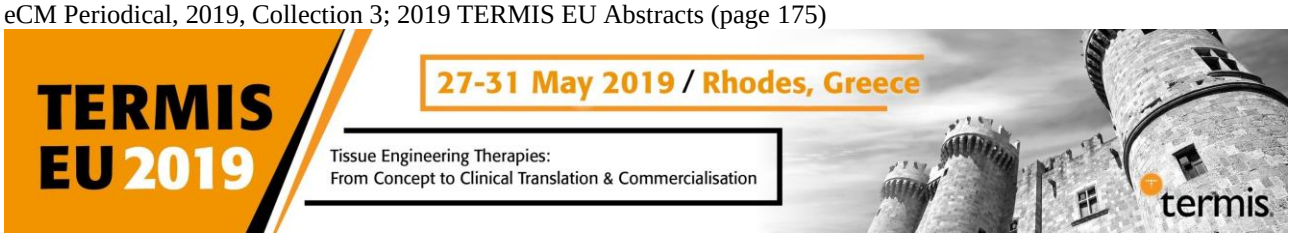
RESULTS & DISCUSSION: The biological investigations on healthy cells demonstrated that 2D substrates (GO and 2D bP) improved cell attachment and proliferation, with the best expression of early marker of osteogenic differentiation (ALP) at day14. Opposite effects were observed on cancer cells with a reduction of cell proliferation and inhibition of ALP activity. Furthermore, 2D bP is able to increase antinflammatory cytokines (i.e IL-10) on co-culture model and to inhibit pro-inflammatory mediator synthesis (i.e. IL-6) thus suggesting the opportunity to prevent cancer-related inflammation.

CONCLUSIONS: This study demonstrated that 2D substrates (GO and 2D bP) enhance the growth and osteogenic differentiation of human healthy cells (HOB and hMSC) and inhibits the proliferation of osteosarcoma cells (SAOS-2). The inhibition of cell proliferation (SAOS-2) of 2D bP reveals its antinflammatory activity on an in vitro co-culture model of cancer-related inflammation.

ACKNOWLEDGEMENTS: Open Lab project and PNR Aging program 2012-2018.

REFERENCES

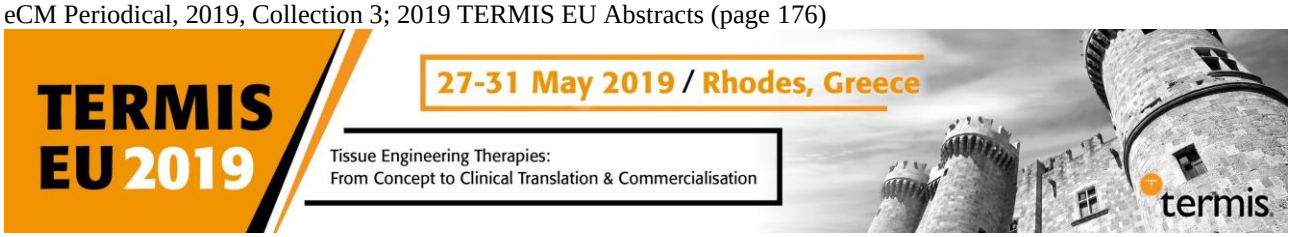
[1] Qiu Meng, et al. Proceedings of the National Academy of Sciences, 2018, 201714421



Using induced pluripotent stem cells to model neurodegenerative diseases

M. Lagarkova
Presenting Author: Maria Lagarkova: lagar@rcpcm.org
Laboratory of Cell Biology, Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russia

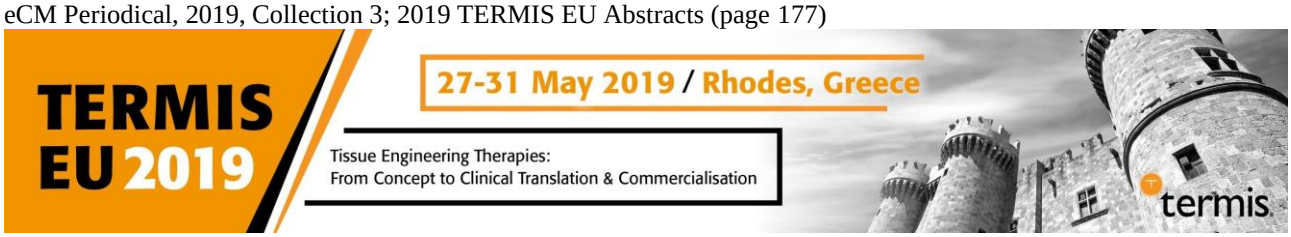
Neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease (HD), spinocerebellar ataxias (SCA), amyotrophic lateral sclerosis (ALS), Alzheimer's disease represent a large group of various disorders that are associated with the gradual degradation and death of certain types of neurons. In most cases, these disorders occur for unknown reasons and are constantly progressing. Understanding and treating neurodegenerative diseases have been constrained by the absence of in vitro models, especially because culturing primary cells affected by the diseases is very challenging. Limitations primarily lie in the access to patient's tissues. However, the development of stem cell studies and the discovery of induced pluripotent stem cells (iPSCs) provided an important source of cells to conduct in vitro studies. The ability to generate patient-specific iPSCs offers a new paradigm for modelling human disease and for individualizing drug testing. Modern genome editing technologies allow for the correction of mutations in IPSCs obtained from the patients with hereditary forms of neurodegenerative diseases, which is important both for obtaining isogenic systems for modeling and for tasks of future personalized therapy. We have successfully established and characterized IPSCs of patients with genetically characterized Parkinson’s disease, Huntington’s disease and spinocerebellar ataxias 1 and 17. We also established reliable protocols of differentiation iPSCs into the cell types affected in each particular disease (dopaminergic neurons, GABA-ergic neurons, Purkinje cells) and 3D brain organoids. Established IPSCs-based models recapitulated disease pathology in vitro, as evidenced by mutant huntingtin protein aggregation, nuclear indentation, enhanced neuronal death during cell aging and dysregulated store operated calcium channels in HD model, and defects in pathways associated with the functioning of mitochondria, phagosomes and lysosomes in PD model. Comparison of models of Huntington's disease and ataxias will provide an opportunity to understand the general mechanisms of the development of polyglutamine neurodegenerative diseases.



Design of advanced polymer and hybrid materials for biomedical applications

M. Vamvakaki
Presenting Author: Maria Vamvakaki, vamvakak@materials.uoc.gr
Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas, 700 13 Heraklion, Crete, Greece and Department of Materials Science and Technology, University of Crete, 710 03 Heraklion, Crete, Greece

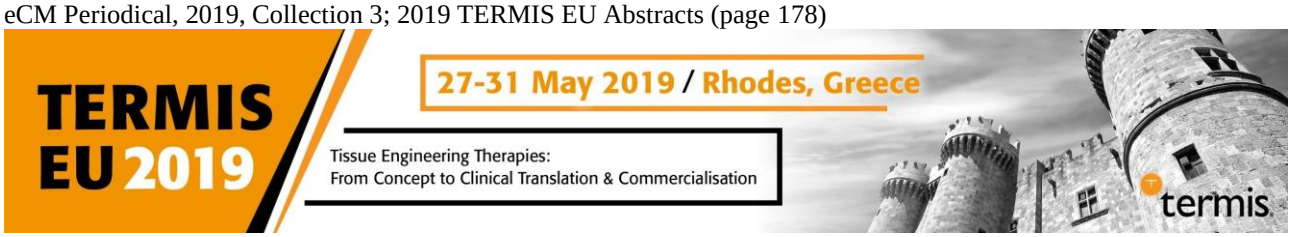
Polymer and hybrid materials have been extensively employed in biomedical applications (i.e. drug delivery, tissue engineering, medical implants). Natural, as well as synthetic polymers, alone or in combination with inorganic materials (ceramics, metals, etc.) have allowed to prepare constructs presenting cell biocompatibility, bioactivity, controllable biodegradability and appropriate mechanical properties. Our approach in this field aims in the sophisticated design and synthesis of both polymeric and hybrid, non-toxic, biomaterials which promote cell adhesion, growth and proliferation, while also presenting multi-functional properties, i.e. anti-inflammatory or antimicrobial action, material induced cell differentiation and others, for use in tissue engineering scaffold fabrication. The hybrid, organic-inorganic materials are developed using sol-gel chemistry followed by multi-photon lithography to fabricate complex 3D architectures. Additional functionalities, such as natural antimicrobial agents have been incorporated to obtain multi-functional cell scaffolds. On the other hand, appropriately modified natural biopolymers (polysaccharides, proteins, etc.) and synthetic polyesters prepared via multi-step chemical processes, are covalently linked to form biodegradable comb copolymers or polymer hydrogels. These materials are evaluated in cell cultures as potential candidates for use in bone and dental tissue engineering. The strong cell attachment and good cell proliferation on the material surface, combined with its anti-inflammatory action on macrophages, underpin their promising use in the field. Current work, focuses on further material development to afford spatiotemporally controlled release of important biological factors from these multi-functional scaffolds aiming at the design of programmable, “smart” biomaterial-cell-bioactive 3D constructs.



Local controlled drug delivery: Tenured translation

M. A. Tryfonidou
Presenting Author: Marianna A. Tryfonidou, M.A.Tryfonidou@uu.nl
Utrecht University, Faculty of Veterinary Medicine, Utrecht, The Netherlands

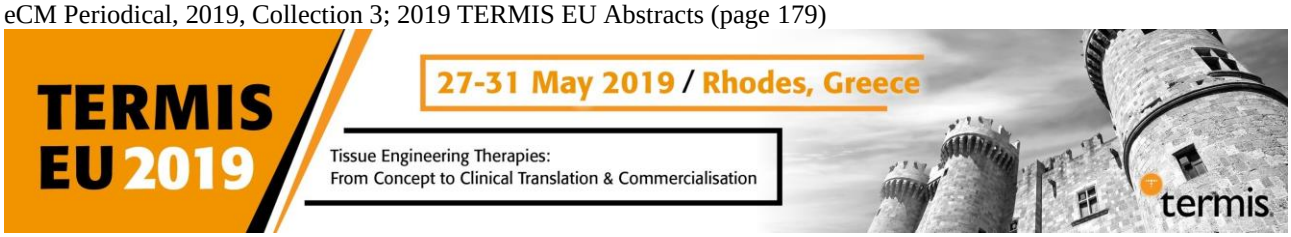
Chronic low back pain patients present an urgent societal problem, as its onset is in the economically productive age. In 40% of the patients, pain related to degeneration of the intervertebral disc (IVD); inflammation mediates the degenerative processes and contributes to pain. Current treatments are initially conservative, with as final option highly invasive surgery such disc arthroplasty or spinal arthrodesis. There are several minimally invasive treatment strategies being developed in order address back pain and at least delay the process of disc degeneration. This keynote will concentrate on the translation steps taken and the challenges phased for local controlled drug delivery focusing on anti-inflammatory drugs. In the course of several public-private partnerships we have obtained data on the safety and efficacy of intradiscal biomaterial-based delivery of a COX-2 inhibitor, celecoxib (CXB), in inhibiting inflammation and pain in disc degeneration. To this end, the canine model was been extensively employed in preclinical studies. Canines develop disc degeneration with similar macroscopic, microscopic, radiological and clinical signs as humans. As such, canines can be employed as experimental animals for development of treatments for both human and veterinary patients. We demonstrated that biomaterials loaded with CXB hold great promise as long acting treatment of disc-associated back pain for both human and veterinary patients. The injection procedure is safe, high loading dosages are effective in reducing tissue inflammation and in halting the degenerative process without systemic and local adverse effects.



Enhance your science writing and publishing

M.E. Barabas[1]
Presenting Author: Marie-Elizabeth Barabas, marie-elizabeth.barabas@us.nature.com
[1]Nature Partner Journals, Springer Nature; New York, USA

Your research is only as strong as the way it is written and published. Editors, reviewers and readers need to understand the merits of your work, no matter where your paper is submitted. At this talk, you will learn about the consideration for choosing a journal as well as ways that you can improve the impact of your writing. Are you curious about how to avoid #1 reason manuscripts are rejected? Join Managing Editor, Dr. Marie-Elizabeth A. Barabas, for an informative discussion about science publishing.



From cell-laden bone tissue engineering to personalized bone organoids

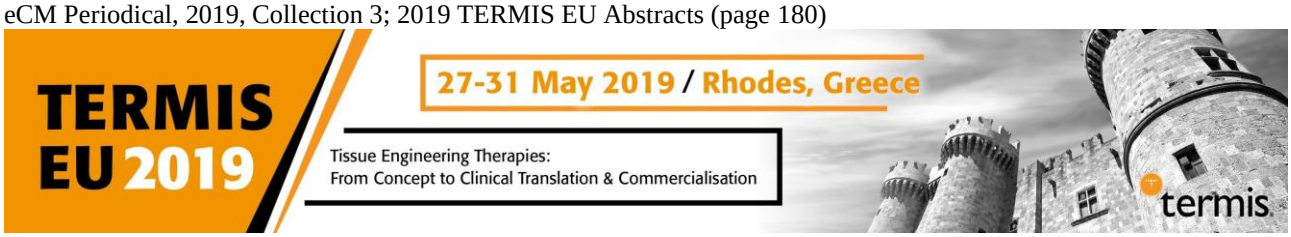
M. Rubert[1]
Presenting Author: Marina Rubert, marina.rubert@hest.ethz.ch
[1]Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

To advance on the development of therapeutics/implants for bone disease treatment, it is imperative to create bone organoids that closely mimic the structural and biological complexity of bone tissues. Models should also provide a better understanding of the molecular mechanism and the evaluation of the structural changes in bone that occur during the bone remodeling process. 3D bioprinting is an attractive approach to overcome the unstructured scaffolds geometries, or the lack of control on cell and matrix organization with limited usage of different cell types governed in the conventional tissue engineering methods. Moreover, given the high automation and reproducibility, the use of 3D bone bioprinting offers a promising tool not only to generate engineered bone tissues for damaged bone replacement but also to create reproducible test bed models for drug or implant development in vitro. One of the main challenges in 3D bioprinting of engineered bone relies on material selection. Inks must provide high fidelity for the printed scaffold geometries while assuring high cell viability and formation of a mineralized extracellular matrix. Efforts have already been made on the development of bioinks for 3D bioprinting of bone. However, these studies rely only on the assessment of rheological parameters of the inks and assessment of the cell viability after the printing process [1]. Mechanical properties of 3D extracellular matrix (ECM) influence stem cell differentiation towards the osteogenic lineage. While stiffer alginate-gelatine bioinks provide higher structural scaffold stability, lower stiffness 3D cell-laden scaffold better support bone-like tissue formation, cell survival, proliferation, differentiation and formation of a mineralized ECM in vitro. To move towards generation of bone organoids, co-culture of osteoblast and osteoclast cells resembling the bone remodeling process is needed. Co-culture models that lead to a coordinated osteoblastic and osteoclastic activity mimicking the human bone remodeling have been established. These models allow for the first time in vitro monitoring of the bone remodeling process non-invasively using micro-computed tomography. Co-cultured cells were seeded manually on salt-leached silk fibroin scaffolds. Translation from manual to 3D bioprinted seeded cells will open new avenues towards better understanding of bone physiology and to advance drug/implant testing in a relevant human bone model. Here, our experience with 3D bone bioprinting and development of organoid bone models using human cells will be discussed. Future prospects towards personalized bone organoids will also be addressed.

ACKNOWLEDGEMENTS: Financial support was received from the European Union Seventh Framework Programme under grant agreement no. 329389 and Chinese Scholarship Council (CSC).

REFERENCES

[1] Bendtsen ST, Quinnell SP, Wei M. Journal of biomedical materials research Part A 2017;105:1457-68.



Development and GMP-compliant manufacturing of EV therapeutics: From preclinical research to clinical application

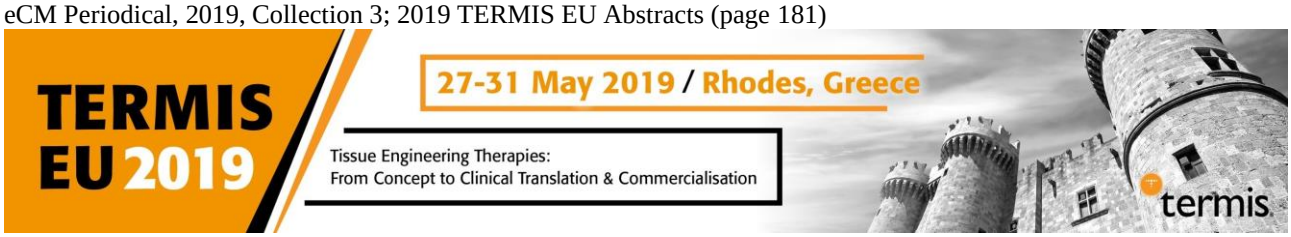
M. Gimona [1,2]
Presenting Author: Mario Gimona, mario.gimona@pmu.ac.at
[1]Spinal Cord Injury & Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, GMP Unit Strubergasse 21, 5020 Salzburg, AUSTRIA [2]Celericon Therapeutics GmbH, Strubergasse 21, 5020 Salzburg, Austria

Extracellular vesicles (EVs) derived from multipotent stromal cells (MSC) have gained increasing popularity as promising cell-free therapeutic agents for the treatment of a variety of medical indications [1]. Despite the huge but largely unexploited potential of EV-based therapeutics in relation to the evident biological and regulatory complexity, it is imperative to continue developing and improving scalable and reproducible purification protocols based on robust risk-based approaches, and to elucidate a potential Mode-of-Action through qualified potency assays in disease-relevant in vitro and in vivo models. Good Manufacturing Practice (GMP) compliance and a well-developed understanding of the benefit of regulatory requirements must be applied to EV-based biological therapeutics development. Regulatory compliance is an integral part of a stable manufacturing process and should guide prospective manufacturers of EV therapeutics at the earliest possible stage for the benefit of the patients. To reach the intermediate goal of clinically testing MSC-derived EVs, we have thus designed and validated a set of manufacturing and quality control strategies [2] to develop safe and efficacious therapeutics that are compliant with current regulatory requirements for pharmaceutical manufacturing. I will also discuss the rationale for testing human umbilical cord-derived MSC-EVs in selected indications with a poorly or unmet clinical need such as non-healing bone defects, enthesiopathies, spinal cord injury and implantation-induced cochlear injury. Preclinical data on tendon and bone regeneration as well as on neuroprotection and immunomodulation will be presented. Our data point in the direction of a multimodal alteration of cell physiology via immunomodulation and mechanosensation / mechanotransduction processes to enhance and support tissue regeneration.

ACKNOWLEDGMENTS: The outstanding contributions from Andreas Traweger, Eva Rohde and Karin Pachler, and the financial support of the EXOTHERA IT-AT 1036 and ExtraNeu Wiss2025 Projects are gratefully acknowledged.

REFERENCES

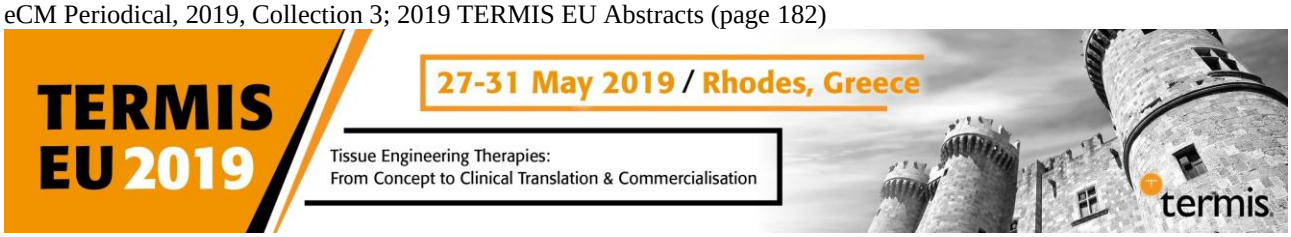
[1] Roy, S., Hochberg, F.H., and Jones, P.S. Extracellular vesicles: the growth as diagnostics and therapeutics; a survey. J. Extracell. Vesicles. 7(1) 2018
[2] Gimona, M., Pachler, K., et al. Manufacturing of Human Extracellular Vesicle-Based Therapeutics for Clinical Use. Int. J. Mol. Sci. 18(6) 2017



Enthesis regeneration using topographical cues and stem cells

M. van Griensven
Presenting author: Martijn van Griensven, Martijn.vanGriensven@tum.de
Experimental Trauma Surgery, Klinikum rechts der Isar, Technical University of Munich (TUM), Munich, Germany

Tissue engineering is an attractive strategy for tendon-to-bone interface repair. The structure and extracellular matrix composition of the interface are complex and allow for a gradual mechanical stress transfer between tendons and bone. We fabricated biphasic silk fibroin scaffolds. The scaffolds had two different pore alignments: anisotropic (tendon) and isotropic (bone). Total porosity ranged from 50 to 80% and pores were <100-300 µm. Young's modulus varied from 689 to 1322 kPa. In addition, adipose-derived mesenchymal stem cells were cultured on the scaffolds. Biphasic scaffolds supported cell attachment and influenced cytoskeleton organization depending on pore alignment. In addition, the gene expression of tendon, enthesis and cartilage markers significantly changed depending on pore alignment in each region of the scaffolds. Subsequently, we administered these scaffolds in a patellar tendon enthesis defect model in the rat. As controls, collagen scaffolds and empty defects were used. The animals (n=12 per group) were observed for 4 and 12 weeks by X-ray, µCT, histology, biomechanics and qRT-PCR. No failure in any of the scaffold groups occurred. The empty groups and the collagen control showed ossifications in the tendon region, whereas the biphasic scaffolds did not. Histology and qPCR indeed confirmed tendon regeneration in the tendon part of the biphasic scaffolds. Furthermore, biomechanic testing showed superior results of the biphasic scaffolds over the control groups. The mechanical parameters were close to those of native patellar tendon enthesis. In conclusion, the biphasic scaffolds fabricated in this study show promising features for tendon-to-bone tissue engineering.



Nanosensitive imaging of tissue constructs and cells

M. Leahy[1,2]
Presenting Author: Martin Leahy, martin.leahy@nuigalway.ie
[1]Tissue Optics and Microcirculation Imaging Laboratory (TOMI), National University of Ireland Galway (NUI Galway), Galway [2]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: There is a clear need for non-invasive and non-destructive methods to map tissue engineered structures and cells at the nanoscale. The accessible spatial frequencies provide information about small, submicron structure and highly sensitive to structural changes such as collagen organization.

METHODS: We have developed nano-sensitive Optical Coherence Tomography (nsOCT) and spectral encoding of spatial frequency (SESF) imaging for visualization of internal structure with nano-sensitivity to structural changes in superficial 2D [1-3] and depth-resolved imaging and for super-resolution imaging.

RESULTS & DISCUSSION: Different modalities of the SESF images of the collagen scaffolds are presented in Figure 1. The size of all images is 11 × 11 μm. Collagen samples after 3, 7 and 21 days of culture, respectively becomes more uniform, and the range of the dominant structure is reduced. The same conclusion can be made from analysis of the SESF images (D, E, F), formed as maps of mean spatial period, and from the sSESF images (G, H, I).

CONCLUSIONS: Collectively, these data clearly illustrate the benefit of nsOCT and SESF for elucidating tissue and cell structure. Even in the presence of noise, a difference in structure of 0.1 nm can be detected.

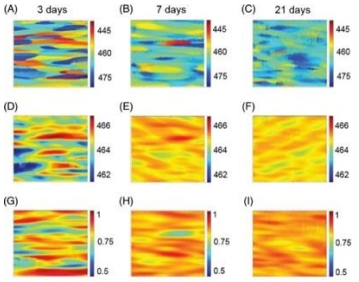
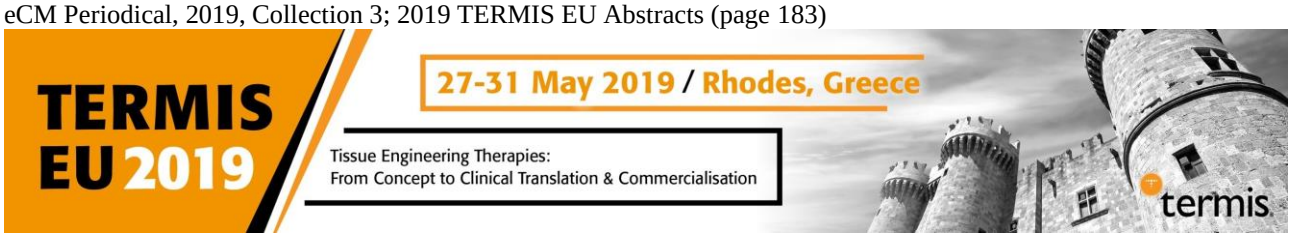


Figure 1: Images of collagen fibres: Imaging of the collagen scaffolds as a function of the day of culture. (A, B, C) SESF images as maps of the spatial period at the maximal signal distributions. (D, E, F) SESF images as maps of mean spatial period. (G, H, I) sSESF images as maps of the correlation coefficient distributions (for other sizes of probing structure see Videos S6-S8 [6]).
All images: 11 × 11 μm.

ACKNOWLEDGEMENTS: EU Horizon 2020 under grant agreement No 761214 STARSTEM.

REFERENCES

[1] S. Alexandrov et al. Opt. Lett. 2011, 36, 3323
[2] S. Alexandrov et al. Opt. Express 2012, 20, 9203
[3] S. Alexandrov et al. Appl. Phys. Lett. 2012, 101, 033702



Role of kinematic load on cell behavior

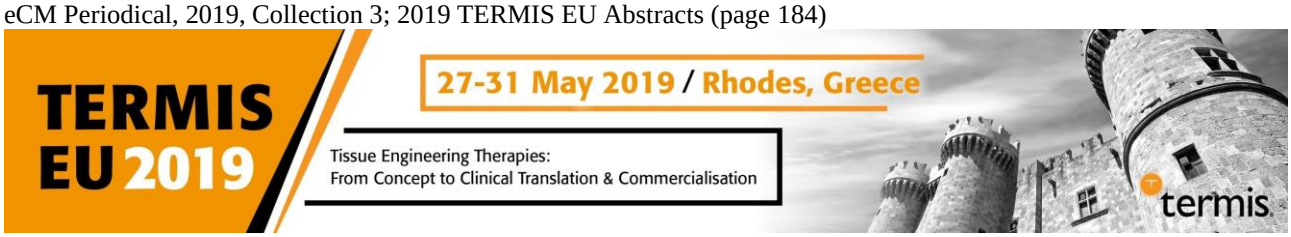
M. J. Stoddart
Presenting author: Martin Stoddart martin.stoddart@aofoundation.org
AO Research Institute, AO Foundation, Davos, Switzerland

INTRODUCTION: The emerging field of regenerative rehabilitation aims to enhance regenerative medicine approaches by harnessing and enhancing the body's endogenous healing response by way of rehabilitation protocols after treatment. It is widely accepted that the musculoskeletal system is strongly regulated by mechanical load, yet most new repair therapies are developed and studied using static culture. However, due to the critical role mechanics plays in vivo, a more physiological loading regime in vitro would be more appropriate, and this can be achieved by the use of bioreactors. Multiaxial load bioreactors can be utilized to this end, by mimicking the articulating joint, thus acting as an in vitro test bed for novel cartilage repair strategies. Bone marrow derived mesenchymal stem cells (BMSCs) are frequently used as a source material for cell-based cartilage repair strategies, they are also present during marrow stimulation treatments such as microfracture. Using a complex, multiaxial load bioreactor, we have demonstrated that superficial shear, superimposed over uniaxial load, can provide a chondrogenic signal in the absence of exogenous growth factors, namely TGF-β [1]. In agreement with other studies, compression alone did not induce chondrogenesis, however shear superimposed on compression lead to a more robust chondrogenic response. As the study did not include exogenous TGF-β, it demonstrates that mechanical stimulation alone is able to direct human MSCs towards a chondrogenic phenotype. In a previous study we had shown that the chondrogenic response is modulated by the frequency and amplitude of the multiaxial load applied, suggesting that this model can be used to pre-screen rehabilitation protocols in vitro. Within this system, the application of multiaxial load leads to an increase in the production, and activation, of endogenous TGF-β by the mechanically stimulated cells [2]. Deposition of both glycosaminoglycan and collagen II are increased in asymmetrically seeded scaffolds when compared to homogenously seeded scaffolds. This is providing further insights into novel material design for cartilage regeneration. The applied load enables the production of growth factor gradients, and this induced anisotropy is an interesting example of naturally changes generated in response to physical loads. In addition to the effect of load on direct differentiation, it is known that biomechanical stimulation can modulate the cell secretome [3]. Investigating these changes may lead to new potential clinical targets, that may be present during articulation, to be identified. This offers new avenues for potential clinical therapies. One such marker is nitric oxide. Normally absent during static culture, it is found after the application of complex load. Whether this has a positive or negative influence, is still under investigation.

ACKNOWLEDGEMENTS: This work was supported by the AO Foundation and the Swiss National Science Foundation (Grant no. 31003A_179438 / 1).

REFERENCES

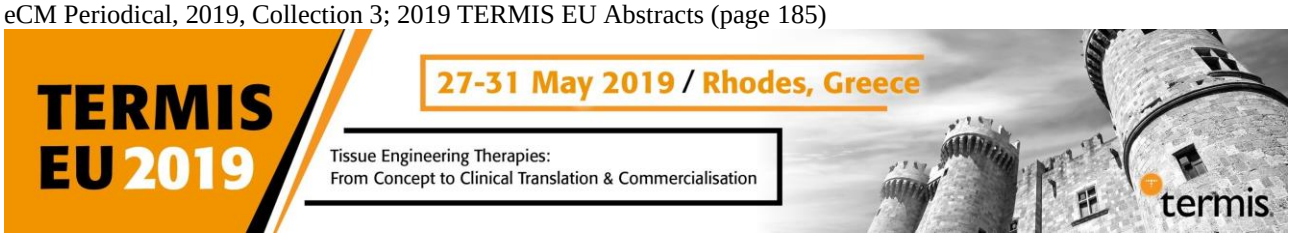
[1] Z. Li, et al. Mechanical Load Modulates Chondrogenesis of Human Mesenchymal Stem Cells through the TGF-beta Pathway, J Cell Mol Med, 14 (2010) 1338-1346.
[2] O.F.W. Gardner, et al. Joint mimicking mechanical load activates TGFbeta1 in fibrin-poly(ester-urethane) scaffolds seeded with mesenchymal stem cells, JTERM, 11 (2017) 2663-2666.
[3] O.F. Gardner, et al., Differences in human mesenchymal stem cell secretomes during chondrogenic induction, Eur Cell Mater, 31 (2016) 221-235.



Mesenchymal stem/stromal cells as a therapy for osteoarthritis: Mechanistic insights

M. Murphy
Presenting author: Mary Murphy, mary.murphy@nuigalway.ie
Regenerative Medicine Institute & Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

Although mesenchymal stromal cells (MSCs) represent an important technology in regenerative medicine, translation to clinical practice has been slow. Recent successes with market approvals slowly emerging have highlighted the potential of the cells as a therapy for multiple diseases. However, mechanisms whereby therapeutic effects are achieved are still unclear. Initial concepts of cell differentiation to various mesenchymal tissues have been replaced by a paracrine model with implanted cells licensed by the in vivo environment and responding by secreting immunomodulatory or tissue reparative factors. Early apoptosis of infused MSCs has also been shown to be involved in the immunomodulatory process. We and others have shown that intraarticular injection of mouse MSCs into osteoarthritic joints modulates the diseased environment through attenuation of inflammatory processes. However, low engraftment is also a feature after local cell injection. A small percentage of cells survive and, when labelled appropriately, can be retrieved from sham and osteoarthritic mouse joints using whole joint digestion and cell sorting. Retrieved MSCs formed colonies, proliferated ex vivo and showed differentiation capacity. Conditioned medium collected from the cells at early passages was capable of shifting the polarisation of macrophages towards an immunomodulatory state. Induction of apoptosis in parental MSCs in vitro also increased their immunosuppressive potential, with effects on both T-cells and macrophages, suggesting that apoptosis may contribute to therapeutic effects of MSCs in osteoarthritis. Molecular analyses of MSCs retrieved from osteoarthritic and sham-injected joints, and apoptotic MSCs was performed to highlight therapeutic factors involved in MSC modulation of osteoarthritis.



Mass production of core/shell nanofibers: Industrial perspective

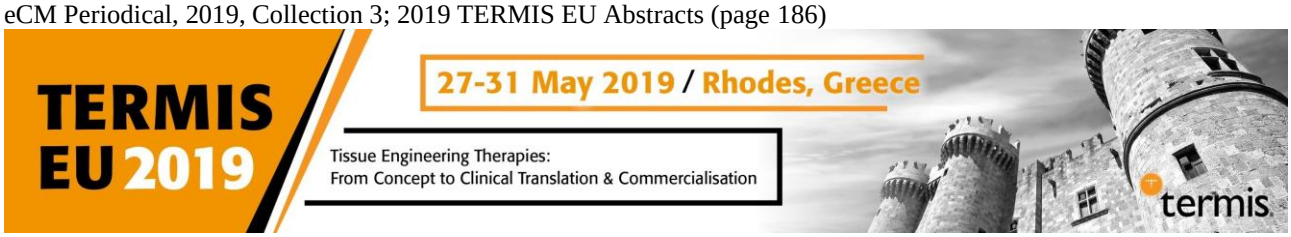
M. Buzgo[1,2]
Presenting Author: Matej Buzgo matej@inocure.cz
[1]InoCure s.r.o., Politických věznů 13, 110 00 Prague, Czech republic [2]Institute of Experimental Medicine, Academy of Sciences, v.v.i, Vídeňská 1083,142 20, Prague 4, Czech Republic

Coaxial nanofibers are among the most promising tissue engineering systems. However, the mass production of core/shell fiber was a challenge from industrial point of view. This contribution reviews the evolution of core/shell electrospinning methods for mass production and industrial application. **Needle based coaxial electrospinning:** The possibility to mimick ECM structure and drug release properties enables stimulation of cells and accelerates tissue regeneration. Needle based core/shell spinning methods were used for encapsulation of wide range of active molecules in TERM field. **Needleless coaxial electrospinning:** Classical needle based coaxial spinneret was modified to enable increase of production. The systems based on pyramid electrode [1], linear cleft electrode [2] and two-chamber electrode [3]. The systems had increaded production capacity. The industrial application is hampered by complex formation of core/shell layers, low stability of process and decreased homogeneity. **Needleless emulsion electrospinning:** In order to enable high-throughput development of core/shell fibers with high quality, we have focused on emulsion spinning systems. They could be used for formation of core/shell fibers from immiscible polymeric liquids (i.e. water-oil, oil-water emulsions) [4]. In the contribution we present results from industrial up-scaling of technology and testing of reproducibility, stability and applicability for use in TERM. Needleless emulsion technology is a viable system for production of industrial-grade core/shell fibers and might be used for drug delivery system development in TERM.

ACKNOWLEDGEMENTS: H2020 NanoBAT (691061), NanoFEED (778098) and IP-OSTEO (824007).

REFERENCES

[1] Jiang G. et al. Mat. Lett, 2013, (106): 56-58
[2] Yan, X. et al., PLOS One, 2015 (10): e0125407
[3] Vyslouzilove L. et al., Int.J.Pharm.,2017,516 (1-2):293-300
[2] Buzgo M. et al., JTRM, 2018, 12 (3):584-597
[3] Buzgo M. et al.,RCS Adv,2017,7(3):1215-28.



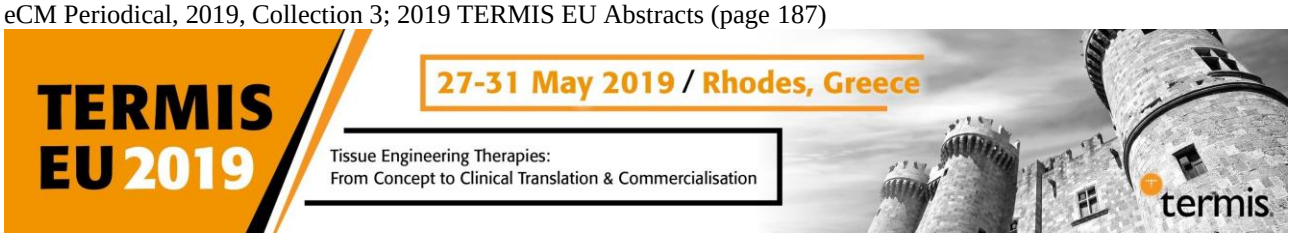
Co-cultured complex 3D miniaturized and vascularized models for metastatic processes and drug investigations

M. Moretti[1,2,3]
Presenting Author: Matteo Moretti, Matteo.moretti@eoc.ch
[1]Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Lugano, Switzerland [2]Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy [3]Fondazione Cardiocentro Ticino, Lugano, Switzerland.

Metastases of a primary tumor develop in specific organs depending on the tumor itself, e.g. breast cancer metastasizes preferentially to bone but not to muscle, through a multi-step process culminating with cancer cells (bCCs) extravasation through the endothelium. To study such a complex phenomenon and to find drugs potentially limiting its occurrence, present models are not adequate. In particular, in vivo models are limited by species-specific differences in biological mechanisms and in vitro models presently fail to mimic the complex microarchitecture and multi-cellular organization of native tissues. In this context, we generated miniaturized tissue-specific vascularized models based on human primary cells [1,2] and exploited them for drug evaluations. Microfluidic models were based on HUVECs embedded in fibrin gel, which formed perfusable microvascular networks (MVNs) during culture in the chip. We developed both bone and muscle mimicking chips, that were then used to study bCCs extravasation. We also investigated the role of platelets and neutrophils in the metastatic process and the effects of platelet □2b□3 integrin inhibition through a clinically used drug (Eptifibatide). Moving to meso-scale contructs, we generated a bone-remodeling model, based on HUVECs embedded in a fibrin gel with human primary osteoblasts, osteoclasts and macrophages, containing hydroxyapatite nanoparticles to mimic the mineral part of the bone. A muscle-like model was created through human myoblasts and muscle fibroblasts with vascular cells in fibrin gel. The meso-scale models were used to investigate bCCs proliferation and migration in different tissue models and the effects of a known anti-proliferative drug (rapamycin) on both bCCs and vascular cells. Using the microfluidic chip, we were able to monitor bCCs extravasation through the microvascular network in both bone and muscle-like environments. Furthermore, the presence of neutrophils and platelets increased bCCs extravasation whilst the addition of □2b□3 inhibitor reduced extravasation, by increasing anti-tumor effects of neutrophils and strengthening HUVECs junctions through decreased VE cadherin nuclear translocation. Within our vascularized mesoscale models, MVNs were found to be more developed in bone than in muscle tissue, and even more in the presence of macrophages. Nanoparticle presence did not affect microvascular network formation, allowing to increase both osteoblastic and osteoclastic activity. Seeding of bCCs in the bone and muscle models decreased significantly their growth in the muscle as compared to the bone model. Finally, addition of rapamycin to the system reduced also bCCs proliferation and affected vascular network organization in a dose-dependent manner. In conclusion, the use of our vascularized 3D human in vitro models allowed to better mimick the complexity and heterogeneity of human physiological systems. In particular, the generation of perfusable vascularized networks and the use of multiple cell types co-cultures enabled to recapitulate organ-specific bCCs metastatic processes from extravasation to invasion and proliferation. Finally letting us use these complex miniaturized human models to evaluate possible biological system responses to known drugs for anti-tumor purposes in an unprecedented manner.

REFERENCES

[1] Jeon J, et al. PNAS, 112, 214-9, 2015
[2] Bongio M, et al. Nanomedicine (Lond), 11,1073-91, 2016

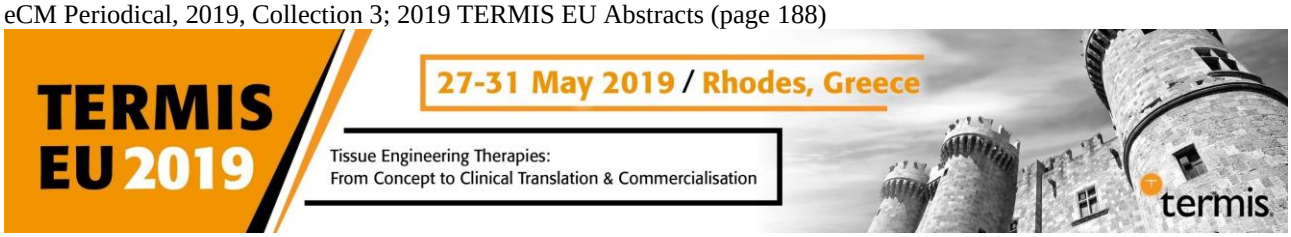


Synthetic basement membrane analogues induce the formation of 3D tissue-like structures in 2D cultures

M. Santin
Presenting author: Matteo Santin, m.santin@brighton.ac.uk
Centre for Regenerative Medicine and Devices, School of Pharmacy and Biomolecular Sciences, University of Brighton, Huxley Building Lewes Road, Brighton BN2 4GJ, UK

The basement membrane (BM) is a specialised form of extracellular matrix that provides the structural support and the phenotypic control of various types of cells including vascular cells, epithelial cells and mesenchymal stem cells (MSCs). These functions are fulfilled through its various proteins; among them Collagen Type IV and laminin. The present paper shows the ability of a novel class of biomimetic biomaterials substrates, PhenoDrive, to mimic the mesh structure of Collagen Type IV while presenting bioligands of the laminin in an orderly spaced manner. Co-cultures of various cell types with human umbelical vascular endothelial cells (HUVEC) at a typical ration of 40/60 were comparatively studied on Matrigel-coated plasticware and PhenoDrive-coated plasticware. Morphological studies of co-cultures of either MSCs or human beta cells or hepatocytes or renal cells and neuroglial cells with HUVEC on Phenodrive showed the formation of an endothelial sprouting more consolidated than that observed on Matrigel with the respective tissue cells being preferentially organised into 3D structures resembling a vascularised tissue originating from the endothelial sprouting crossing points. When the tested tissue cells, of primary origin or cell lines, were co-cultured with HUVEC on PhenoDrive, they expressed higher levels of their phenotypic markers and acquired improved paracrine activities. Such tissue engineering constructs has potential for clinical applications as well as organotypic cultures for the testing of novel drugs and nanomedicines.

ACKNOWLEDGEMENTS: The EC HORIZON 2020 project REFINE, H2020-NMBP-2016-017/H2020-NMBP-2017-two-stage contract n. 761104. The EC FP7 project NEXT, contract n. HEALTH.2013.1.3-2-602235**.** PhenoDrive-Y has kindly been donated by Tissue Click Ltd, UK.

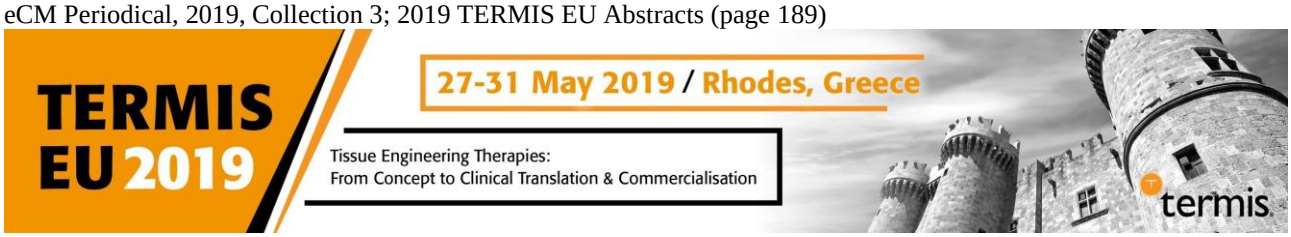


Development of dynamic polymeric hydrogels for tissue engineering

M.B. Baker[1]
Presenting Author: Matthew Baker, m.baker@maastrichtuniversity.nl
[1]Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

Tissue engineering and regenerative medicine require the development of artificial environments to provide a niche for new tissue. A quintessential source of inspiration, the native extracellular matrix (ECM). Made from a complex mixture of proteinaceous macromolecules, non-covalent and dynamic interactions allow for unique mechanical, responsive, and biochemical functions. Inspired by these dynamic interactions, our lab focuses on utilizing supramolecular interactions and dynamic covalent bonds to create hydrogels with inherent dynamics and reversibility. During this talk, I will tell the story of development behind dynamic hydrogels, going from an idea on paper to a material to a cell-culture platform. These hydrogels can be rationally controlled in terms of stiffness and bioactivity, yet these dynamic hydrogels impart several unique properties including self-healing, viscoelasticity, modular biofunctionalization, and stress-relaxation. Consequently, they can provide both a biomimetic environment and a tool for exploring fundamental biomaterial development. Due in part to their uniquely dynamic networks, these gels have found initial success in applications ranging from simple cell encapsulation to 3D bioprinting to spheroid encapsulation. In a majority of cases, the dynamics of the network can be tuned to control not only the suitability of the material for its application, but also the way in which cells respond to the material.

ACKNOWLEDGEMENTS: Financial support was received from the Netherlands Organization for Scientific Research, (731.016.202 “DynAM”), the Province of Limburg, Brightlands Materials Center, and RegMedXB.



Engineered fibre and gel composite materials as potential bioinks

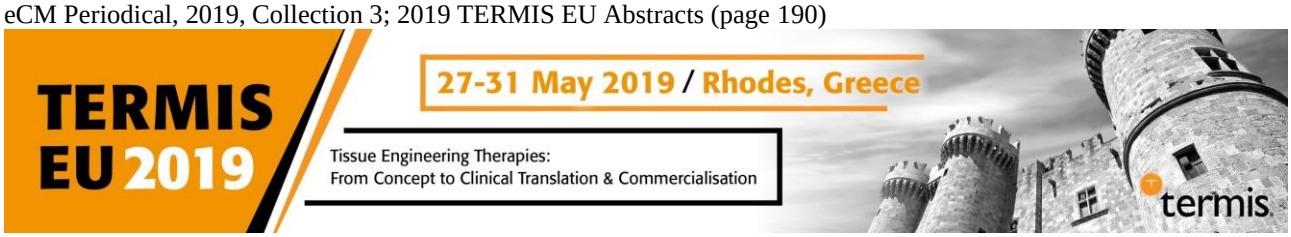
M. N. Collins [1,2]
Presenting Author: Maurice N Collins, maurice.collins@ul.ie
[1]School of Engineering, Bernal Institute, University of Limerick, Ireland. [2]Health Research Institute, University of Limerick, Ireland

3D printing is a well-established technology across numerous fields. It has attracted attention in biomedical engineering due to its ability to produce well-defined structures in three dimensions at high spatial resolution. While 3D bioprinting builds on these principles and offers the potential to create 3D functional tissues with complex and hierarchical structures through the controlled assembly of biocompatible materials in conjunction with living cells. Cells and biological components are encapsulated in a hydrogel matrix, which collectively is known as the bioink allowing extracellular matrices and cells to be deposited with high precision mimicking the actual cellular arrangement of the tissue it is replicating. Acellular inks are also being developed to print complex porous scaffolds which can be later seeded with cells [1]. However, in order to realise the potential of bioprinting there is an urgent requirement to develop bioinks for a range of potential applications. This lecture presents some initial printing trials for recently developed acellular inks which are capable of being printed in relatively cheap commercially available printers. The systems discussed include: pneumatic micro-extrusion utilising subsequent visible light curing (gelatin methacrylate, Methacrylated Hyaluronan), thermally extruded (PolyLactic acid, polycaprolactone) and their combinations with novel nanofibre reinforcement and extracellular molecules (Chondroitin Sulphate). The concept of self-assembling bioinks through novel thiol coupling will be introduced. Particular attention will be paid to print optimisation using viscometric techniques and crosslinking optimisation to ensure structural integrity. Initial biological assessment of the developed inks will also be discussed taking the meniscus as a potential application while providing an outlook for these materials as future bioinks.

ACKNOWLEDGEMENTS: Maurice N Collins acknowledges received funding from the BioBased Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No 720707

REFERENCES

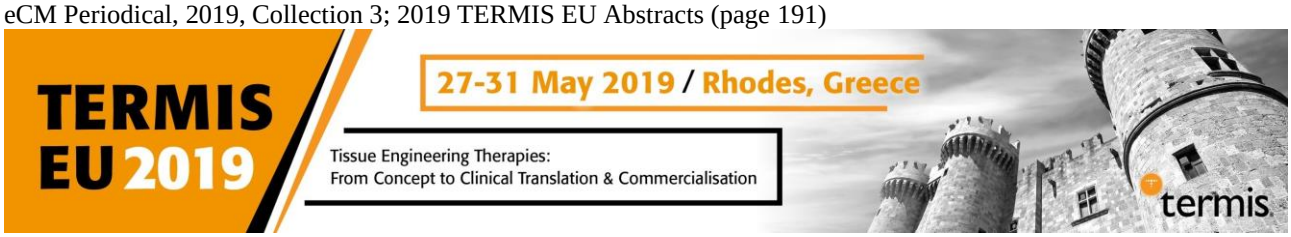
[1] Murphy C et al. Appl. Mater. Today 2018; 12:51-71



Hyaluronan hydrogel platform for musculoskeletal regeneration

M. Alini
Presenting Author: Mauro Alini, mauro.alini@aofoundation.org
AO Research Institute, Musculoskeletal Regeneration Program, Davos Platz, Switzerland

HA is a key extracellular matrix component involved in tissue development and regeneration, cell motility and viscoelasticity. HA is commonly employed for fabricating drug delivery and regenerative medicine constructs. Using an unconventional conjugation method, here we present a series of derivatives providing HA with tunable pseudoplasticity, temperature-induced gelation and dual enzymatic/light-induced gelation. HA was grafted via DMTMM-mediated amidation with: 1. an array of substrates for validating the method; 2. short alkyl chains; 3. Thermo-responsive moieties; 4. tyramine. Products were characterized via rheology, ^{1}H-NMR and UV-vis spectrophotometry. Reaction kinetics and coupling yields were determined under a spectrum of conditions. The thermo-responsive derivative was employed to deliver chemokines in vivo. The HA-tyramine derivative was optimized for additive manufacturing with a dual enzymatic and light gelation. The thermo-responsive derivative was employed for in situ tissue engineering. We found increased cell density at one week within a rabbit osteochondral defect upon the delivery of chemokines compared to chemokine-free gel. This approach is particularly attractive as it may facilitate endogenous cell recruitment, eliminating the need of tissue harvesting and cell expansion. The use of HA-tyramine for AM revealed that: the dual gelation allowed good extrusion properties; cells can attach and spread; to obtain gelation, a low concentration of polymer and low degree of substitutions can be used. Furthermore, this approach negates the use of UV light, eliminating safety concerns regarding UV-induced DNA damage and tumorigenesis. We have demonstrated how chemical modification can improve properties of HA including viscoelastic profile, drug delivery and 3D printing capability.



Designing biosynthetic corneal implants for clinical application

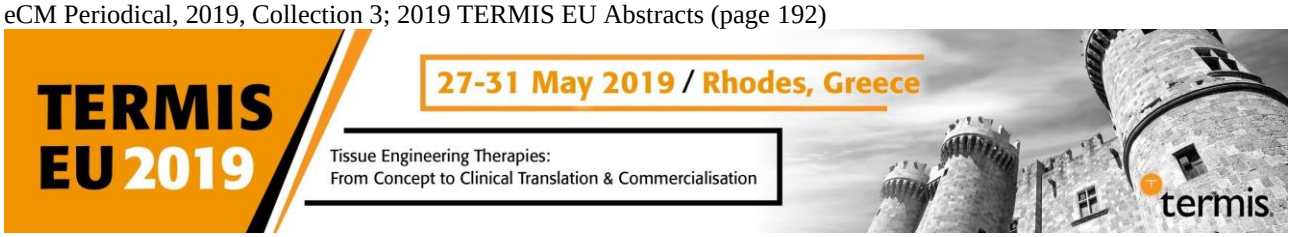
M. Griffith[1,2]
Presenting Author: May Griffith, May.Griffith@umontreal.ca
[1]Department of Ophthalmology & Maisonneuve-Rosemont Hospital Research Centre, University of Montreal, Montreal, Canada

Corneal blindness affects 23 million individuals worldwide [1]. An estimated 12.7 million individuals await transplantation with only 1 in 70 being treated due to a severe dearth of donor human corneas [2]. Artificial corneas have been developed as prostheses but the non-reversible surgical implantation and side effects have necessitated their use only as last resorts [3]. A range of stem cell-based techniques including expansion and grafting of epithelium [4], stroma [5] and endothelium [6] have successfully tested in clinical and pre-clinical trials. In addition, self-assembled scaffold-free techniques have successfully produced tissue equivalents but requires significant time or a cleanroom operating to Good Manufacturing Practice guidelines for advanced therapy medicinal products (ATMPs). For clinical application, particularly in low to medium income countries where majority of corneal blind patients are located, mass produced, off-the-shelf implants are therefore desirable. As such, a cell-free, full synthetic low-cost implant that can be easily customized is advantageous. Our team has developed cell-free implants comprising recombinant human collagen (RHC) and tested them successfully in first-in-human clinical trials [7-8]. The use of RHC was planned at the outset to avoid possible batch-to-batch heterogeneity, pathogen transmission [9] and severe allergies that can occur [10] with xenogeneic animal collagen sources. For patients with inflammation and severe pathologies, a synthetic phosphorylcholine-mimicking lipid with inflammation suppressing properties was incorporated leading to stable regeneration of the cornea in patients at high risk of rejecting conventional donor corneal transplantation [8]. However, for scale-up, full-length RHC is expensive to produce. Hence, short collagen-like peptides (CLP) conjugated to mechanically tough polymers, and shorter self-assembling artificial collagens have been developed by our team and others. An example of a CLP-based collagen analog in the corneas of mini-pigs has shown that such analogs can be functional equivalent to RHC-based implants. We have since tested several CLPs for potential use as implants. The use of CLPs and the design of implants to ensure immune compatibility will be discussed.

ACKNOWLEDGEMENTS: Financial support was received from an Indo-Sweden DBT-Vinnova grant, a Canadian Institutes of Health Research grant (PJT 155924), and the Caroline Durand Foundation Chair in Cellular Therapy, University of Montreal and the Maisonneuve-Rosemont Hospital Research Centre.

REFERENCES

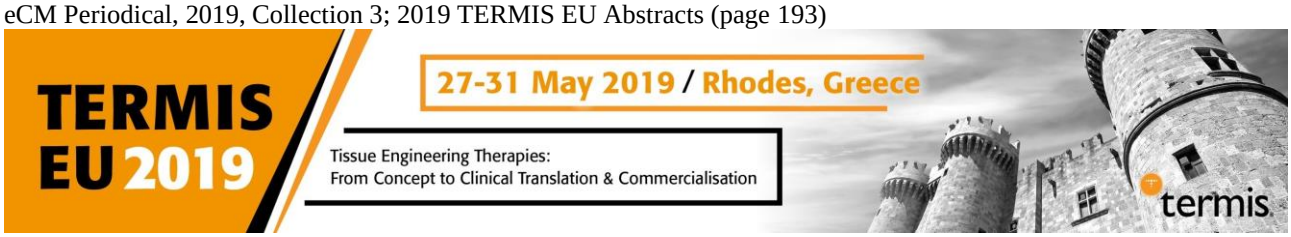
[1] Oliva MS et al. Indian J Ophthalmol 2012; 60: 423-7
[2] Gain P et al. JAMA Ophthalmol 2016; 134 : 167-73
[3] Avadhanam VS & Liu CS. Br J Ophthalmol 2015; 99: 878-87
[4] Rama P et al. N Engl J Med 2010; 363:147-55
[5] Basu S et al. Sci Transl Med. 2014 6(266):266ra172.
[6] Okumura N et al. Cornea 2018 37(9):1081-86
[7] Fagerholm P et al. Science Transl Med 2010; 2: 46ra61
[8] Islam MM et al. npj Regen Med 2018; 3 (2): doi:10.1038/s41536-017-0038-8
[9] Silvipriya KS et al. J Appl Pharm Sci 2015; 5: 123-7
[10] Mullins RJ et al. Aust NZ J Ophthalmol 1996; 24(3): 257-60



Will chondroinductive materials revolutionize cartilage regeneration?

M. Detamore
Presenting Author: Michael Detamore, detamore@ou.edu
The University of Oklahoma, Carson Engineering Center, Norman, OK, USA

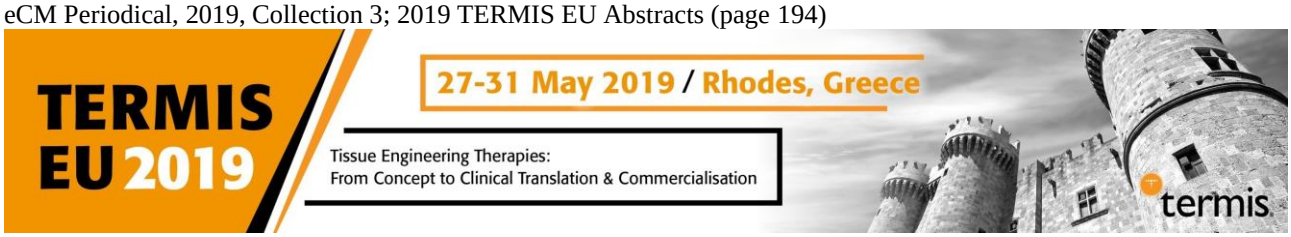
One of the core tenets of our philosophy for tissue regeneration include the use of "raw materials," where biomaterials themselves serve as both building blocks and bioactive signals. In recent years, a few groups around the world have gravitated toward cartilage matrix as a potentially chondroinductive material for cartilage regeneration. The major challenge to date in cartilage injury has been creating a biomaterial-only strategy that is capable of regenerating true hyaline-like cartilage without the addition of growth factors or exogenous cells. In the past few years, we have focused our efforts on establishing chondroinductivity in vitro, and in developing new materials synthesis strategies to provide ease of application for orthopedic surgeons in the operating room. By leveraging nanotechnology, we have developed a paste-like material constructed from cartilage matrix with encouraging mechanical performance post-crosslinking, and which avoids contraction after extended time. Looking to the future, we are working on next-generation approaches to chondroinductive materials. We have encouraging preliminary data which suggest the possibility of a chondroinductive response to a novel peptide sequence in vitro, which may be enhanced by simultaneous inclusion of adhesion peptides. Initial in vivo data in regeneration of rabbit femoral condyle cartilage defects may suggest promising regenerative capabilities with hydrogels based on these peptides. If indeed chondroinductive materials exist, and if they can be delivered easily, are safe, and can be provided at reasonable cost and with a reasonable regulatory strategy, chondroinductive materials may hold the potential to revolutionize cartilage regeneration.



Mechanobiology revisited: From phenomena to design of the biomaterial-tissue interface

M. Gasik
Presenting Author: Michael Gasik, michael.gasik@aalto.fi
School of Chemical Engineering, Aalto University Foundation, Espoo, Finland; Seqvera Ltd., Helsinki, Finland

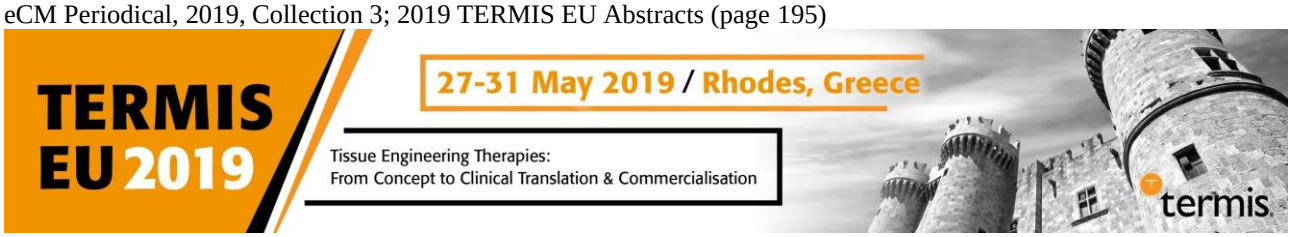
Tissue engineering applications often rely on biomaterials as scaffolds, substrates or implants assisting in regeneration. It is now widely acknowledged that there are no "inert" biomaterials and all the features like surface parameters, porosity, chemical and biochemical interactions play critical role in the tissue-biomaterial interface formation, quality, and development. Cells seeded in traditional in vitro cultures often seen losing their phenotype, function and therapeutic potential. The expansion to 3D and more complex cultures requires a better understanding of biomaterial-related microenvironment modulators (e.g. surface topography, substrate rigidity, mechanical stimulation) to control cell function in vitro. One of the topics of interest is to measure, guide and predict the effect of modulation of biomaterial surface and applied mechanical stimuli on realistic fluids exchange and mechano-transduction on cell and sub-cell levels. Here we discuss these phenomena on two major cases: auto-mechano-transduction caused by cell cultures themselves (without explicitly applied external mechanical and biochemical stimuli) and mechano-transduction for cells seeded on specific scaffolds. The possibilities of utilizing multiplexing signalling are presented for a translation to cartilage regeneration engineering and some other applications. The benefits and limitations of the mechanobiology applications for these cases is also discussed.



Hybrid electroconductive platforms for the generation of mature cardiac organoids

M. Monaghan
Presenting Author: Michael Monaghan, MONAGHMI@tcd.ie
Trinity College Dublin, the University of Dublin, Ireland

Conductive polymers have emerged as a class of smart biomaterials that can harness flexibility and facile manipulation with intrinsic conductivity that can enable electrostimulation. Amongst such materials, poly (3, 4-ethylenedioxythiophene) (PEDOT) exhibits appropriate stability, biocompatibility and conductivity and is usually combined with polystyrene sulfonate (PSS) as primary dopant, rendering it highly soluble in aqueous environments and other polar solvents. Modification with crosslinkers and secondary dopants can provide an appropriate degradation resistance and high conductivity without compromising the biocompatibility of the material. To this end, our group has achieved the fabrication and optimization of 3D scaffolds of PEDOT: PSS crosslinked with a novel PEGylated moiety that can possesses suitable electroconductive, mechanical and biocompatibility properties in tissue engineering applications. This smart biomaterial has also been tailored to act as a reservoir in non-viral gene therapy applications. Using lyophilization approaches we achieve scaffolds with defined pore geometries and eccentricities, whereby rat neonatal cardiomyocytes and human IPS derived pre-cardiomyocytes have been seeded and matured using mild pulsatile electric field stimulation we achieve robust organized cardiac organoids.



Macromolecular crowding to tune microenvironmental ECM architecture: From ultraflat 3D to bioprinted structure

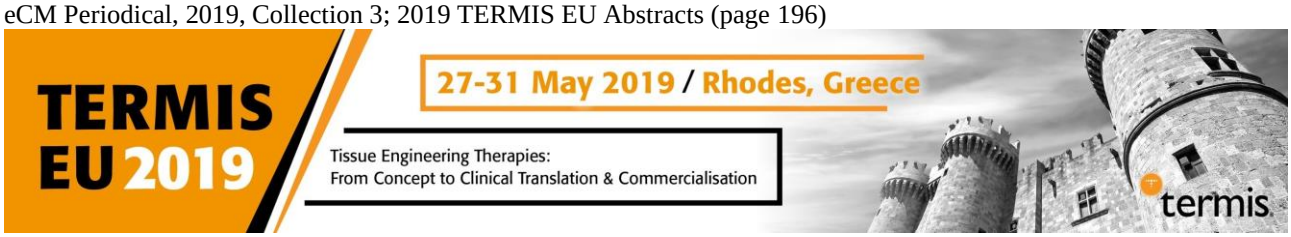
M. Raghunath[1,2]
Presenting Author: Michael Raghunath, ragh@zhaw.ch
[1]Center for Cell Biology and Tissue Engineering [2]Competence Center Tissue Engineering for Drug Development (TEDD), Zurich University of Applied Sciences, Switzerland

The extracellular matrix (ECM) arose with the evolution of multicellular organisms and their needs to contain cells into tissue compartments and organize them by function. Thus, the ECM is the microenvironment of differentiated or progenitor cells, including the fabled stem cell niches. Of note, these microenvironments are built by the cells themselves, either by those residing locally in a given tissue, or by cells that once have been there to do the job. The importance of the cellular microenvironment in controlling cell behavior and fate has been gaining attention in the fields of tissue engineering, regenerative medicine, and mechanobiology. This interest is reflected by intensive efforts to design and sell artificial or semiartificial microenvironments with the aim to mimic native ECM. During these activities, it has become apparently forgotten that cells have the intrinsic capability to make their own specific ECM in which they reside prior to their extraction for cell culture. However, standard culture conditions are highly aqueous, thus impairing efficient ECM deposition. For example, the deposition of collagen is dependent on the swift proteolytic cleavage of N- and C- propeptides from procollagen the very moment it is secreted into the extracellular space. Only then collagen triplehelices can assemble to form nascent fibrils and thus an insoluble scaffold around cells. Aqueous conditions make this and other enzymatic steps inefficient and tardy. This can be overcome by introducing macromolecular crowding (MMC) into the culture medium of monolayer cultures or hydrogels [1]. A variety of enzymatic steps is substantially accelerated, not only procollagen conversion, but also crosslinking and remodeling. Even more powerful is mixed MMC, the combination of two or more crowders with different molecular weights [2]. Crowders we use in this context are carbohydrate-based polymers of glucose (dextran), or sucrose (Ficoll). The resulting excluded volume effects restores some physiology in the system by accelerating enzymatic key steps in ECM formation, and by driving the supramolecular assembly of ECM components such as fibronectin, collagen and fibrillin. Thus, MMC empowers cells to construct rich microenvironments comprising a multitude of structural components and associated ligands and stored growth factors. Two years after we published the Scar in a Jar [3], a platform to identify anti-scarring agents, it was adopted by the pharma industry. Using MMC, we could empower human bone marrow-derived stem cells to build their own ultra-flat 3D ECM and thus unleashed a dormant brown fat differentiation potential. Interestingly, exposure of 3D skin models to MMC in the submerged phase boosts the formation of the dermo-epidermal junction as evident by the expression of collagen VII. Internationally adopted applications are the production of stem cell matrix, and tuning the microarchitecture of bioprinted collagen hydrogels. This bodes well for the application of MMC also in the framework of bioprinting to mature printed tissues

ACKNOWLEDGEMENTS: Financial support from Biotechnet (NTN Swiss Biotech) and the Zurich University of Applied Sciences (ZHAW) is gratefully acknowledged.

REFERENCES

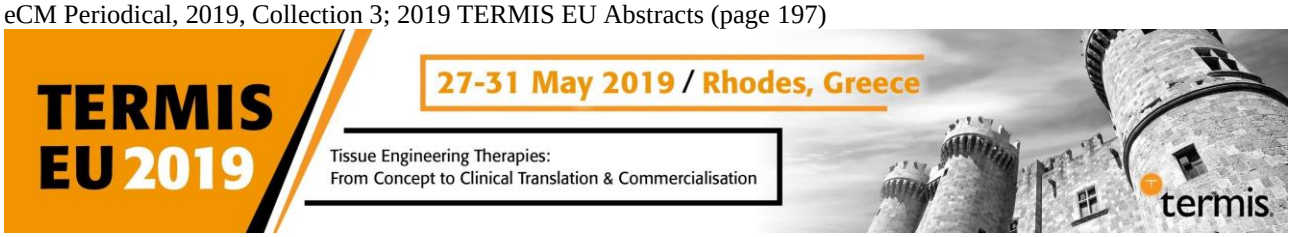
[1] Chen C et al. Adv. Drug Deliv. Rev. 2011, 63(4-5):277-90
[2] Dewavrin J-Y et al. J. Phys. Chem. B 2015, 119 (12):4350-8
[3] Chen et al. Brit. J. Pharmacol. 2009, 158(5):1196-209.



Advanced tissue manufacturing strategies and applications in soft tissue repair

M.D. Castilho[1,2]
Presenting Author: Miguel Dias Castilho, M.DiasCastilho@umcutrecht.nl
[1]Department of Orthopaedics, University Medical Center Utrecht, the Netherlands [2]Department of Biomedical Engineering, Eindhoven University of Technology, the Netherlands

(Bio) fabrication technologies are evolving rapidly, focusing on one of the main current challenges in tissue engineering (TE); the creation of support materials at the multi-scale that could elicit both an optimal cell behavior and an adequate mechanical environment. Composite constructs composed of soft hydrogels reinforced with micro-fibre scaffolds obtained by a Melt-Electrospinning Writing (MEW) process has demonstrated great promise for developing tissue engineered constructs with compressive properties compatible to native tissues. Although, cell differentiation and abundant matrix formation was observed, these novel composite constructs are not able to address the interplay of compressive, shear, and tensile stresses that tissues in the human body are subjected to. Here, additional strategies for the design and fabrication of fibre-reinforcement are reviewed. Also, new insights into the real structural deformation and reinforcement mechanism of these novel reinforced hydrogels are presented.



Engineering protein- and polysaccharide-based bioinks for bioprinting of personalised implants

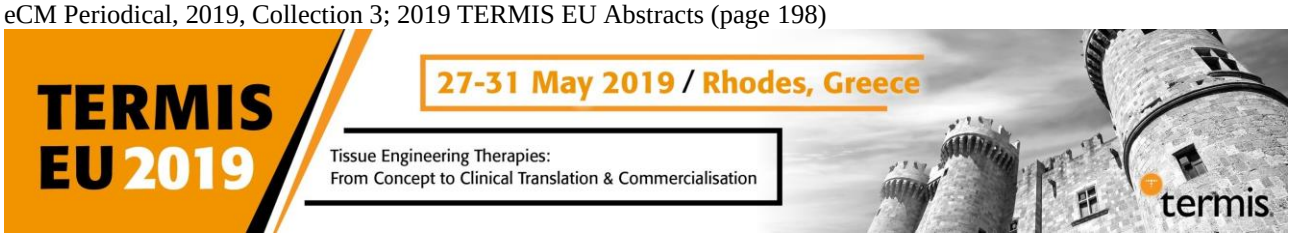
J.M. Oliveira[1,2,3]
Presenting Author: J. Miguel Oliveira, miguel.oliveira@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, AvePark, 4805-017 Barco, Guimarães, Portugal

The combination of reverse engineering, 3D printing and novel mimetic bioinks can be the perfect solution to address many important challenges that we still face in the fields of musculoskeletal tissue engineering and personalized medicine. This lecture overviews our recent advances in the development of protein- and polysaccharide-based biomaterials and bioinks of natural origin. A fast setting enzymatic-crosslinked silk fibroin (SF) bioink has been developed allowing the production of patient-specific implants [1], including human meniscus implant and human intervertebral disc. The tyrosine groups (5 mol%) present in silk fibroin allowed obtaining a hydrogel formation through enzymatic crosslinking. Horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) were used and an optimization of the Enzyme/oxidizer ratio was performed to obtain a good printability. These bioinks presented good reliability, and the produced scaffolds showed promising features in terms of biocompatibility and memory-shape properties. We also recently developed Gellan gum-based bioinks that can be ionically-crosslinked [2] for tissue engineering and 3D printing of implants that can respect the patient anatomy. In brief, an novel generation of natural-based bioinks were developed for biofabrication of patient-specific and memory-shape implants aimed at finding applications in personalized medicine.

ACKNOWLEDGEMENTS: The FCT distinction attributed to J. Miguel Oliveira under the Investigator FCT program (IF/01285/2015) is greatly acknowledged.

REFERENCES

[1] Costa JB et al, Adv. Healthcare Mater., 2017, 6(22), 1701021.
[2] Silva-Correia J et al., J. Biomedical Mater. Res. Part A, 2013, 101(12):3438-3446



Controlling the immune system to promote tissue regeneration – From inflammatory cytokines to regulatory T cells

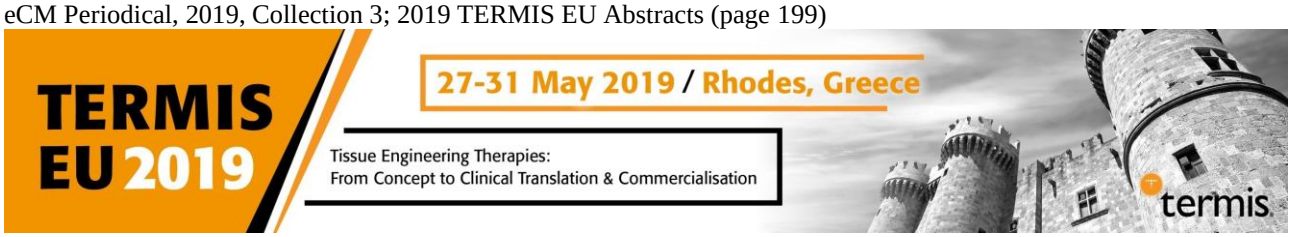
M.M. Martino[1]
Presenting Author: Mikaël Martino, mikael.martino@monash.edu
[1]European Molecular Biology Laboratory (EMBL) Australia, Australian Regenerative Medicine Institute, Melbourne, Australia

Although it is now well recognized that the tissue healing process is tightly regulated by the immune system, we still have little understanding of the key cellular and molecular mechanisms involved. Therefore, great efforts are required to reveal how immunity governs tissue regeneration, in order to design better regenerative strategies [1]. The role of inflammation driven by inflammatory immune cells and cytokines has been explored to some extent. For instance, we found that macrophage-derived interleukin (IL)-1β negatively regulate bone regeneration via inhibiting the regenerative function of mesenchymal stem cells (MSCs) and osteoblasts [2]. Based on these findings we engineered a stem cell delivery system based on a fibrin matrix which integrate an inhibitor of IL-1β signaling. Using this approach in a mouse model, we could significantly improve MSC-driven bone regeneration. More recently, we found that IL-1β also inhibits tissue repair and regeneration driven by growth factors. For instance, the inflammatory cytokine inhibits the signaling of bone morphogenetic protein-2 and platelet-derived growth factor-BB during bone regeneration. In addition, wound healing in diabetic mouse is impaired by an imbalance between IL-1β and IL-1 receptor antagonist (IL-1Ra). To overcome the negative effect of IL-1β in various tissues, we have engineered an IL-1Ra variant that displays very high affinity for the endogenous extracellular matrix (ECM). Using the engineered IL-1Ra we could significantly improve bone regeneration and diabetic wound healing in mouse models. In addition to the innate immune system, we also focus on how the adaptive immune system – in particular T cells – controls tissue repair and regeneration. Using various adoptive transfer and T cell depletion models in the mouse, we found that regulatory T cells (Tregs) are critical for skin, bone and muscle healing. Very interestingly, Tregs seem to promote tissue repair and regeneration in a tissue-specific manner not only via immune regulation (anti-inflammatory cytokines) but also by directly promoting morphogenesis. Based on these findings, we engineered various systems aiming at promoting accumulation of Tregs at sites of tissue injury and we could demonstrate that those strategies greatly improve tissue regeneration in mouse models of skin, bone and muscle. For instance, we have engineered the signaling of a novel Treg-derived cytokine to improve its capacity to support Treg migration and immunoregulatory function. Delivering the engineered cytokine at sites of injury in wild-type mice is able promote accumulation of naturally occurring Tregs, which induce tissue regeneration. Overall, designing strategies that control both the innate and adaptive immune systems holds great promises for regenerative medicine applications – in particular, novel systems which harness the pro-regenerative function of T cells.

ACKNOWLEDGEMENTS: Financial support was received from the Australian Research Council (DE170100398) and the National Health and Medical Research Council (APP1140229). The Australian Regenerative Medicine Institute is supported by grants from the State Government of Victoria and the Australian Government.

REFERENCES

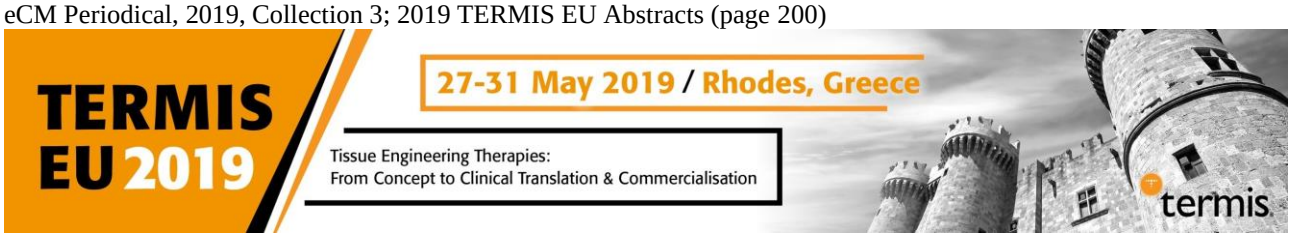
[1] Julier Z et al. Acta Biomaterialia. 2017;53:13-28
[2] Martino M.M. et al. Nature Communications. 2016;7:11051



Small animal models in bone regeneration research

N. Weissenbacher[1,2]
Presenting Author: Nadja Weissenbacher, nadja.weissenbacher@pmu.ac.at
[1]Institute of Tendon and Bone Regeneration, Spinal Cord Injury and Tissue Regeneration Center, Paracelsus Medical University, Salzburg, Austria [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria

For understanding basic mechanisms of bone healing as well as for the development of novel treatment strategies, animal models of bone defects are needed. The choice, which model to use is crucial for any successful research attempt. To study biological properties of implants, particularly regarding osteoinductivity, subcutaneous implantation models are used, allowing large numbers of test items to be screened. More complex models are required to simulate healing in a clinically relevant setting. Simple osteotomies, segmental bone defects of increasing size up to critical size are under investigation. Bone defects can be created in a number of locations: to reflect intramembranous ossification, defects in the cranium may be chosen, whereas to investigate endochondral ossification, lesions in long bones are used. The healing process following a lesion in a small animal strongly depends on the defect model and the animal species and strain chosen. In many models of long bone defects, fracture fixation is required to allow the animal to use the injured limb. No fracture fixation is required for defects in the cranium, in some species in the radius, the flat pelvic bones and the ribs. For choosing the optimum site for a bone defect, several aspects need to be considered: the mechanical load the bone needs to bear , the predominant mechanism of ossification in the bone chosen (intramembranous like in the calvarium or endochondral like in extremities), or if cortical or cancellous bones are to be used. The duration of bone healing depends on the distance of the bone ends, on the degree of mobilization or distraction, on vascular supply, and potential infection. Various rodent strains with a wide array of genetic attributes or defects are available. For the investigation of fracture repair, the femur of rodents is the most commonly used model. Both internal and external fracture fixation devices are available for stabilization, thus allowing experimental animals to maintain function of their limbs. Within studies and amongst published results commercially existing implants obviate the need of experimental implant design and help reducing the associated inconsistency of data. Other than large animal models, which are more costly but resemble more the situation in human patients, small animal models allow larger experimental approaches and offer the use of a wide array of molecular screening tools.



Collagen scaffolds with sustained insulin release and controlled pore structure for tissue engineering

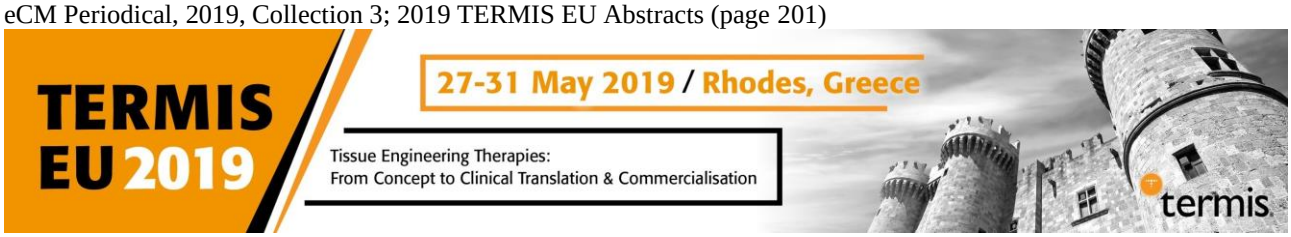
N. Kawazoe
Presenting Author: Naoki Kawazoe, Kawazoe.Naoki@nims.go.jp
Research Center for Functional Materials, National Institute for Materials Science, Tsukuba, Japan

Numerous studies have been conducted on the design and preparation of porous scaffolds for regeneration of various tissues such as skin, cartilage, and bone. In particular, controlled and local release of bioactive molecules like growth factors from porous scaffolds can provide an effective strategy to control the complex and dynamic regulation of cellular processes such as cell proliferation and differentiation in a three-dimensional microenvironment and, as a result, to regenerate functional tissues. In the present study, hybrid scaffolds of biodegradable poly(lactic co- glycolic acid) (PLGA) microbeads and collagen were prepared by embedding insulin-loaded PLGA microbeads in collagen sponges. The hybrid scaffolds were cultured with human dermal fibroblasts to investigate the effect of locally released insulin on cell functions. The hybrid scaffolds were prepared as the following procedure. First, human insulin molecules were loaded into PLGA microbeads with a water-in-oil-in-water (w-o-w) double emulsion solvent evaporation method [1]. In the emulsification process, two different homogenization speeds (1,000 and 8,000 rpm) were used to control the microbead diameters. Each group of the resulting microbeads were mixed with collagen solution and pre-prepared ice particulates with diameters of 150 - 250 μm as a porogen [2]. The mixtures were freeze-dried and after then crosslinked via carbodiimide chemistry. The laser particle analysis demonstrated the two types of microbeads had diameters of 19.4 ± 1.6 μm (large) and 4.4 ± 0.9 μm (small), which were prepared at emulsification speeds of 1000 and 8000 rpm, respectively. The loading efficiency of insulin was 87.0 ± 2.0% and 85.1 ± 2.8% for the large and small size microbeads. The SEM observation confirmed that evenly distributed large pores ranging from 150 to 250 μm were formed in the hybrid scaffolds. The result indicated that the large pores replicated the same morphology and diameter range as the ice particulates. The large pores were interconnected, which is beneficial for cell penetration and distribution. Microbeads were homogeneously distributed on the wall of the scaffolds. In vitro insulin release from the scaffolds was studied under shaking at 37°C. The scaffolds had a lower initial burst and a more prolonged insulin release than did the free microbeads. A low initial release of 3.3 ± 2.6% and 5.7 ± 2.9%, respectively, was obtained from the hybrid scaffolds incorporated with large and small microbeads. The hybrid scaffolds exhibited cumulative release of approximately 70% insulin for 4 weeks. The bioactivity of the locally released insulin was evaluated by in vitro culture of human neonatal dermal fibroblasts. The DNA assay indicated that insulin released from the hybrid scaffolds promoted cell proliferation more effectively than did the insulin supplemented in the culture medium as a control. In particular, the hybrid scaffold prepared with large microbeads had a more linear release profile of insulin and a higher promotion effect on cell proliferation than that with small microbeads. The hybrid scaffold should be useful for skin tissue engineering.

ACKNOWLEDGEMENTS: This work was supported in part by JSPS KAKENHI Grant Number 18K19945.

REFERENCES

[1] Cui F et al. J Control Release. 2005; 107:310-9
[2] Zhang Q et al. J Bioact Compat Polym. 2013;28:426-38.



Hands-on tissue engineering course for students from various educational backgrounds – A Fulbright-funded experience in Yerevan, Armenia

N. Sarvazyan
Presenting Author: Narine Sarvazyan, phynas@gwu.edu
George Washington University, School of Medicine and Health Sciences, Department of Pharmacology and Physiology, USA

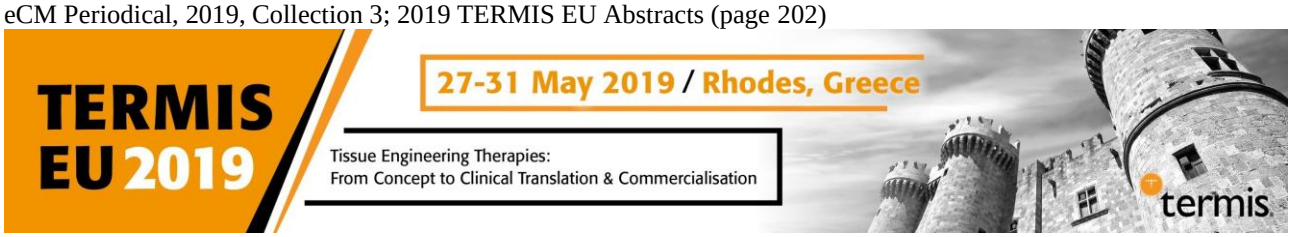
INTRODUCTION: The goal of this presentation is to share the author's educational experience conducting an introductory, hands-on course in tissue engineering in Yerevan, Armenia. The course aimed to spark students' interest in tissue engineering field giving them basic knowledge of field's principles and terminology. The experience was highly rated by the students and was complementary to their future careers including medicine, biology, physics or engineering.

METHODS: Multiple social media channels were used to inform potential applicants. The main goal of these wide advertisement efforts was to recruit an interdisciplinary group of students from any STEM field as well as of different career stages: from high school students to recent PhDs. Applicants were asked to submit their CV and a short letter of interest. During a follow-up interview, student's level of interest, time commitment, and his/her English language skills were tested. Twelve selected applicants were then organized into four interdisciplinary teams with the goal of forming maximally diverse teams. Two-hour long in-class mandatory sessions were held twice a week. Laboratory was available for any additional hours needed to perform team assignments between the sessions. During the last weeks of the course teams were given a complete freedom to create the simplest version of engineered tissue of their choice.

RESULTS & DISCUSSIONS: The structure of the course included a concise overview of main tissue engineering topics followed by demos and students exercises – all designed to be performed using minimal specialized equipment and supplies. The developed course can be conducted as a one-month intensive summer course, one-semester 4-5 credit hour course, or a two semester 2-3 credit hour course. Each section included a didactic and a practical part. During the didactic part, the instructor went over key material and answered any questions students had about it. This was followed by a demonstration that covered the basic steps of that week experiment. During the practical, hands-on part, students conducted experiments of their own with the instructor serving only an advisory role. During the last three weeks of the course teams were given a task to put together a simple prototype of their tissue of choice. During 2017 course, students decided to create tissue engineered versions of heart, liver, cartilage and skin. Course ended with teams presenting their efforts to general public using oral and poster presentations.

CONCLUSIONS: The course was extremely well perceived by the students and interested public. To help spreading interest in tissue engineering throughout Armenia and the Caucasus, participants of the course were asked to pick their favorite exercise and to write a draft of the experimental protocol in their native language. After editing and corrections, a handbook of tissue engineering protocols was then created to help conduct similar courses in the language of the host country.

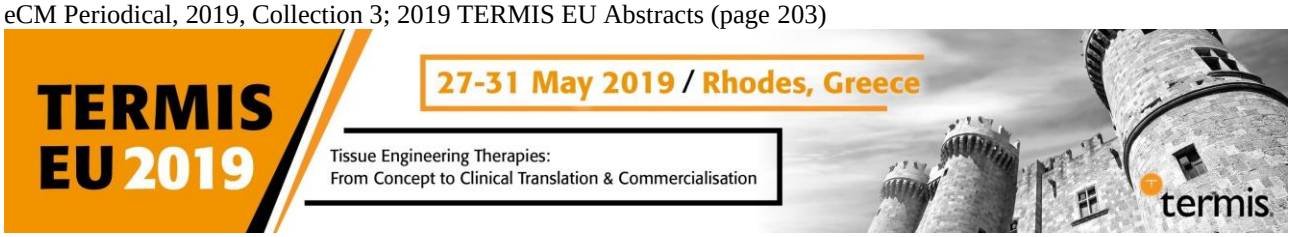
ACKNOWLEDGEMENTS: Financial support from the United States State Department is gratefully acknowledged (Fulbright US Scholar Award to N. Sarvazyan). Author also want to thank the scientific staff and administration of the Institute of Physiology after L.A. Orbeli, National Academy of Sciences of the Republic of Armenia for hosting the course.



Transforming scaffold designs from university to industry – A bench-to-bedside perspective

N. Khani[1]
Presenting Author: Navid Khani, Navid.khani@bellaseno.com
[1]BellaSeno GmbH, Leipzig, Germany

Convey scaffold design from university to industry is such a challenging road in medical device communities. This road is limited by several factors, including the need to understand native tissue behavior and incorporate this behavior into scaffold design and the ability to manufacture scaffold to those design specifications. Scaffold needs to be characterized in relationship to the behaviour of the tissue that is intended to replace. The ability to bring clinical requirements earlier into the scaffold development process is a critical issue for study sections and investigators. For investigators developing scaffolds, the ability to get clinical insight for specific applications and to look towards developing scalable manufacturing methods is critical to easing the path to the clinic. Furthermore, it is necessary to evaluate scaffold characteristics with respect to specific applications, ASTM standards, and FDA guidance documents. For study sections and funding agencies, it is also important to prioritize funding of interdisciplinary research teams that bring clinical issues early into the scaffold-development process. furthermore, it will be important for funding agencies and study sections to fund development issues in addition to basic research.



It takes two to tango: The promises of hybrid 3D bioprinting

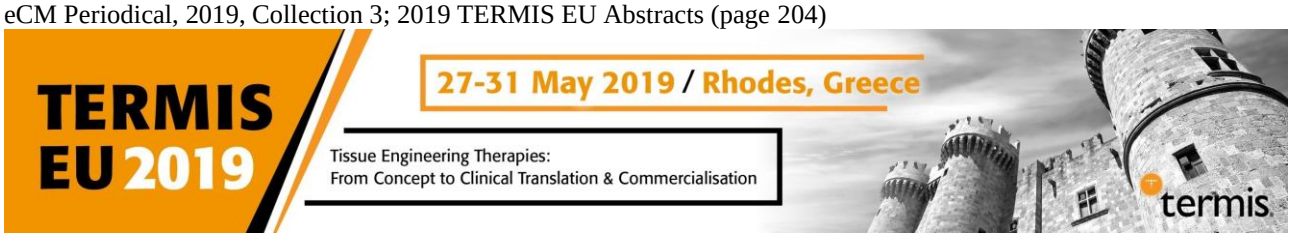
N. I. Moldovan[1,2,3]
Presenting Author: Nicanor Moldovan, nimoldov@iupui.edu
[1]3D-Bioprinting Core Laboratory, 'Richard L. Roudebush' VA Medical Center, and Departments of [2]Biomedical Engineering & [3]Ophthalmology, Indiana University-Purdue University Indianapolis, USA

Bioprinting is set to become one of the most disruptive next-generation biofabrication technologies. It aims to revolutionize tissue engineering and thus to improve the way we study disease mechanisms, discover new drugs or assess the effects of chemicals on living tissues, and eventually change how we treat diseases. So far, bioprinting mainly rode the wave of additive manufacturing revolution, imitating the technically similar 3D printing (i.e., layer-by-layer deposition of a material). However, this approach is dependent on the printability of the 'scaffold' biomaterial (bioink), which also has to accommodate and support the live cells. In spite of the constant progress in this area, a universal bioink capable to serve all the requirements of the bioprinted constructs, is still to be found. Therefore, an increasingly sophisticated array of technological add-ons is incorporated into the bioprinting process, some exploiting the resources of biomaterials, while others trying to minimize their involvement. A radically different variant of biofabrication uses only cells for creation of complex 3D tissue similars. These scaffold-free methods mostly rely on the properties of spheroidal cell aggregates. Correspondingly, scaffold-free bioprinting methods emerged, capable to assembly multiple spheroids into larger construct [1]. One of the most significant development is the microneedle-assisted spheroids skewering ('Kenzan' method), enabled by the commercialization of the robot Regenova [2]. Our University recently acquired one of the few such instruments outside Japan. However, scaffold-free bioprinting is not without its own limitations, and for this reason the field is looking back to the secondary incorporation of biomaterials within the bioprinting workflow. In my talk, I will illustrate opportunities [3], problems and current and contemplated solutions for the bioprinting of useful tissue models, arguing that an ultimately successful technology is likely to be 'hybrid' [4], thus emulating the modular-combinatorial nature of biological processes themselves.

ACKNOWLEDGEMENTS: The IUPUI Regenova bioprinter was funded by the NIH 'Shared Instrument Grant' S10 OD23595.

REFERENCES

[1] Moldovan NI, J. Cell. Mol. Med., 2018. 22(6):2964-2969
[2] Moldovan NI et al., Tissue Eng. Part B Rev., 2017. 23(3):237-244.
[3] Moldovan L et al., Biotechnol. J, 2017. 12 (2).
[4] Moldovan L et al., Curr. Opin. Biomed. Eng., 2017. 2: p. 124–131.



Nanosilicate clay promotes healing in a murine model of chronic skin wounding

N. D. Evans[1]
Presenting Author: Nicholas Evans, n.d.evans@soton.ac.uk
[1]Centre for Human Development, Stem Cells and Regeneration, University of Southampton Faculty of Medicine, Tremona Road, Southampton, SO16 6YD, United Kingdom

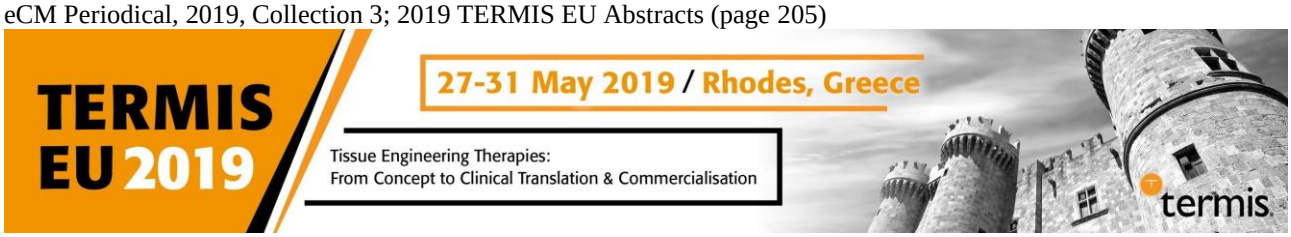
INTRODUCTION: Sorptive clays have been used since prehistory by humans as a material to treat skin abrasions and wounds. Despite this, there is a paucity of studies that systematically address the efficacy of defined clays in improving skin wound healing. In this study, we tested the hypothesis that a well-defined, synthetic smectite clay, Laponite, could increase the rate and degree of healing in chronic skin wounds in the non-obese diabetic mouse strain db/db.

METHODS: Full thickness skin wounds of 6 mm diameter were made in the back skin of db/db and wild type mice with a biopsy punch. 50 µL Laponite clay gels (3% w/v in H_2O) or controls of phosphate buffered saline (PBS) or alginate (1.1% w/v) were applied 24 h post-wounding and held in place with a semi-occlusive dressing. Wound size and re-epithelialisation was measured for up to 18 days post-wounding using photographic or morphometric analysis. Blood vessel infiltration, epithelial thickness, neutrophil count and cell density were measured using immunohistochemistry at d14 and d18.

RESULTS & DISCUSSION: Laponite treatment significantly promoted the rate of re-epithelialisation (100% vs 47 ± 43 µm), epithelial cell division, epithelial thickness (and fibroblast invasion compared to PBS or alginate-treated db/db mice ($p < 0.01$; $n = 3$). Moreover, in Laponite-treated mice, hair follicle anagen was stimulated in proximity to the wound concurrent with wound healing improvement, but was absent in PBS- or alginate treated wounds. Neutrophil infiltration was reduced in Laponite compared to alginate or PBS-treated controls. We suggest that Laponite may act to localise host proteins or growth factors at injury sites and promote skin wound healing processes.

CONCLUSIONS: We suggest that Laponite may act to localise host proteins or growth factors at injury sites and promote skin wound healing processes. Our data indicate a role for well-defined nanosillicate clays in wound healing and hair restoration.

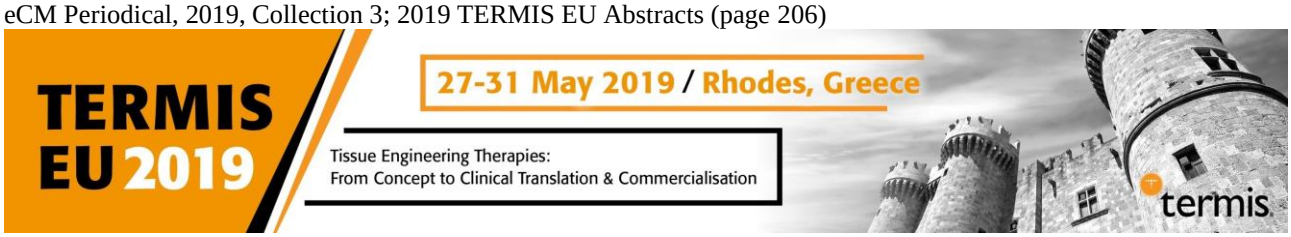
ACKNOWLEDGEMENTS: Financial support was received from the University of Southampton Eustice Award, a Grundy Educational Scholarship to D Page, and from a small grant from NovoNordisk.



Human textile: The next generation vascular grafts

N. L'Heureux
Presenting Author: Nicolas L'Heureux: nicolas.lheureux@inserm.fr
Laboratory for the Bioengineering of Tissues (BioTis) U1026 INSERM/Université de Bordeaux, Bordeaux, France

Sheets of Cell-Assembled extracellular Matrix (CAM), produced by cultured cells in vitro, have been rolled to produce completely biological tissue-engineered vascular grafts (TEVGs). Despite a rapid and successful progression to clinical trials, this approach remains complex. Our novel strategy is to produce TEVG with yarns produced from CAM sheets using a textile-based approach, which is faster, more reliable, more versatile and more easily automated. CAM sheets can be cut in ribbons of various width, which can also be twisted to make threads. Yarns (ribbons or threads) can be processed in different ways: devitalized (freezing/dehydration//freezing/rehydration cycle), decellularized (8 mM CHAPS, 1 M NaCl, 25 mM EDTA, 0.12 M NaOH) and/or gamma-sterilized (25 kGy). We have evaluated the effect of these processing steps on the histological and mechanical properties of the yarn. Also, we have assessed the impact of these steps on the remodeling (histological and mechanical) of the yarn after subcutaneous implantation in a nude rat for up to 6 months. Results showed that the CAM-based yarn creates very little inflammatory reaction and is essentially only mildly remodeled by the host response. However, some processing steps do lead to increased reactivity and decreased performance in vivo. Because of its structure and versatility or use, the CAM can truly be called a "bio-material". More importantly, because of its native-like structure, this material has the potential to be accepted by the host without significant immune responses and to support a long-term "physiological" remodeling process.



Immunoprofiling in tissue engineering and immune assisted tissue engineering via macrophage containing scaffolds

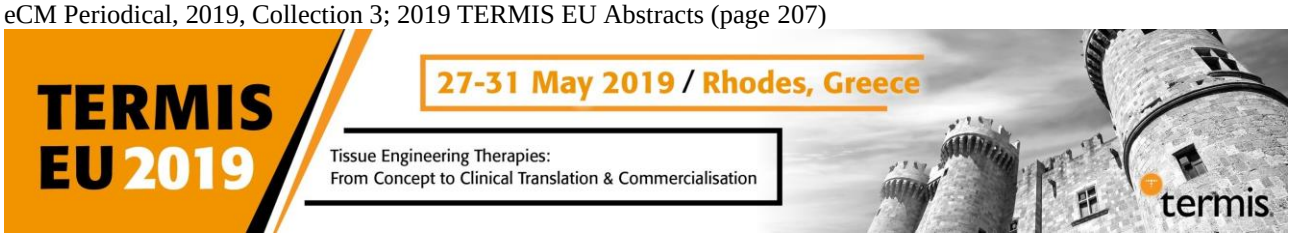
N.E. Vrana[1,2]
Presenting Author: Nihal Engin Vrana, e.vrana@protipmedical.com
[1]Protip Medical, 8 Place de l'Hopital Strasbourg, France [2]INSERM UMR 1121, Biomaterials and Bioengineering, Université de Strasbourg, 11 rue Humann, 67000 Strasbourg, France

The replacement of complex organs with multiple functions such as larynx (swallowing, speech, prevention of aspiration) requires hybrid systems of mechanical and biological components. Following our initial positive results after clinical application of a completely mechanical, valve-based, titanium artificial larynx system [1]; we are currently developing tissue engineered connectors that will enable faster integration of our artificial larynx. However, the significant variation of the shape, size and mechanical properties, particularly after radiotherapy, of larynx between the patients requires the development of personalized solutions that are specifically designed for a patient which can diminish the level of reaction by the host due to a better mechanical and anatomical match between the implant and the surrounding tissue. Beyond the anatomical match, the patient's immunological profile (where our efforts are focused on mimotope variation analysis based immunoprofiling) and the specific reactions to a given material (where we use transcriptomic approaches to detect patient specific reactions to materials such as titanium as a model) must also be considered for better clinical outcomes. The determination of patient specific components of the adverse immune reactions enables the development of personalized immunomodulatory solutions [2]. In this context, the control over macrophage phenotype in the microenvironment of implanted artificial tissues has strong therapeutic potential. In this talk, the surface modification of the implants and engineered tissues with autologous macrophage and/or immunomodulatory cytokine containing hydrogels for improving the physicochemical interface and biological crosstalk between the implanted structures and the host immune system will be covered. Our most recent efforts are focused on direct incorporation of phenotype controlled innate immune cells in hydrogels in the presence of connective tissue cells for more rapid maturation of vascularized connective tissues as support structures in artificial organ development. The potential advantages of such systems in the specific case of an artificial larynx will be elaborated. Also, with the potential testing of such structures in dedicated robotic simulation systems, where biomechanical environment of larynx/pharynx can be mimicked, will be discussed with the demonstration of a first version of such a robot (SWALL-E).

ACKNOWLEDGEMENTS: This project has received funding from the European Union's FP7 programme 602694 (IMMODGEL) and Horizon 2020 research and innovation programme under grant agreement No 760921 (PANBioRA).

REFERENCES

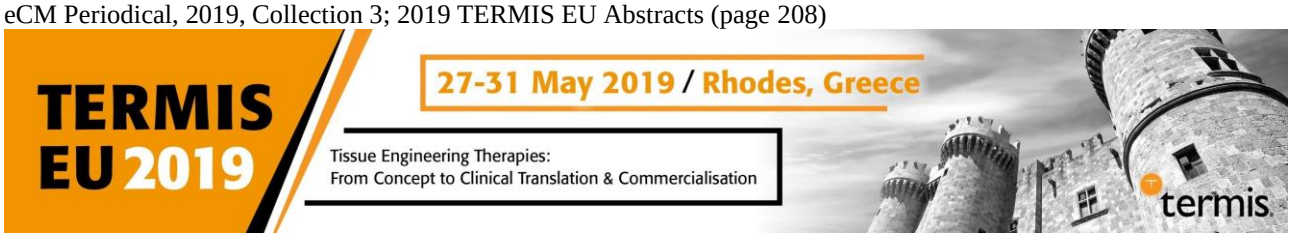
[1] Debry C et al. NEJM 2017; 376(1):97-98.
[2] Vrana NE. Future Science OA. 2016; 2 (4), FSO146.
[3] Barthes J et al. Front. Biotech. Bioeng. 2018;6 :108.



Biomaterials combined with stem cells for bone and cartilage tissue engineering

N.M. Neves
Presenting Author: Nuno M. Neves, nuno@i3bs.uminho.pt
[1]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark 4805-017 Barco, Guimarães, Portugal

Many biomaterials have been proposed to produce scaffolds aiming the regeneration of many tissues. We have a particular interest in developing systems combining natural polymers and synthetic biodegradable polymers. By proposing those systems for those demanding applications, we aim at obtaining biomaterial systems with enhanced properties namely mechanical properties, processability, cell-friendly surfaces and tunable biodegradability. Our biomaterials may be processed by melting routes (solvent-free) into devices with wide applications such as biodegradable scaffolds, films or particles and adaptable to many biomedical applications. As an example of processing technologies, electrospinning has recently gained popularity as a simple and versatile technique to produce synthetic polymeric ultrafine fibers. This technique allows the production of non-woven meshes with fiber diameters in the nanometer range, which results in a high surface area-to-volume ratio and high porosity. Additionaly, these nanofiber meshes can mimic the extracellular matrix of human tissues and, therefore, can be used as scaffolds for Tissue Engineering (TE) applications. Many sources of cells were considered for tissue engineering. Embryonic, iPS and adult stem cells are among the most promising to achieve the cell numbers required to have therapeutic relevance. We have been proposing adult stem cells from different sources for bone and cartilage tissue engineering applications. This talk will review our latest developments using natural-based biomaterials and nanofibre meshes in the context of bone and cartilage tissue engineering applications.



Development of 3D cellular microenvironments as potential remedy

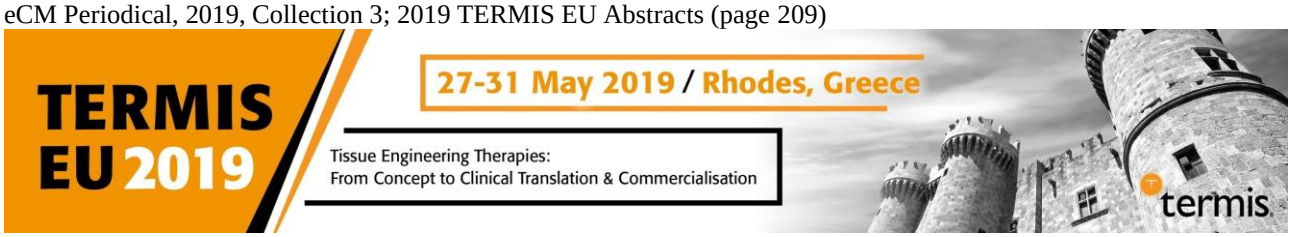
O. Tsigkou[1]
Presenting Author: Olga Tsigkou, olga.tsigkou@manchester.ac.uk
[1]School of Materials, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

Current clinical protocols for tissue regeneration incorporate the use of a supporting material along with transplantation of stem cells at the site[1]. This approach aims to repair the damaged tissue by replenishing the lost tissue. However, clinical translation of stem cell-based interventions is prevented by poor functional integration and cell survival into the host tissue in accordance to the restraint of large constructs to rely on interstitial fluid diffusion and blood perfusion for nutrients and oxygen supply and waste removal[2]. Extracellular matrix based hydrogels can be used to promote cell integration into the host by providing spatial and mechanical support for cell adhesion and survival. In a large graft, the core is under hypoxic conditions that lead to cell death. An engineered functional three-dimensional (3D) vascular network within the graft will support and induce tissue regeneration. Assembly of the tissue specific cells (i.e., bone, tumour, neuronal or adipose tissue), mesenchymal stem cells as a source of pericyte precursors[3], and vascular cell populations into functional tube like structures infers that communication between cell types is required for tissue homeostasis. Based on the bidirectional communication between cells, it is hypothesized that a hydrogel scaffold composed of natural biopolymers such as collagen and hyaluronic acid, will support functional integration of endothelial cells, and tissue specific cell populations to re-establish vascular networks and promote the reconstruction of the local vasculature. This engineered network will inosculate with the host vasculature upon implantation and reduce the time required for a true functional graft vasculature to form and consequently will support and induce superior tissue regeneration[4]. Herein, our efforts and experience with co-culture systems to develop vascularized tissues used to in vitro model tissues for fundamental studies related to drug screening, disease modeling, as well as tissue repair and regeneration will be discussed.

ACKNOWLEDGEMENTS: Financial support was received from School of Materials, University of Manchester, EPSRC-MRC Centre for Doctoral Training at The University of Manchester (EP/L014904/1) and Marie Skłodowska-Curie Actions, IEF (302804).

REFERENCES

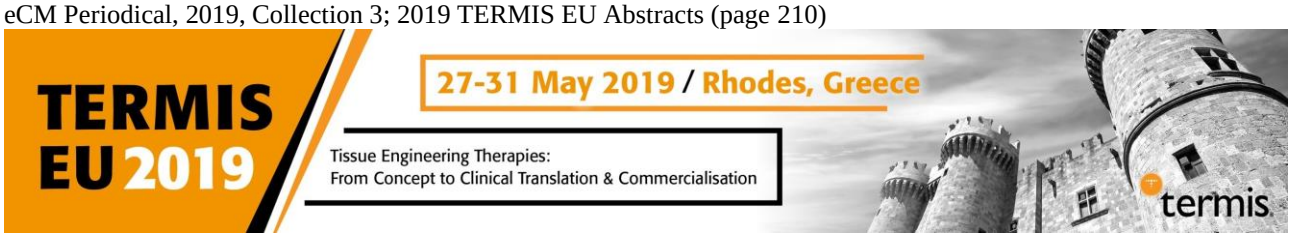
[1] Muschler GF et al. J Bone Joint Surg Am. 2004;86-A(7):1541-58.
[2] Folkman J, Hochberg M. J Exp Med. 1973;138(4):745-53.
[3] Au P et al. Blood. 2008;111(9), 4551-58.
[4] Tsigkou O et al. PNAS. 2010;107(8), 3311-16.



Modeling myogenesis in vitro using directly reprogramed induced myogenic progenitor cells

O. Bar-Nur
Presenting Author: Ori Bar-Nur, ori.bar-nur@hest.ethz
Department of Health Sciences and Technology, ETH Zurich, 16 Schorenstrasse, 8603 Schwerzenbach, Switzerland

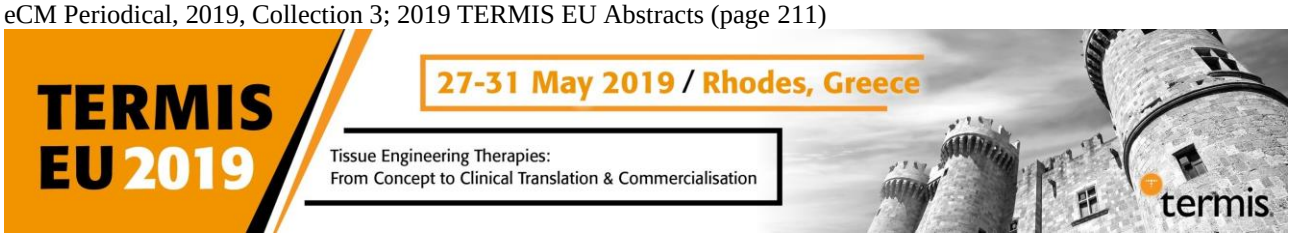
Transplantation of satellite cells offers an attractive approach to treat degenerative loss of muscle mass, however ex vivo expansion of these cells has proved challenging. We recently described a method that enable direct lineage reprogramming of mouse fibroblasts into expandable satellite cell-like “induced myogenic progenitor cells” (iMPCs) by transient MyoD overexpression combined with treatment of three small molecules. Undifferentiated iMPCs propagate extensively in vitro while expressing markers of skeletal muscle stem and progenitor cells, including Pax7 and Myf5, and can further differentiate into contractile myotubes that express mature skeletal muscle markers. Furthermore, iMPCs differentiate into dystrophin-expressing myofibers upon transplantation into a mouse model of Duchenne Muscular Dystrophy, and a subset of transplanted cells contribute to the satellite cell pool and maintain Pax7 expression in vivo. In this talk I will present our recent efforts to reprogram various defined somatic cells into expandable myogenic stem/progenitor cells, and the pivotal role small molecules play during this cellular transition. I will further discuss our efforts to generate novel in vitro stem cell-based models for Muscular Dystrophies using this unique myogenic cell culture system.



Two decades of research on amniotic cells: What have we learned?

O. Parolini[1,2]
Prsenting Author: Ornella Parolini, ornella.parolini@unicatt.it
[1]Istituto di Anatomia Umana e Biologia Cellulare, Università Cattolica del Sacro Cuore Facoltà di Medicina e Chirurgia, Largo Francesco Vito, Rome, Italy [2]Centro di Ricerca "E. Menni" Fondazione Poliambulanza Istituto Ospedaliero, Via Bissolati, Brescia, Italy

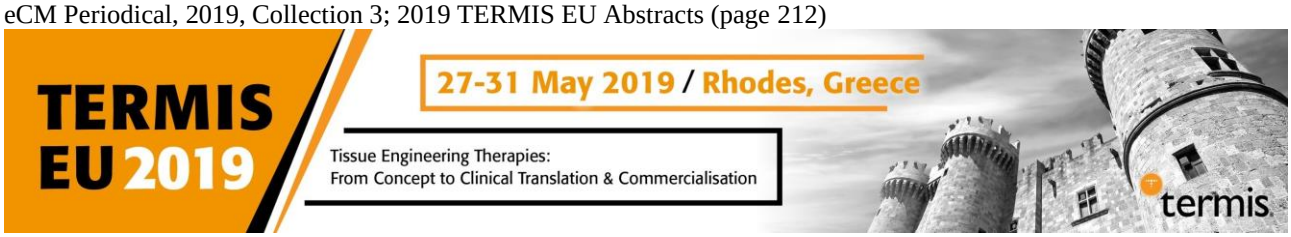
Perinatal cells can be isolated from different birth-related tissues, including different regions of placenta and amniotic fluid. In addition to hematopoietic stem cells, other tissue-specific cells with stem/progenitor characteristics have been identified. The First International Workshop on Placenta-Derived Stem Cells defined stem/progenitor cell populations from four major placenta regions: human amniotic epithelial cells, human amniotic mesenchymal stromal cells, human chorionic mesenchymal stromal cells, and human chorionic trophoblastic cells. Cells with MSC properties have also been isolated from other placental tissues, such as the chorionic villi, the maternal decidua basalis, from different compartments of the umbilical cord, such as the Wharton's jelly. The notable interest that perinatal cells have received in the past decade is due to their low immunogenicity and high immunomodulatory potential, in addition to practical reasons such as their easy procurement. The uniqueness of perinatal MSC is their intrinsic immunomodulatory potential that doesn't require pre-conditioning with inflammatory stimuli; this is likely due to their origin – a battlefield of the fetal-maternal immune crosstalk. In the field of regenerative medicine, we have learned that immunomodulation plays a relevant role to favor tissue homeostasis after injury. As a matter of fact, immunomodulation, rather than direct cell differentiation, has been shown to be a key mechanism contributing to the regenerative capability of perinatal cells, whereby they activate endogenous tissue stem cells that in turn contrast degenerative phenomena. Our group has pioneered the understanding of the immunomodulatory potential of mesenchymal stromal cells isolated from the amniotic membrane (hAMSC) of human term placenta. We have described the ability of hAMSC and their conditioned medium (CM-hAMSC) to reduce in vitro T cell proliferation induced by alloantigens, via T-cell receptor or mitogens. hAMSC and CM-hAMSC significantly reduce the expression of markers associated to Th1 and Th17 populations, and significantly induce the regulatory T-cell compartment. Amniotic cells are able to block the differentiation of monocytes into both dendritic cells (DC) and inflammatory M1-macrophages, and skew monocyte differentiation toward anti-inflammatory M2 macrophages. The therapeutic effects of amniotic cells and their conditioned medium (which contain secreted bioactive factors) have been reported in other preclinical models of diseases based on inflammatory processes and with altered immune reactions, such as lung and liver fibrosis, sepsis, inflammatory bowel disease, cardiac ischemia, autoimmune encephalomyelitis, rheumatoid arthritis and Huntington's disease. In these diseases, the modulation of inflammation seems to be a key element underlying the restoration of tissue integrity promoted by amniotic cells and their bioactive factors. The multifaceted, immunomodulatory properties of cells isolated from the amniotic membrane of human term placenta, and conditioned medium derived from their culture, render them precious cells for a variety of therapeutic applications, especially inflammatory-based diseases. The future will unveil the underlying mechanisms of action of amniotic cells and, in addition, novel therapeutic applications.



The lymph node niche as an ex vivo tool for T cell priming

Ø. Halaas [1,2]
Presenting Author: Øyvind Halaas, oyvind.halaas@ntnu.no
[1]Department of Clinical and Molecular Medicine, NTNU The Norwegian University of Science and Technology, Trondheim, Norway.

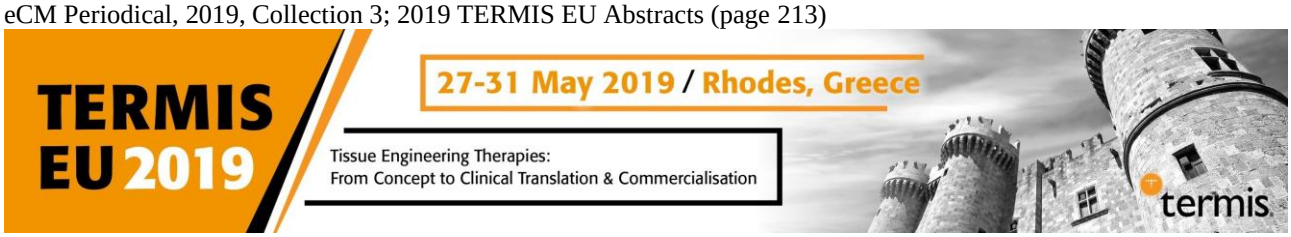
Vaccinations have prevented more human suffering than any other medical technology, and will continue to serve in therapy of cancers of infectious diseases for the foreseeable future. Today´s immunization regimes is based on injecting immunogens into the body, whereupon dendritic cells (DCs) having acquired the immunogens, as well as the immunogen itself, is translocated to the lymph node where T cells (and B cells) from blood and lymph scavenge the DCs in search of cognate epitopes. For T cells, this occur in a densely cell populated area in the lymph node paracortex, the T cell zone, resulting in priming and activation of a diverse set of T cell subtypes subsequently responding to any cell presenting the epitope in the context of MHC (HLA). The possibility of creating immune responses in vitro opens a whole new range of opportunities in immunotherapy, but this is technically and physiologically difficult. We approach this challenge by creating 3D networks of reticular fibroblasts in microbioreactors, and administer DCs and T cells by perfusion, by this aiming to recreate the dynamic microenvironment of the lymph node. We show shear stress-affected antigen specific adhesion of T cells to DCs. We show migration of DCs and T cells on the scaffold. We further describe the cell phenotypes affected by the microenvironment and the mechanical cues, create 3D models of T cell priming process and perform functional testing of the T cells.



3D goes clinical - Modified islet transplantation as blueprint for future 3D cell transplantation

P. Kugelmeier
Presenting Author: Patrick Kugelmeier, patrick@kugelmeiers.com
Kugelmeiers Ltd., Roswiesstrasse 3, CH-8125 Zollikerberg, Switzerland

We are at a tipping point where nature is revealing us top secrets of life. We can reveal them when we look at life how it really is - in 3D. 3D cell culture systems reflecting real life processes thus provide not only sound scientific data, but they also enable regenerative medicine with stem cells. Almost every life begins with cell clusters. 3D cell clusters are therefore an ideal scientific model that offers additional therapeutic options. But for full functionality, clusters need to be size-controlled in a physiological, “cosy” environment. Unfortunately, current technology is limited to either quality or scalability, and therefore many desired applications are not possible. Because of this unmet medical need, we therefore developed the Sphericalplate 5D, enabling the full translation from lab to clinics with freely scalable, size-controlled spheroids in clinical grade quality. A human multicenter trial for the treatment of Diabetes is beginning in 2020.



Getting on nerves: In vitro models of engineered tissue

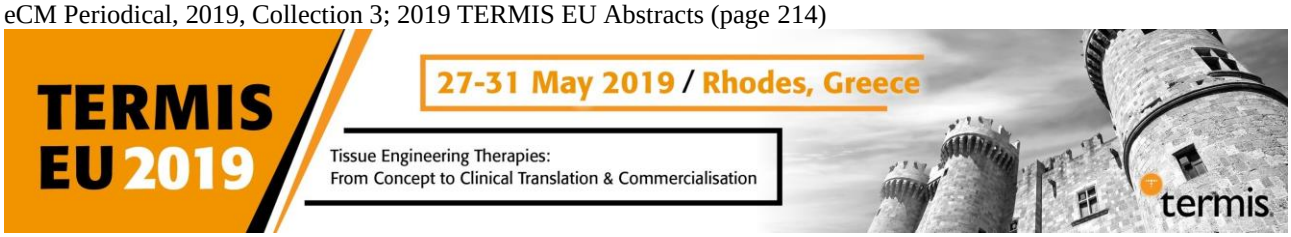
P.A. Wieringa[1]
Presenting Author: Paul Wieringa, p.wieringa@maastrichtuniversity.nl
[1]Complex Tissue Regeneration Department, MERLN Institute of Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands

The peripheral nervous system (PNS) is critical in regulating tissue function and homeostasis, with supporting evidence that innervation also plays a role in tissue repair [1]. Furthermore, innervation can impact the effectiveness of organ transplantation and influence pathological conditions [2]. However, innervation is rarely considered in regenerative medicine strategies. While in vivo studies have been critical to show that the nervous system plays a role in these processes, there are also conflicting findings [3]. This can be attributed to the various targets which are simultaneously contacted by the nervous system, triggering activity in other regions of the body which may also subsequently influence the tissue of interest through the release of hormones or paracrine signals. This complex interplay between these tissues and the PNS can result in confusion and controversy and is a contributing factor to the marginalization of innervation in tissue engineering approaches. In vitro models can effectively eliminate uncontrolled confounding factors and provide a defined context to explore nerve/tissue interactions. However, classical 2D cell cultures often cannot support the formation of representative tissues, limiting the types of interactions and functional readouts that can be assessed. Discussed here is our development of a 3D in vitro platform that supports directed innervation of 3D cultures and can be reconfigured to support various target tissues. The approaches described intend to provide a universal tool that clarifies the role of the PNS within regenerative medicine and that provides strategies to achieve meaningful innervation.

ACKNOWLEDGEMENTS: Financial support was received from the Dutch Province of Limburg and the Netherlands Organisation for Scientific Research (NWO) as part of the VENI research programme (project# 15900)

REFERENCES

[1] Buckley G et al. J. Anat. 2012; 220: 3-12.
[2] Bengel FM et al. N Eng. J. Med. 2001; 345(10): 731-738.
[3] Taborsky GJ Cell Metab. 2011; 14: 5-6.



Engineered and devitalized cartilage extracellular matrix as a bone substitute material

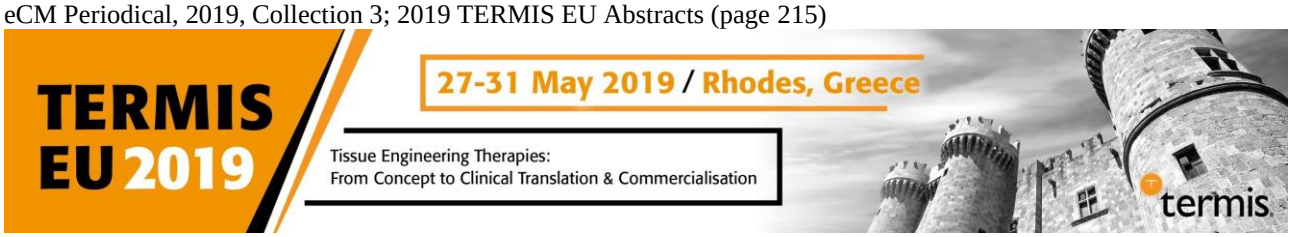
P. Bourgine[1]
Presenting Author: Paul E. Bourgine, paul.bourgine@med.lu.se
[1]Laboratory for Cell, Tissue and Organ Engineering, Department of Clinical Sciences, Wallenberg Centre for Molecular Medicine, Stem Cell Centre, Lund University, Sweden

According to reference books, our bones can form by intramembranous and/or endochondral ossification. The endochondral route involves the formation of cartilage tissue as primordial template, initiating the development of most bones and their healing upon fractures. Several groups in the past years have demonstrated that by engineering cartilage in vitro using different mesenchymal stromal cells (MSC), the tissue has the capacity to robustly remodel into bone and bone marrow. In this context, hypertrophic chondrocytes contribute to the bone formation, following the natural process of endochondral ossification. The successful recapitulation of the endochondral approach has opened new avenues in tissue engineering, with the increasing exploitation of cartilage tissue for bone repair. This lecture will offer evidences that not only living cartilage can be used to form bone, but also devitalized templates. Using both human primary and immortalized MSC, cartilage matrix can be engineered and subsequently devitalized by apoptosis induction. Despite being cell-free, the templates show strong bone inducing stimuli by retaining essential osteoinductive signals. This has been demonstrated at ectopic (subcutaneous) and orthotopic (calvarial defect) sites, using static scaffold-free culture or scaled-up systems based on perfusion and 3D porous scaffolds. Bone formation was also assessed in the presence of co-delivered stromal vascular fraction cells freshly isolated from adipose tissue, which could be intra-operatively available for enhanced matrix remodeling. Although inspired from development, the described approach hijacks the natural endochondral process; forming bone via a cartilage intermediate, but without involving hypertrophic chondrocytes. The findings will be further discussed in light of the challenges to understand and control the activated biological processes, in order to achieve a superior level of robustness, standardization and possibly customization depending on the specific demands of defined clinical settings.

ACKNOWLEDGEMENTS: Financial support was received from the AO Research Foundation, the Eurostars program (Grant E!7865), the Swiss National Science Foundation (Grant 310030-133110) and the Wallenberg family.

REFERENCES

[1] Bourgine PE et al. Proc Natl Acad Sci USA. 2014;111:17426-17431
[2] Papadimitropoulos A et al. Bone. 2015; 70:66-72
[3] Haumer A et al. Adv Drug Deliv Rev. 2018;129:285-294



Intuitive design of melt electrowritten products for tissue engineering and regenerative medicine

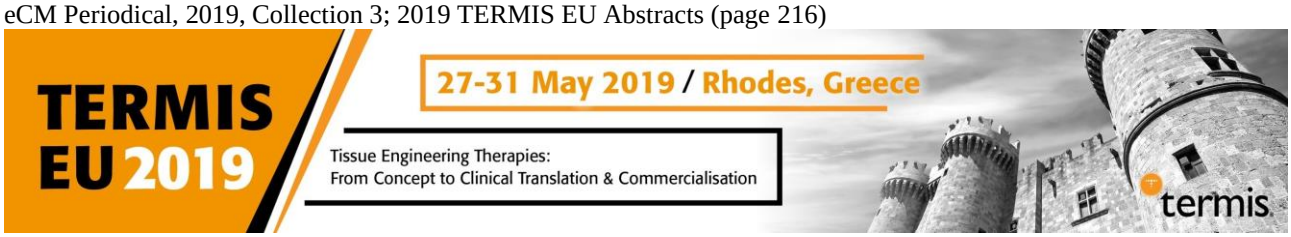
P.D. Dalton[1]
Presenting Author: Paul Dalton, paul.dalton@fmz.uni-wuerzburg.de
[1]Functional Materials in Medicine and Dentistry, University Clinic Würzburg, Germany

Melt processing has been used for decades to manufacture medical materials including sutures, hernia meshes and bone screws [1]. Also using melt processing, scaffolds made by 3D printing are used to fill maxillofacial defects [2]. In this lecture, the intuitive design of soft-tissue implants made from medical-grade poly(ε-caprolactone) (PCL) using a technology termed melt electrowriting (MEW) is described [3]. Briefly, MEW operates with air-pressure control, driving molten PCL to a flat-tipped nozzle with 5-6kV applied. Calibration curves for both air pressure and speed were performed so that the direct-writing of multi-phasic and multimodal scaffolds performed [4]. MEW scaffolds consist of small filament diameter structures in the low-micron resolutions. The diameter of the fibers within the scaffold can also be controlled. There are many possibilities for scaffold design, Since the diameter of the fiber can be discretely changed, multiphasic and multimodal scaffolds can be designed and 3D printed. All scaffolds are melt processed with methods friendly to the medical device regulatory process. In summary, MEW, is a robust, reproducible and solvent-free approach to manufacture 3D printed materials for soft tissue applications. It bridges the divide between solution electrospinning and fused deposition and is an inexpensive approach that permits clinically translational research to be performed in university settings.

ACKNOWLEDGEMENTS: Financial support was received from the German Research Foundation (DFG) – Project number 322483321.

REFERENCES

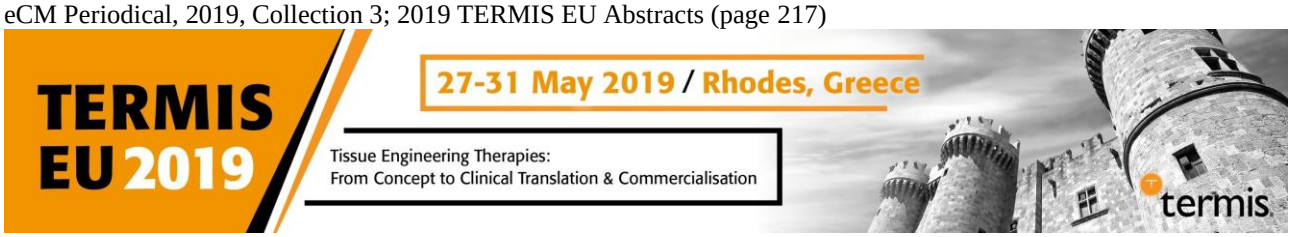
[1] Youssef, A.B. et al. Biofabrication, 2017. 9(1): 012002.
[2] Probst, F.A., et al., Handchir Mikrochir Plastische Chir, 2010. 42(6): 369-373.
[3] Dalton, P.D., Current Opin Biomed Eng, 2017. 2: 49-57.
[4] Hrynevich, A., et al., Small, 2018. 14(22): e1800232.



3D bioprinting with adult stem cells. Roadmap for translation to clinic

P. Gatenholm
Presenting Author: Paul Gatenholm, paul.gatenholm@chalmers.se
3D Bioprinting Center, Chalmers University of Technology, Göteborg, Sweden

3D Bioprinting has a potential to revolutionize regenerative medicine due to unique ability to place multiple cell types in predetermined position and build bottom up the microenvironment to control cellular fate processes. We have investigated the effect of bioink and bioprinting conditions of stem cell-laden biopolymer hydrogels on cell viability. We found multiple factors such as; cell mixing process, printer head design and most important; rheological properties of the biopolymeric bioinks affecting cell viability. We discover that nanofibrillar hydrogels were most beneficial due to orientation of fibrils in the nozzle and decreased shear forces. Bone marrow derived mesenchymal stem cells and adipose derived stem cells, ASC were successful 3D bioprinted with high viability and implanted directly subcutaneously in nude mice. We were not able to observe neither proliferation nor differentiation during 30 days in vivo. In contrast, we have seen significant effect of stem cells onto human chondrocytes when 3D bioprinted co-culture. Stem cells contributed to proliferation of chondrocytes and increased production of cartilage. This trophic effect of stem cells is enhanced in 3D bioprinted constructs due to controlled microenvironment. Our studies together with plastic surgeons have been expanded to up to 1 year implantation and use of different protocols for cell isolation. We have also showed that 3D bioprinted mechanically disintegrated adipose tissue constructs survived engraftment in vivo and displayed macroscopic and microscopic evidence of neovascularization. The roadmap for translation of this technology to clinic which includes cell isolation protocols and use of regulatory compliant bioinks will be presented.



Mechanisms and characteristics of extracorporeal shock wave therapy

P. Slezak[1]
Presenting Author: Paul Slezak, paul.slezak@trauma.lbg.ac.at
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Austrian Cluster for Tissue Regeneration, Vienna, Austria

Extracorporeal shockwave treatments have been shown to accelerate tissue regeneration in diverse clinical situations, ranging from soft to nerve and bone tissue applications. The underlying mechanisms of these mechanically induced beneficial effects on cells and whole tissue volumes are not yet fully understood. Furthermore, the physical properties of the acoustic shockwaves that are produced by various generating techniques (electro hydraulic, electro-magnetic) are not yet properly characterized. In a co-operation with the major manufacturers of therapeutic shock wave applicators we have performed analysis of various clinically used devices, evaluated their physical properties as well as their biologic effects on cells. Optical phase contrast imaging was used to visualize shockwaves in various in vitro test setups and a computational model has been established to calculate the propagation of shockwaves and to simulate treatment options. This allows planning, prediction and optimization of complete therapeutic sessions. Beyond the computational work, different types of shockwaves have been tested with various cell types, ranging from animal derived cells to fungal biofilms. Increased cell proliferation as well as augmented resistance of fungal biofilms to antimycotics was shown in vitro, outline the protective and regenerative potential of acoustic shock waves in a broad range of indications once more.

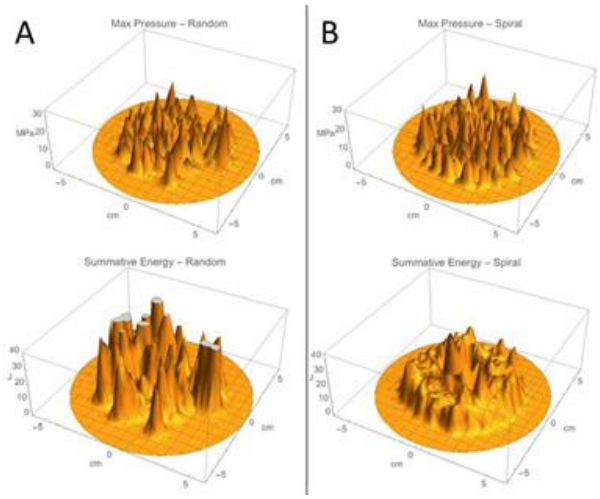
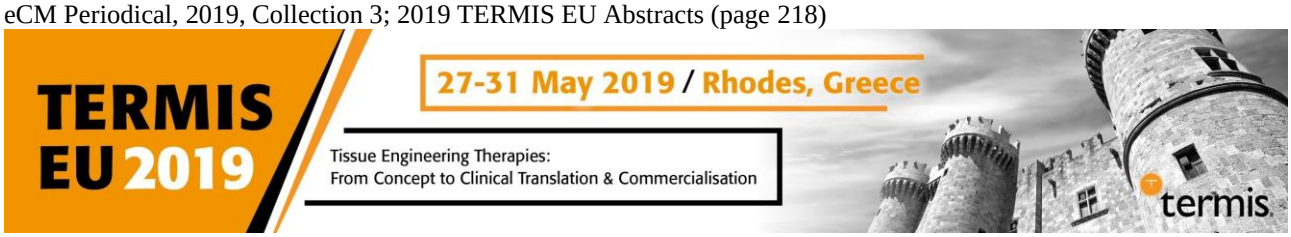


Figure 1: Simulation of the peak pressures and summative energy applied to a tissue volume during an extracorporeal shockwave therapy session with an electo-hydraulic device; 300 pulses, 4cm penetration depth. Comparison between a random (A) and spiral (B) application pattern.

ACKNOWLEDGEMENTS: The author would like to thank Cyrill Slezak, Anna Weihs, Christiane Fuchs, Roland Rose, David Hercher, Kyle Hutchings, and Dr. Olga Kopp and her team for the support of this project.



Functional and multi-functional devices and scaffolds for musculoskeletal tissue regeneration

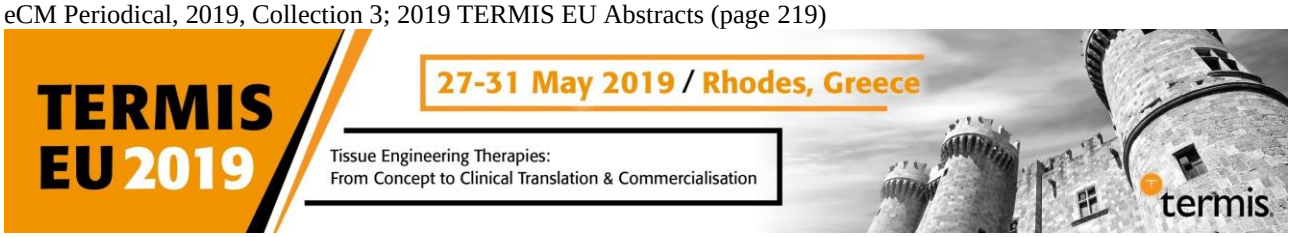
P. V. Hatton[1,2]
Presenting Author: Paul Hatton, paul.hatton@sheffield.ac.uk
[1]EPSRC Centre for Innovative Manufacturing in Medical Devices (MeDe Innovation), [2]University of Sheffield, Sheffield S10 2TA, UK

Despite considerable progress in biomaterials science and regenerative medicine over the past 20 years, numerous challenges and unmet clinical needs remain. This is undoubtedly the case in musculoskeletal repair, where issues related to the ageing population and an increased prevalence in co-morbidities have placed considerable burdens on healthcare systems while reducing the quality of life for millions of people worldwide. Two persistent challenges today are poor or inconsistent musculoskeletal tissue regeneration, and deep bone infection (that is frequently device-associated). Moreover, these two major issues are increasingly combined in elderly patients where – especially when combined with the increase in antimicrobial resistance - they present clinicians with one of the greatest challenges in medicine today. Research at Sheffield, frequently undertaken with academic and industrial partners, has identified a number of highly promising innovations that could both enhance musculoskeletal tissue regeneration and combat infection (directly, and/or indirectly via a reduction in risk). Some of these technologies are relatively simple and therefore close to translation to a useful product, whereas others are more complex but could underpin a new approach to clinical regenerative medicine. At the most straightforward level, there are inorganic modifications to existing technologies, for example the substitution of metal oxides in bioactive glasses to enhance bone tissue response and/or introduce antimicrobial activity. Substitution of calcium with strontium in Hench's classical 45S5 bioactive glass composition was previously shown to stimulate bone tissue regeneration [1], and we now have a good understanding of both the materials science and biological mechanisms responsible [2]. Modification of bioactive glass composition to enhance biocompatibility and antimicrobial activity has been extended to different systems [3,4], and in parallel work an important discovery was that nanoscale calcium phosphates could also be modified - in this case with silver - to introduce potent antimicrobial activity against opportunistic pathogens. More technically complex approaches include the multilayer nanoscale encapsulation of therapeutic molecules including antibiotics. To summarise, recent research has provided a range of highly promising technologies that have the potential to enhance musculoskeletal tissue regeneration while reducing the risk of deep bone infection. These represent the first generation of truly multi-biofunctional biomaterials, and this paper will conclude with an analysis of the translational pathways that are best suited to bringing these innovations to clinical trial and adoption.

ACKNOWLEDGEMENTS: Financial support was received from EC, UK EPSRC, and industrial partners including Ceramisys Ltd. Collaborators and members of the group who made a substantial contribution to this work include Cheryl Miller, Aileen Crawford, and Graham Stafford from the University of Sheffield, Piergiorgio Gentile (now at Newcastle University), Rui Reis (3B's Group), and Ricardo Pires (3B's Group). Postdoctoral and PhD researchers include Caroline Wilcock, Martin Santocildes-Romero, Joao Fernandes, and Joss Atkinson.

REFERENCES

[1] O'Donnell et al. J Mater Chem 2010; 20: 8934
[2] Santocildes-Romero et al. J Tiss Eng Regen Med 2015; 9:619
[3] Fernandes et al 2016; Acta Biomat 44:168
[4] Fernandes et al 2017; Acta Biomat59:2

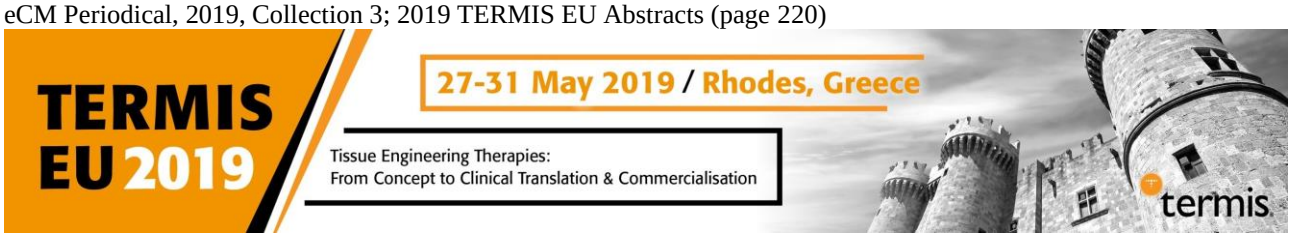


Bringing TERM to the market by employing hybrid approaches

P.F. Costa[1]
Presenting Author: Pedro Costa, pedro.costa@biofabics.com
[1]BIOFABICS – 3D Biotissue Analogues, Porto, Portugal

The translation of science to the market continues to be a rather long and painstaking process. Nonetheless, scientists are becoming increasingly aware of it and more often considering this path for their newly developed technologies as well as their own careers. This trend may be explained by the current influence of entrepreneurial culture in society, by a greater visibility of successful scientists turned entrepreneurs as well as by an increased awareness among the scientific community towards the meager availability of exciting career progression opportunities in academia. The field of tissue engineering and regenerative medicine (TERM) is no exception to this. Despite being a relatively young field, large numbers of highly skilled researchers have been trained in this area of knowledge in the past few decades, at the cost of substantial investment and fueled by the promise of being able to replace and regenerate tissues, organs and body parts in the near future. Nearly thirty years past, and despite many breakthrough advances, the promise did not yet fully turn into reality. However, this does not mean that opportunities were not created. In particular, many new research labs and companies were created since then, taking advantage of the newly trained human resources and the new know-how generated. Such labs and companies may have remained focused solely on TERM or also spun out to many other fields of application. The past decades have shown us that it may be wiser for TERM-based companies to keep an open eye towards other fields where TERM-based technologies can be more easily applied and where markets are more mature or simply easier to reach. This talk will mainly discuss how TERM-based companies can and should embrace the ability to collaborate and combine various technologies and know-how across disciplines, fields, sectors and borders in order to reach broader markets and therefore more successfully translating TERM to the market, whatever and wherever it may be.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 798014.



Biofabrication techniques for scaffolds manufacturing: The complex development for osteochondral regeneration

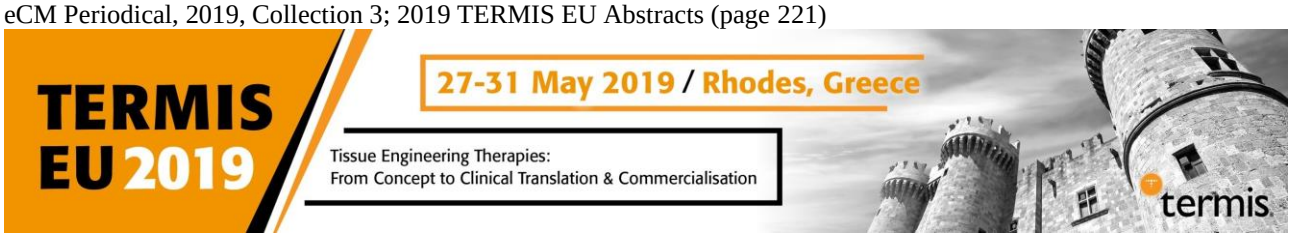
P.G. Morouço[1]
Presenting Author: Pedro Morouço, pedro.morouco@ipleiria.pt
[1]Biofabrication Research Group, Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Portugal

Biofabrication allows the formation of 3D scaffolds through a precise spatial control. This is of foremost importance when aiming to mimic heterogeneous and anisotropic organization, as wished for the osteochondral tissue. However, osteochondral defects keep being a supreme challenge for TE of the musculoskeletal system. It is quite challenging to design and fabricate TE constructs resembling the different compositions and mechanical properties of bone and cartilage, as well as their interface. Due to its nanostructure of complex stratified architecture and contrasting biomechanical properties, attempts to mimic integration at the cartilage-bone interface have failed. Furthermore, most of the current research focus on repairing focal cartilage damage, failing in to address the entire osteoarthritic joint. The recent advancements in 3D biofabrication, has permitted the design and fabrication of patient-specific scaffolds that possess structural and functional features comparable to the native tissue. It allows for design and fabrication using tissue images captured with commonly used medical imaging techniques such as computer tomography (CT) and magnetic resonance imaging (MRI) that are readily available in hospitals, something that conventional fabrication techniques lack. The mechanical properties of developed scaffolds should be directly related to their microarchitectural topology. As such, the permeability of a porous scaffold could be manipulated to mimic the native tissue and to facilitate cell and nutrient movement and allow for a better host-tissue integration as in osteointegration [1]. Accordingly, scaffolds have a determinant role for providing the proper 3D environment, so that cells can proliferate and differentiate in adequate lineages. To do so, both the used biomaterials/ bioinks, as the fabrication techniques should be evaluated. Regarding the biomaterials, there are advantages and drawbacks on using synthetic or natural polymers for the scaffolds production [2]. On the fabrication techniques, there is a trend to accept additive manufacturing techniques as advantageous procedures, as it enables a customized product design, with development and fabrication for patient-specific applications. With this presentation we will discuss the effects on boosting an effective synergy between mechanical engineering (including robotics automatization) and biomedical sciences. That will benefit TE from the revolutionary steps in manufacturing at industry 4.0. Revolutionizing the available procedures for tissue repair and regeneration: bringing the robotics expertise to the biofabrication domain, it will be possible to promote an outstanding precision with the ability to develop stimuli-responsive implant, employing a combination of responsive materials and novel construct geometries to amplify the consequence of the material response. We will provide up-to-date examples on significant steps on tailored implants for osteochondral tissue regeneration, as well as demonstrate what we should expect in the near future.

ACKNOWLEDGEMENTS: Financial support was received from COMPETE2020, ANI (POCI-01-0247-FEDER-017771; POCI-01-0247-FEDER-033877); Portuguese Foundation for Science and Technology (PTDC/EMS-SIS/7032/2014; UID/Multi/04044/2013); and CENTRO2020 (ROTEIRO/0328/2013 (Nº 022158)).

REFERENCES

[1] Liu X et al. Biomaterials. 2011; 32(2):330–8
[2] Lutolf M & Hubbell J. Nat Biotechnol. 2005;23(1):47–55



Landscape of regenerative medicine and tissue engineering in Russia

P. Timashev
Presenting Author: Peter Timashev, timashev.peter@gmail.com
[1]Institute for Regenerative Medicine, Sechenov University, Moscow, Russia [2]Institute of Photonic Technologies, Research Center "Crystallography and Photonics", RAS, Troitsk, Moscow, Russia [3]Department of Polymers and Composites, N.N. Semenov Institute of Chemical Physics, RAS, Moscow, Russia

INTRODUCTION: In the last decade, Russia has become an active player in the field of tissue engineering and regenerative medicine. While this field is of high priority and intensively developing all around the world, it is essential to provide a framework of the place and role of Russia. In this study, we analyzed the local research pattern, revealed trends in publication and clinical trial activity and discussed the background determining them.

METHODS: Publication and clinical trial activity was studied using Scopus and clinicaltrials.gov databases respectively.

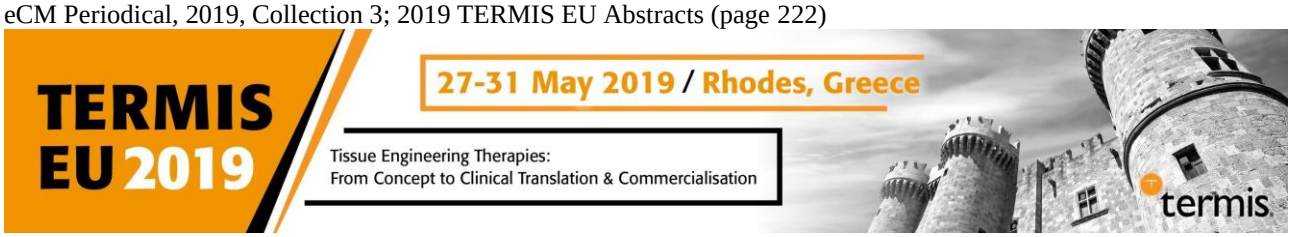
RESULTS & DISCUSSION: We revealed that the amount of publications in the field of regenerative medicine and tissue engineering written by Russian scientists annually increased from 1998 to 2018, and during this period it rose almost in 24 times. However, last two years the publication activity was approximately on the same level. The same trend was noticed for clinical trials. The government has started to create the required environment to enhance research in this field and its clinical translation by passing the new law regulating the production of biomedical cell products and acting from 2017. Due to historical background, tissue engineering and regenerative medicine in Russia differ from other countries and are strongly connected with physics (especially laser technologies), material science, and mathematics. The highest amount of research centers working in this field are concentrated in the central part of Russia, particularly in Moscow and Saint Petersburg. However, there are strong research teams located in Tomsk and Novosibirsk, which are placed in Siberia.

CONCLUSIONS: Today, Russia with its distinctiveness mainly formed in the USSR during the Iron Curtain period is opened to collaborations with scientists from all over the world giving a birth to the new generation of scientists who work in the field of tissue engineering and regenerative medicine and creating non-trivial approaches [1-4].

ACKNOWLEDGEMENTS: Financial support was received from the Russian Science Foundation (18-15-00401; analysis of publication and clinical trial activity) and Russian academic excellence project 5-100 (background analysis).

REFERENCES

[1] Kuznetsova DS et al. Biofabrication. 2017;9(2): 025009
[1] Zurina IM et al. Biomedical Materials. 2018;15(5):054104
[2] Grebenik EA et al. Biomedical Materials. 2018;13(5):054103
[3] Gorkun A et al. Biomedical Materials. 2018;13(4): 044108



Biofabrication of different tissue models based on methacrylated gelatine

P.J. Kluger[1]
Presenting Author: Petra J. Kluger, petra.kluger@reutlingen-university.de
[1]Reutlingen University, Reutlingen, Germany

INTRODUCTION: Additive manufacturing techniques are increasingly used in tissue engineering to generate complex tissue structures. Therefore, bioinks are needed to build up 3D-geometries, maintain cell viability and function to enable the maturation of the tissue after printing.

METHODS: Human primary cells (ASCs, Adipocytes and endothelial cells) were isolated from different biopsies as described in [1-3]. Hydrogels based on methacrylated gelatin (Fraunhofer IGB) were used and modified with hyaluronic acid, hydroxyapatite particles [4,5,1]. Cells were encapsulated into these inks to achieve tissue specific bioinks that can be printed by microextrusion in different geometries. Moreover, constructs containing two different bioinks were manufactured. These hydrogels were cultured under static or dynamic conditions in a perfusion reactor [6], for up to 28 days. Tissue constructs were characterized e.g. by using different staining techniques or rheological measurements.

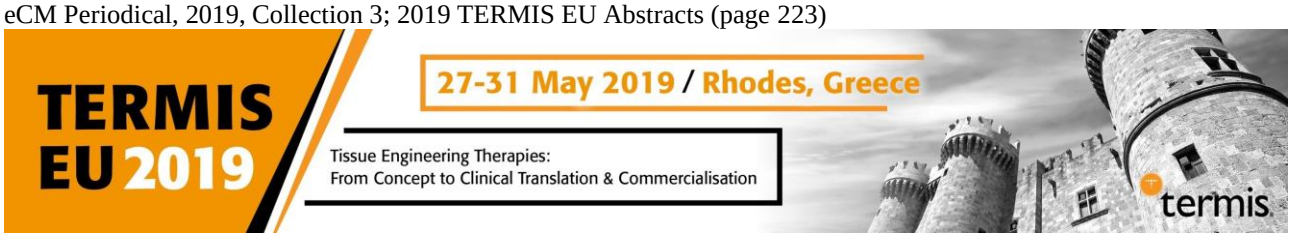
RESULTS & DISCUSSION: The encapsulation of different primary cells in methacrylated gelatin was successfully established. Numerous geometries for various tissue models were fabricated including viable and functional cell, proved e.g. by different immuno fluorescent stainings. The use of our tailor made tissue specific bioinks lead to an improved differentiation of e.g. ASCs to the osteogenic lineage measured by rheological methods [7]. Even more physiological performance of the cells were found in coculture approaches especially in the formation and stability of new formed vessel like structures [2]. Other models were directly printed in our perfusion bioreactors and cultured there for up to 28 days. Thereafter, a maturation of the tissue models could be visualized by detecting proteins like ALP or Osteopontin.

CONCLUSIONS: Different tissue models were successfully manufactured based on methacrylated gelatin. More physiologi-cal behavior can be achieved by adding tissue specific cues like tailored bioinks/components, coculture of cells and adapted culture conditions.

ACKNOWLEDGEMENTS: We thank the Dr. Kirsten Borchers (Fraunhofer IGB), the European Commission, the Carl Zeiss Stiftung and the Ministry of Science, Research and the Arts in Baden-Württemberg.

REFERENCES

[1] Huber B. et al. Tissue Eng Part C Methods 2015 21(12):1237-45
[2] Wenz A. et al. Biotechnol Bioeng. 2018 115(10):2643-2653
[3] Volz A-C. et al. ALTEX. 2018;35(4):464-476
[4] Hoch E et al. J. Mater. Chem. B, 2013; 1:5675–5685, 2013
[5] Wenz A. et al. BioNanoMaterials, 2016; 17(3–4):179–184
[6] Huber B. et al. Biomed Tech. 2018
[7] Wenz A. et al. Biofabrication 2017 14; 9(4):044103



From implant making & screws to material science & glues - An unexpected journey!

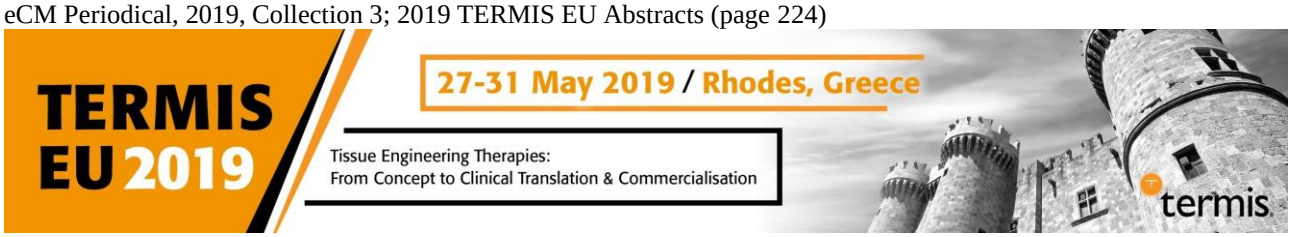
P Procter [1,2]
Presenting Author: Philip Procter, philip.procter@angstrom.uu.se
[1]Division of Applied Materials Science, Department of Engineering Sciences, Uppsala University, Box 523, 75120, Uppsala Sweden. [2]GPBio Ltd., Shannon, Ireland.

When you realise that you are actually trying to imagine what it is like to be an orthopaedic bone screw inside living bone, is the diagnosis midlife crisis or perhaps an epiphany? As a young engineer I escaped industry into biomedical engineering to take on the ultimate challenge of repairing people rather than machines. At that time the orthopaedic implant industry left it to surgeons to decide what healing bone needed. We implant makers gave them whatever metal implant they asked us to make. Much of what passed for implant design in that era was empirical and mechanical. With the empirical approach we developed an innovative hip fracture implant, the Gamma locking nail, that eventually becomes a world-wide standard of care. However, no matter how we modify the metal lag-screw part of this implant it still migrates through poor-quality bone at a similar rate to earlier devices. In my moment of epiphany, inside the lag-screw hole in cancellous bone, I visualize the many trabecular struts in contact with the implant and realise how little of the surface is actually solid in poor quality bone. It becomes obvious in an instant that a biomaterial is needed that can effectively bridge between implant and the many weak bony struts. The biomaterial could be a calcium phosphate cement or, to dream, even an adhesive. When I test the idea of a bone glue with surgeons, they list many clinical situations where they too dream of a bone glue. This changes the direction of my life: from development engineer to scientist, from employee to entrepreneur and from metal implant making to biomaterials formulation. After many travails we eventually develop a bone glue [1], and in an evidence-based world where no such biomaterial exists, there are many hurdles in qualifying this as both safe and effective for human use. In this talk to the TERMIS learned society I will share some of hard lessons learned in translating from bone screws to bone glues.

ACKNOWLEDGEMENTS: Financial support for the OsStic bone adhesive project was received in part from EIT Health HeadStart grant (2018 HS PoC 2018-HS-0046).

REFERENCES

[1] Pujari-Palmer M et al. Materials. 2018 Dec 7;11(12).



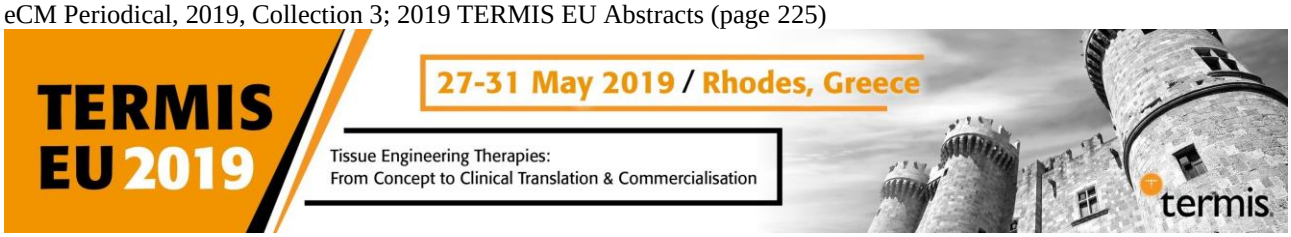
Immunomodulatory programming and antimicrobial activity by new biomaterials

P. Lavalle[1]
Presenting Author: Philippe Lavalle philippe.lavalle@inserm.fr
[1]Inserm, Biomaterials and Bioengineering, University of Strasbourg, France

All implantable biomedical systems face several risks once in contact with the host tissue: excessive immune response to the implant and development of bacterial biofilms. A multifunctional surface coating that can address all these two issues concomitantly would significantly improve clinical outcomes. We hypothesized that polyarginine (PAR), a synthetic highly cationic polypeptide, can act on macrophages to control innate immune response because arginine is an important component of macrophage metabolism. Moreover, PAR is susceptible to act as an antimicrobial agent due to its positive charges. We developed a new polyelectrolyte multilayer film based on PAR and hyaluronic acid (HA). The layer-by-layer PAR/HA films have a strong inhibitory effect on the production of inflammatory cytokines released by human primary macrophages subpopulations [1]. This could reduce potential chronic inflammatory reaction following implantation. Next, we show that PAR/HA films were very effective to inhibit Gram-positive and Gram-negative pathogenic bacteria associated with infections of medical devices [2] [3]. We demonstrate that exclusively films constructed with poly(arginine) composed of 30 residues (PAR30) acquire a strong antimicrobial activity. Moreover, changing HA by another synthetic or natural polyanion did not provide any more antimicrobial activity. HA is a key component of the system and the mechanism behind this property has been elucidated. The cytocompatibility of the PAR/HA films was assessed with several cell types playing a major role in tissue regeneration. This all-in-one system that limits strong inflammation and prevent pathogens infections on implants constitutes a powerful material easy to scale up.

REFERENCES

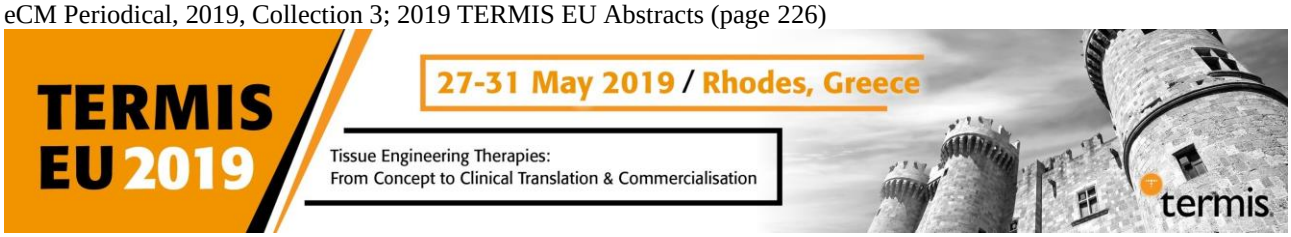
[1] Özçelik, H. et al. Adv. Healthc. Mater. 2015; 4, 2026-36.
[2] Mutschler, A. et al. Chem. Mater. 2016; 28, 8700-09.
[3] Mutschler A. et al. Chem. Matter., 2017; 29, 3195–01.



Bioactive nanocoatings via layer-by-layer technique to improve antibacterial properties of polymeric membranes for guided bone regeneration

P. Gentile
Presenting Author: Piergiorgio Gentile, piergiorgio.gentile@newcastle.ac.uk
School of Engineering, Newcastle University, Newcastle upon Tyne, United Kingdom

The use of antimicrobial biomaterials is attractive in medicine and dentistry field, where the elimination of bacteria and device-associated biofilms is necessary for an effective treatment. For instance, the conventional periodontitis treatment is helpfully complemented by the additional administration of antibiotics, which can be applied by local or systemic administration. Compared to systemic drug delivery, in periodontology local administration is considered to be more effective, since the pathogen-specific drug can be positioned in the periodontal pocket with suitable concentrations for an adequately long time period. Moreover, the risk of undesired side effects caused by high systemic doses or resistance development can be drastically reduced. Thus, it is beneficial to use local delivery systems that control the release of their agents and guarantee lasting drug concentrations in the pocket. Therefore, innovative localised and controlled delivery system will be discussed in the treatment of dental and periodontal infections for: (1) reducing the released antimicrobials amount, preventing both drug-related systemic side effects and bacteria resistance, and (2) preserving the physico-chemical properties of the medical device without incorporating the antibiotic into the material bulk. Specifically, we propose the Layer-by-Layer (LbL) technique for coating biodegradable membrane by to obtain discrete nanoscale layers to (1) incorporate and control the release of the antibiotic drug with minimal interaction with the biomaterial substrate, or (2) propose a strong antibacterial material, Manuka honey, as negative-charged polyelectrolyte for the manufacturing of the nanocoating.



Maxillo-facial bone regeneration with stem cells and biomaterials in a comparative clinical trial

P. Layrolle[1]
Presenting Author: Pierre Layrolle, pierre.layrolle@inserm.fr
[1]INSERM UMR 1238, PHY-OS, Bone sarcomas and remodeling of calcified tissues, Faculty of Medicine, University of Nantes, Nantes, France

INTRODUCTION: After loss of teeth, alveolar bone significantly resorbs over years and implant supported rehabilitation is often not possible. Autologous bone grafting, despite some disadvantages, is still considered the gold standard for reconstruction of maxillofacial bone defects. During the previous European project REBORNE, a novel bone augmentation protocol has been introduced (EudraCT, 2012-003139-50). It consists of mandible bone augmentation with autologous culture expanded mesenchymal stem cells and biphasic calcium phosphate granules prior to dental implants. The aim of this study is to compare bone regeneration by using cell therapy and biomaterials with autologous bone grafting in 150 edentulous patients.

METHODS: This clinical trial has received ethical and medicinal agency approvals. 30 ml of bone marrow stem cells (BMSC) were aspirated from the posterior iliac crest and plastic adherent mesenchymal stem cells were expanded in culture medium containing human platelet lysate for 2 weeks. During surgery, 100 million of BMSC were seeded on 5 cm^3 of biphasic calcium phosphate (BCP) granules and inserted on the alveolar bone. After 4-6 months, bone regeneration was assessed by clinical examinations, cone beam computed tomography (CBCT) and histology of core biopsies.

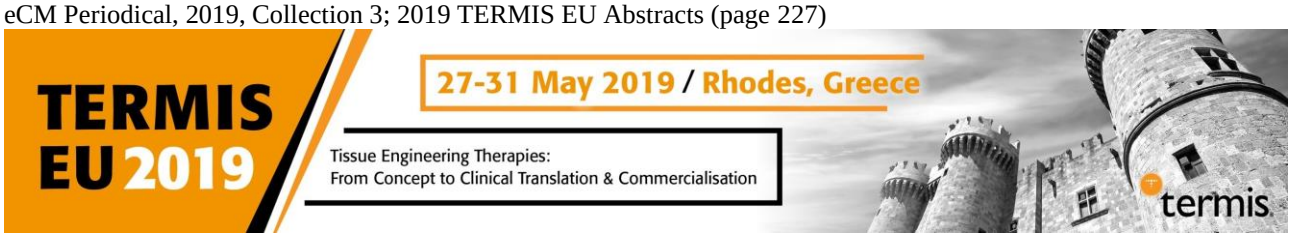
RESULTS & DISCUSSION: Eleven patients have been successfully treated without adverse events. The combination of autologous cells and biomaterials induced significant new bone formation. After 4-6 months, the mean increase in bone width and volume was 4.05 mm and 887.2 mm^3, respectively ($n=14$, $p < 0.001$). Histological analysis revealed that BCP granules were well integrated with deposition of newly formed lamellar bone tissue on the surface of the BCP particles in the entire core biopsies. The regenerated bone volume was adequate for dental implant installation. The patients were satisfied with the esthetic and functional outcomes.

CONCLUSIONS: These results warrant further clinical investigation in the MAXIBONE project challenging the current gold standard in bone augmentation such as autologous bone transplantation.

ACKNOWLEDGEMENTS: This clinical study has received financial support from the European Commission through the FP7 and H2020 projects REBORNE (241879) and MAXIBONE (779322).

REFERENCES

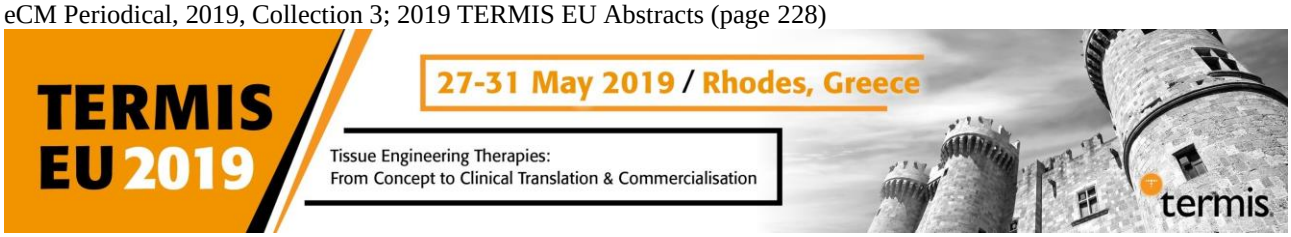
[1] Gjerde et al. Cell therapy induced regeneration of severely atrophied mandibular bone in a clinical trial. Stem Cell Research & Therapy. 2018; 9:213.



Injectable biomaterials for bone regeneration

P. Weiss[1]
Presenting Author: Pierre Weiss, pierre.weiss@univ-nantes.fr
[1]UMR 1229, RMeS, Regenerative Medicine and Skeleton (Regos Team), Université de Nantes, Inserm, ONIRIS, Nantes, F-44042, France

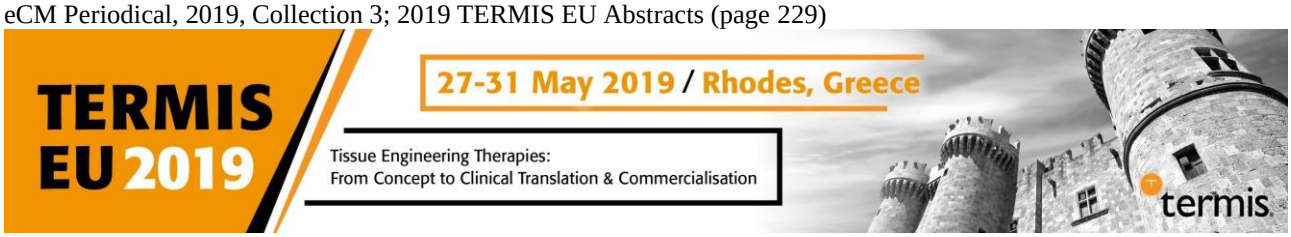
20 years ago, we designed injectable bioactive suspensions in water of calcium phosphate ceramics for bone and periapical regenerations. Because of leakage of these suspensions, we focused on injectable hydrogels before to set in situ by chemical crosslinking to form 3D scaffolds. We set up a platform to develop a series of innovative hydrogels for bone, cartilages and periodontal tissue regeneration. We based our strategy on polysaccharides macromolecules because they are renewable materials, that originate from biological sources and generally are biocompatible, non-toxic and biodegradable. We developed a family of silated macromolecules able to react forming biocompatible hydrogels. The silated polymers are self-setting hydrogels able to covalently crosslink under pH variation, without addition of toxic crosslinking agent. All these macromolecules could be combined in multicomponent hydrogels, representing a strategy for improving mechanical properties of biomaterials or to tailor particular properties to meet specific needs. For mineral scaffolding, we realized composites of calcium phosphates particles or cements with hydrogel, increasing the ductility and creating macroporous scaffold to propose foam bone cements well adapted to bone biomaterials and Bone tissue engineering. Perspectives are 3D printing and bio printing techniques. We will use our hydrogels platform to prepare tunable (bio)inks in skeletal medicine.



Deciphering fate choices in mesenchymal stems cells

V. P. Shastri[1,2]
Presenting Author: V. Prasad Shastri, prasad.shastri@gmail.com or prasad.shastri@makro.uni-freiburg.de
[1]Institute for Macromolecular Chemistry, University of Freiburg, Freiburg, Germany [2]BIOSS, Centre of Biological Signalling Studies, University of Freiburg, Germany

Mesenchymal stem cells (MSCs) have been long studied for their role in skeletal development. In adult physiology, MSCs are unique in that they exhibit pluripotency and differentiate into cells that can evolve into various skeletal tissue. As a result, they have been extensively employed as a viable alternative to terminally differentiated cells in engineering of cartilage and bone tissue ex vivo and in vivo. In spite of decades of effort in this direction, our understanding of what drives MSC fate choices is rather narrow in that it places heavy emphasis on a role for morphogens and cytokines (TGF-beta super family, FGF-2). In recent years it has become evident that MSCs also play an important role in wound healing, immunomodulation (immune suppression) and in tumor progression. While this points to a more ubiquitous role for MSCs in regenerative processes and tissue repair, what factors affect MSC fate choices remains to be fully unraveled. We hypothesize that activation of differentiation programs in MSCs have an autocrine and paracrine component involving interplay between MSC-MSC (cell-cell contact) and MSC-(environment), and in this signaling paradigm the biophysical aspects of their microenvironment play a dominant role. We have tested this premise in several aspects of MSC behavior (proliferation, migration, differentiation, chondrogenesis) and have gathered compelling evidence for biophysics and mechanobiology in MSC fate decisions. This talk will present some of our latest findings in this area.



Micro-3D printing of osteocyte organoid models

R. Mueller
Presenting Author: Ralph Müller, E-mail: ram@ethz.ch
Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

INTRODUCTION: Osteocytes are deeply embedded in the bone matrix in lacunae and connected by dendritic cell processes through sub-micron canalicular channels. The morphology of osteocytes and their surrounding lacuno-canalicular network (LCN) are hypothesized to regulate cellular mechanosensing [1]. Furthermore, it has been shown that morphological parameters like osteocyte shape, volume and number of dendrites are altered with aging and in diseases such as osteoarthritis and osteoporosis [2]. The role of these changes however remains elusive. Yet, it has been demonstrated in 2D that rounded osteocytes are more mechanosensitive than osteocytes spread on flat surfaces [3]. In 3D however, it has so far not been possible to manipulate the morphology of osteocytes and the LCN on the micrometer level to assess their effects on mechanosensing. We therefore aim to control LCN and cell morphology, particularly dendrite number, using a micro-3D printed osteocyte organoid model to mimic dendrite loss reported during aging / age-related disease.

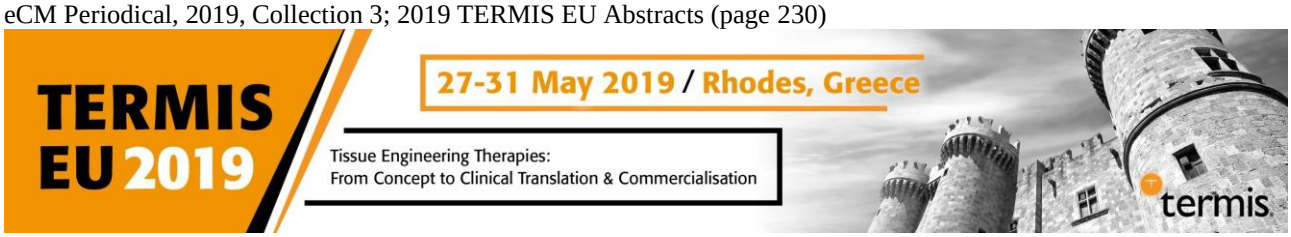
METHODS: LCN-mimetic structures were micro-3D printed by two-photon-polymerization with a Nanoscribe GT 3D microprinter using inorganic-organic-hybrid polymer OrmoComp® (micro resist technology GmbH) and coated with Collagen I. Following 35 days of differentiation in tissue culture flasks, IDG-SW3 osteocytes (Kerafast Inc.) were dissociated using Trypsin-EDTA and Collagenase type II and reseeded on printed structures. 24 hours after reseeding, cells were fixed, stained with Phalloidin-TRITC, and mounted with Fluoroshield™ containing DAPI. Images were acquired by scanning confocal microscopy (Zeiss LSM880) and cells (n=92) forming dendrites in the artificial canaliculi were analyzed using FIJI. Dendrite length (n=333) was determined as the distance from the end of the dendrite to an ellipse approximating the cell body. Dendrite branches were disregarded in the analysis.

RESULTS & DISCUSSION: IDG-SW3 osteocytes in two micro-3D printed structures allowing for 10 or 6 dendrites per cell to be formed were investigated. The difference in the LCN-mimetic structures induced a major difference in the number of dendrites per osteocytes. Average length of dendrites and their length distribution were not affected, indicating undisturbed dendrogenesis.

CONCLUSIONS: We were able to mimic dendrite loss in 3D, one of the hallmarks of aging in bone. In the future, we will expand this concept to manipulate osteocyte cell volume and aspect ratio and assess their effect under mechanical loading. We believe that a micro-3D printed organoid is versatile tools to further our understanding of osteocyte mechanobiology.

REFERENCES

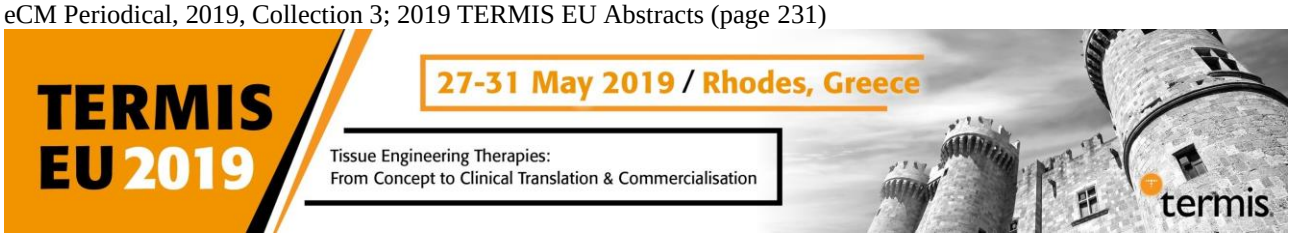
[1] van Oers RF et al. Curr Osteoporos Rep. 2015; 13:61-66
[2] Hemmatian H et al. Curr Osteoporos Rep. 2017;15:401-411
[3] Bacabac RG et al. J Biomech. 2008;41:1590-8



Immunomodulation in intervertebral disc degeneration

R. M. Gonçalves
Presenting Author: Raquel M. Gonçalves, raquelg@ineb.up.pt
i3S-Instituto de Investigação e Inovação em Saúde; INEB-Institute of Biomedical Engineering, Porto, Portugal

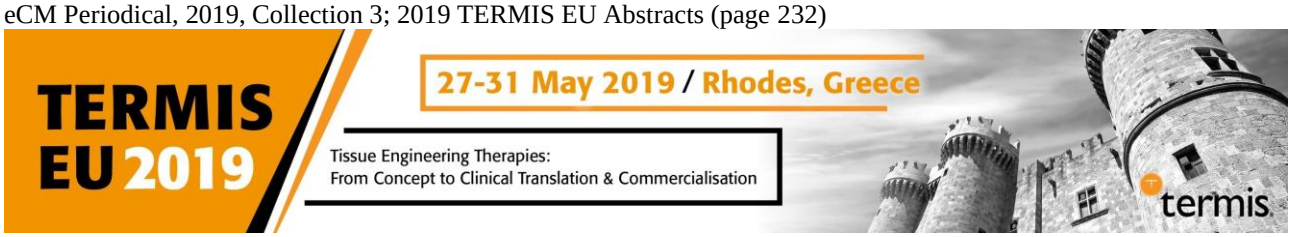
Aging and degeneration of intervertebral disc impact on tissue function leading to low back pain, the first disorder in years lived with disability and a major burden in healthcare systems. Frequently, the inflammatory microenvironment has been strongly associated with disc degeneration. Besides native nucleus pulposus cells, immune cells namely macrophages, are known to be recruited to degenerated disc. However, it is still not clear the mechanisms behind the crosstalk of these cells and their contribution to the disease pathomechanism. To improve tissue regeneration, immunomodulatory strategies are gaining attention worldwide, either using biomaterials or cell therapies. In this work, anti-inflammatory nanoparticles based on chitosan and poly-gamma-glutamic acid with an anti-inflammatory drug, previously developed by our group, were able to reduce inflammatory markers in disc degeneration while promote remodelling of cartilaginous extracellular matrix (ECM), in a single intradiscal injection. We also demonstrated that mesenchymal stem/stromal cells (MSCs) reduce the pro-inflammatory gene expression of nucleus pulposus, but not anulus fibrosus, cells, under a pro-inflammatory microenvironment, which might explain the success of MSCs-based therapies in the context of low back pain. In the end, this presentation expects to highlight new ex vivo models of disc degeneration and associated inflammatory response and to disclose how immunomodulatory strategies can impact on degeneration of intervertebral disc.



Implant biomaterials in the age of tissue engineering and regenerative medicine

R. Bizios
Presenting Author: Rena Bizios, rena.bizios@utsa.edu
Department of Biomedical Engineering, The University of Texas at San Antonio, San Antonio, Texas, USA

Major scientific developments in several bio-related fields during the last three decades compelled new thinking and practices regarding implant biomaterials. Novel and seminal advances in the biological/physiological/medical fields have redefined the criteria and specifications for the design, synthesis, and formulation of biomaterials used for various implantable devices. Such approaches require close attention to advances at the tissue, organ**,** molecular, cellular and gene biology/physiology levels, keeping track of clinical needs and developments at the frontiers of medicine, as well as creative translation and application of the latest developments in the aforementioned biomedical fields to biomaterial designs and formulations pertinent to applications in tissue engineering and regenerative medicine. Currently design and development of novel biomaterials has reached a “steady state” plateau. Pertinent developments have been either incremental or “refinements” of existing biomaterials (e.g., those approved by federal regulatory agencies). How can the biomaterials field meet present challenges and benefit from future scientific advances? Undoubtedly, novel ways of thinking and practice are needed to assure a bright future for biomaterials in the age of tissue engineering and regenerative medicine. Pertinent approaches include (but are not limited to) the following: (1) redefinition of biomaterials-related criteria and specifications to address needs of specific biomedical applications; (2) training the next generations of biomaterial scientists/engineers to include basic biological/physiological sciences; (3) interdisciplinary teams of collaborating scientists, clinicians and engineers whose complementary expertise has the potential to provide solutions to current scientific problems/limitations, explore and advance new scientific frontiers, and thus develop the implant biomaterials of the future.



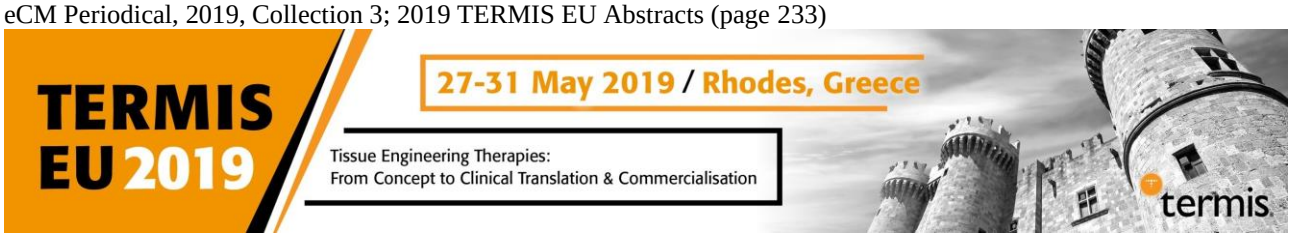
Where civil engineering and biology meet: articular cartilage

P.R. van Weeren[1]
Presenting Author: René van Weeren, r.vanweeren@uu.nl
[1]Department of Equine Sciences and Department of Clinical Care of Companion Animals), Faculty of Veterinary Medicine, Utrecht University, The Netherlands

The main function of the non-contractile tissues of the musculoskeletal system (i.e. bone, cartilage and tendons/ligaments) is mechanical. These tissues give structural support to the body and they accommodate, transmit and to a certain extent dampen the (often considerable) forces generated by terrestrial locomotion. If those external challenges exceed the strength of the tissues, failure will occur. This may be caused by single major insult, but is more often an insidious process that is the consequence of wear and tear and gradual degradation. All of these tissues have a collagenous template as basis which is modified according to the demands made to the specific tissue. Calcium hydroxyapatite is deposited on the underlying collagen network of bone to provide rigidity, in ligaments and tendon collagen fibrils are arranged in parallel form complying with the tensile stress they are subjected to and may have more or less additional components such as elastin to increase elasticity in case of energy storing tendons, such as the Achilles tendon in humans or the superficial digital flexor tendon in horses. In cartilage it is the intrinsic tension, generated by the collagen network and the interspersed hydrophilic proteoglycan aggregates, that gives the tissue its unique resilience and resistance against both compressive and shear loading. In this sense, cartilage is by far the most challenged tissue of the three as it is not only loaded in various directions but it also must transmit the same forces while still being resilient, so without having recourse to the solution of rigidity. In the perception of the term “tissue engineering” the word “engineering” often has a connotation of “fabricating”, “producing” or “making”. That is fine for organoids, but in musculoskeletal tissue engineering the word “engineering” should be interpreted differently. In civil engineering it is all about the combination of materials and their spatial arrangement or architecture to meet the mechanical demands. The Pons Fabricius in Rome that connects the left Tiber embankment to the Tiber island, constructed in 63BC and in continuous use ever since, is a good example of durable civil engineering. The architecture of the arches, taking into account loading by traffic and the forces generated by intermittent flooding of the river, together with solid material choice gave the structure its impressive durability. In living beings the situation is not essentially different. Nevertheless we often tend to forget especially the architecture, even where we are well aware of the fact that this architecture is essentially loading driven during development. That has been known for long in bone [1], but it applies to articular cartilage too [2]. It was Benninghoff who described the essence of the architecture of the collagen network of articular cartilage already almost a century ago [3]. However, where articular cartilage tissue engineering has strongly focused on production of the major extracellular matrix components (proteoglycans and collagen type II), architecture has been largely neglected. As biological engineers we’re far yet in terms of durability from the achievements of the Roman civil engineers, but are fortunate that our durability goal is not more than a lifetime. We will only reach that goal, though, if we take into account that in tissue engineering of musculoskeletal tissues the same applies as in civil engineering: it is all about the mechanics and they are dictated by components and architecture.

REFERENCES

[1] Wollf J. Das Gesetz der Knochen. 1893.
[2] Brama PAJ et al. J Orthop Res. 2009; 27(9):1226-1234.
[3] Benninghoff A. Zsch Zelforsch Mikroskop Anat. 1925;2:783-862



ECM mimics using modular minimalistic supramolecular systems

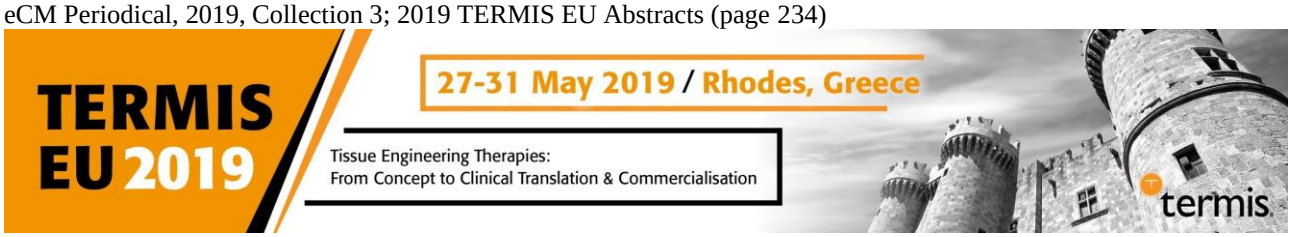
R.A. Pires[1,2,3]
Presenting Author: Ricardo A. Pires, rpires@i3bs.uminho.pt
[1]3B's Research Group, I3Bs, University of Minho, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

Supramolecular gels made of peptide amphiphiles (PA) are close biomimics of the extracellular matrix (ECM) at functional and structural level. On one hand, PA represent the targeted bioinformation of ECM proteins through the incorporation of specific aminoacid sequences. On the other hand, the PAs are designed to generate nanofibers via non-covalent interactions (i.e. Pi-Pi stacking, hydrogen bonding and hydrophobic effects). These nanofibers can further generate hydrogels that are stable under physiological conditions and stimuli responsive (e.g. pH, temperature). Despite the advantages that these systems have, supramolecular hydrogels generated by one type of PA lack chemical diversity and functional complexity present in the ECM. [1] As an example, there is a large set of bioinformation that is added in biological systems via post-translational modifications, such as protein glycosylation. Functionalization of PAs with carbohydrates to better mimic proteoglycan has been recently proposed. [2] However, this involves a laborious synthetic work, and the conjugation of the carbohydrate can modify the PA's self-assembly propensity. Multicomponent co-assembly offers a possibility to expand the chemical complexity and diversify the functionality of the supramolecular systems without compromising the simplicity of the building blocks. [3] Thus, we developed a modular system to generate proteoglycan mimics, which is based on the combination of two different amphiphiles (modules): a PA and a carbohydrate amphiphile (CA). We explored the capacity of these amphiphiles to self-assemble and generate structures that display the biofunctionality of glycans and proteins. We observed that a PA (e.g. fmoc-FF) and a CA (e.g. fmoc-glucosamine-6-sulfate or fmoc-glucosamine-6-phosphate), if properly designed, are able to interact cooperatively and form nanofibers with a structural PA core and a functional CA shell – a structural arrangement that resembles the proteoglycans in the ECM. We proved that this system is versatile: different carbohydrates can be used to generate the outer shell of the nanofibers. The generated nanofibers can further form hydrogels at physiological conditions via supramolecular crosslinking with Ca ions from the culture medium. These supramolecular gels are non-cytotoxic and biofunctional, as demonstrated by studies with growth factors, lectins and cells. They can be regarded as mimics of the ECM at another complexity level as they mimic two of the most important classes of biomolecules present in the ECM: proteins and glycans. Using this modular system it is possible to create nanofibers and supramolecular hydrogels with different peptides and carbohydrates in a high throughput manner that can be used to mimic different proteoglycans present in the ECM. Such systems will be useful either for fundamental studies targeting proteoglycans or as scaffolds for tissue engineering and regenerative medicine.

ACKNOWLEDGEMENTS: The financial support by the EC under grant agreement numbers 692333–CHEM2NATURE, 668983–FORECAST and 739572-THE DISCOVERIES CTR is acknowledged.

REFERENCES

[1] Makam et al, Chem Soc Rev 2018, 47, 3406-3420;
[2] Lee et al, Nat Nanotech 2017, 12, 821-829;
[3] Brito et al, Chem Sci 2019, DOI: 10.1039/C8SC04361B.



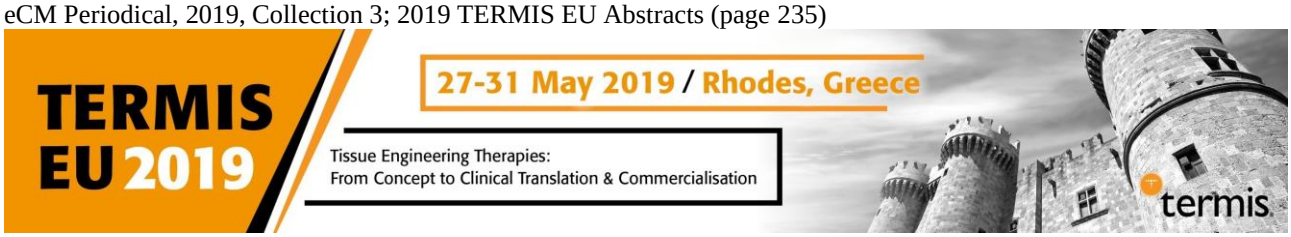
Translation of early healing principles into implant surface design for bone regeneration

R. Tejero[1,2]
Presenting Author: Ricardo Tejero, ricardo@minin.es
[1]Materials Science and Surface Engineering Laboratory, BTI Biotechnology Institute (Miñano, Spain) [2]Institute for Regenerative Medicine and Oral Implantology (UIRMI), University of the Basque Country (UPV-EHU, Vitoria, Spain)

Implant integration is a complex process between the implant surface and the surrounding ions, proteins, bacteria and tissue cells. Ideally, more bone-like implant surfaces with an extracellular matrix structure and composition would enable earlier implant integration and function. Typically, these biomimetic modifications consist in calcium phosphates containing oxides or coatings, with or without collagen and other non-collagenous proteins. However, these developments face limitations such as lack of structural integrity, absence of epitaxial matching, low solubility, elevated extraction and purification costs, high sourcing variability, potential immunogenicity or complex pre-processing steps that compromise their bioactivity. [1] Unsurprisingly, few if any of these developments make its way to the clinic. Rather than focusing on the final steps of bone regeneration, an alternative strategy consists in exploiting some of the principles of the early healing phases around implants. Bone-tissue regeneration starts at implant placement with the establishment of an ionic equilibrium at the interface, modulated by the surface and the local ionic composition. Protein adsorption and exchange at this new interface ensues and commands the processes of hemostasis and inflammation. A fast stabilization of the implant within the neighboring tissues is crucial for the success of implantation. It relies on the formation of the provisional matrix: a three-dimensional network of fibrin with embedded activated platelets and growth factors. Harnessing the development of this structure at the implant surface opens up the possibility to improve angiogenesis, reduce inflammation and attract, shelter and induce the activity of osteoprogenitor cells in charge of tissue regeneration [2,3]. Herein we present our experience modifying implant surfaces with soluble bioinorganic ions with relevant roles in intra- and extracellular processes relevant for bone regeneration. We investigate how this modulates the ionic equilibrium of the interface, the protein adsorption pattern and the ultimate impact of this approach all the way down to bone-implant integration in vivo.

REFERENCES

[1] Tejero R et al. Prog Polym Sci. 2014; 39:1406-47
[2] Anitua E et al. JPeriodontol. 2013;84(8):1180-90
[3] Tejero R et al. Langmuir 2013;29(3):902-12



Mechanical and microtopological environments to drive chondrogenesis

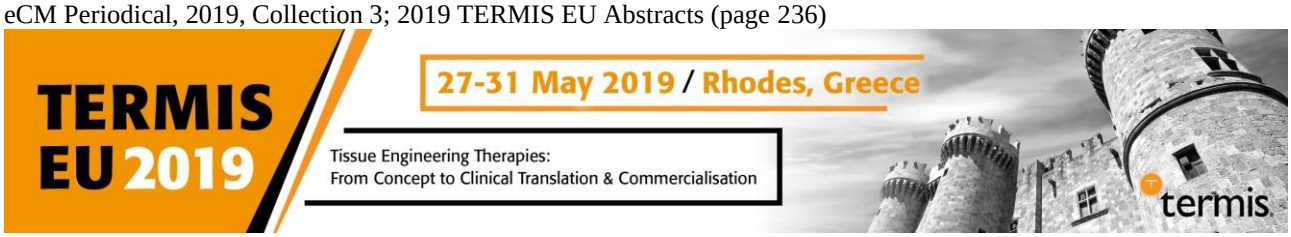
R. Gottardi[1,2]
Presenting Author: Riccardo Gottardi, gottardir@email.chop.edu
[1]Pediatric Bioengineering and Biomaterials Laboratory (Bio[2] Lab), Department of Pediatrics, The Children's Hospital of Philadelphia (CHOP), Philadelphia, USA [2]Ri.MED Foundation, Palermo, Italy

Several regenerative medicine approaches are currently adopted to repair small focal lesions of articular cartilage, from microfracture, widely used but highly unsatisfactory, to matrix assisted chondrocytes implantation, to allografts (primarily in the US). All these techniques, are followed by extensive rehabilitation, that in general start with early passive motion followed by limited weight bearing exercises. However, there is limited (if any) scientific evidence of how these different mechanical regimes affect cell differentiation at the defect site, cartilaginous tissue formation, and integration with the surrounding cartilage [1]. To study this process, we employed a reductionist approach based on in vitro model of cartilage repair, subjected to different loading regimens to simulate the different rehabilitation exercise sessions. Ultimately, we aim at identifying the specific effects on regeneration of each exercise regime by applying multiple combinations of mechanical stresses in order to guide the optimization of post-op rehabilitation. When devising tissue engineering solutions for larger defects, a number of other factors need to be considered. Cartilage and bone are intimately and developmentally connected in articular joints [2]. To provide effective and translatable regenerative approaches, scaffolds need to include both simplicity and complexity. Simplicity to lower the translational, regulatory barrier; complexity to provide mechanical strength, mimic the local bone and cartilage microenvironment, and support and guide cell fate and differentiation. We tuned a thermally induced phase separation (TIPS) [3] approach to produce a porous poly-L-lactide (PLLA) scaffold for osteochondral tissue engineering that meets these criteria. The overall scaffold properties are suitable for implantation, while the local topology of the porous microenvironment supports and guides specific cellular differentiation towards a chondrogenic or osteogenic phenotype.

ACKNOWLEDGEMENTS: Financial support was received from the EC Horizon 2020 project OACTIVE: Advanced personalised, multi-scale computer models preventing OsteoArthritis, Grant Agreement No. 777159; CHOP Frontiers Programs; Ri.MED Foundation.

REFERENCES

[1] Gottardi R, Stoddart M. J. Am. Ac. Orth. Surg. 2018, 26(15): e321-e323.
[2] Nichols DA, et al. Biom. Microdev. 2018, 20: 18
[3] Mannella GA et al. Mater Letts. 2015, 160: 31-33.



Towards the biofabrication of complex, heterogeneous bone and cartilage structures with multiple printing technologies

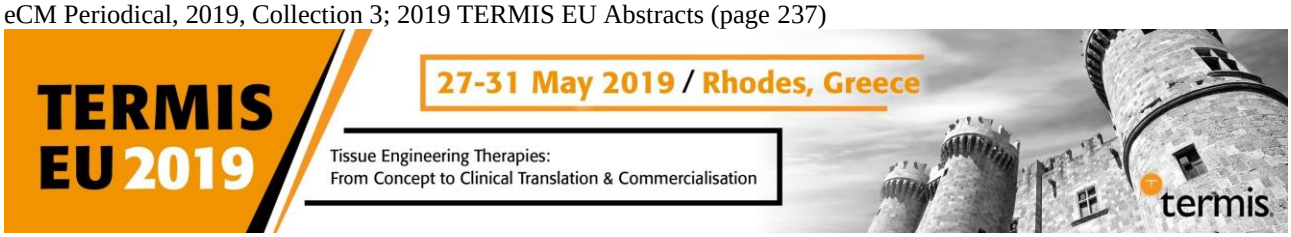
R. Levato[1,2]
Presenting Author: Riccardo Levato, r.levato-2@umcutrecht.nl
[1]Regenerative Medicine Center and [2]Department of Orthopedics, Biofabrication in Translational Regenerative Medicine group, University Medical Center Utrecht (UMCU), Utrecht, The Netherlands

Major challenges in musculoskeletal tissue engineering revolve around recapitulating the architecture, and therefore the function, of native tissues. Present strategies to treat chondral and osteochondral defects, including tissue engineering and cell implantation, prevalently result in repair tissue with poor mechanical properties, which is prone to degeneration, and can only delay the insurgence of severe pathologies like osteoarthritis. Biofabrication is opening new avenues for the restoration of impaired joints tissues. Multi-material bioprinting enables to fabricate composite structures combining cell-laden, soft hydrogels with mechanically strong polymers for structural support [1]. By the accurate 3D patterning of stem and progenitor cells with different chondrogenic potential, salient features of the native zonal and depth dependent organization of articular cartilage can be replicated [2]. Alongside hydrogel extrusion and bioprinting, different additive manufacturing technologies, such as melt electrowriting of polymeric microfibers, ceramic plotting and digital light processing lithographic printing of hydrogels [4], can be combined to create composite, cell-laden constructs that enable integration between engineered cartilage hydrogels and bone scaffolds. These bioartificial osteochondral grafts exhibit improved interfacial mechanical strength, favouring their integration in vivo. Herein, the latest development in the field of bioink printing to create zonal-biomimetic cartilage constructs will be discussed, together with the integration of multiple (bio)printing strategies (i.e. co-fabrication of hydrogels, reinforcing polymers and bioceramics), and the impact of these technologies towards the generation of fully biofabricated, high-performance engineered osteochondral grafts, with potential application for regenerative medicine.

ACKNOWLEDGEMENTS: Financial support was received from the Dutch Arthritis Foundation (LLP-12, LLP-22, and CO-14-1-001), and the AO Foundation (ReInforce project).

REFERENCES

[1] Mouser et al. Biofabrication. 2017; 9 (1), 015026
[2] Levato et al. Acta Biomater. 2017; 61, 41-53
[3] Lim KS et al. Biofabrication. 2018; 10 (3), 034101



Regulation of cartilage phenotype during MSC differentiation

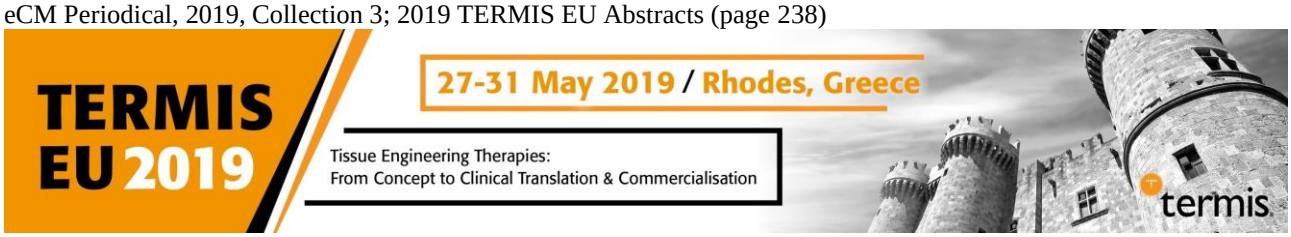
R. Narcisi[1]
Presenting Author: Roberto Narcisi, r.narcisi@erasmusmc.nl
[1]Department of Orthopedics, Erasmus MC, University medical Center, Rotterdam, The Netherlands

Adult mesenchymal stem cells (MSCs) are potential source of chondrogenic cells for the treatment of cartilage disorders, but loss of chondrogenic capacity during in vitro expansion and the propensity of the cartilage derived from those cells to undergo hypertrophic maturation limit their therapeutic application. In the last years, often taking inspiration from the process of cartilage and bone formation during development, we aimed to identify the factors that regulate MSC expansion and their hypertrophic maturation. What we observed highlighted the importance of the FGF, WNT and TGFβ signaling interplay during the chondrogenic differentiation processes. By manipulating the expression of the TGFβ signaling components , for example, we showed how the expression of specific ALKs (TGFβ receptors) and SMADs (TGFβ intracellular effectors) during differentiation of MSCs is essential to allow chondrogenesis to take place [1-2]. We also observed that WNT3A, specifically when added in combination with FGF2, supports extensive expansion of MSCs while maintaining their chondrogenic capacity. Interestingly, the administration of WNT3A during expansion combined with the inhibition of WNT signals during differentiation was the best strategy to prevent hypertrophy maturation and calcification in vivo [3]. More recently we identify in mir-221 an interesting regulatory molecule to stimulate chondrogenic differentiation of MSC while preventing the production of the hypertrophic marker collagen type-10 in the extracellular matrix of the generated cartilage [4]. Notably, there are evidences that mir-221 can exert part of its function by regulating WNT and TGFβ signaling. In parallel, studies we conducted on developing mouse limbs allow us to identify in the transcription factor TWIST1 (a WNT target gene) an important factor in regulating proliferative and chondrogenic capacity of MSC [5], and now a new research is on-going to understand the possible involvement of TWIST1 in hypertrophic maturation. Given our recent interest in TWIST1, we are currently using a mRNA-base strategy to sort living MSCs based on their TWIST1 expression in order to verify whether the expression of this transcription factor can be used as selection marker for MSCs with improved proliferative and differentiation capacity. In our view all the acquired knowledge will facilitate the generation of new strategies to improve the applicability of MSC-based approaches in regenerative medicine, also beyond the cartilage field.

ACKNOWLEDGEMENTS: Financial support was received from the VENI grant of NWO (#13659); the Dutch Arthritis Foundation (ReumaNetherlands; #16-1-201) and the STW Perspectief Grant (#P15-23).

REFERENCES

[1] de Kroon L et al. PLos One. 2015; 10(12):e0146124.
[2] de Kroon L et al. Sci Rep. 2017;7:43164.
[3] Narcisi R et al. Stem Cell Reports. 2015; 4(3):459-72.
[4] Lolli A et al. Stem Cells. 2016; 34(7):1801-11.
[5] Cleary MA et al. Stem Cell Dev. 2017; 26(10):751-761.



Rejuvenation of regenerative capacity in aged cells

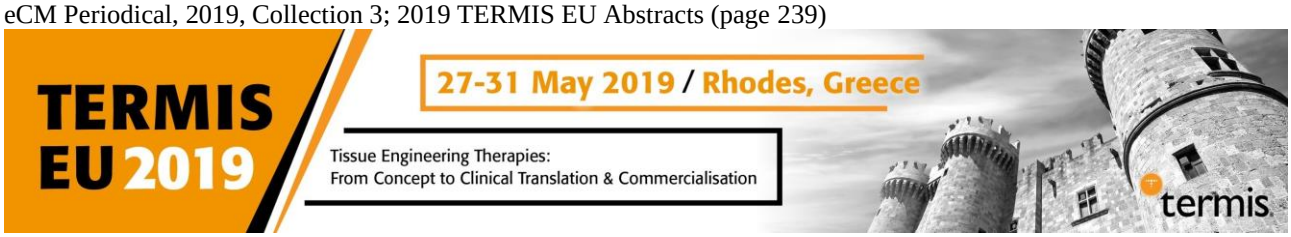
R. S. Tuan[1,2]
Presenting Author: Rocky S. Tuan, tuanr@cuhk.edu.hk
[1]Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong S.A.R., China [2]Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, U.S.A

The bio-activities of bone marrow derived mesenchymal stem cells (BMSCs) generally decline with increasing biological and in vitro culture age, possibly affecting in vivo maintenance of tissue homeostasis and regenerative application. However, the exact nature of the link between stem cell senescence and natural aging is not known. Interleukin-6 (IL-6) is a multi-functional pro-inflammatory cytokine involved in inflammation and infection responses, as well as in the regulation of metabolic, regenerative, and aging processes [1]. Our results showed that BMSCs from older donors exhibited higher level of cellular senescence as a result of replication during the process of culture expansion, and IL-6 treatment may be applied to reduce the degree of senescence with the potential as an anti-aging agent. Recently, FOXO4-DRI, a FOXO4 peptide that perturbs the FOXO4 interaction with p53, was reported to selectively kill the senescent cells through triggering apoptosis [2]. Our results showed that chondrocytes for Autologous chondrocyte implantation (ACI) exhibited a higher level of cellular senescence as a result of replication during in vitro expansion, and the treatment of FOXO4-DRI was able to selectively kill senescent cells in PDL9, which reduced the SASP in chondrocytes, enhanced their proliferation capacity, and led a cartilage formation with superior quality. In view of the encouraging in vitro results, we are currently testing the chondrogenic capacity of FOXO-DRI treated chondrocyte in rabbit model.

ACKNOWLEDGEMENTS: Financial support was received from the National Institutes of Health (R21AG056819) and Pepper Grant P30 AG024827.

REFERENCES

[1] Pricola KL et al. J Cell Biochem. 2009;108(3):577-88.
[2] Peter L.J. et al. Cell, 2017, 169(1): 132-147.



Progress towards a new biomimetic adhesive inspired by sea urchins

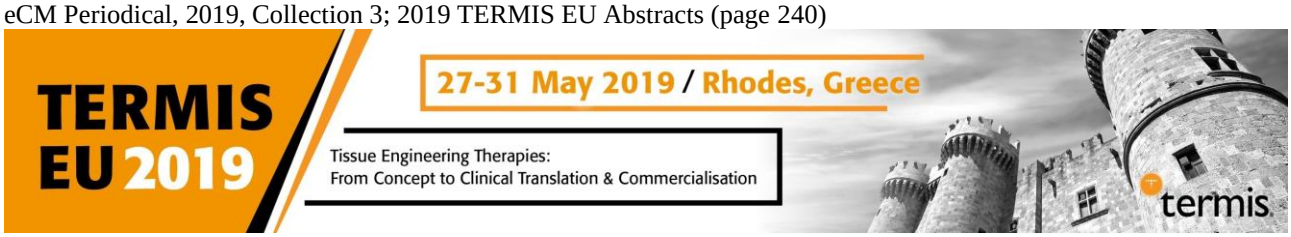
R. Santos[1]
Presenting Author: Romana Santos, rlasantos@fc.ul.pt
[1] MARE - Marine and Environmental Sciences Centre, Science Faculty of the University of Lisbon, Portugal

Most surgical adhesives are based on synthetic cyanoacrylates, fibrin, albumin/glutaraldehyde or PEG polymer but despite their availability, these products are either harmful or toxic, not biodegradable, provide low adhesive strength or have a high risk of disease contaminations. Bioadhesives, on the other hand, are a new class of biological molecules optimized over millions of years of evolution, combining high biocompatibility and biodegradability with strong adhesive forces. Being inhabitants of wave-swept shores, sea urchins rely on specialized adhesive organs, the tube feet, to move and attach to the substrate. These organs possess a proximal motile stem and a distal attaching disc. The later, encloses a duo-gland adhesive system that produces separately adhesive and de-adhesive secretions. After detachment, the adhesive remains on the substratum as a footprint, but the de-adhesive secretion is not incorporated in the adhesive, indicating that it might act as an enzyme [1]. Despite having reversible adhesion, tube feet from several sea urchin species attach with tenacities around 0.5 MPa (force per unit area), which are similar to other marine permanent adhesives and superior to commercial surgical adhesives like fibrin. Sea urchin adhesive is also effective on several substrates, with variable chemistry and roughness, re-enforcing its versatility and biotechnological potential [1]. In terms of biochemical composition, sea urchin adhesives are made of inorganic residues, proteins, sugars and lipids. Recent proteomic studies produced a list of highly over-expressed disc proteins, bringing new knowledge on the key elements involved in sea urchin reversible adhesion. Within these proteins, Nectin, was found to be not only highly expressed in tube feet discs (up to 13-fold), but is an actual component of the secreted adhesive, constituting the first report on a sea urchin footprint adhesive protein [2]. At present, the gene coding for this protein has been fully sequenced [3], and recombinant technology is being used to mass-produce soluble Nectin and test its adhesive properties and biocompatibility.

ACKNOWLEDGEMENTS: Financial support was received from Portuguese Foundation for Science and Technology, (IF/00006/2015) and COST Action CA15216 “European Network of BioAdhesives” ENBA

REFERENCES

[1] Flammang P et al. 2016. Biological Adhesives, 2nd ed. Springer-Verlag
[2] Lebesgue N et al. J Proteomics. 2016;138:61-71
[3] Toubarro D et al. Mar Biotechnol. 2016; 18(3):372-83.



Kidney bioengineering: Can we sufficiently replace its function?

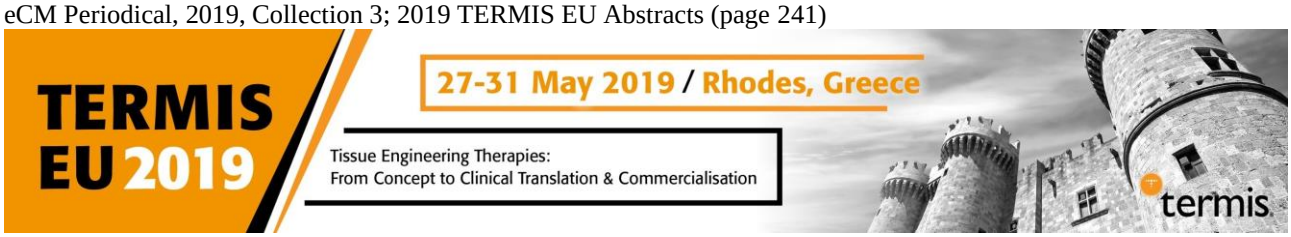
R. Masereeuw[1]
Presenting Author: Rosalinde Masereeuw, r.masereeuw@uu.nl
[1]Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands

In patients with chronic kidney disease (CKD) adequate renal clearance is compromised, resulting in the accumulation of a plethora of uremic solutes. These uremic retention solutes, also named uremic toxins, are a heterogeneous group of organic compounds with intrinsic biological activities, of which many are too large to be filtered and/or are protein-bound. Their renal secretion depends largely on active tubular secretion, which shifts the binding and allows for active secretion of the free fraction. To facilitate this process, renal proximal tubule cells are equipped with a range of transporters that cooperate in basolateral uptake and luminal excretion. In recent years, we and others have invested in the development of bioengineered kidneys that could potentially restore this essential function. For this, well characterized renal cells were combined with functionalized membranes.
This presentation addresses these developments in the context of renal tubular clearance mechanisms for uremic toxins. Furthermore, hurdles to take before a safe implementation of bioengineered kidneys in clinics becomes a realistic option will be discussed.

ACKNOWLEDGEMENTS: Financial support was received from the Dutch Kidney Foundation (17PHD16, 17OI13); Gambro Dialysatoren GmbH (Baxter); Marie Skłodowska-Curie Actions, Innovative Training Networks (Bioart-2012-ITN).

REFERENCES

[1] Legallais C et al. Adv. Healthcare Mat. 2018; in press
[2] Mihajlovic M et al. Sci. Rep. UK., 2017; 7:7103.
[3] Jansen J et al. Sci. Rep. UK, 2016; 6:26715.



Gene therapy: From the nano to the macro scale

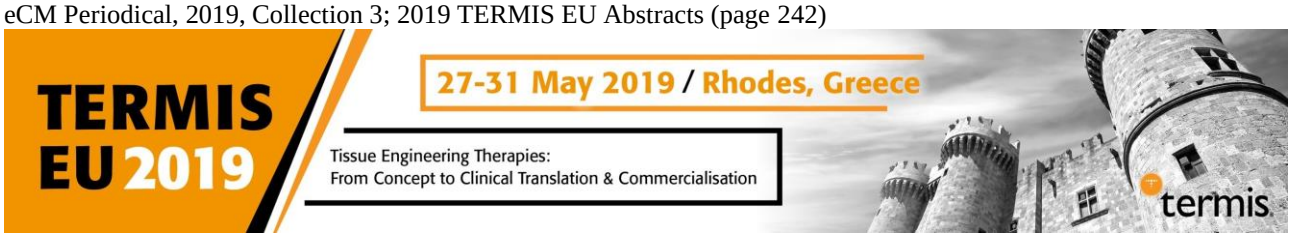
S. A. Cryan[1-5]
Presenting Author: Sally-Ann Cryan, scryan@rcsi.ie
[1]Drug Delivery & Advanced Materials Team & [2]Tissue Engineering Research Group, RCSI, Dublin, Ireland [3]Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Dublin, Ireland [4]Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI & TCD [5]Centre for Research in Medical Devices (CURAM), RCSI, Dublin and National University of Ireland, Galway, Ireland

The field of tissue engineering (TE) is increasingly using biomaterial scaffolds, which are augmented with therapeutics to facilitate enhanced tissue regeneration. The formation of a "gene activated" scaffold, an advanced construct containing gene therapeutics within a 3D scaffold is recognised as a safe method to provide improved spatiotemporal control of growth factor release at a defect site via the in-situ transfection of host cells. A wide range of non-viral gene vectors are being explored for application in tissue engineering to enable the transfection of host cells with gene cargoes in a 3D environment [1,2]. The development of gene therapies from the nano- to the macro scale requires careful design, formulation and screening of the gene delivery systems with a specific focus on the molecular pharmaceutics of the nanotechnologies at a cellular level and the spatiotemporal requirements of the growth factor being delivered in the context of the clinical application of the scaffold. Overall, versatile and biocompatible gene delivery vectors which are capable of functionalising 3D scaffolds for the efficient in vivo delivery of nucleic acids are going to be critical to the successful clinical translation of gene-activated scaffolds. To this end we have developed innovative, bio-inspired gene delivery vectors in the form of star-shaped poly(L-lysine) polypeptides (star-PLLs) [3] capable of effective host cell transfection in vivo and harnessed advanced imaging tools to develop and screen these vectors. Herein, our experience in the development of this novel gene delivery vector for application in tissue engineering will be discussed.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland, (13/IA/1840 and 13/RC/2073).

REFERENCES

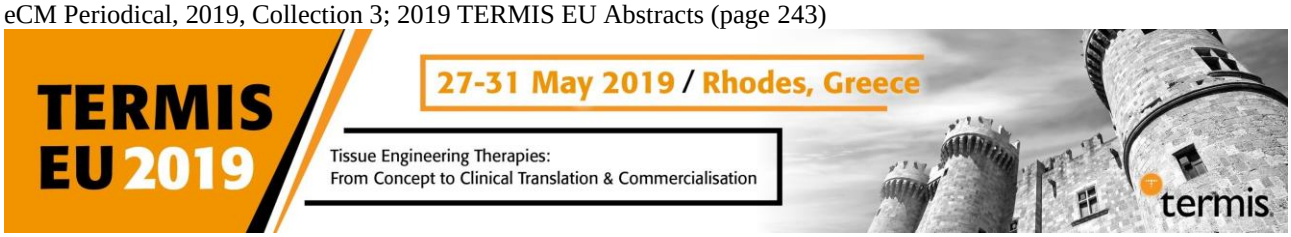
[1] Curtin et al. Adv. Health. Mat. 2015;4(2): 223-227
[2] Raftery et. al. Adv.Mat. 2016;28(27): 5447-69 [3] Walsh et al. Mol Pharmaceutics 2018; 15 (5), 1878-1891.



Tissue engineering of bone - As close as we can get?

S. Hofmann
Presenting Author: Sandra Hofmann, s.hofmann.boss@tue.nl
Orthopaedic Biomechanics Group, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands

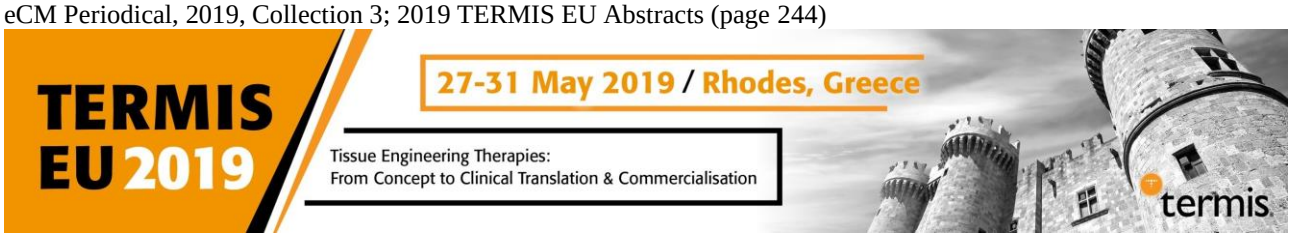
Bone tissue engineering has the intent to grow bone copies in the laboratory that could be used either for bone regeneration or as model systems to study bone physiology and pathology. Bone marrow- or adipose derived derived mesenchymal stromal cells are commonly used as they have been shown to be capable to differentiate into osteoblasts and depositing a calcium phosphate rich extracellular matrix. However, real bone is more than that: there are commonly three cell types described that are essential contributors to the tissue's native function: osteoblasts, osteocytes and osteoclasts. While all three cell types are being investigated separately, co-cultures of them including their precursors and inactive forms still provide a huge challenge these days, both in terms of culturing and (quantitative) evaluation. In addition, the matrix deposited by the osteoblasts in vitro is still far from bone's hierarchical organization in vivo that contributes to bone's impressive mechanical properties. Using a large set of microscopic tools (micro-computed tomography, SEM, 3D FIB/SEM, TEM and fluorescence), combined with spectroscopic (FTIR) and molecular tools (qPCR) we show that our 3D model system develops the main features of bone by human stromal cells differentiating first into osteoblasts who further embed themselves to become osteocytes. In their right environment and when stimulated mechanically, the cells are embedded within a collagenous matrix which is mineralized with carbonated hydroxyapatite. While this system still needs the addition of osteoclasts to represent 'real' bone, it allows to study the interaction between osteoblasts and osteocytes and to invest parameters contributing to collagen mineralization in high resolution and cryogenic conditions.



Synthetic versus bio-inspired approaches for adipose tissue engineering

S. Van Vlierberghe[1,2]
Presenting Author: Sandra Van Vlierberghe, sandra.vanvlierberghe@ugent.be
[1]Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Ghent, Belgium [2]Brussels Photonics, Department of Applied Physics and Photonics, Vrije Universiteit Brussel, Brussels, Belgium

There is a clear and urgent clinical need to develop soft tissue fillers that outperform the materials currently used for adipose tissue reconstruction. Recently, extensive research has been performed within this field of adipose tissue engineering as the commercially available products and the currently existing techniques are concomitant with several disadvantages. Commercial products are highly expensive and associated with an imposing need for repeated injections. Lipofilling or free fat transfer has an unpredictable outcome with respect to cell survival and potential resorption of the fat grafts. Therefore, researchers are predominantly investigating two challenging adipose tissue engineering strategies: in situ injectable materials and porous 3D printed scaffolds. The present work provides an overview of current research encompassing synthetic, biopolymer-based and extracellular matrix-derived materials with a clear focus on emerging fabrication technologies and developments realized throughout the last decade. Moreover, clinical relevance of the most promising materials will be discussed, together with potential concerns associated with their application in the clinic.



Use of electrical stimulation for tissue engineering

S.H. Cartmell[1]
Presenting Author: Sarah Cartmell, sarah.cartmell@manchester.ac.uk
[1]School of Materials, The University of Manchester, UK

The growth of new bone tissue in vitro requires a variety of different factors that need to be controlled and optimised. One of these factors that has previously not been considered for bone tissue engineering is electrical stimuli. Given that bone is piezoelectric in nature, it is feasible to assume that local electrical regimes have an effect on osteogenesis. There are clinical products currently on the market that deliver electrical currents locally via a cathode to fracture sites. These products demonstrate significant clinical improvements in bone repair. We have recently designed a variety of different bioreactors to both house the developing tissue and also control the applied electrical stimuli in either capacitive or direct contact methods to in vitro cultures. These bioreactors have enabled us to assess the potential use of this stimuli for in vitro bone tissue engineering purposes. It has also allowed us to further study the mechanism by which the activity of primary human mesenchymal stem cells are altered both in terms of cell proliferation and differentiation. A novel finding of the importance of the faradic by product of H_2O_2 proximal to the cathode as result of the direct electrical stimulus will be presented and its subsequent role in influencing primary mesenchymal stem cell proliferation. The morphology of primary cilia on these cells after electrical stimulation has been applied will also be discussed, in addition to the effect of varying electrical regime on cell response.

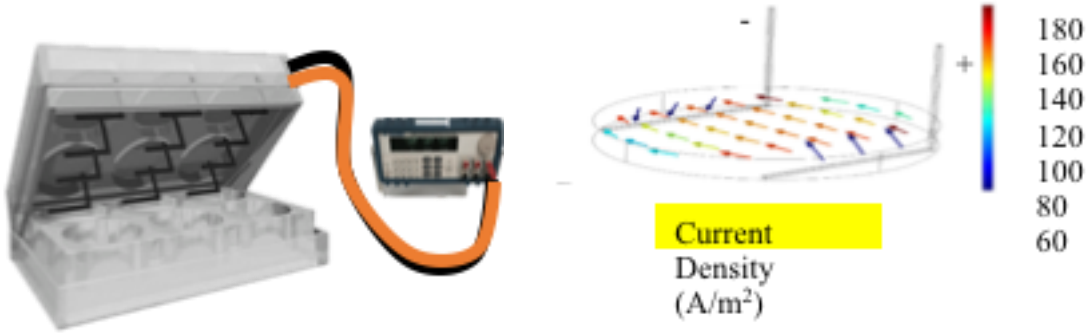
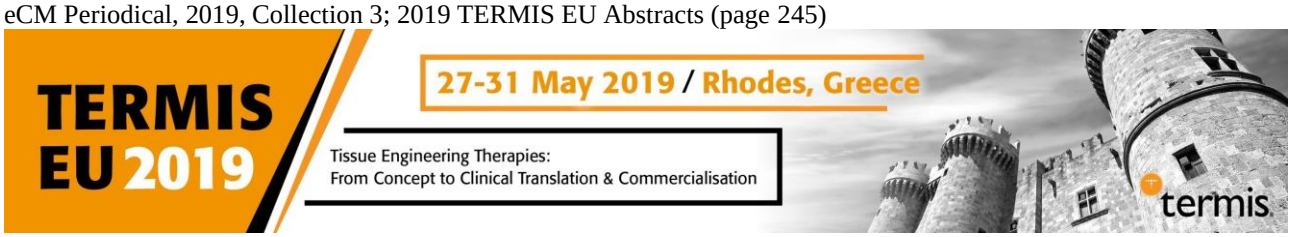


Figure 1: (left) Image demonstrating one of the direct electrode bioreactors; (right) steady state model of current density in in vitro set up.

ACKNOWLEDGEMENTS: Financial support was received from BBSRC.

REFERENCES

[1] S Mobini, ÜL Talts, R Xue, NJ Cassidy, SH Cartmell J. Biomat. and Tiss. Eng. 2017 7(9):829-833
[2] Balint, R., Cassidy, N.J., Cartmell, S.H. (2014) Acta Biomaterialia 10(6) 2341-2353.. Tissue Eng Part C Methods. 2015;21(7):660-70
[3] Balint, R., Cassidy, N., Hidalgo-Bastida. L.A. and Cartmell, S.H. (2013) Journal of Biomaterials and Tissue Engineering 3:212-221



Implementing design control for 3D printed patient specific resorbable implants: From concept to clinic

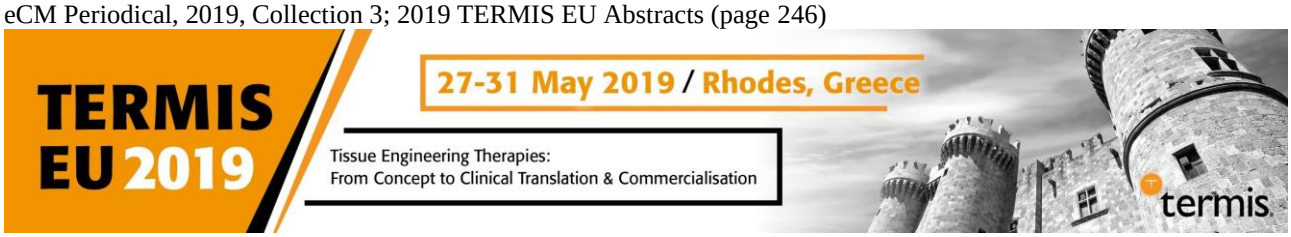
S.J. Hollister[1]
Presenting Author: Scott J. Hollister, scott.hollister@bme.gatech.edu
[1]Center for 3D Medical Fabrication and the Tissue Engineering and Mechanics Laboratory, Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA USA

Resorbable scaffolds delivering biologics has been a cornerstone of regenerative medicine research for over three decades, promising personalized medicine in the form of patient specific reconstructive approaches. However, translation of this research to clinical care, even resorbable materials alone minus biologics, has occurred at an extremely slow pace. This is due in part to the economics of manufacturing patient specific devices and in part due to the regulatory requirements that must be addressed to achieve clinical translation. This is especially difficulty in academics given the model of academic research is orthogonal in many ways to the steps necessary for translation. Specifically, regulatory bodies like the FDA require all device development be done in a design control framework that is a centerpiece of quality control. There are, however, few published examples of academic design control approaches. In this talk, we present an example of design control process for a 3D printed patient specific resorbable device that has been implanted in 17 patients to date from follow-up from 2 to 90 months[1, 2]. In this talk, we first outline the design control components required for any device: user needs, design inputs, design process, design outputs, design verification and design validation[3]. We then detail how we have implemented each design control component for the airway splint, from developing user needs to design validation in clinical use. We specifically focus on challenges in the design control process that result from patient specific devices created through 3D printing, including the increased use of computational modeling to assess devices to specific batch testing of 3D printed devices to assess manufacturing quality of the 3D printing process. We then suggest a template that can be used as a basis for design control of general 3D printed patient specific resorbable devices, including how this design control template can fit into a general quality control process. Finally, we relate clinical results in patients reported to date with device design requirements.

ACKNOWLEDGEMENTS: This work was supported by funding from the NIH NICHD R01 086201.

REFERENCES

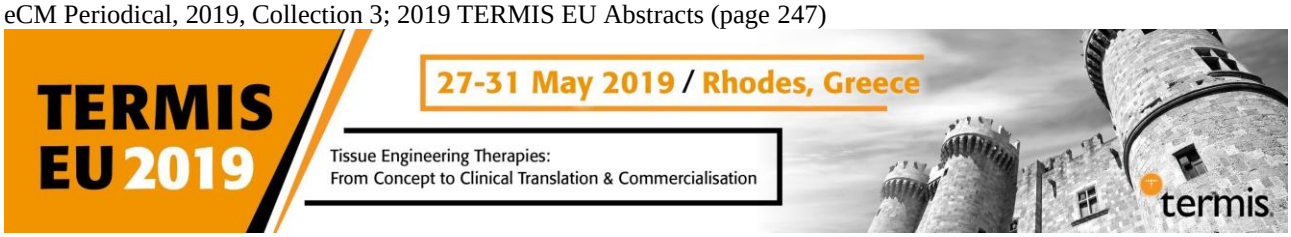
[1] R.J. Morrison, et al., Mitigation of tracheobronchomalacia with 3D-printed personalized medical devices in pediatric patients, Sci Transl Med 7(285) (2015) 285ra64.
[2] D.A. Zopf et al., Bioresorbable airway splint created with a three-dimensional printer, N Engl J Med 368(21) (2013) 2043-5.
[3] S.J. Hollister, et al., Integrating Image-Based Design and 3D Biomaterial Printing to Create Patient Specific Devices within a Design Control Framework for Clinical Translation, ACS Biomaterials Science & Engineering 2 (2016) 1827-1836.



Zirconia/Alumina ceramics with cellular structure for biomedical applications: Experience for sintering and real practice

S. Kulkov
Presenting Author: Sergei Kulkov, kulkov@ms.tsc.ru
Tomsk State University and Institute of Strength Physics and Material Sciences RAS, Russia

It has been studied a porous ceramics sintered from nanopowders obtained by plasma spray technique. The porosity of ceramic was up to 75 %. The aim of the work is the investigation of densification, structure and mechanical properties of materials based on zirconia-based powders produced by plasma spray synthesis and sintered at different temperatures. It has been shown that structure of the sintered ceramic has a system of cell and rod elements. These structures formed by stacking hollow powder particles. There were three types of pores in ceramics: large cellular hollow spaces, small interparticle pores which are not filled with powder particles and the smallest pores in the shells of cells. The cells generally did not have regular shapes. The size of the interior of the cells many times exceeded the thickness of the walls which was a single-layer packing grains. The increase of the pore space in the ceramics was accompanied by the decrease of the average size of voids inside the cells and the average grain size. The stress-strain diagrams for ceramics with porosity higher than 20 % are non-liner, and sintered ceramic with a high porosity has a very similar behavior as compare with natural bone and can be used as perspective material for bone replacement. These materials are already used for real medical practice.

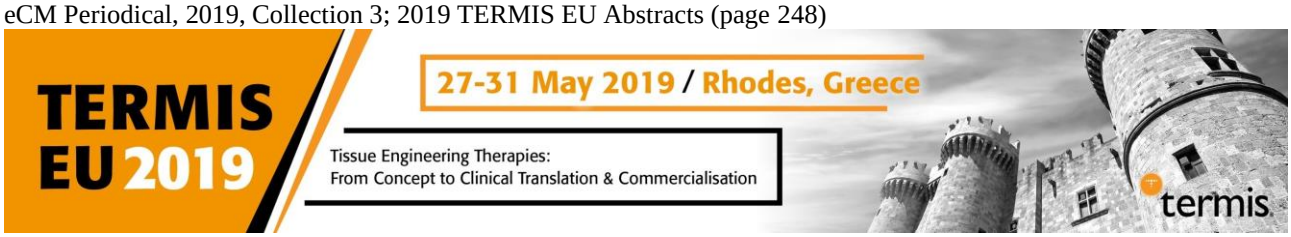


Skeletal disease-in-a dish: Using induced pluripotent stem cells to model human skeletal disorders

S. R. Lamandé[1,2]
Presenting Author: Shireen Lamandé, shireen.lamande@mcri.edu.au
[1]Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia [2]Department of Paediatrics, University of Melbourne, Australia

Genetic skeletal disorders are a significant disease burden. The lifelong disabilities suffered by patients, which range from short stature and precocious arthritis to severe bone fragility and premature death, make these an important clinical and societal problem in their own right, but they also offer insights into more common conditions such as osteoarthritis and osteoporosis. Although many genes and mutations underlying genetic skeletal disorders have been defined, our knowledge about the cellular and extracellular pathological mechanisms is incomplete, and is likely to be disease and mutation specific. A major challenge to advancing effective therapies that appropriately target disease mechanisms has been developing accurate human in vitro disease models that can be used to fully define the molecular pathology and are suitable for drug screening. A powerful emerging approach is using induced pluripotent stem cells (iPSCs) and differentiating them into chondrocytes to produce in vitro cartilage, or into osteoblasts to model bone disease. This innovative "disease in a dish" approach is driven by the importance of studying patient-specific mutations in the context of the affected human skeletal tissues. This has not been possible previously because cells from human bone and cartilage are not readily accessible and are therefore only ethically available in rare cases if a patient undergoes surgery. We have developed and optimised iPSC differentiation protocols to model cartilage and bone disorders caused by mutations in COL1A1, COL2A1 and TRPV4 and are using them to define mutation specific pathogenic pathways and, ultimately, test candidate drugs.

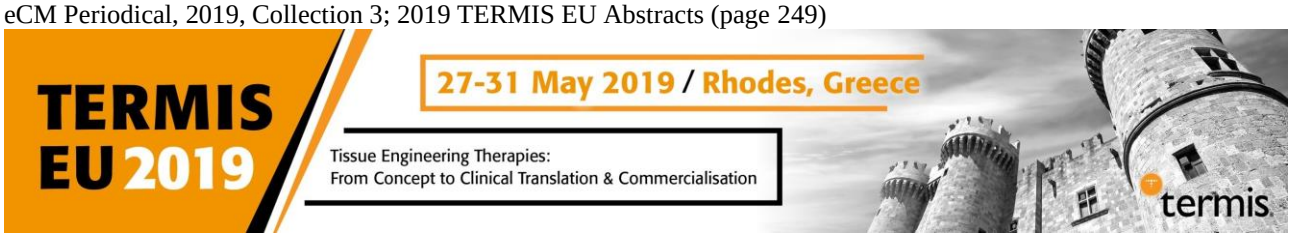
ACKNOWLEDGEMENTS: Financial support was received from The National Health and Medical Research Council of Australia (GNT1144807, GNT1146952); the Financial Markets Foundation for Children; H2020 Marie Skłodowska-Curie Actions, RISE 2015 RUBICON (690850); the Murdoch Children's Research Institute; the Victorian Government's Operational Infrastructure Support Program.



A clinical perspective on the future of natural polymer-based regenerative medicine

S. Shah
Presenting Author: Siegfried Shah, siegfried.shah@ct-west.de
Department for General and Abdominal Surgery, St. Marien Hospital Dueren, Dueren, Germany

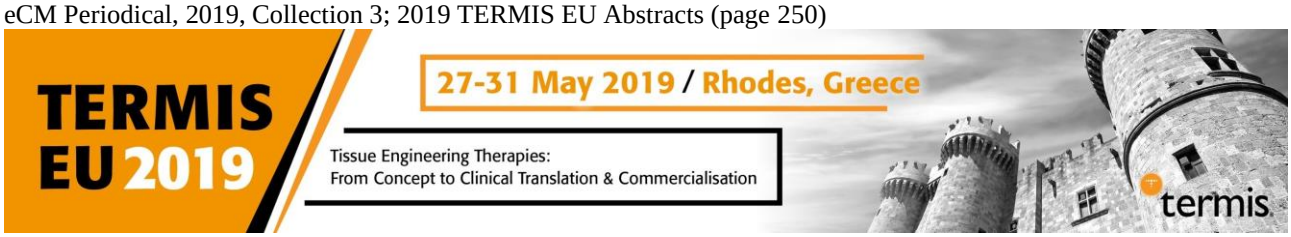
Since disciplines like Tissue Engineering (TE) and Regenerative Medicine (RM) are getting more into the focus of interventional clinicians and surgeons the demand for biodegradable and bioresorbable polymer-based biomaterials fulfilling highest biocompatibility requirements and including tuneable mechanical properties for specific applications is strongly increasing.
Such polymer-based biomaterials can be of synthetic or natural origin. The synthetic, non-degradable polymers PTFE (VascuGraft® SOFT), PET (Vascutek®) and the synthetic, degradable polyesters PLA (Absorb™ BVS) and PGA (Valtrac®) are giving relative positive and negative clinical examples as well. In case of regenerative medicine approaches clinicians are dreaming of shifting post-pathological situations in the healing phases from reparation to more “restitutio-ad-integrum”, which means **true** regeneration. Natural polymers like fibrin, collagen, polysaccharides (e.g. hyaluronic acid, alginate) are typical candidates, but comprise several disadvantages in case of their application to regenerative medicine. A very promising class of natural polymers are the polyhydroxyalkanoates (PHAs), biotechnologically synthesized in bacteria. Many of them are “biocompatible”, s.th. which is still under critical evaluation with a very promising perspective for some of the PHA candidates. Additionally, their mechanical properties are highly tuneable and thereby a very good processability is given. More specified potential application areas for PHA-based biomaterials are regenerative wound dressings, biodegradable and -resorbable enteral anastomosis stents and in situ-regenerative vascular grafts to name only a few. Finally, we are convinced that not every natural polymer is a promising biomaterial for regenerative medicine applications. But amongst different types of natural polymers the PHAs have a huge potential for translational regenerative approaches.



Versatile crosslinked gelatin hydrogel for regenerative medicine applications

S. Farè
Presenting Author: Silvia Farè, silvia.fare@polimi.it
Department of Chemistry, Materials and Chemical Engineering “G. Natta”, Politecnico di Milano, Milan, Italy & National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, Italy

Gelatin is widely investigated in regenerative medicine for its solubility in water, lower antigenic and immunogenic response compared to collagen, readily availability, and exposure of cell-binding motives and metalloproteinase target-sites for degradation. Crosslinking is necessary to produce gelatin hydrogels stable at 37 °C. In this work crosslinking is based on a Michael-type addition between gelatin and methylenebisacrylamide, the crosslinker. Gelatin concentration and reaction stoichiometry were varied to tune hydrogels chemico-physical and mechanical properties. All the produced hydrogels were stable in water at 37 °C, proving the efficiency of the crosslinking reaction; different mechanical and rheological properties were exhibited, as well as the in vitro cell activity. Using sacrificial alginate microbeads and 3D printed structures a porous gelatin hydrogel with a controlled vasculature was obtained. The so-produced scaffolds showed good porosity, mechanical compressive response, and enzymatic degradability. The hollow channels allowed fluids flow and cells adhesion to the hollow channels walls, promising aspects for a promoted vascularisation. Microspheres for cell and drug delivery were prepared in a controlled dimension range (40-135 □m). Metabolic activity of cells on the microspheres increases up to 14 days of incubation and Live/Dead staining showed vital cells on the surface of the MSs and a reduced presence of dead cells. A preliminary study showed the possibility to spray the microspheres with cells or loaded with drug, representing an innovative and possible approach. In conclusion, the versatility of the innovative crosslinked gelatin hydrogels was demonstrated by using advanced fabrication technologies.



Innovation and commercialisation in the field of regenerative medicine: Learning from the past and lessons for the future

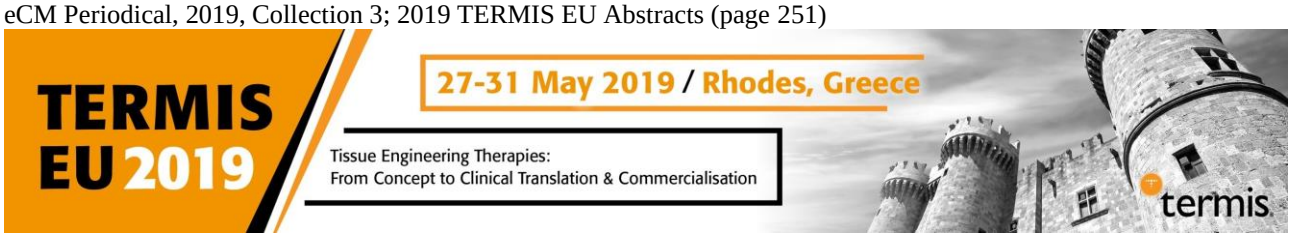
S. Champ[1]
Presenting Author: Simon Champ, simon.champ@bellaseno.com
[1]BellaSeno GmbH, Deutscher Platz 5a, 04103 Leipzig, Germany

The resources required to develop and market a regenerative therapy are substantial, as is the time to market, and the risk of failure is high. However, the potential benefits, both social and financial, of regenerative medicine are enormous. Turning this potential into reality will be challenging and companies need to develop and implement innovative business strategies in order to succeed. In this presentation, business strategies, market and competitive environments will be discussed in the context of several case studies of successful as well as unsuccessful companies in the field of regenerative medicine. A number of important lessons from these cases shall be discussed. From a management perspective the most critical lesson is the importance of effective financial planning and management of costs, and in particular R&D costs, including the significant costs associated with clinical trials. In addition, a clear strategic focus is extremely important due to the significant resources required in the development of a new therapy. From an investor's perspective the lessons to be gathered from the case studies are related to the risk involved in investing in the field of regenerative medicine. The importance of a clear and focused strategy based on long-term investor commitment shall be emphasised for the successful commercialization of regenerative medicine products.

ACKNOWLEDGEMENTS: Financial support was received from Saxony Development Bank (SAB) and European Funds for Regional Development (EFRE).

REFERENCES

[1] Pangarkar N et al. Regen Med. 2010; 5(5)



Recent developments to utilize induced pluripotent stem cells for cartilage regeneration

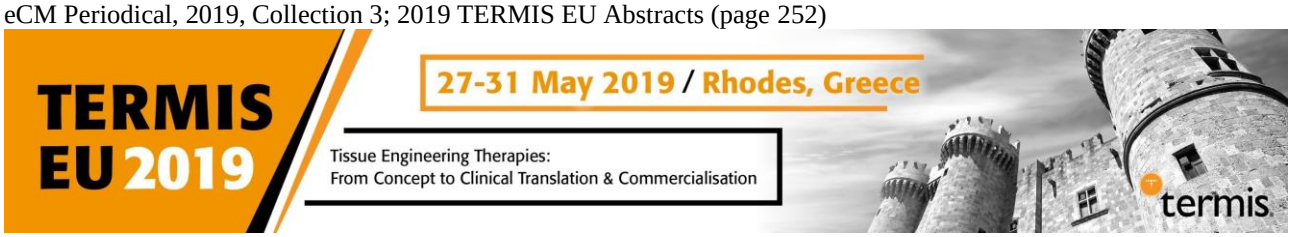
S. Diederichs[1]
Presenting Author: Solvig Diederichs, solvig.diederichs@med.uni-heidelberg.de
[1]Research Center for Experimental Orthopaedics, Center for Orthopaedics, Trauma Surgery and Paraplegiology, Heidelberg University Hospital, Heidelberg, Germany

As a potential non-invasive cell source for a large amount of chondrocytes, induced pluripotent stem cells (iPSCs) are highly attractive for cartilage regeneration. This field currently lacks an optimal cell source, because the only applicable cells –autologous chondrocytes – are highly invasive to harvest and extremely limited in number. Although mesenchymal stromal cells (MSCs) from bone marrow or adipose tissue have been hoped to overcome cell shortage, they differentiate along the endochondral pathway when induced into chondrocytes in vitro and develop an inherent mineralization activity leading to bone formation at ectopic sites. iPSCs promise to overcome both these limitations, since they possess a virtually unlimited expansion capacity and are intrinsically capable to form any adult tissue including stable hyaline cartilage. Their primitivity, however, makes in vitro differentiation of iPSCs into chondrocytes highly challenging. So far, varying methods have been reported and there is currently no generally accepted standard protocol. A common basis for many attempts is to recapitulate embryonic cartilage development. This requires sequential induction of multiple developmental phases and, thus, production of an intermediate phenotype representing a metastable state of mesenchymal progenitors. These progenitors are subsequently induced into the chondrogenic lineage. We and others have shown that such iPS-derived mesenchymal progenitor cells (iMPCs) resemble MSCs by expressing a similar surface marker profile and a general capacity for in vitro differentiation into the three mesenchymal lineages (cartilage, bone, fat) [1]. Yet, in direct comparison with MSCs, striking differences became apparent, including their inducibility to differentiate in vitro [2] and their DNA methylation pattern which indicated that iMPCs have a juvenile rather than an adult stem cell activity. Here, current concepts for iMPC generation and chondrogenic differentiation will be discussed and specific characteristics, efficiency, reproducibility and robustness will be compared with MSC chondrogenesis. Moreover, potential applications beyond cell therapeutics for cartilage regeneration as well as clinical safety aspects will be highlighted.

ACKNOWLEDGEMENTS: Financial support was received from the German Research Foundation (DI_1684/4).

REFERENCES

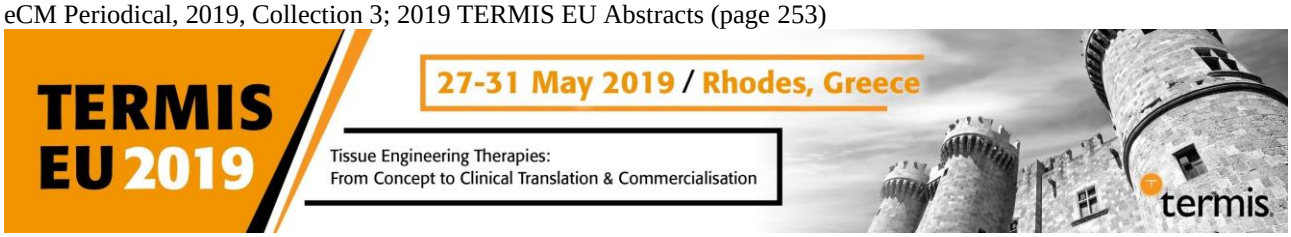
[1] Diederichs S et al. Stem Cells Dev 2014;23: 1594-610.
[2] Diederichs S et al. Stem Cells Dev 2016;25: 598-609.
[3] Diederichs S et al. Cartilage: Vol 3 Repair Strategies and Regeneration. Springer; 2017. p. 73-93.



Advanced formulations based on multifunctional mesoporous glasses for highly targeted therapies in tissue regeneration

S. Fiorilli
Presenting Author: Sonia Fiorilli, sonia.fiorilli@polito.it
Applied Science and Technology Department, Politecnico di Torino, Italy

In the field of bioceramics, mesoporous bioactive glasses (MBGs) have received significant interest in the past decade as powerful bone regeneration systems. However, in the presence of compromised remodelling process (e.g. delayed bone healing, osteoporosis), the effective treatment of bone defects still represents a challenging clinical issue. In addition, for these compromised clinical situations, the risk of bacterial infections is very high and increases the number of unsuccessful therapies. To address this challenge, the design of novel and multifunctional MBG formulations able to synergistically combine the biological functions of specific therapeutic elements (pro-osteogenesis, pro-angiogenesis and bactericidal effects) with bacterial anti-adhesive properties would represent a big step towards timely and effective therapies. In addition, due to their well-defined and accessible porous structure MBGs are able to store and release different drug molecules (i.e. anti-inflammatory, anti-oxidant and antibiotic agents) and their surface functionalization can impart stimuli-responsive and/or specific targeting properties. In this contribution, SiO_2-CaO MBGs containing therapeutic elements (i.e. Sr, Cu) were prepared in the form of nano- and micro-particles, and functionalised through different routes (i.e silane chemistry, polymer grafting) to provide their surface with anti-adhesive properties, in order to prevent bacteria attachment and colonization. The ion/drug release properties of functionalized MBGs have been assessed and the obtained multifunctional systems incorporated into a vehicle phase based on a thermo-sensitive polymer to design an injectable device for prolonged and localised release.



Towards the development of a biohybrid lung

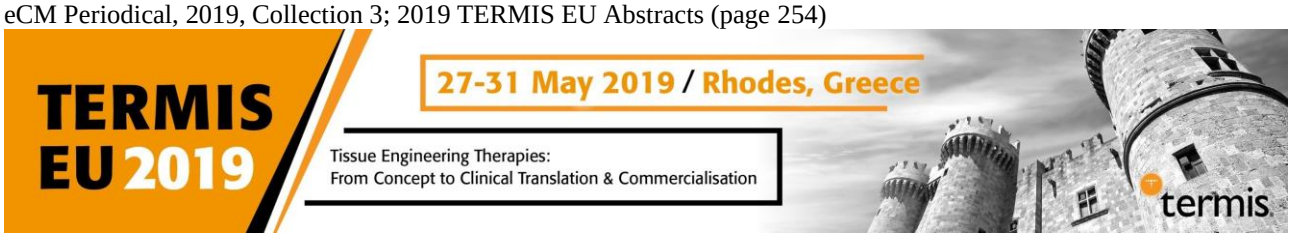
S. Korossis[1,2]
Presenting Author: Sotiris Korossis, s.korossis@lboro.ac.uk
[1]Lower Saxony Centre for Biomedical Engineering Implant Research and Development, Department of Cardiothoracic, Transplantation and Vascular Surgery Hannover Medical School, Hannover, Germany [2]Cardiopulmonary Regenerative Engineering (CARE) Group, Centre for Biological Engineering, Loughborough University, United Kingdom

Lung disease is the third largest cause of death, behind cardiovascular disease and cancer. In the US alone, 35 million patients suffer from chronic lung disease, with a yearly fatality rate of nearly 340,000 patients [1]. Chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome (ARDS) are two of the most common respiratory conditions worldwide. Currently, end-stage and chronic lung disease can only be treated by lung transplantation. However, the low donor lung availability and patient ineligibility limit the number of worldwide lung transplantations to below 1,500 per year [2], whereas the waiting time and death rate of the patients in lung transplantation lists is >14 months and 11.3%, respectively. Moreover, the half-life survival after lung transplantation is about 5.4 years [3]. Patients with irreversible lung disease have no reliable bridge-to-transplantation option, having a poor quality of life, often confined in intensive care units (ICUs). Current therapies used for respiratory failure include mechanical ventilation, and artificial lung devices, such as extracorporeal membrane oxygenation (ECMO) and lung assist (ECLA) for decarboxylation. Mechanical ventilation offers effective short-term support, but is associated with barotrauma, volutrauma and other injuries, further exacerbating respiratory deficiency. Artificial lung devices provide respiratory support independent of injured lungs and allow them to rest and heal. ECMO and ECLA are established therapeutic tools in modern ICUs, and are primarily employed as short-term bridges to transplantation. Although these systems are attractive due to simulating physiological decarboxylation/oxygenation, they are limited by the complexity of operation, reduced patient mobility, thrombosis and associated thromboembolism, plasma weeping through the membrane pores, flow-path-induced blood trauma, and inflammatory reaction due to direct contact of blood with artificial components. These complications limit artificial lung usage to between 4-6 weeks and in specialized ICUs. The development of a fully-haemocompatible and partially- or fully-implantable artificial lung that can be used as a longer-term bridge to transplantation or a destination therapy, and allows ambulation and hospital discharge, could significantly improve the prospects and quality of life of patients. In the past few years, artificial lung endothelialization has emerged as a promising alternative for providing a natural barrier between the artificial gas-exchange membranes and the patient's blood. In this talk, our experience with the functionalisation, endothelialization and haemodynamical optimization of hollow-fiber membrane oxygenators will be discussed.

ACKNOWLEDGEMENTS: Financial support was received from the German Research Foundation through the SPP2014 (348028075) and the Cluster of Excellence REBIRTH (EXC 62).

REFERENCES

[1] Vos T et al. Lancet. 2012; 380:2163-96.
[2] Udobi KF et al. Am. Fam. Physician. 2003; 315-22
[3] Pflaum et al. Acta Biomater. 2017; 50:510-521



Nerve repair and axonal guidance: Trophic and topographical cues

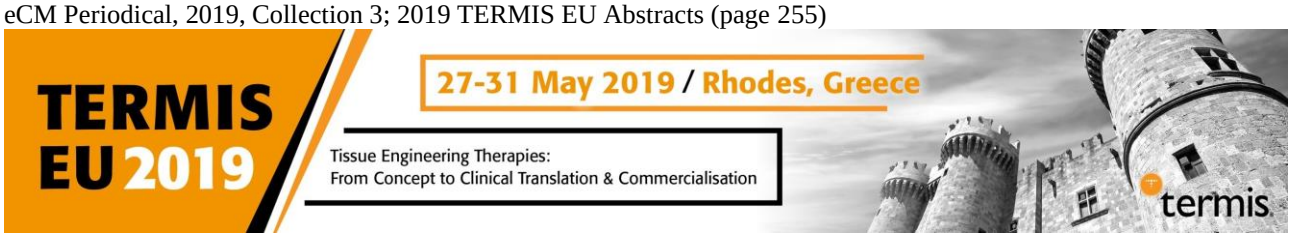
S. Madduri
Presenting Author: Srinivas Madduri, srinivas.madduri@usb.ch
[1]Department of Plastic, Reconstructive Surgery, University Hospital Basel, Spitalstrasse 21, 4021, Basel, Switzerland [2]Department of Biomedical Engineering, University of Basel, Gewerbestrasse 14, 4123 Allschwil, Switzerland

Peripheral nerve injuries affect more than one million people each year and often result in life-long disabilities due to lack of efficient therapeutic options. Currently available treatment options are associated with several drawbacks and lack the ability to provide dynamic microenvironment required for axonal path finding following injuries. Here, we report on bioengineered nerve conduits addressing both trophic and topographical requirements of adult axonal regeneration. For this, we generated genetically enriched Schwann cells for delivering multiple neurotrophic factors with discrete release kinetics. Resulting Schwann cells, proved to be viable and efficacious both in vitro and in vivo in a nerve injury model. Further, collagen or silk fibroin based nerve conduits scaffolds with controlled nano-topography were fabricated using a novel technique and repopulated with genetically enriched Schwann cells. Resulting conduit scaffolds varying in trophic and topographical functions were studied in10 mm nerve gap model in rats. The anatomical and functional outcomes differed significantly among the various animal groups. Furthermore, serum analysis indicated absence of undesired immune response against the genetically modified Schwann cells. Importantly, bioengineered collagen and silk fibroin NCs carrying most important biological functions showed potential to overcome the present hurdles of nerve regeneration and achieved the level of autograft performance. This study not only revealed the important role of trophic and topographical functions for effective axonal regeneration, but also demonstrated the impact of biomaterial composition in mounting selective axonal growth response.

ACKNOWLEDGEMENTS: Financial support was received from Swiss National Science Foundatation under grant agreement nº 31003A_133076/1; Commission for Technology and Innovation under the grant agreement nº 16168.1 PFLS-LS.

REFERENCES

[1] Madduri. et al., Biomaterials. 2010, 31:2323-34
[2] Madduri. et al., Biomaterials. 2010, 31:8402-09
[3] Madduri. et al., Journal of controlled release. 2010, 143:168-174



Microfluidic systems for in vitro morphogenesis models

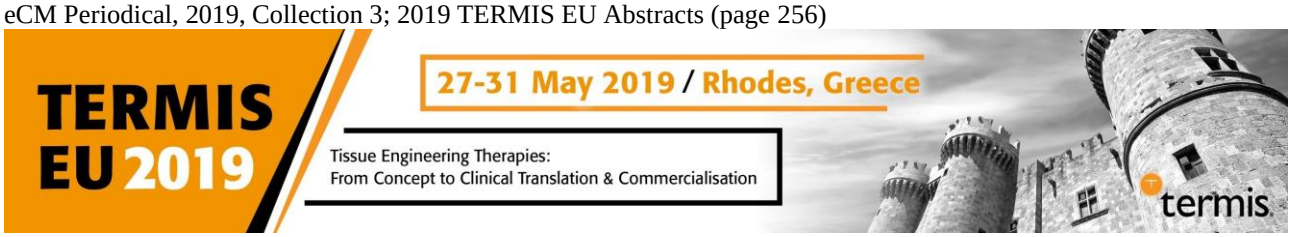
S. Giselbrecht[1]
Presenting Author: Stefan Giselbrecht, s.giselbrecht@maastrichtuniversity.nl
[1]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

Tissue patterning and organogenesis are key processes in a developing embryo, which are tightly regulated by autonomous and non-autonomous mechanisms. Several of these underlying mechanisms have been elucidated in the past, but numerous details remain still elusive. Various research groups have recently shown that under certain conditions aspects of early embryonic patterning, such as symmetry breaking and germ layer specification, can be recapitulated in vitro. [1] Stem cell-derived, embryo-like entities, so-called 'synthetic embryos', were created by a bioengineering approach that will help us to shed more light on essential cell-cell interactions in the embryo and to study mechanisms of implantation in future. [2] We are focusing on the development of non-adherent cultures of pluripotent stem cell aggregates in microfluidic systems to study early embryonic patterning, such as collective cell migration and axis formation. The systems allow us to observe and manipulate developmental phenomena on a cellular level which normally occur in vivo within the uterus, making them difficult to study. The heart of our technology platform is based on our proprietary 'substrate modification and replication by thermoforming' (SMART) technology. This technology is used to fabricate functionalized polymer film-based microcavity arrays to control cell patterning and differentiation in embryoid bodies under static and actively perfused 3D cell culture conditions, e.g. by integrating them into microfluidic bioreactors. We used these microcavity arrays for studying the effects of various parameters including cell seeding density, fluid flow, and microcavity size and shape in order to increase control and reproducibility of pattern formation processes. Our results showed an influence of microcavity dimensions and the rate of refreshing medium on patterns of paracrine signals secreted by cells (COMSOL simulations), which might influence patterning events in 3D in vitro morphogenesis models. We also showed that some of the in vitro morphogenesis models can be cultured in microcavity-based systems in a high-throughput manner. Further, the degree of (axis) elongation and orientation in pluripotent stem cell aggregates can be controlled by changing the size of the microcavities. The development of innovative microfluidic tools in this new research area, also called 'Morphogenetic Micro Engineering', will allow us to interfere and eventually control pattern formation in well-described in vitro models of developmental biology and will help us to gain deeper insights into the mechanisms underlying organogenesis. [3] In future, this, in turn, will facilitate us to develop new and more sophisticated tools for biomedical applications and to improve regeneration in adult organisms.

ACKNOWLEDGEMENTS: Financial support was received from the Dutch Province of Limburg (program 'Limburg INvesteert in haar Kenniseconomie/LINK').

REFERENCES

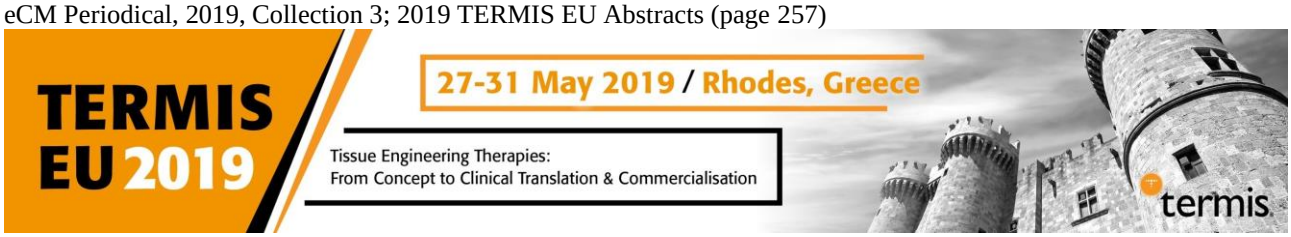
[1] van den Brink SC et al. Development 2014; 141: 4231-4242
[2] Rivron NC et al. Nature 2018; 557:106-111
[3] Samal P et al. Adv Mater. 2019; Article in Press



Resetting the aging clock: Reprogramming stem cell rejuvenation for vascular and neural tissue regeneration

S. T. Andreadis
Presenting Author: Stylianos Andreadis, sandread@buffalo.edu
Department of Chemical and Biological Engineering, University at Buffalo, State University of New York, USA

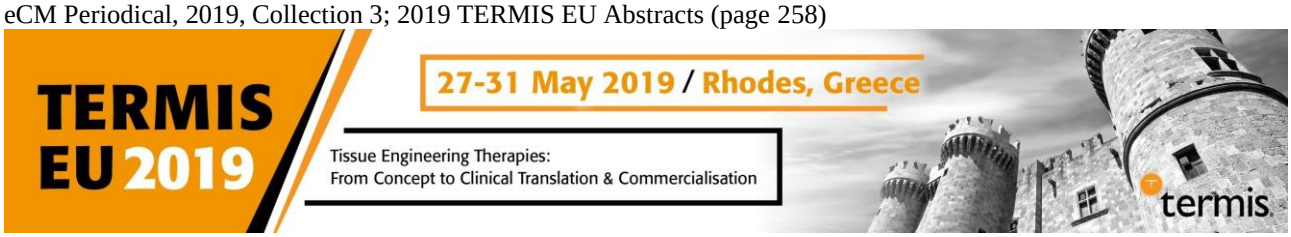
Cardiovascular disease is the leading cause of mortality worldwide. Regarded as the therapeutic gold standard, treatment with autologous grafts suffers from several technical and patient-related risks. Tissue engineered small diameter blood vessels may provide a promising alternative solution as replacement grafts. In this study, we employed adult and induced pluripotent stem cells to engineer fully functional vascular grafts that were implanted into the arterial circulation of a physiologically relevant ovine animal model, where they remained patent and underwent successful remodeling. In addition, we engineered cell-free (off-the-shelf) vascular grafts that were implanted successfully into the arterial system of adult as well as neonatal sheep. Most notably, as the animals grew, the grafts increased in size (length and diameter), demonstrating their potential application for treatment of congenital pediatric disorders. During the course of our studies using mesenchymal stem cells (MSC) to engineer vascular grafts, we observed MSC originating from older donors suffer from limited proliferative capacity and significantly reduced myogenic differentiation potential. This is a major concern, as the patients most likely to suffer from cardiovascular disease and therefore in need of vascular grafts are elderly. Notably, we developed a strategy to reverse the proliferation and differentiation potential of MSC from adult donors as well as to restore of ECM synthesis and contractile function. In addition to MSC, we demonstrated that our strategy could “rejuvenate” senescent skeletal muscle cells (SkMC) and identified molecular pathways that are involved in this process. Our work demonstrate the potential of molecular engineering strategies to reverse the effects of organismal aging and restore the potential of adult stem cells for use in cellular therapies and tissue regeneration. In the second part of my presentation, I will focus on deriving neural crest stem cells (NC) and their derivatives from neonatal and adult epidermis. NC cells are induced by signaling events at the neural plate border during development of vertebrate embryos. Initially arising within the central nervous system, NC cells subsequently undergo epithelial-to-mesenchymal transition and migrate into the periphery, where they differentiate into diverse cell types. We discovered that NC can be derived from postnatal human epidermal keratinocytes (KC) without genetic modification or reprogramming to the pluripotent state. Genome-wide transcriptome analyses showed that KC-derived NC cells are similar to NC cells derived from human embryonic stem cells. Moreover, KC-NC give rise in vitro and in vivo to NC derivatives such as peripheral neurons, melanocytes, Schwann cells and mesenchymal cells (osteocytes, chondrocytes, adipocytes, and smooth muscle cells). Lineage tracing studies by implantation of KC-NC into chick embryos confirmed the NC phenotype of these cells in an in vivo setting. This work represents a paradigm shift as it demonstrates that the epidermis is a novel source of abundant, readily accessible, autologous stem cells for treatment of neurodegenerative diseases, for which cell sourcing remains a severe impediment hampering cell therapy approaches.



Development of adipose stem cell-based approaches for intervertebral disc regeneration and treatment of low back pain

S.M. Richardson
Presenting Author: Stephen M. Richardson s.richardson@manchester.ac.uk
Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom

Low back pain (LBP) represents one of the leading causes of disability in the Western world, affecting over 80% of the population at some during their lifetime. In the majority of cases intervertebral disc (IVD) degeneration is the cause of LBP. Current treatments for LBP associated with IVD degeneration offer poor long-term efficacy and this has led to an increasing interest in the development of cell-based regenerative therapies. IVD degeneration originates in the central nucleus pulposus (NP) region, where increases in proinflammatory cytokines drives a catabolic phenotype in resident NP cells leading to degradation of the aggrecan and type II collagen rich matrix. We have focussed on the development of adult stem cell-based approaches for NP regeneration and compared the anti-inflammatory and regenerative potential of human donor-matched bone marrow mesenchymal stem cells (MSCs) and adipose stem cells (ASCs). Here I will discuss our recent findings on the anti-inflammatory properties of MSCs/ASCs, their directed differentiation to NP cells (specifically with the growth factor GDF6), and the signalling mechanisms which underpin these responses. I will also discuss the development of biomaterials to deliver growth factors and support implanted cells within the harsh microenvironment of the degenerate IVD, which will enable future clinical translation of the next generation of regenerative therapies for IVD degeneration and associated LBP.



Hydrogels that support cell adhesion

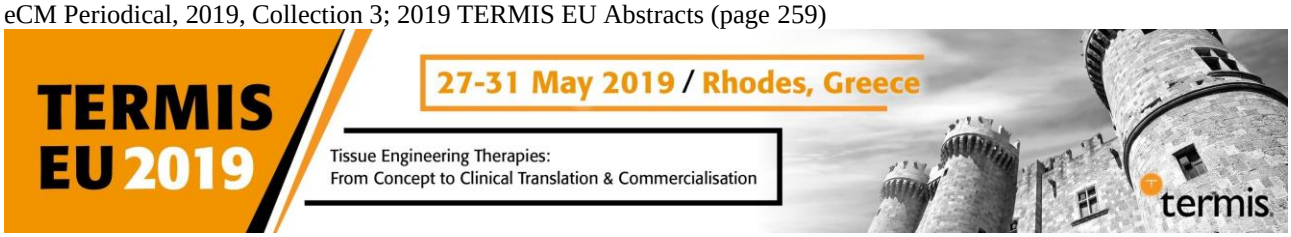
S. Rimmer
Presenting Author: Stephen Rimmer s.rimmer@bradford.ac.uk
Polymer and Biomaterials Chemistry Labs., School of Chemistry and Biosciences, University of Bradford, Bradford, West Yorkshire, BD1 7DP, UK

Hydrogel materials provide the closest match to the physical properties of the extra-cellular matrix. However, this is obtained because of their high-water content and this high degree of swelling produces materials that are very non-cell adhesive. In natural systems this allows for specific cell-adhesion interactions without general and non-specifc adhesion. Chiefly, interactions between extracelluar proteins and integrins provide the signals for adhesion. Amine functional surfaces have been studied as charged modifications of biomaterials for improving cell adhesion. However, only recently have we realized that alkyl primary amine functionality can provide interfaces that are highly selective for epithelial cells.[1] In this initial work on human corneal epithelial cells we had assumed that coculture with stromal cells would be required as is in traditional in vitro culture. However, later we showed that culture of the epithelial cells alone was successful and that these hydroxylated hydrogels with alkyl primary amine-functionality were, in fact, poor substrates for fibroblasts.[2] Extensive assessment of these 2D culture substrates showed that they did not initiate a significant response from murine peritoneal macrophages[3]; giving them potential as implantable materials to enhance wound healing. With this in mind we showed that implantation in an explanted corneal model supported the re-epithelization of the cornea.[4] To be useful as supports for epithelial cells these hydrogels require functionalization with primary amines; secondary and tertiary amines do not support epithelization. It is necessary to attach the amine via an alkyl chain of at least 3 methylene units. The materials do not support chondrocytes, MSCs or osteoblasts. Finally, protein adsorption experiments showed that there was little difference in general protein adsorption between non-adhesive hydrogels (not alkyl primary amine-functional) and cells adhesive hydrogels (alkyl primary amine-functional). These observations indicated a possible enzymatic mechanism and given that a primary alkyl amine interface has the same functionality as lysine units on collagen it seems reasonable to provide the hypothesis that tissue transglutaminases can modify these interfaces during culture to provide an interface that can support these cells. Another approach used by us and many others has been to functionalize hydrogels with integrin binding peptides. A new approach adopted by us is to prepare peptide functional polymers that can blended into hydrogels during manufacture. To do this we have developed semi-interpenetrating hydrogel networks in which the soluble component is branched. The use of polymeric additives in this has major advantages in processing because they can be easily dosed into production procedures such as additive manufacturing. The use of branched polymers with peptide end groups (GRGDS in this case) allows for maximum availability of the peptide ligands and the branched architecture ensured that the functional polymer cannot be leached from the network.

ACKNOWLEDGEMENTS: Financial support was received from EPSRC (UK, GR/T19773/01,), BBSRC (UK, BBS/B/01162) and MRC (UK, 77508)

REFERENCES

[1] Rimmer et al Biomater 2007;28 (35):5319-31.
[2] Hassan et al Acta Biomat 2014; 10(7):3029-37.
[3] Rimmer et al Biomater 2009;30 (13):2468-78.
[4] Ma et al J. Mat. Sci:Mat. Med. 2011, 22(3):663-70.



3D bioprinting iPSCs to recapitulate human limb bud development - An osteoarthritis disease model

S. Simonsson[1]
Presenting Author: Stina Simonsson, stina.simonsson@gu.se
[1]Institute of Biomedicine, Department of Clinical Chemistry and Transfusion Medicine, University of Gothenburg, Sweden

Cartilage lesions that can develop into degenerated osteoarthritis (OA) cartilage are a worldwide burden. As a prospective treatment for such lesions, we have previously shown that human-derived induced pluripotent stem cells (iPSCs) can be 3D bioprinted into cartilage-mimics [1]. The advantages by using an established iPSC line developed from chondrocytes are unlimited, immortal characterized cell source with a differentiation bias towards cartilage [2].
Designing protocols that generates hyaline cartilage from pluripotent cells in vitro is still a challenge, due to that joint formation are late in development and far from the pluripotent state. There are recent protocols for hyaline-like cartilage generation from iPSCs. Herein our 3D differentiation of bio-printed iPSC line experience that resemble limb bud formation (Figure 1) will be discussed.

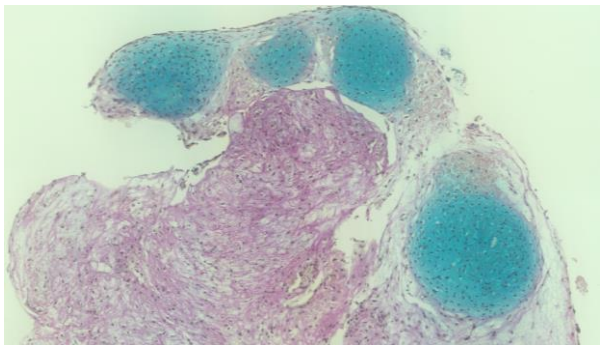
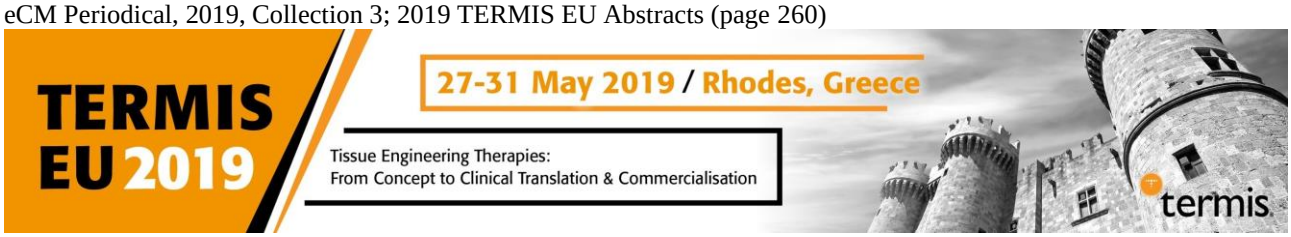


Figure 1: Limb bud like condensed structures are formed after in vitro differentiation of iPSCs.

ACKNOWLEDGEMENTS: Financial support was received from Swedish research council VR 2015-02560, granted Stina Simonsson

REFERENCES

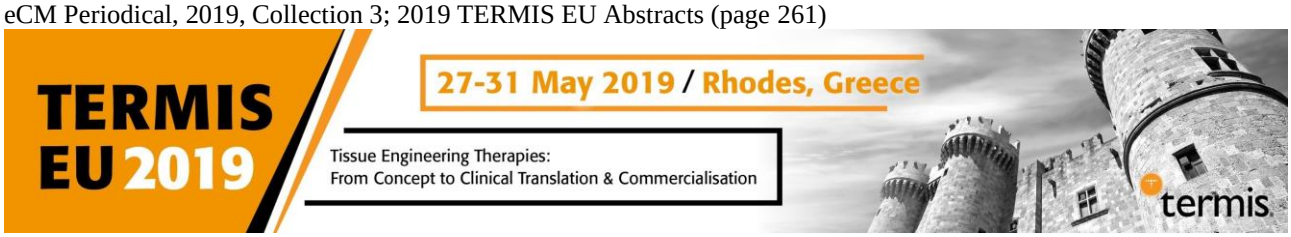
[1] Nguyen, D., et al., Sci Rep, 2017. **7**(1): 658.
[2] Borestrom, C., et al., Stem cells translational medicine, 2014; **3**(4): 433-47.



Inflammation and bone regeneration

S. B. Goodman
Presenting Author: Stuart B. Goodman, goodbone@stanford.edu
Department of Orthopaedic Surgery and Bioengineering, Stanford University, Stanford, CA, USA

Inflammation is the first stage of tissue healing and regeneration after traumatic injuries, exposure to infectious agents, and other adverse stimuli. If the natural sequence of events comprising tissue healing is interfered with or dysregulated, repair and regeneration of host tissue is impaired. Furthermore, if the injurious stimulus is not quickly mitigated or completely extinguished, either the organism as a whole will succumb (if the injury or response is overwhelming), or the local tissues will progress to a state of chronic inflammation, in which ongoing injury and attempts at repair persist. In the context of orthopaedic surgery, we have studied important interactions among cells of the innate immune system, i.e. the monocyte-macrophage-foreign body giant cell-osteoclast lineage, and the mesenchymal stem cell (MSC)-osteoblast lineage using in vitro and in vivo models. Based on this work, we have confirmed several important principles that underlie how bone is formed, destroyed and remodelled, that are applicable to the engineering of new bone. First, for optimal bone repair, the inflammatory phase of healing cannot be omitted. Second, there exists a definite period of time for the inflammatory phase to occur; shortening this time period mitigates bone healing. Third, during the initial inflammatory phase, pro-inflammatory cytokines and other macrophage-derived molecules precondition MSCs to be more efficient at bone formation. Fourth, if chronic inflammation occurs, the timely introduction of an anti-inflammatory cytokine such as IL-4 by direct infusion, release from a scaffold or via genetically modified MSCs can be effective in quenching inflammation and facilitating bone formation.



Designing natural biomaterial-based functional 3D scaffolds for cancer modelling

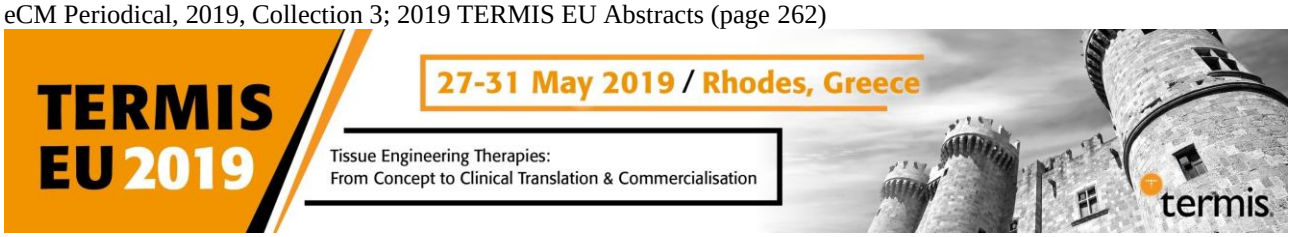
S. C. Kundu
Presenting Author: Subhas Kundu, kundu@i3bs.uminho.pt
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal

Cells and non-cellular players compose cancer microenvironment. In particular, cancer cells interact with fibroblasts, mesenchymal, endothelial and immune system cells. Extracellular matrix (ECM) supports cancer progression, migration and metastasis. Biomaterials are engineered for different matrices for cell-based tissue regeneration as they satisfy most of the qualities of ideal scaffolds. Solid evidences have shown that these protein/polysaccharides-based matrices support the growth, viability and proliferation of various mammalian cell lines and the differentiation of primary cells. Biomaterials can easily be functionalized according to the target application, in particular cancer modelling and therapeutic screening [1]. Three-dimensional cell culture is employed to achieve a comprehensive understanding of the molecular mechanisms underpinning cancer development [2]. In this work, different experimental approaches are used to develop 3D tumor models (breast, bone and lung). Naturally-derived polysaccharides (gellan gum), hyaluronic acid or proteins (silk and collagen) are employed to mimic the tumor microenvironment. The surfaces are designed using hyaluronic acid being a component of ECM for mimicking the extracellular microenvironment of cancer cells. The crosstalk between cancer cells, fibroblasts, stem cells and endothelial cells is reproduced in the 3D cancer models, to mimic the tumor microenvironment. The blended hydrogels composed of silk/gellan gum are developed with different ratios, to modulate the capability of bone cancer cells to self-organize into spheroid-like structures. The interaction between stem cells and osteosarcoma cells is investigated using blended silk/gellan gum freeze-dried hydrogels. Moreover, silk fibroin freeze-dried scaffolds are characterized in terms of porosity and porous interconnectivity. Silk fibroin scaffolds are used as ECM models to seed breast cancer cells (MCF-7) and normal mammary fibroblasts. The 3D microtumor tissues are analyzed using biochemical assays (alamar blue, live-dead, DNA quantification), microscopic techniques (phase-contrast, confocal and SEM), gene analysis, histology, immuno-histochemistry. The 3D microtumors are used as testing platform for anti-cancer drug compounds, in particular doxorubicin. Finally, lung cancer (A549) and endothelial (EA.hy926) cells are incorporated into a tumor-on-chip microfluidic device leading to a more realistic model to investigate intravasation. Time-lapse microscopy is used to monitor the role of protrusions in the mechanism of invasion, and the effect of doxorubicin and Rho inhibitor (C3 transferase) in tumor dissemination is investigated. These results shed light on the mechanisms of cancer development, invasion and strategies to treat the disease.

ACKNOWLEDGEMENTS: In this work David Caballero, Virginia Brancato, Banani Kundu, Ricardo Pires, Miguel Oliveira and Vitor Correlo have contributed. The financial supports are received from European Union Framework Programme for Research and Innovation Horizon 2020 under ERA-Chair grant agreement nº 668983-FoReCaST, the FCT projects BREAST-IT (IF/028168/2017) and 2MATCH (IF/028070/2017)

REFERENCES

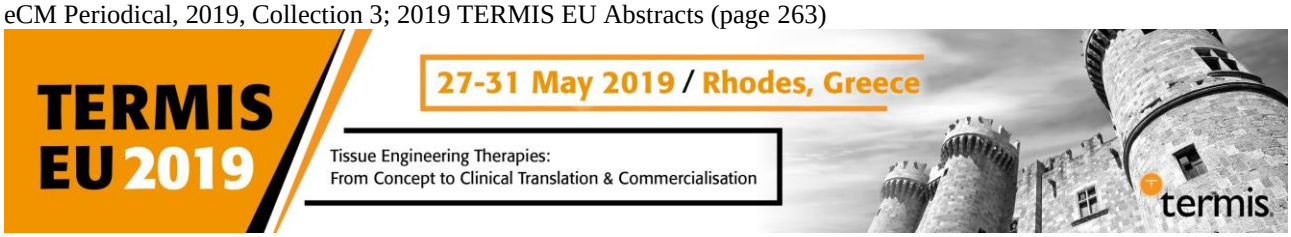
[1] Rodrigues, T. et al. Pharmacol Ther. 2018;184:201–211
[2] Caballero, D. et al. Biomat. 2017;149:98–115.



New approach to regeneration of osteochondral defects by using Agili-C cell-free scaffold

S. Chubinskaya
Presenting Author: Susanna Chubinskaya, susanna_chubinskaya@rush.edu
Department of Pediatrics, Rush University Medical Center, Chicago, IL 60612, USA

The Agili-C^{TM} is a first-in-class acellular implant designed for simultaneous regeneration of cartilage and subchondral bone. Here we provide the evidence of the ability of human adult articular chondrocytes to migrate from cartilage explants into the Agili-C^{TM} scaffold forming hyaline-like cartilage. Normal cartilage was collected from the knee and ankle joints of 13 human donors. The chondral phase of the implant was placed inside donut-shaped cartilage plugs and cultured in 20% serum. Chondrocyte survival, migration, PG synthesis, histology, and collagen II and aggrecan gene and protein expression were used to characterize cellular responses and confirm hyaline-type structure. The data were analyzed with two-tailed t-test comparing the mean of samples cultured with Agili-C^{TM} construct to the mean of control. The P values <0.05 were considered statistically significant. Chondrocyte migration into the Agili-C^{TM} constructs was independent of the joint, gender or donor's age. Cells remained viable inside 3D construct and deposited ECM enriched in collagen II and aggrecan. By day 60, PG synthesis was 3-fold higher, Agg gene expression was 4-fold higher ($p<0.0001$) and ColII gene expression was 8-fold higher ($p<0.001$) respectively in explants containing Agili-C^{TM} ($p=0.007$) in comparison with control. Additionally, we showed a layer formed on the surface of the implant populated by progenitor-like cells. Concluding, the Agili-C^{TM} implant is capable of inducing hyaline cartilage evidenced by the expression of specific markers and appropriate structural organization suggesting a migration and proliferation of chondrocytes and progenitor cells from the periphery and the surface of the tissue with the formation of ECM.



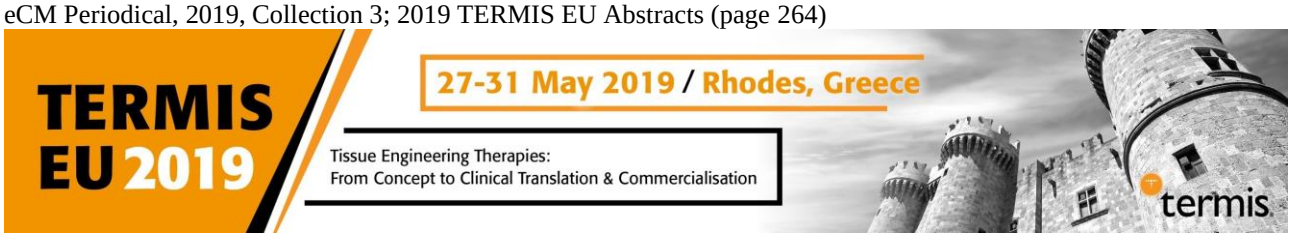
Immunocompetent skin models to emulate sensitation and allergy formation

S. Gibbs[1,2]
Presenting Author: Susan Gibbs, s.gibbs@vumc.nl
[1]Department of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, The Netherlands [2]Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, The Netherlands

Approximately 15-20% of the general population have Allergic Contact Dermatitis (ACD) resulting from environmental chemical exposure, making this skin disease a major health problem. Development occurs in two phases: in the first (induction) phase exposure to a chemical allergen causes immunological priming known as skin sensitization. The second (elicitation) phase is triggered if a sensitized person is again exposed to the same allergen. Therefore, prevention of ACD should be prioritized, warranting extensive efforts into understanding the cellular mechanisms of sensitization in order to identify sensitizers, predict the potency of a sensitizer and most importantly to predict the safe (no response) concentration of a potential sensitizer. This has led to an adverse outcome pathway for sensitization which identifies the 4 Key Events: Key Event 1 – chemical penetration through the skins stratum corneum; Key Event 2 – keratinocyte activation (cytokine secretion); Key Event 3 – antigen loaded Langerhans cell (LC) maturation and migration from epidermis to dermis and then to lymph nodes; and Key Event 4 - antigen presentation by LC to antigen-responsive T-cells in the local lymph node which results in T-cell priming (memory) [1]. Here we describe a unique reconstructed human skin model with integrated MUTZ-3 derived Langerhans Cells (RhS-LC) which enables key events 1, 2 and 3 to be investigated in a single skin model [2,3]. MUTZ-LC display similar phenotypic plasticity as their primary counterparts when incorporated into RhS. We describe differences and similarities in the mechanisms regulating LC migration and plasticity upon allergen or irritant exposure enabling contact sensitizers to be distinguished from irritants. The model therefore provides a unique and relevant research tool to study human LC biology in situ under controlled in vitro conditions, and will provide a powerful tool for hazard identification, testing novel therapeutics and identifying new drug targets. Currently the RhS-LC is being incorporated into a microfluidics device to further investigate immune mediated skin disease and systemic toxicity.

REFERENCES

[1] Rodrigues Neves C et al. Curr Top Microbiol Immunol. 2018; doi: 10.1007/82_2018_88. Epub 2018.
[2] Gibbs S et al. Toxicology. 2018;doi: 10.1016/j.tox.2017.10.014. Epub 2017
[3] Kosten I et al. TAAP. 2015;287:35-42



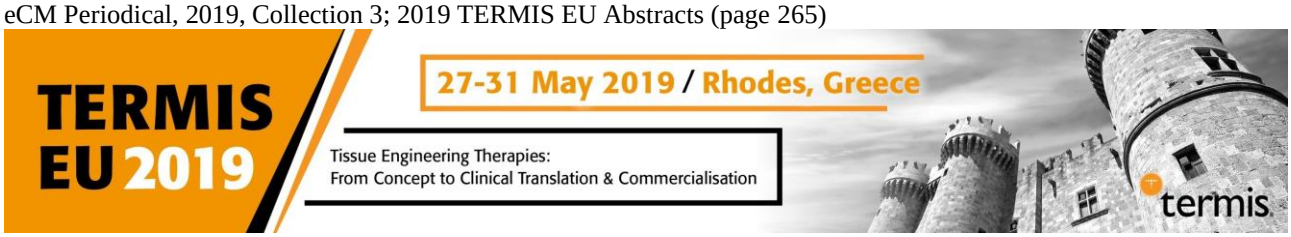
Approaching cartilage repair from several direction using pluripotent stem cells

S. J. Kimber
Presenting Author: Susan J Kimber, sue.kimber@manchester.ac.uk
Division of Cell Matrix Biology and Regenerative Medicine, Faculty of Biology Medicine and Health
University of Manchester M13 9PT, UK

Osteoarthritis (OA) affects the mobility and life-quality of millions of people worldwide with prevalence increasing during ageing and with risk factors e.g. injury and obesity. Thus, the incidence of OA is set to rise steeply with an ageing and increasingly obese population. Hyaline cartilage lining the joints consists mainly of extracellular matrix containing proteins, proteoglycans, glycosaminoglycans and water, with chondrocytes making up less than 3% of the tissue volume. The matrix produced by the chondrocytes contains region-specific orientation of collagen fibres and is rich in proteoglycans especially chondroitin sulphate proteoglycan and hyaluronan. Aggrecan with associated glycosaminoglycans forms the major proteoglycan. Disease and trauma of articular cartilage result in progressive damage to this matrix. Since cartilage has poor intrinsic repair mechanisms due to the lack of blood vessels and nerves, this damage commonly leads to OA accompanied by pain and loss of mobility. Due to the limitations of joint replacement and restrictions in adoption of autologous chondrocyte implantation there is an unmet clinical need to provide new ways to repair articular cartilage. Clinical grade human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs), generated under Good Manufacturing Practice (GMP) conditions, are a promising cell source for chondrocytes. They can be maintained as an inexhaustible, expandable cell population which retain their ability to differentiate into all body cell types. hESCs and iPSCs can be induced to form chondrocytes using standardised protocols based on the temporal course of signaling during mesodermal development (1) and generating cells of consistent and reproducible repair potential. We showed that these cells can repair cartilage in an osteochondral defect in the hind limb femoral head cartilage of immunocompromised rats (2). We refined this protocol (e.g. 3) extended it into a 3D pellet or 3D scaffold formats in which cartilage extracellular matrix protein is laid down in vitro. However, although the cells make increasing amounts of collagen II, until recently they have generated only modest amounts of the major proteoglycan of cartilage, aggrecan. As aggrecan is crucially important for cartilage compressive properties, this was a weakness in using such cells therapeutically. In recent work we have shown that epigenetic modifiers can enhance aggrecan formation over and above other methods such as TGFB family modulation. By histone deacetylase activation, we achieved a 64 fold increase in aggrecan transcript with parallel upregulation of the transcription factor SOX 5 (18fold), co-regulator with SOX9 of chondrogenic genes. This occurred without inducing expression of COLX (indicative of pre-hypertrophic chondrocytes) or increased COL1. We are now identifying the regulatory pathways associated with these changes. To improve chondrogenesis in vitro further we have investigated encapsulation in hydrogels. In collaboration with the Karperien lab (University of Twente) we have identified hydrogels which support chondrogenesis from hESC far better than our previous scaffold, fibrin. The results suggest that hydrogels based on carbohydrate chains may be superior to those based on peptide gels for maturing hESC-prechondrocytes to chondrocytes and maintaining phenotype.

REFERENCES

[1] Oldershaw R et al 2010 The directed differentiation of human embryonic stem cells. Nature Biotech 28, 1187-1193
[2] Cheng A, et al **(**2014) Cartilage Repair Using Human Embryonic Stem Cell-derived Chondroprogenitors. Stem Cell Translational Medicine 3, 1287-1294



Bone injury response and the extracellular vesicle-mediated crosstalk between immune and mesenchymal stem/stromal cells

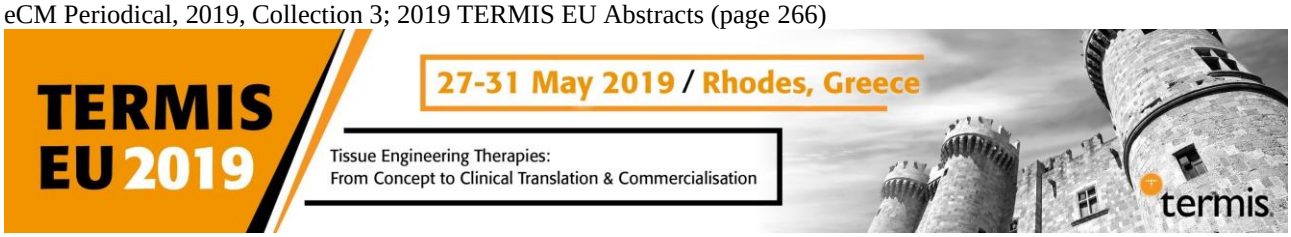
S.G. Santos[1,2]
Presenting Author: Susana G. Santos, Susana.santos@ineb.up.pt
[1]i3S - Instituto de Investigação e Inovação em Saúde and INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal [2]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

Tissue response to injury is initiated with acute inflammation, which defends the host against pathogens and prepares the injury microenvironment for tissue repair and remodelling. Coordination of the different stages requires intercellular communication, cell recruitment, proliferation, activation and differentiation. As part of their communication strategies cells secrete Extracellular Vesicles (EV), including exosomes and microvesicles. EV, and particularly exosomes, are reported to play important roles for the different stages of tissue repair and regeneration [1]. Our work has been addressing the communication between immune cells and Mesenchymal Stem/Stromal Cells (MSC), in the context of bone repair. Herein, we will discuss our results and approaches using primary human cells and a rat bone defect model. Using human primary cells, we showed that monocyte-derived Dendritic Cells (DC) can recruit MSC via paracrine action, and that EV are the main effectors of that recruitment, being enriched in chemoattractant mediators [2]. Analyzing the rat femoral bone defect model at 3 days (acute inflammation) and 14 days (inflammation resolution) after bone injury, we characterized the temporal regulation of the systemic response at the levels of immune cell populations and plasma micro(mi)RNA profile. We encountered changes in systemic immune cell populations in blood, draining lymph nodes and spleen at day 3 and a return to baseline by day 14. At the miRNA level, bone injury led to a general down-regulation of miRNA levels in plasma at 3 days, and an up-regulation of let-7 family and miR-21 at day 14 after injury. In silico pathway analysis predicted that most miRNAs temporally affected were involved in miRNA processing, cellular development, proliferation and movement [3]. Additionally, we are analyzing the impact of bone injury microenvironment on bone marrow (BM) precursors of DC and macrophages, and their EV-mediated crosstalk with MSC. Interestingly, the capacity of BM-DC-secreted EV to recruit MSC changes significantly with time after bone injury. Finally, the potential of EV as therapeutic tools, and our work on delivery strategies to preserve their biological action will be discussed.

ACKNOWLEDGEMENTS: Work funded by project NORTE-01-0145-FEDER-000012, Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through European Regional Development Fund (ERDF); AO Foundation, Switzerland (S-15-83S); and Portuguese funds through FCT, including PhD and Post-Doc fellowships.

REFERENCES

[1] Silva et al., European Journal of Pharmaceutical Sciences. 2017; 98: 86–95
[2] Silva et al., Scientific Reports. 2017; 7:1667
[3] Silva et al., Theranostics. 2018; 8:3902-3917



Impact of the sensory nervous system and its neuropeptides on osteoarthritis pathology

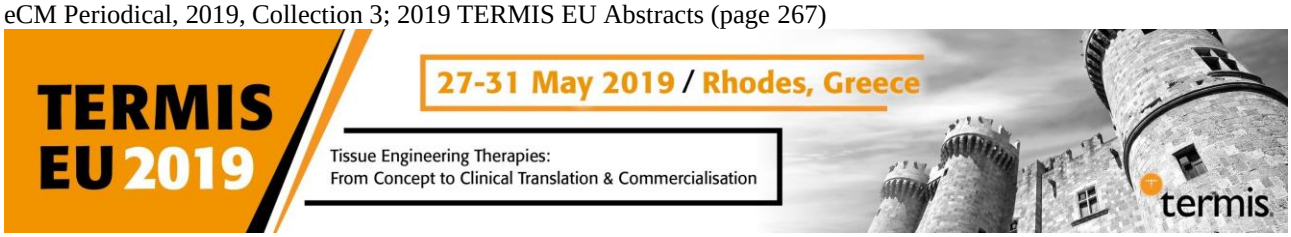
S. Grässel[1,2]
Presenting Author: Susanne Grässel, susanne.graessel@ukr.de
[1]Experimental Orthopedics, Centre for Medical Biotechnology (ZMB), BioPark 1, University of Regensburg, Germany [2]Department of Orthopedic Surgery, University of Regensburg, Germany

Osteoarthritis (OA) is the most common joint disorder and the leading cause of disability and chronic pain in the elderly with a major adverse socio-economic impact. To date pharmacological interventions can only relieve pain, whilst cell- and compound based therapies have limited success in the regeneration of damaged tissues. Thus, there is a need for identification of novel targets for diagnostic and therapeutic approaches. The joints are innervated by calcitonin gene-related peptide (CGRP) - and substance P (SP) positive sensory nerve fibers which are a potential source of tibial-femoral pain during OA pathogenesis. Alteration of sensory joint innervation might be partly responsible for degenerative changes that contribute to development of OA [1-3]. OA was induced in wildtype (WT), SP-knockout (Tachykinin1-/-) and CGRP-knockout mice by surgical destabilization of the medial meniscus (DMM). The medial subchondral bone structure was analyzed using µCT and nano-CT techniques. Cartilage matrix alterations were evaluated by scoring of Safranin O-stained sections of knee joints according to OARSI guidelines and by stiffness analysis using atomic force microscopy (AFM). Human chondrocytes obtained from OA patients after joint replacement and stimulated with SP or CGRP in 2D- and 3D-cell culture systems. Subsequently, proliferation, apoptosis, senescence, adhesion ability, GAG concentration, gene expression of marker genes and activation of signaling pathways was analysed. SP-deficient mice revealed a cartilage phenotype which might contribute to the considerable cartilage alterations early after induction of murine OA, ultimately leading to subchondral bone sclerosis at the early stage. In general, loss of sensory neuropeptides induced subchondral bone sclerosis in conjunction with advanced aging and not with OA. In addition, we conclude for both neurotransmitters dose-dependent effects on OA-chondrocyte metabolism and signalling via ERK and cAMP pathways. Underlying mechanisms remain elusive but changes in cartilage and bone cell composition might contribute to OA bone and cartilage phenotype which will be discussed in the presentation.

ACKNOWLEDGEMENTS: Financial support was received from the German Science Foundation (DFG) (GR1301/18-1; GR1301/19-1).

REFERENCES

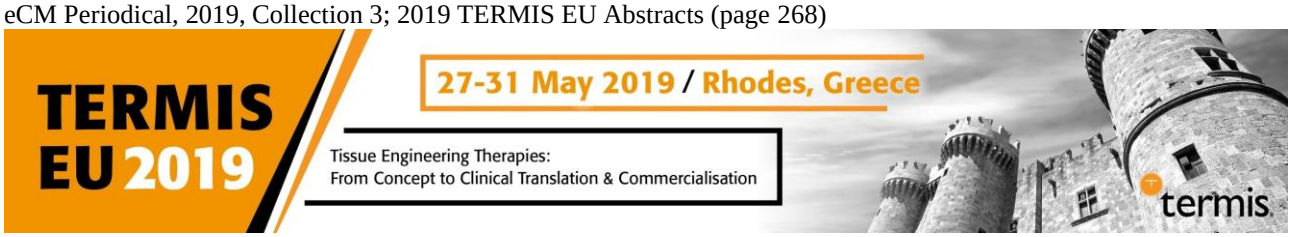
[1] Goldring MB, Goldring SR. Ann NY Acad Sci. 2010;1192:230-7
[2] Grässel S. Arthritis Res Ther. 2014;16(6):485
[3] Grässel S, Muschter D. Int. J. Mol. Sci. 2018, 19(2), E367.



The human amniotic membrane for regenerative medicine - A critical overview

S. Wolbank[1,2]
Presenting Author: Susanne Wolbank, susanne.wolbank@trauma.lbg.ac.at
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in the AUVA trauma research center, Vienna, Austria [2] Austrian Cluster for Tissue Regeneration, Austria

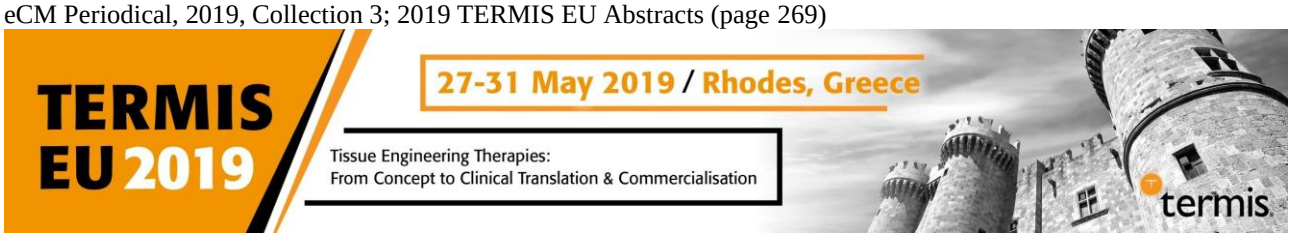
Human amniotic membrane (hAM), one of the perinatal tissues arising as a waste material during birth, is well established as therapeutic biomaterial in the clinics. The clinical application is mostly concentrated within ophthalmology and wound care, both of which use devitalized/denuded hAM as barrier in homologous use, as classified by the regulatory authorities. Novel applications in regenerative medicine are currently emerging including stroke, lung damage, liver disease, chronic ulcers or myocardial infarction. On the other hand, a variety of novel hAM formulations have been developed that depend on processing such as drying, lyophilizing, homogenization, or the use of isolated amniotic cells. It is hence crucial to identify the modes of action that justify application for treatment of a specific condition, and in consequence to identify the most suitable formulation, especially since this will reflect on the regulatory classification of the product. Within this presentation different examples for applying hAM derivatives in the field of regenerative medicine will be discussed, comparing the applied formulations, the outcomes and in consequence potential modes of action. One focus will be the potential of hAM and the cells thereof for musculoskeletal regeneration, the second one will be anti-fibrotic applications of hAM derivatives. We and others have previously demonstrated that hAM is a promising gliding and anti-fibrotic material, preventing adhesions in the peripheral nerve, in hernia repair and a model of liver fibrosis. Another crucial point to be discussed is the heterogeneity of natural biomatrices. In hAM we have identified regional differences on various functional levels, including metabolic status, differentiation potential, secretion of cytokines, growth factors, vesicles and lipid components, which led us to conclude that depending on the application the origin of the tissue should be considered.



Systemically administered, yet target-specific therapeutic recombinant proteins for regenerative medicine

T.A.H. Järvinen
Presenting Author: Tero A.H Järvinen, blteja@uta.fi
Faculty of Medicine and Life Sciences, University of Tampere and Department of Orthopedics and Traumatology, Tampere University Hospital, Tampere, Finland

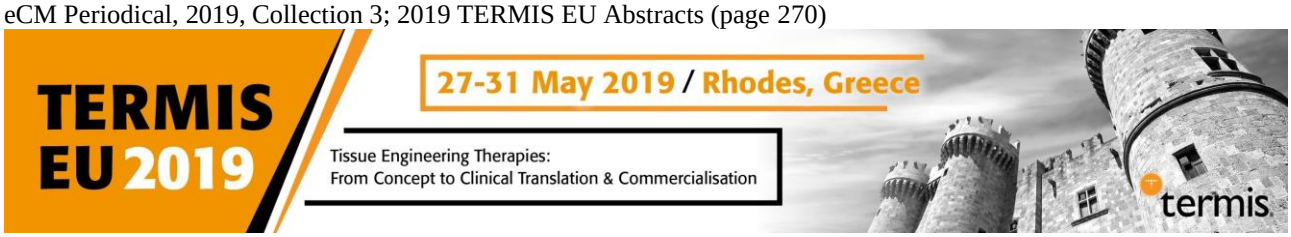
Growth factors, chemokines, and cytokines responsible for tissue regeneration have been identified. Their therapeutic usage in humans is almost non-existent because of the difficulty in maintaining their bioactivity in the protease-rich milieu of injured tissues. Safety concerns related to unwanted accumulation of potent growth factors in healthy organs have ruled out their use as systemic administration. Systemic, yet target-specific delivery of therapeutic recombinant proteins could be the solution for their translation to the clinical medicine. Angiogenic vasculature forming in the regenerating tissues after injury/surgery provides an opportunity for doing that. Angiogenic blood vessels has unique molecular structures specifically expressed by them, essentially forming a “zip/postal code” for injured tissue. This unique vascular zip code specific for injury/surgery can be utilized as a molecular target for target-specific delivery of systemically administered therapeutics to tissue injuries by ligands (peptides or antibodies identified by in vivo phage display) binding to this specific structure. Molecules with therapeutic potential together with targeting device can be expressed as a part of multi-functional recombinant protein. The desired outcomes of the targeted delivery of therapeutic recombinant proteins are increased drug concentrations in the target and lowered accumulation of the therapeutic payload in the healthy organs. The targeted delivery of systemically administered recombinant proteins to the injured tissue is hopefully rapidly advancing to clinical trials to provide new therapeutics to regenerative medicine.



Two decades of commercializing nanomedicine to aid in human health: Real patient success stories

T.J. Webster
Presenting Author: Thomas J. Webster, th.webster@neu.edu / websterthomas02@gmail.com
Chemical Engineering, Northeastern University, Boston, MA, USA

There is an acute shortage of organs due to disease, trauma, congenital defects, and most importantly, age related maladies. The synthetic materials used in tissue engineering applications today are typically composed of millimeter or micron sized particles and/or fiber dimensions with nanometer smooth features. Although human cells are on the micron scale, their individual components, e.g. proteins, are composed of nanometer features. By modifying only the nanofeatures on material surfaces without changing surface chemistry, it is possible to increase tissue growth of any human tissue by controlling the endogenous adsorption of adhesive proteins onto the material surface. In addition, our group has shown that these same nanofeatures and nano-modifications can reduce bacterial growth without using antibiotics, which may further accelerate the growth of antibiotic resistant microbes. Inflammation can also be decreased through the use of nanomaterials. Finally, nanomedicine has been shown to stimulate the growth and differentiation of stem cells, which may someday be used to treat incurable disorders, such as neural damage. This strategy also accelerates US FDA approval and commercialization efforts since new chemistries are not proposed, rather chemistries already approved by the US FDA with altered nanoscale features. This invited talk will highlight some of the advancements and emphasize current nanomaterials approved by the FDA for human implantation. Most importantly, it will highlight real patient success stories from patients who have received nanomedicine products created in my lab.

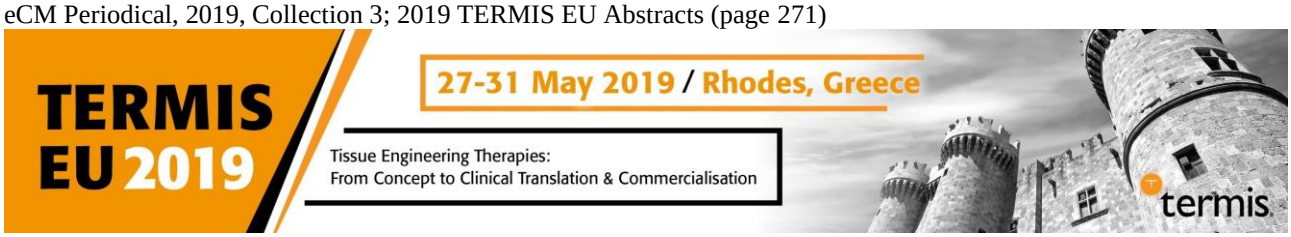


Exercise enhances the treatment of volumetric muscle loss by stem cell transplantation

T.A. Rando[1,2]
Presenting Author: Thomas A. Rando, rando@stanford.edu
[1]Center for Tissue Regeneration, Repair, and Restoration, Veterans Affairs Health Care System, Palo Alto, California, USA [2]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California, USA

Stem cell mediated regeneration of injured or diseased tissue holds great promise as a means to restore tissue structure and function. Our research focuses on the use of muscle stem cells (MuSCs) for the treatment of muscle injuries, particularly those characterized as volumetric muscle loss (VML), using a mouse model of VML. Toward this end, we have studied the characteristics of MuSCs as well as other muscle resident mononucleated cells isolated by fluorescence activated cell sorting (FACS) from mice and from humans to understand the properties that maintain or enhance their potency following transplantation to restore normal structure and function to diseased or injured muscle. Our studies of treatment of VML have focused on the use of MuSCs engrafted onto decellularized muscle tissue employed as a scaffold for the transplantation of cells, and how best to enhance the regenerative potential of the MuSCs transplanted within those scaffolds. Whereas we have found negligible benefit of transplanting the scaffold alone, we have found marked improvement in the structure and functional restoration of injured muscle when scaffolds are seeded with MuSCs. Interestingly, we have found that those improvements are enhanced when MuSCs are transplanted along with other muscle resident cells, particularly cells of the endothelial lineage. More recently, we have focused on the benefits of exercise, in this case voluntary wheel running or forced treadmill running, on the efficacy of muscle regeneration after acute injury and on the efficacy of MuSC therapy for VML. Our studies show a marked enhancement of tissue restoration when mice engage in physical activity (voluntary wheel running) after cell transplantation for VML injuries. In particular, we observe an improvement in both vascularization and innervation of the newly formed muscle fibers. There is an increase in capillary density and an increase in the rate of formation of de novo, mature neuromuscular junctions in the muscles of mice that have engaged in physical exercise compared with those that have not been provided with a running wheel. Our long-term goals are to optimize multiple aspects of the process, ranging from the cells to the scaffolds to the physical activity regimen, to generate a scalable process that will lead to effective stem cell-mediated treatments of VML in humans.

ACKNOWLEDGEMENTS: Financial support was received from the Department of Veterans Affairs, USA (I01RX001222, I01BX002324), and the National Institutes of Health, USA (AG036695, AG047820).



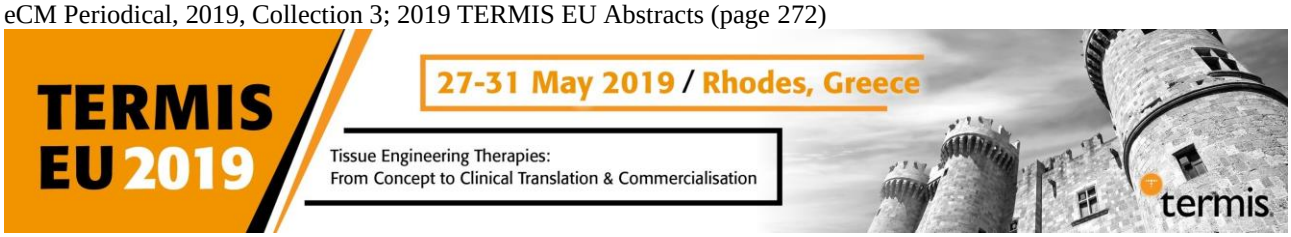
From academia to industry: A translational research approach for soft tissue regeneration

T. Steinberg[1]
Presenting Author: Thorsten Steinberg, thorsten.steinberg@uniklinik-freiburg.de
[1]Division of Oral Biotechnology, Center for Dental Medicine, University Medical Center Freiburg, Freiburg, Germany

Translational clinical research is an issue everybody is talking about. But the definition in the scientific community is somehow inconclusive, because the translational aspect in clinical research is not unidirectional, but rather means taking into account both point of views: the translation of knowledge from bench-to-bedside and from bed/chair-side to bench as well. Hence, we started a translational research approach for soft tissue regeneration in the oral cavity and here especially for large and full-thickness wound situations, because there is still no satisfying treatment solution on the market. Coming from this dual strategy, we developed a new wound healing medical device by implementing own basic research data and experiences from clinicians, which are facing the clinical need for specific wound situation day by day. The result was a biohybrid membrane, consisting out of two different polymers, gelatin and polycaprolactone, which mediate by an innovative production procedure, the electrospinning technology, and the hierarchic nano-fiber configuration, outstanding biomechanical, regenerative and handling characteristics. The key feature of the membrane is a nano-fiber gradient with increasing fiber diameters reaching from 100nm-800nm which mimics a soft tissue specific biomechanical nano-environment, enabling cells to migrate on those fibers, like a trafficking system, from the wound margins into the membrane [1-3]. To prepare this new developed wound healing device for commercialization, we performed extensive preclinical investigations to proof the safety and efficacy, firstly in vitro, by using human and tissue specific 3D cell cultures and in a further step with a pilot animal study in a mini pig model. Finally, we were able to perform a few individual health trails in patients suffering progressed oral carcinogenesis. All these promising and conclusive data were collected to bring this innovative project on a higher level that the medical device industry got interested to bring this product on the market. But the reality at Universities is that it is very hard or nearly impossible to transfer such a project from academia to the industry, because of the missing clinical data and the high risk from the viewpoint of the industry to fail. For this low readiness to assume a risk, we failed to find a strategic investor in Europe and the US for almost 6 years and finally found a very committed group of investors in China, who were willing to spend the risk money to found a new “medtec” company with the respective production infrastructure and support us on the way to get the regulatory approval for our innovative biohybrid membrane for soft tissue regeneration in China.

REFERENCES

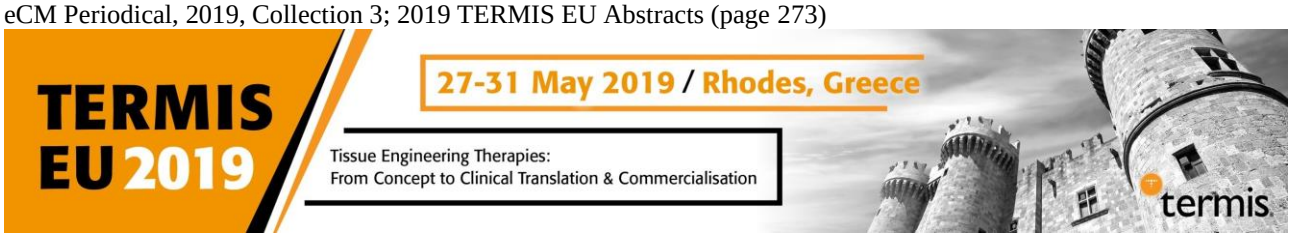
[1] Angarano M et al. Advanced Functional Materials. 23 (2013) 3277-3285.
[2] Schulz S et al. Tissue Eng Part A, 20 (2014) 1935-1947.
[3] Jedrusik N et al. Advanced Healthcare Materials. 7 (2018) e1700895



Considerations for manufacture of MSCs for clinical development

T. O'Brien
Presenting Author: Timothy O'Brien Timothy.Obrien@nuigalway.ie
REMEDI and CURAM, NUI Galway, Ireland

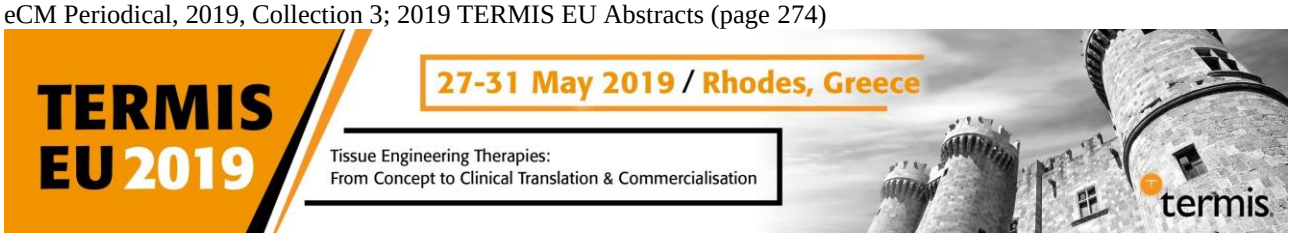
MSCs are currently being investigated for use in a wide range of clinical disorders. The therapeutic potential is related to their direct differentiation potential, the secretome capable of paracrine effects and the recently described immune reprogramming associated with their use. While a large number of clinical trials are underway worldwide there is wide variation in the methods of cell manufacture. This will make interpretation of trial outcome challenging and comparison between the outcomes of different trials problematic. A number of questions arise when an MSC product is under development including: cell source – bone marrow, adipose tissue, umbilical cord; the use of sorted versus bulk manufactured cells; allogeneic versus autologous; if allogeneic pooled versus individual donor; cell passage and population doubling; choice of bioreactor; conditioned versus unconditioned cells; and choice of xeno free media. Additional challenges reside around potency assays, mechanism of action, repeat dosing and the effect of disease on potential outcomes. A single product for development would have advantages and an off the shelf, allogeneic product cultured in a bioreactor under xeno free conditions would be ideal. These issues will be discussed kin the context of developing an MSC therapeutic for the treatment of diabetic complications.



Glycosaminoglycan-based hydrogels for musculoskeletal regeneration

U. Freudenberg
Presenting Author: Uwe Freudenberg; freudenberg@ipfdd.de
Leibniz Institute of Polymer Research Dresden (IPF), Max Bergmann Center of Biomaterials Dresden (MBC), Hohe Str. 6, 01069 Dresden, Germany

Glycosaminoglycans (GAGs) govern important functional characteristics of the extracellular matrix in living tissues. Incorporation of GAGs into biomaterials opens up new routes for the presentation of signaling molecules, providing control over development, homeostasis, inflammation, and tumor formation and progression. Recent approaches to GAG-based materials are reported, highlighting the formation of modular, tunable biohybrid hydrogels, including both theory-driven design concepts and advanced processing technologies. Special emphasis is put on the independent tuning of molecular and physical cues within the GAG-based materials in order to control cell-fate decisions of embedded cells. Based on multi-armed poly(ethylene glycol), heparin, and selectively desulfated derivatives thereof we have developed a modular platform of cell-instructive hydrogels for various applications in the field of musculoskeletal regeneration. Angiogenesis promoting materials have been used for promotion of cardiac regeneration by sustained release of SDF-1a. Moreover, matrixmetalloprotease responsive starPEG-heparin hydrogels provided well-adjusted biomolecular and physical cues in order to guide MSC- and chondrocyte-based cartilage regeneration in vitro and in vivo. Finally, zonal hydrogel constructs allowed in vivo growth of bizonal cartilage with a stable calcified cartilage layer. As a perspective, multiphasic GAG-based hydrogel materials fabricated using microfluidic and additive manufacturing techniques shall allow for a fare-going control of cellular-co-cultures and finally may pave the way for innovative regenerative approaches in the musculoskeletal field and beyond.



Direct reprogramming of human fibroblasts as a novel tool in cardiac tissue engineering

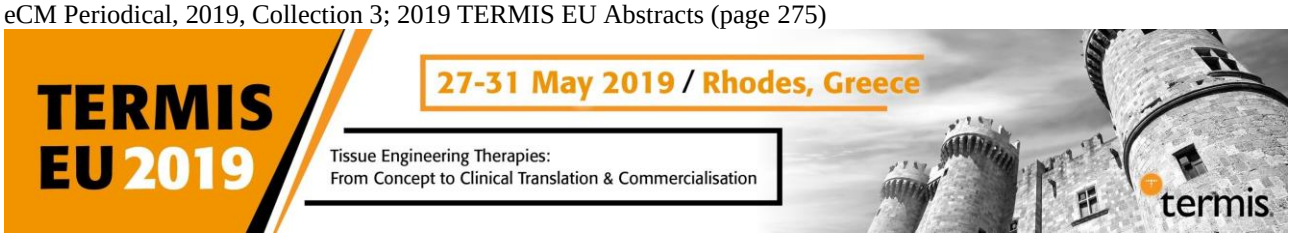
V. Chiono [1]
Presenting Author: Valeria Chiono, valeria.chiono@polito.it
[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy

Heart failure following myocardial infarction is the current leading cause of mortality in the industrialized world.
Currently, the only clinical therapy addressing the irreversible loss of cardiomyocytes is heart transplantation. On the other hand, many tissue engineering approaches and cell therapies are under investigation to regenerate myocardial tissue. However, such approaches are limited by the poor grafting and survival ability of implanted cells and by the limited endogenous regenerative potential of human adult heart. Since 2010, a new promising strategy has emerged based on direct reprogramming of CFs into induced cardiomyocytes (iCMs) using transcriptional factors and/or microRNAs (miRNAs) (miR-combo) [1-4]. Early studies have demonstrated the in vitro and in vivo direct conversion of mouse CFs into iCMs and the in vitro direct reprogramming of human CFs [1-4]. However, before prospecting a translation of the approach into the clinics, direct reprogramming efficiency and iCM maturation level should be greatly improved. Particularly, the role of 3D scaffolds/hydrogels in direct cardiac reprogramming needs to be further investigated [3-4]. In this work, the miR-combo mediated direct reprogramming of human cardiac fibroblasts was studied for the first time, by culturing cells in 2D tissue culture plates and in 3D microenvironments consisting of fibrin-based hydrogels. Untransfected cardiac fibroblasts expressed cardiac genes, hence they are "primed" for direct reprogramming respect to other types of fibroblasts. In 2D cultures, after 3 days from transfection, miR-combo treatment increased cardiac gene expression while decreased vimentin gene expression respect to NegmiR control and untrasfected cells. On the other hand, cells cultured in 3D fibrin-based hydrogels as NegmiR control and untransfected cells showed enhanced cardiac gene expression than their 2D counterparts. However, miRcombo transfection did not enhance cardiac gene expression in cells cultured in 3D hydrogels respect to controls after 3 days. At higher times (7 and 15 days), CFs cultured in 3D hydrogels showed a strongly enhanced expression of cardiac genes such as cardiac troponin I and alpha myosin heavy chain 6. Immunocytochemistry data confirmed PCR analysis results. Hence, a 3D biomimetic environment was found to play a key role in enhancing direct reprogramming of human CFs which cannot be simply explained by an enhancement of early cardiac genes. Based on that gene expression profile of reprogrammed cells in different 3D microenvironments is under investigation.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 772168; BIORECAR project).

REFERENCES

[1] Batty J.A. et al. Eur. J. Heart Failure 2016; 18: 145
[2] Jayawardena T.M. et al. Circ. Res. 2015; 116:418-24.
[3] Li Y. et al. Scientific Reports 2016; 6: 38815.
[4] Paoletti C. et al. Cells 2018, 7(9), 114.



Redox lipidomics deciphering of lipid signaling in tissue re-engineering and regeneration

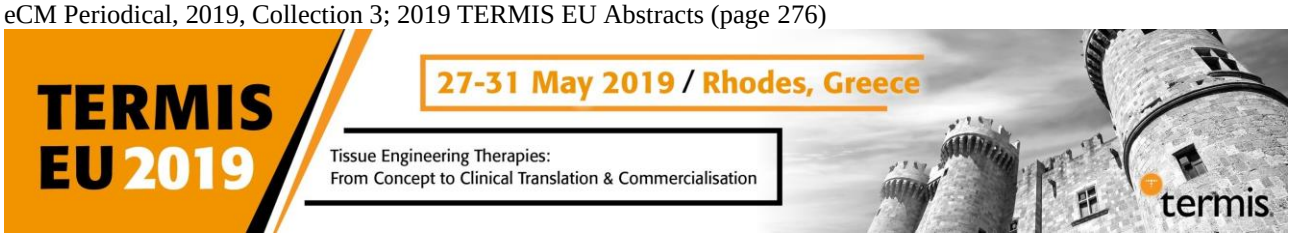
V.E. Kagan[1,2]
Presenting Author: Valerian E. Kagan, kagan@pitt.edu
[1]Center for Free Radical and Antioxidant Health, University of Pittsburgh, PA, USA [2]Laboratory of Navigational Redox Lipidomics, Institute of Regenerative Medicine, IM Sechenov Moscow State Medical University, Russia

Harmonized coordination of the myriads of metabolic reactions within a cell as well as regulation of cell-cell interactions in the context of optimized tissue homeostasis and even safe programmed death requires a highly sophisticated and rich communication language. Replacement of injured tissues and organs and their re-engineering with a purpose of successful regeneration may represent an even more complex task with a larger variety of refined signals. There is a compelling need for a more profound understanding and characterization of the signaling landscape by identifying and deciphering the complex interactive signals/markers leading to the balanced regulation of pro- and inti-inflammatory responses, immune reactions, cell differentiation, proliferation and death engaged by the re-engineering and transplantation therapy using suitable cells, biomaterials (including nanomaterials), and/or endogenous and exogenous factors and signals required for translating the great technological promise to clinical success. Historically, much research attention has been focused on cytokines and growth factors. For more than seven decades, lipid mediators - oxygenated free polyunsaturated fatty acids (PUFA) of several major classes such as octadecanoids, eicosanoids, docosapentanoids and docosahexanoids - have been studied and identified as essential components of the overall signaling system [1]. Latest developments of soft-ionization mass-spectrometry combined with high resolution liquid chromatography (LC) brought to life new opportunities offered by Redox Lipidomics. In this talk, I will discuss how this new technological breakthrough has lead to the discovery of a vastly richer communication/ signaling opportunities by oxygenated PUFA-phospholipids encompassing hundreds of thousands of possible words in the language composed of individual molecular species of peroxidized phospholipids, their oxidatively-truncated electrophilic derivatives and their adducts with nucleophilic protein targets. Among the unearthing accomplishments in this emerging field of research, are the unique signals of programmed cell death. Oxygenated forms of mitochondria-specific phospholipids, cardio-lipins (CLs), have been identified as intermediates required for the execution of apoptosis. These signals are enzymatically produced by complexes of CLs with an intermembrane space hemo-protein, cytochrome c (cyt c). New classes of regulators of apoptosis have been designed and successful tested in vitro and in vivo - mitochondria-targeted electron scavenging nitroxides conjugated with specific peptides and imidazole-substituted fatty acids. Selective and specific oxidation of PUFA-phosphatidylethanolamines (PE) catalyzed by complexes of 15-lipoxygenase with a scaffold protein, PE-binding protein 1 (PEBP1) has been found critical for the execution of ferroptotic death program occurring in the endoplasmic reticulum and its specific domains, mitochondria-associated membranes. Deciphering of new signals formed from PUFA-neutral lipids and PUFA-phospholipids are important for understanding of the immune response and signaling by extra-cellular vesicles emitted by cells during the transplantation and tissue remodeling.

ACKNOWLEDGEMENTS: Financial support from NIH (U19AI068021, HL114453-06, NS076511, NS061817, HL136143, HL086884, and HL123771) and Russian academic excellence project “5-100”.

REFERENCES

[1] Serhan CN Nature 2014; 92

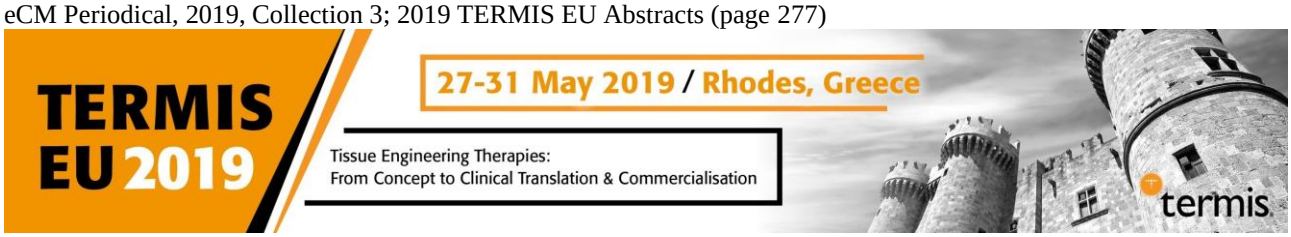


Emerging role of extracellular vesicles in bone formation and application to bone regeneration

V. Geoffroy[1,2]
Presenting Author: Valérie Geoffroy, valerie.geoffroy@inserm.fr
[1]Inserm, UMR 1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS, Nantes, F-44042, France [2]Université de Nantes, UFR Odontologie, Nantes, F-44042, France

Abundant extracellular vesicles (EVs) are produced in the bone matrix throughout life, in response to many stimuli (mechanical load, inflammation…). It is known since the seventies that these EVs play a role in the mineralization of skeletal tissue. EV-based cell-free therapy appears to be a promising strategy for the restoration of bone defects. This novel system based on Mesenchymal Stem Cells (MSC)-derived EVs/exosomes provides a new therapeutic option for bone tissue engineering and showed promising potential in joint repair that is mediated by their immunosuppressive as well as pro-regenerative activities. MSC-EVs exert immunomodulatory as well as pro-regenerative effects and mimic therapeutic effects of MSCs. MSC-EVs carrying certain proteins and nucleic acids that can support healing of injured tissues. Strategies for advancing MSC-EV-based therapies are already ongoing. All these data indicate that EV-mediated transfer of mRNAs, miRNAs, or proteins from MSC may be used to reprogram surrounding cells and to support tissue repair. But EV-based exchange of genetic information may be bi-directional. Our working hypothesis is based on the transfer of active molecules (miRNAs, proteins…) associated to EVs derived from the injured cells (e.g. the cells in bone that experienced mechanical stress), to MSCs or other bone cells in order to induce their differentiation or activation, which may then contribute to the bone repair. The role of EVs and their associated molecules in different cells in bones and neighboring tissues (cartilage, blood vessels...) will be discussed in the context of bone regeneration.

ACKNOWLEDGEMENTS: Financial support was received from Inserm (Institut National de la Santé et de la Recherche Médicale) and ANR (Agence Nationale de Recherche) (ANR-13-EPIG-0001-04).



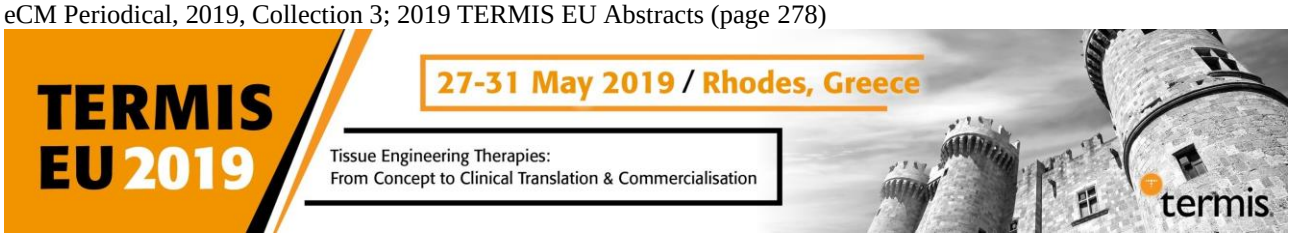
Rapid expansion of mesenchymal stem cells using media supplemented with human platelet lysate, suitable for cGMP expansion at large scale

V. Alonso-Camino[1]
Presenting Author: Vanesa Alonso-Camino, alonso-camino.vanesa@millcreekls.com
[1]Mill Creek Life Sciences, LLC, Rochester, Minnesota, USA

Mesenchymal Stem Cells (MSCs) are a promising candidate for tissue engineering and regenerative medicine applications due to their capacity of self-renewal and multilineage differentiation, ease of isolation and ability to grow in vitro. They are currently being used in hundreds of clinical trials and have been safely administered to thousands of patients with an expanding body of evidence of therapeutic efficacy. Mill Creek Life Sciences' PLTMax®, derived from normal human donor platelets, arose as an alternative to FBS to be used as a supplement for in vitro expansion of human cells used in translational medicine [1]. It is the only media supplement to date demonstrating clinical cultures of MSCs with long term genetic fidelity [2], rapid expansion and potent clinical activity. PLTMax® is being used worldwide in over 30 clinical trials including Phase I, II and III [3]. The effective transfer into the clinic of allogenic cell therapies using MSCs will depend predominantly on the development of large scale and cost effective manufacturing platforms that allow production of functional cells at the scale required to meet clinical demand. Here we will present the development of an optimized protocol for the establishment of large scale expansion of MSCs using PLTMax® and PLTGold®, a second-generation human platelet lysate that does not require the addition of heparin to remain clot free, thus providing a fully xenogeneic free alternative supplement to grow cells that will be used in clinical applications.

REFERENCES

[1] Karnieli O et al. Cytotherapy. 2017 Feb;19(2):155-169.3
[2] Crespo-Diaz R et al. Cell Transplant. 2011;20(6):797-811.
[3] Dietz AB et al. Gastroenterology. 2017 Apr 8. pii: S0016-5085(17)35411-2.

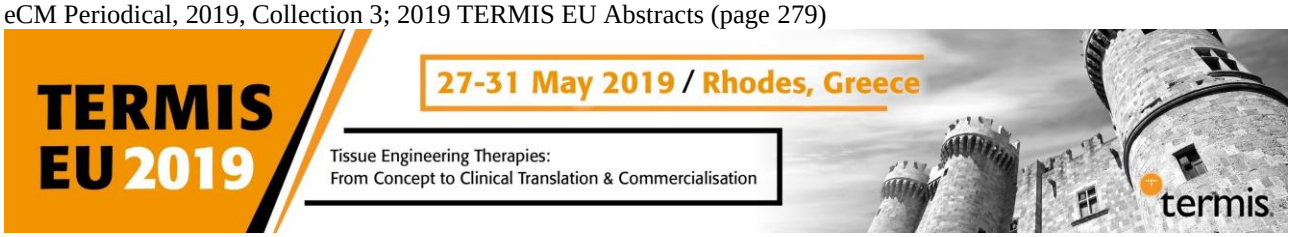


Regulation of cell fate and differentiation by SOX transcription factors in skeletal development, homeostasis and disease

V. Lefebvre[1]
Presenting Author: Véronique Lefebvre, lefebvrev1@email.chop.edu
[1]Translational Program in Pediatric Orthopaedics, Department of Surgery/Division of Orthopaedic Surgery, Children's Hospital of Philadelphia, Philadelphia, USA

The development and adult homeostasis of our skeleton require proper specification and coordinated, fully functional activities of skeletal progenitor/stem cells and downstream cell types, namely growth plate chondrocytes, articular chondrocytes, synovial fibroblasts, and osteoblasts. The cellular and molecular mechanisms involved in the governance of these processes are extremely complex, as are the anatomical and functional properties of our skeleton and as are the many diseases that can affect our skeleton and seriously impact our lives at any age. Research conducted worldwide over the last decades has uncovered major regulatory players in these processes, such that it is now becoming possible to decode the molecular basis of various diseases and to rationally design preventive and therapeutic strategies for these diseases. Research in the Lefebvre laboratory has focused on transcriptional mechanisms that rank high in the hierarchical regulation of cell lineage determination and differentiation in the skeletal system. Several members of the SOX transcription factor family have been discovered to be pivotal. This talk will review milestones achieved in recent years and research directions being currently pursued. It will include an overview of the cellular and molecular activities of the master chondrogenic factor SOX9 in the early step of specification of progenitor/stem cells to the chondrocyte lineage as well as in the ultimate steps of adult articular chondrocyte homeostasis and fight against osteoarthritis. It will newly reveal a key partner of SOX9 in the chondrocyte lineage and will identify highly conserved motifs in the proteins that are critical for their transcriptional activation functions. The presentation will also highlight the critical importance of SOX4 and SOX11 in specifying skeletal progenitor/stem cells and in controlling timely differentiation of downstream lineages, such as osteoblasts. Finally, a discussion will be opened on the implication of these findings for translational and clinical applications, including tissue engineering strategies.

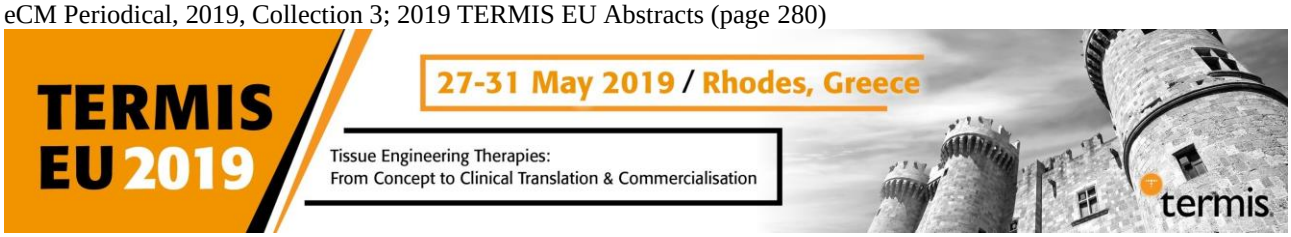
ACKNOWLEDGEMENTS: Financial support was received from The Children's Hospital of Philadelphia and The National Institute of Arthritis and Musculoskeletal and Skin Diseases (R01 AR068308 and AR072649 grants to VL).



Development of enabling platform technology for bioprinting of functional and vascularized endocrine human organ constructs

V.A. Mironov
Presenting Author: Vladimir A. Mironov, vladimir.mironov54@gmail.com
Laboratory of Biotechnology Research of 3D Bioprinting Solutions, Moscow, Russia & Regenerative Medicine Institute of Sechenov Medical University, Moscow, Russia

Organ printing could be defined as an automated robotic 3D biofabrication of functional human tissue and organs from living cells and bioprintable biomaterials according to digital model. Here we report first time about the development of novel platform technology for bioprinting of functional and vascularized human endocrine organs using tissue spheroid as buiding blocks. Bioprinting platform technology includes development of viscous porcine collagen (VisCol) as a bioprintable hydrogel and bioprinter FABION capable to print tissue and organ constructs using viscous colagen collagen scaffold and tissue spheroids. Organo-specific tissue spheroids have been proposed to use for bioprinting of organ parenchyme and endothelial tissue spheroids for bioprinting organ vasculature. Commercially available non-adhesive multiwells (Corning, USA) have been used for biofabrication of tissue spheroids. Viscous porcine collagen have been used for bioprinting porous scaffold with pores suitable for placing tissue spheroids. Tissue spheroids have been precisely automatically placed one spheroid a time into bioprinted scaffold using microfluidic device incorporated into multifunctional bioprinter FABION. The bioprinted tissue and organ contructs have been transplanted on chick chorio-allantoic membrane for estimation their post-printed vascularization, viability and functionality. It have been shown using human ovarian tissue spheroids that bioprinting of functional and vascularized human ovarian tissue is technologically feasible. Thus, our data clearly illustrate a potential of using proposed platform technology for 3D bioprinting of several endocine functional and vascularized human organs such as thyroid gland, parathyroid gland, ovary, testis, adrenal grand, endocrine part of pancreas and thymus.



Sheep and goat as model animals for cartilage repair in biomedical research

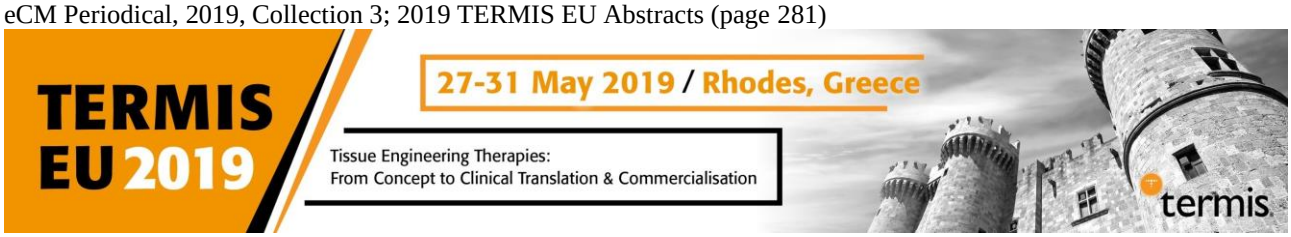
W. Brehm[1,2]
Presenting Author: Walter Brehm, brehm@vetmed.uni-leipzig.de
[1]Department for Horses, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany
[2]Saxonian Incubator for Clinical Translation (SIKT), University of Leipzig, Leipzig, Germany

Sheep and Goat have been widely used for translational research in various fields with an emphasis on orthopedic research. Bone regeneration, tendon healing, vertebral surgery particularly cartilage repair are important topics using these species. Recently, the fact that the Sheep usually give birth to twins was used to study the healing of osteochondral defects in ovine fetuses. While the size and the biomechanical features of the Sheep and the Goat together with their availablility, cost of purchase and cost of keeping are frequently cited arguments for the choice of both species for experimental approaches, it is of paramount importance to be aware of the specific behavioural, physiologic and immunologic characteristics, which have to be respected when planning and performing studies in these species. To name a few spots, the behavior of Sheep and Goat after experimental surgery is fundamentally different from Human, in that these animals will fully load their locomotor system very shortly after anaesthesia. Considering this is of fundamental importance upon planning as study focused on cartilage repair. Sheep and Goat both have a strong immune system, which needs to be respected when planning the application of potentially immunogenic materials. As an example, the application of bovine fibrin glue has led to massive invasion of immune cells in a osteochondral defect model in the Goat. Experimental design has to respect the anatomy, biomechanics, physiology and immunology of the model species to avoid false negative results of potentially beneficial treatment strategies, and even more to reduce the unnecessary use and suffering of animals. We will therefore present an overview of ovine and caprine cartilage defect models pointing out pros and cons, how to improve the outcome and how to avoid pitfalls.

ACKNOWLEDGEMENTS: Thanks to all the colleagues with whom I had the pleasure to work.

REFERENCES

[1] Repair of superficial osteochondral defects with an autologous scaffold-free cartilage construct in a caprine model: Implantation method and short-term results; Osteoarthritis and Cartilage 14(12):1214-26, January 2007, DOI: 10.1016/j.joca.2006.05.002
[2] The Role of Cells in Meniscal Guided Tissue Regeneration: A Proof of Concept Study in a Goat Model; Cartilage 6(1):20-29, June 2015, DOI: 10.1177/1947603514548213
[3] Longitudinal Evaluation of Effects of Intra-Articular Mesenchymal Stromal Cell Administration for the Treatment of Osteoarthritis in an Ovine Model; Cell Transplantation 24(11), January 2015, DOI: 10.3727/096368915X686193
[4] Is sheep lumbar spine a suitable alternative model for human spinal researches? Morphometrical comparison study; Lab Anim Res 2013: 29(4), 183-189, DOI: 10.5625/lar.2013.29.4.183



Regeneration of functional human lung by distal airway stem cells: From benchside to bedside

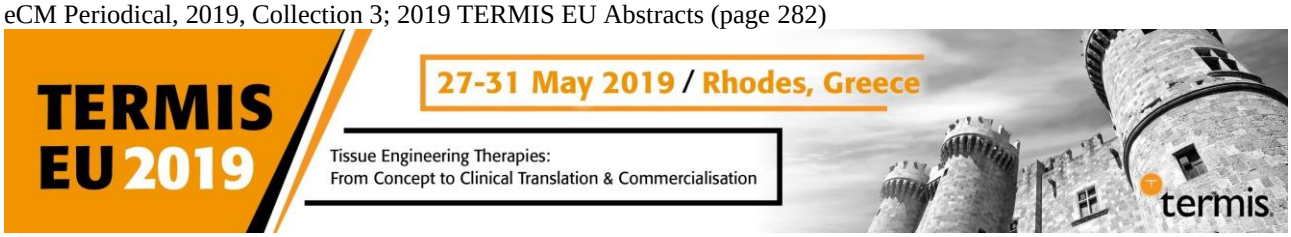
Wei Zuo[1,2]
Presenting Author: Wei Zuo, zuow@tongji.edu.cn
[1]Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai [2]Kiangnan Stem Cell Institute, Zhejiang, China

Irreversible destruction of bronchi and alveoli can lead to multiple incurable lung diseases. Identifying lung stem/progenitor cells with regenerative capacity and utilizing them to reconstruct functional tissue is one of the biggest hopes to cure such diseases. Previously we demonstrated lung regeneration in mice following H1N1 influenza virus infection, and linked distal airway stem cells (DASCs) expressing Trp63 (p63) and keratin 5, to this process. Pre-existing, intrinsically committed DASC(p63/Krt5) undergo a proliferative expansion in response to lung damage, and assemble into nascent alveoli at sites of interstitial lung inflammation. Selective ablation of DASC in vivo prevents this regeneration, leading to pre-fibrotic lesions and deficient oxygen exchange. Recently we showed that a rare population of SOX9+ DASCs located at airway epithelium rugae can regenerate adult human lung. SOX9+ DASCs can be readily isolated by bronchoscopic brushing and indefinitely expanded in feeder-free condition. Expanded SOX9+ DASCs can differentiate into alveolar cells in a monolayer in vitro culture system. Such cells can also give rise to alveolar and bronchiolar epithelium after being transplanted into injured mouse lung, with air-blood exchange system reconstructed and recipient's lung function improved. Moreover, we conducted the first autologous DASC transplantation clinical trials in patients with bronchiectasis, COPD and IPF, respectively. Lung tissue repair and pulmonary function enhancement was observed in patients 3~12 months after cell transplantation. Altogether our current work indicated that functional adult human lung structure can be reconstituted by orthotopic transplantation of tissue-specific stem/progenitor cells, which could be translated into a mature regenerative therapeutic strategy in near future.

ACKNOWLEDGEMENTS: This work was funded by The National Key Research and Development Program of China (2017YFA0104600), Youth 1000 Talent Plan of China, National Science Fund Committee of China (81570091 and 81770073).

REFERENCES

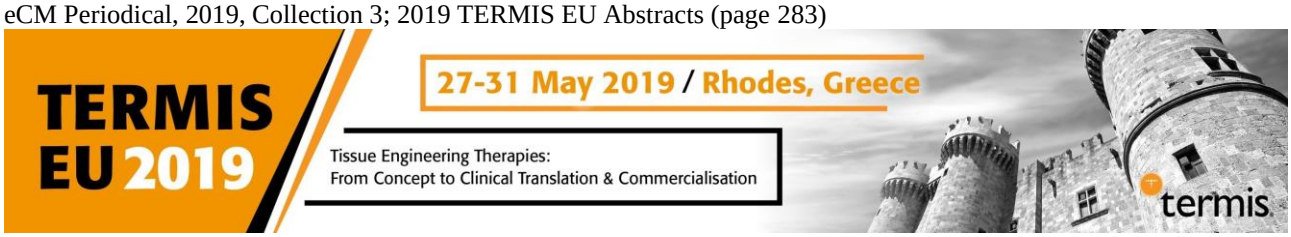
[1] Zuo et al. Nature. 2015; 517:616-620
[2] Ma et al. Protein Cell. 2018; 9(3):267-282
[3] Wang et al. Protein Cell. 2018; 9(3):244-245



Injectable scaffolds for wound healing

W.X. Wang
Presenting Author: Wenxin Wang, wenxin.wang@ucd.ie
Skin Research and Wound Healing Group, Charles Institute of Dermatology, School of Medicine, University College Dublin (UCD), Dublin, Ireland

Adjusting biomaterial degradation profile to match tissue regeneration is a challenging issue. Poly(β-amino ester)s (PBAEs) and their networks have attracted great attention as biodegradable polymers in the field of tissue scaffolds and gene delivery. However, the mechanical properties and biological effects were limited due to the linear structure and poor solubility. Therefore, a series of novel hyperbranched PBAEs (HP-PBAEs) with three-dimensional architecture have been developed as the improved construct for generation of biocompatible and injectable hydrogel. The polymers displayed fast gelation with thiolated hyaluronic acid (HA-SH) via “click” thiol-ene reaction. HP- PBAE/HA-SH hydrogels showed tuneable degradation profiles both in vitro and in vivo by using diamines with different alkyl chain lengths and poly(ethylene glycol) diacrylates with varied PEG spacers. The hydrogels with optimized degradation profiles encapsulating ADSCs were used as injectable hydrogels to treat two different types of humanized excisional wounds - acute wounds with faster healing rate and diabetic wounds with slower healing and neo-tissue formation. The fast-degrading hydrogel had the accelerated wound closure in acute wounds, while the slow-degrading hydrogel showed better wound healing for diabetic wounds. The results demonstrate that the new HP-PBAE-based hydrogels in combination with ADSCs can be used as a well-controlled biodegradable skin substitute, which demonstrates a promising approach in the treatment of various types of skin wounds.



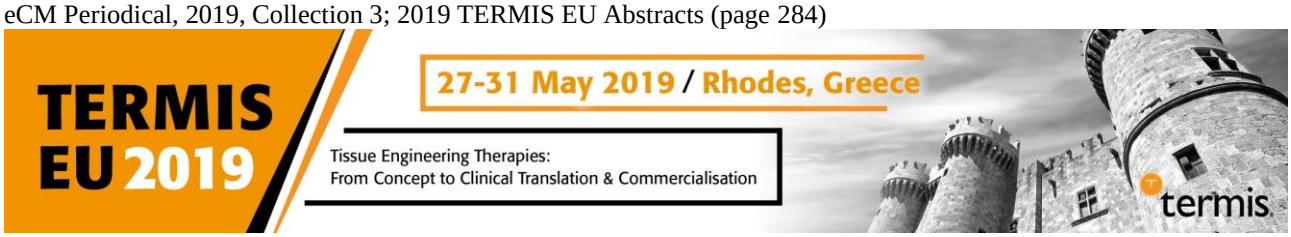
Biomaterials and biofabrication methods for musculoskeletal tissue engineering

W. Swieszkowski
Presenting Author: W. Swieszkowski, wojciech.swieszkowski@pw.edu.pl
Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland

This study shows a biofabrication technology which relies on the simultaneous deposition of cells and biomaterials in a layer-by-layer fashion, to form 3D well-organized heterogeneous structures that can mirror relevant complex biological architectures both physiologically and morphologically. Our 3D biofabrication approach is based on a microfluidic system coupled to a co-axial needle extruder for high-resolution computer-controlled 3D deposition of hydrogel fibers laden with different cells. By formulating tailored hydrogel based bioink and precisely controlling the 3D spatial organization of the extruded hydrogel fibers, the novel 3D bioprinting method has been tested for the fabrication of advanced engineered constructs for the regeneration of musculoskeletal tissues [1,2]. Depending on application, the biomimetic hydrogels were composed of modified biopolymers like gelatin, alginate, hyaluronic acid, or PEG-fibrinogen. The gels were laden with different types of cells including bone marrow-derived human mesenchymal stem cells, muscle precursor cells or chondrocytes. The obtained with high resolution (~ 100 □m), a fiber-based 3D printed living constructs mimic organized musculoskeletal tissues like bone, cartilage, tendon or muscle.

REFERENCES

[1] Costantini M, et al. 3D bioprinting of BM-MSCs-loaded ECM biomimetic hydrogels for in vitro neocartilage formation. Biofabrication, 2016, 8 35002.
[2] Costantini M et al. Microfluidic-enhanced 3D bioprinting of aligned myoblast-laden hydrogels leads to functionally organized myofibers in vitro and in vivo. Biomaterials, 2017, 131 98–110.

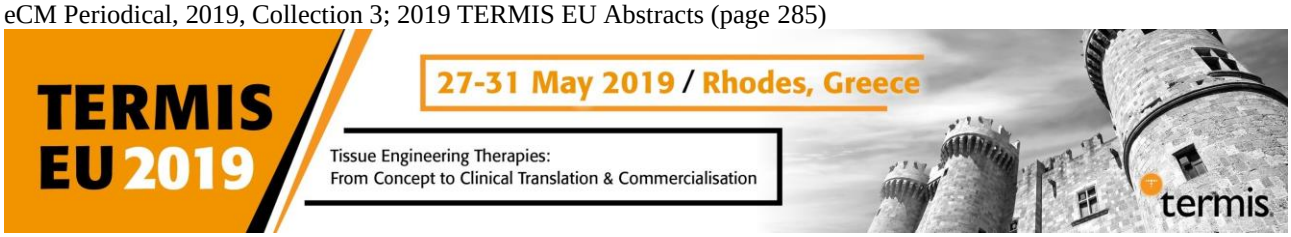


Extracellular vesicles in blood and lymphatic vasculature

W. Holnthoner
Presenting Author: Wolfgang Holnthoner, wolfgang.holnthoner@trauma.lbg.ac.at
Ludwig-Boltzmann-Institute for Experimental and Clinical Traumatology, Austrian Cluster for Tissue Regeneration, Vienna, Austria

Extracellular vesicles (EVs) include exosomes, microparticles, and apoptotic bodies and are phospholipid bilayer-enclosed vesicles that have once been considered as cell debris lacking biological functions. Recently, however, these structures gained tremendous interest, since they are believed to be implicated in a variety of pathological settings. EVs, which are secrected by endothelial cells, have been found to play diverse roles in disease by promoting cardiovascular diseases, but also survival of endothelial cells. One of the main challenges in this field is the isolation and characterization of EVs. Different methods have been developed, yet, standardization of the protocols is still lacking behind. This calls for clinically approved, standardized, and efficient isolation and characterization protocols to harvest and purify endothelial extracellular vesicles. However, such methods and techniques to fulfill stringent requirements for clinical trials have yet to be developed or are not harmonized internationally. The focus of our lab lies in the enrichment and characterization of endothelial EVs. We analyze the obtained fractions by thromboelastometry, nanoparticle tracking analysis and flow cytometry. Our aim is a better understanding of the role of endothelial cell-derived EVs in coagulation, but also their function in cell-cell communication between endothelial and their surrounding stromal cells in tissue engineered vasculature.

ACKNOWLEDGEMENTS: Financial support comes from the Austrian worker's compensation board (AUVA) and the Austrian Research Promotion agency (FFG).



Molecular and functional analysis of MSC-derived extracellular vesicles in the context of osteoarthritis

X.-N. Wang
Presenting Author: Xiao-nong Wang, x.n.wang@newcastle.ac.uk
[1]Institute of Cellular Medicine, Newcastle University, UK

INTRODUCTION: MSC secreted extracellular vesicles (MSC-EVs) showed chondroprotection and osteoarthritis attenuation in animal models. Our previous work observed a distinctive miRNA expression profile in MSC-EVs compared to their parental MSCs. We further investigated the chondrogenesis miRNAs in MSC-EVs and their impact on human osteoarthritic chondrocytes.

METHODS: MSC-EVs were isolated from MSC-conditioned media by differential ultracentrifugation. MSC-EV enclosed chondrogenesis miRNAs were assessed by qRT-PCR. Human articular chondrocytes **(**HACs) were generated from the articular cartilage from patients with osteoarthritis. Following co-culture, the effect of MSC-EVs on HAC migration was examined by scratch assay and chemotaxis assay.

RESULTS & DISCUSSION: MSC-EVs expressed EV signature markers CD63, CD9, CD81, Alix and Flotillin-1 with characteristic particle size range and cup-shaped morphology. MSC-EVs contained well-recognised chondrogenesis microRNAs, with miR-140-5p, miR-142-3p, miR-145-5p, miR-630, miR-29b-3p expression significantly higher and miR-125b expression lower than their parental MSCs. HACs were capable of acquiring MSC-EVs (Figure 1). The HACs exposed to MSC-EVs showed a faster wound closure and high migration capacity compared to those not exposed to MSC-EVs (Figure 1).

CONCLUSIONS: MSC-EVs encapsulate well-recognised chondrogenesis microRNAs. MSC-EVs have functional impact on human osteoarthritic articular chondrocytes. These finding may have implications in developing cell-free therapy for degenerative diseases such as osteoarthritis.

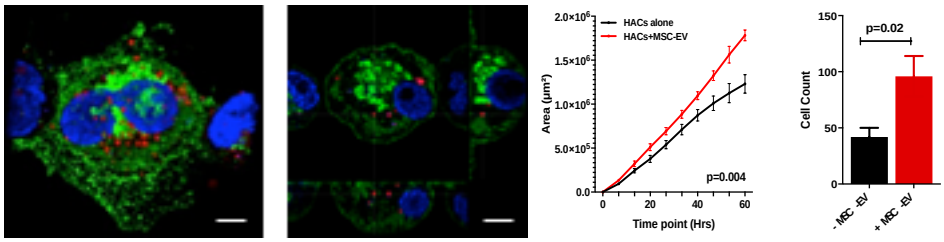
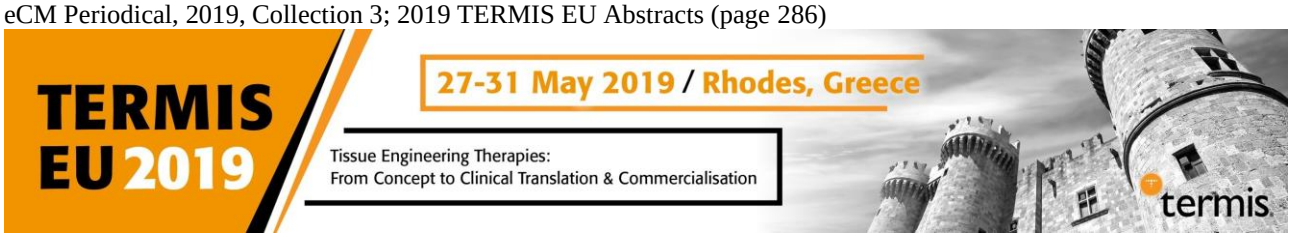


Figure 1: Confocal microscopy images of HACs uptake MSC-EVs. Red=PKH26 labelled MSC-EVs, green =WGA-488 stained cell membrane, blue=DAPI stained cell nucleus. The scale bars indicate 5um. Effect of MSC-EV on HACs wound closure (left, n=12) and transwell migration (right, n=6). Error bars indicate mean +/- SEM.

ACKNOWLEDGEMENTS: This work is funded by Arthritis Research UK TEC (21156), JGWP Foundation, and Newcastle Healthcare Charities.

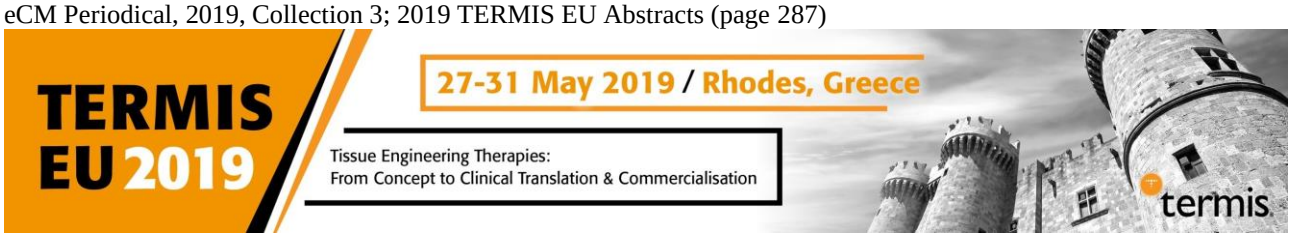


Bone tissue engineering in vitro and in vivo using 3D printed scaffolds

X.B. Yang
Presenting Author: Xue Bin Yang, x.b.yang@leeds.ac.uk
Biomaterials & Tissue Engineering Group, Division of Oral Biology, School of Dentistry, University of Leeds, UK

The ability to generate functional bone tissue is still a major challenge for clinical translation. Although many different types of biomimetic scaffolds are available, however to date, many researchers are still working on the development of 'functional' biomaterials for bone tissue engineering applications. Since bone formation is a dynamic process, it may require different properties of the scaffolds during the entire period of the bone regeneration. This talk will start with an overview of the natural procedure of bone development, fracture healing, as well as bone remodeling during lifetime, which may provide evidence-based guide for the design and manufacture of novel biomaterial scaffolds for functional bone tissue regeneration. It has been general accepted that an ideal scaffold for bone formation should be biocompatible, biodegradable, osteoinductive, osteoconductive, cost effective and supporting angiogenesis, extracellular matrix production and mineralisation. This talk will demonstrate the use of different commonly available 3D scaffolds (such as PLA/PLGA, BioGlass, Glass ceramics, and so on) for bone tissue engineering both in vitro and in vivo, and discuss the advantages and limitations of different scaffolds. Over the last a few years, 3D bioprinting have become one of the most advanced technologies for biomaterials design and fabrications. The 3D printed poly-lactic acid (PLA) scaffolds has great potential to tailor different geometrical designs for improved cellular attachment and growth. In collaboration with Dr Liu at University College London, we have investigated the effect of fibre angle in 3D printed PLA scaffolds on human dental pulp stem cells (hDPSC) attachment, growth and osteogenic differentiation, both in vitro and in vivo. PLA scaffolds containing fibres angled at either 45º or 90º were prepared via 3D printing (UCL). hDPSCs were pre-labeled with Cell Tracker Green prior to be seeded on the 3D printed scaffolds for 4 hours. Fluorescence microscopic images showed that 45º angled PLA scaffolds significantly enhanced the cell attachment compared to that on the 90º angled PLA group ($p<0.0001$). After 3 weeks of ostegenic culture in vitro, SEM confirmed that almost all macro-pores were fully covered with cell sheets in the 45º angled PLA group. In contrast, only a limited amount of cellular bridging was present in the 90º angled PLA group. The majority of cells on the 3D printed scaffolds in both groups are viable with only a few dead cells at weeks 4. After 5 weeks, histology showed marked cellular growth and new collagen matrix formation, with positive immunohistochemical staining to bone specific markers in both groups. In a separated study, a novel histone deacetylases inhibitor (HDACi) - MI192 were used to pre-treat human bone marrow stromal cells (hBMSCs) for two days prior to be cultured as pellets and assembled in a 3D printed PEGT/PBT scaffold to form the Micro-Tissue (In collaboration with Dr Woodfield in Otago University). Then the Micro-Tissues were cultured in osteogenic medium for 6 weeks. Results showed that MI192 pre-treatment extensively enhanced hBMSCs ALP, OCN and Col1α protein expression and extracellular matrix mineralisation within the in Micro-Tissues, which indicate the potential of using MI192 pre-treated cells and 3D printed scaffolds for bone tissue regeneration.

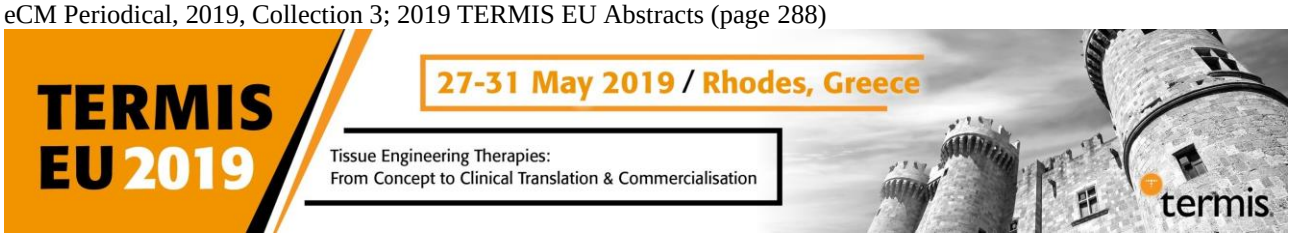
ACKNOWLEDGEMENTS: Rasha Albannaa (funded by Iraq government) and Kenny Man (funded by EPSRC) are PhD students who did the experiments. XY is funded by UKIERI, and EU FP7 ([FP7/2007–2013])No[318553]—SkelGEN.



Regenerative therapy based on in vivo recruitment of cells

Y. Tabata
Presenting Author: Yasuhiko Tabata, yasuhiko@infront.kyoto-u.ac.jp
Laboratory of Biomaterials, Department of Regenerative Sciences and Engineering, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

Regenerative therapy is a medical treatment based on the natural-healing potential of body itself. To realize the regenerative therapy, it is necessary to enhance the cell ability of proliferation and differentiation which contributes to the healing potential. To this end, several biomaterial technologies have been investigated to give cells a local environment for their ability enhancement. If a cell scaffold or a key bio-signalling molecule is supplied to the right place at the right time or concentration by the release technology of biomaterials, the body system initiates to physiologically function, resulting in the natural induction of cell-based tissue regeneration. With the recent scientific development of stem cell biology, various molecules to enhance in vivo cell recruitment have been available for cell-based regenerative therapy. For example, the controlled release of a cell recruitment molecule can enhance the in vivo recruitment of stem cells, followed by the local functional activation of cells recruited by another drug released, resulting in a promoted cell-based tissue regeneration. On the other hand, inflammation is one of the essential host responses to pathologically modify the process of tissue regeneration. Without inflammation, no tissue regeneration takes place. The inflammation process was modified through the local release technology of an anti-inflammatory drug. The positive regulation of inflammation further enhanced the therapeutic efficacy of tissue regeneration induced by the release technology. Tissue regeneration based on the in vivo cell recruitment is a new and promising therapeutic strategy of regenerative medicine.



Development of dynamic culture systems to investigate the topographic influence on the limbal epithelial stem cells' fate

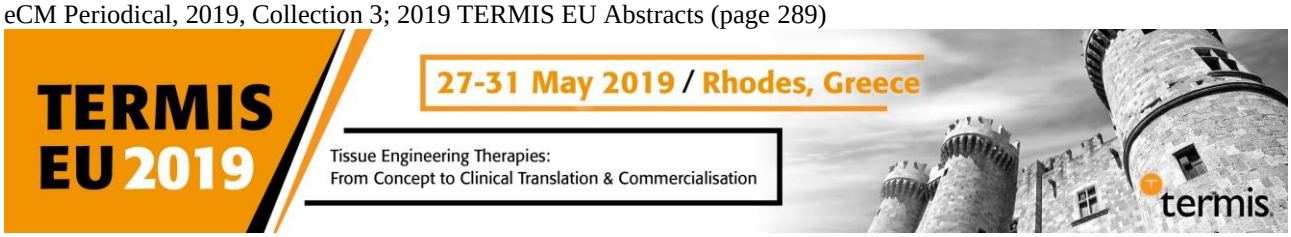
Y. Yang
Presenting Author: Ying Yang, y.yang@keele.ac.uk
Institute for Science & Technology in Medicine, School of Medicine, Keele University, Stoke-on-Trent, UK

The limbus lying between cornea and conjunctiva is an important tissue for the cornea maintenance and repair. The limbal stem cells are found to reside at the basal of Palisades of Vogt (POV). Observing closely at POV, it is understood that limbal stem cells share the common anatomic feature of compartmental locations as other adult epithelial stem cells, such as in skin [1], intestine or airway tract (alveoli). However, the role and types of insoluble molecules, extracellular matrix (ECM) composition and especially topographic features of ECM acting as stem cell niche have not been fully elicited. In this project, two different bioreactors, including suction bioreactor and compression bioreactor, were designed to mimic the limbal stem cell niche of POV in limbus. The suction bioreactor and compression bioreactor can change the cell culture substrate from flat to curved morphology after cells attaching on the substrates. We hypothesized that the curved substrates would change the aggregation and migration state of the limbal stem cells, and the change would be curvature size dependent. Limbal stem cells were extracted from porcine limbus tissues and identified by their unique surface marker, p63 antibody. After 48 hours culture of the corneal limbal stem cells on the bioreactors, several p63 positive cells were aggregated heterogeneously depending on the curvature of the substrate. Furthermore, higher density of p63 positive cells were maintained and aggregated more in stem cell culture media. Thus, the two new bioreactors offer a unique tool to mimic the limbal structure enabling deeper understanding of limbus function for better corneal disease treatment.

ACKNOWLEDGEMENTS: Thanks for the experimental and finance support by Helling AL, Watt FM, El Haj AJ, Wang YX and BBSRC (BB/M007219/1).

REFERENCES

[1] Helling AL et al. Tissue Eng. In press



Adhesive growth factors for tissue engineering

Y. Ito[1,2]
Presenting Author: Yoshihiro Ito, y-ito@riken.jp
[1]Emergent Bioengineering Matter Research Team, RIKEN Center for Emergent Matter Science, Japan [2]Nano Medical Engineering Laboratory, RIKEN Cluster for Pioneering Research, Japan

INTRODUCTION: There is a growing interest in the development of biomaterials involving the immobilization of growth factors, which would allow these artificial materials to regulate specific cellular functions, including the gene expression processes associated with cell growth and differentiation. To design such proteins as a strategy for the surface modification of metals and ceramics, biomimetic approaches inspired by underwater adhesive proteins have been employed with biological materials. One of the proteins is the salivary stathelin protein which is a multifunctional molecule that possesses a high affinity for calcium phosphate minerals such as hydroxyapatite (HA), maintains the appropriate mineral solution dynamics of enamel, promotes selective initial bacterial colonization, and functions as a boundary lubricant on the enamel surface.[1] Another is mussel foot protein which is involved in a sticky pad at the end of threads which stick firmly to rock, or any other hard surface. It is known that the active sites for the adhesion of both proteins consist of post-translationally modified amino acids such as phosphoserine and 3,4-dihydroxyalanine (DOPA). Here two kinds of growth factor epidermal growth factor (EGF) and insulin-like growth factor (IGF) were modified with the motif.

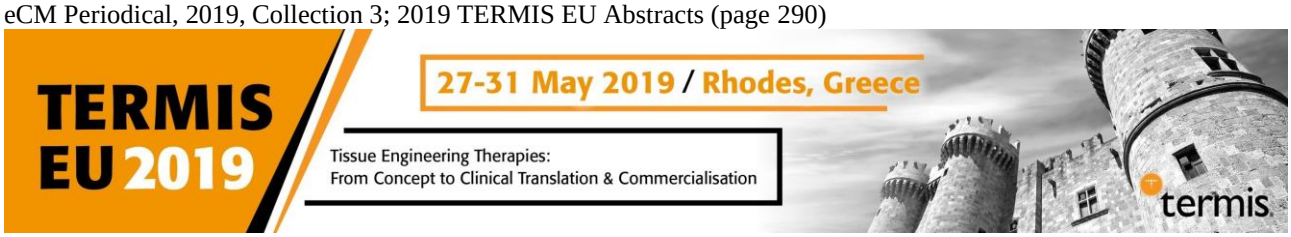
METHODS: To prepare phosphoserine-incorporated EGF, we employed the solid phase synthesis method. The minimum portion of bio-active EGF was conjugated with the short peptide with the binding sequence in statherin. Tyrosinase treatment was employed for incorporation of DOPA into IGF by converting the tyrosine residues to DOPA. In the procedure, a tyrosine-lysine-tyrosine-lysine-tyrosine sequence was added to the C-terminal of IGF using conventional genetic recombinant technology. Subsequently, the tyrosine residues in the product were converted to DOPA residues by tyrosinase.

RESULTS & DISCUSSION: The EGF derivative bound to HA and titanium (Ti), whereas unmodified EGF did not. In addition, smaller amounts of bound EGF had higher effects than soluble EGF. As a conclusion, the bound EGF has higher activity than the soluble form because of its immobilization. The binding affinity of IGF was also significantly higher than that of IGF. Furthermore, the bound IGF did not dissociate even after it had been washed with phosphate buffered saline. The cell growth assay using NIH 3T3 cells also showed that the bound IGF produced a significant enhancement of cell growth, compared with soluble IGF. The relative activities of immobilized or bound growth factor have also been observed for other growth factors. The activity is attributed to high local concentrations of immobilized growth factor, as well as the multivalency and inhibition of down regulation by the bound growth factor.

CONCLUSIONS: We have provided a summary of new adhesive growth factors prepared by site-specific incorporation of non-natural amino acids which play a key role in underwater adhesive proteins. In future, the combination of these approaches will provide new design tools for protein engineering and biomaterials.

REFERENCES

[1] Kang, J. et al., Biomaterials 34:9747, 2013

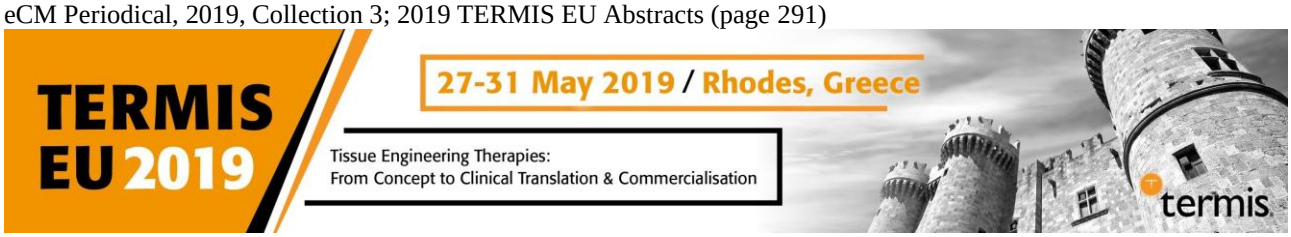


Advanced bioprinting strategies for tissue and tissue model fabrication

Y.S. Zhang[1]
Presenting Author: Y. Shrike Zhang, yszhang@research.bwh.harvard.edu
[1]Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, USA

Bioprinting has recently emerged as an enabling technology in tissue biofabrication at high fidelity. This talk will discuss our recent efforts on developing a series of advanced bioprinting strategies, including sacrificial bioprinting that allows generation of perfusable microchannels embedded in hydrogel microchannels, microfluidic and hollow fiber bioprinting that achieves production of standalone cannular tissues, and multi-material bioprinting based on both extrusion and stereolithograpic modalities that enables creation of complex hierarchical tissue microstructures. Innovations in various cytocompatible and bioactive bioink formulations will also be presented. These platform bioprinting methods have been demonstrated to facilitate faithful fabrication of biomimetic tissues and their models spanning from the heart, liver, and musculoskeletal system to blood vessels and beyond, as well as their diseased forms, for applications in regenerative medicine and in accurate screening of therapeutic agents.

ACKNOWLEDGEMENTS: Financial support was received from the National Institutes of Health (K99CA201603, R21EB025270, R21EB026175).



Design, fabrication and properties of smart thermoresponsive biomaterials

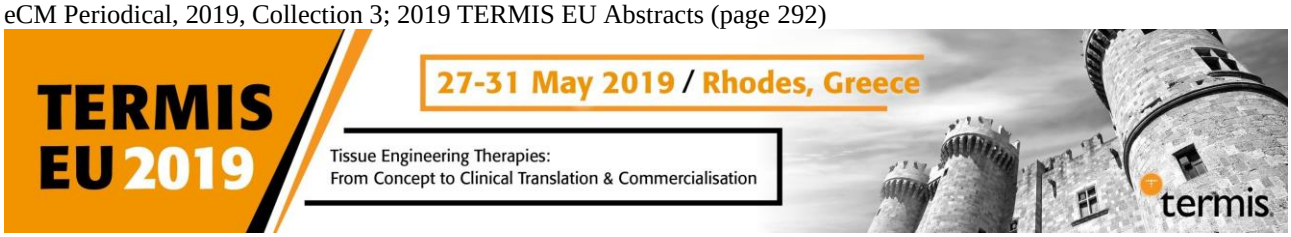
Y. Rochev[1,2]
Presenting Author: Yury Rochev, yury.rochev@nuigalway.ie
[1]National University of Ireland, Galway, Ireland [2]Institute for Regenerative Medicine, Sechenov First Moscow State Medical University, Moscow, Russia

Smart biomaterials are increasingly used in tissue engineering and regenerative medicine. Based on smart biomaterials, three-dimensional scaffolds, cell sheets, theranostic systems, as well as numerous drug delivery systems had been developed. The key factors in the design and development of smart biomaterials and intelligent structures are molecular design and fabrication techniques. Moreover, in recent years, interest in the principles of the fabrication of smart biomaterials has grown particularly. Attention to various methods of fabrication is primarily due to the minimization of products. In addition, it is necessary to obtain hierarchically organized structures at various characteristic lengths from nm to cm. In this report, we consider the concept of design and fabrication of biomaterials on the example of thermoresponsive polymers with low critical solution temperature. Over the last couple of decades a number of innovative approaches to manufacturing themoresponsive films for advanced cell culture applications have been developed and refined. Unlike traditional cell culture detachment methods, detachment from thermoresponsive substrates allows for the detachment of cells without the disruption of cell-to-cell junctions [1-3]. Therefore this approach can facilitate the harvesting of cell sheets, which can be applied for tissue engineering purposes. Thermoresponsive films have been successfully employed to host mammalian cells with a view to tissue reconstruction, such as corneal, endothelial and many others. In this report, we will analyze various methods for obtaining thermosensitive thin and ultrathin films. Among them: grafting, spin coating [1], photo crosslinking [2], physical sorption [3] and others. We will demonstrate, that method of preparation of the themoresponsive materials seems to have a considerable influence on biocompatibility [1]. In conclusion, we consider the relatively new use of the thermosensitive materials for the fabrication of three-dimensional scaffolds. In particular, we give examples of the use of thermosensitive polymers for electrospinning and 3D printing.

ACKNOWLEDGEMENTS: This work was supported by the Russian Science Foundation (grant 15-15-00132) and by the Russian Foundation for Basic Research (grant 17-02-01248).

REFERENCES

[1] Nash M et al. ACS Applied Materials and Interfaces. 2011, 3(6): 1980-1990
[2] Nash M et al. Soft Matter. 2012: 8(14):3889-3899
[3] C Healy D. et al., Macromolecular Bioscience, 2017: 17 (2), 1600175



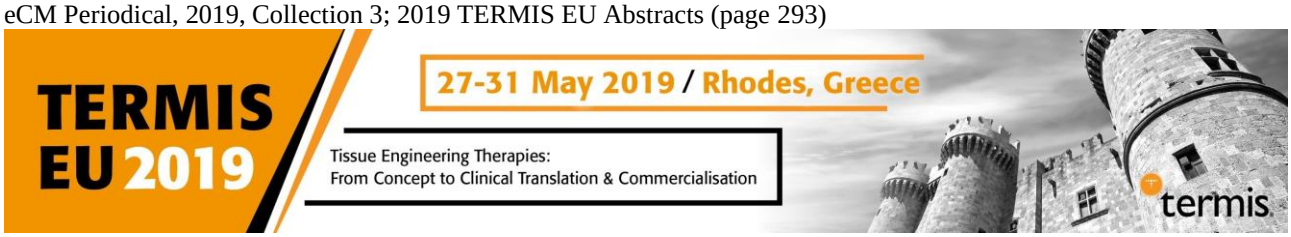
Macrophages as important players in regeneration of osteochondral defects

Y.M. Bastiaansen-Jenniskens[1]
Presenting Author: Yvonne M. Bastiaansen-Jenniskens, y.bastiaansen@erasmusmc.nl
[1]Department of Orthopedics, Erasmus MC, University Medical Center Rotterdam, The Netherlands

Joint injuries may result in cartilage defects and inflammation of the joint. The immune response and inflammatory environment are known to directly influence the repair tissue produced. Macrophages are one of the cell types residing in synovium and intra-articular fat tissues and can acquire a spectrum of phenotypes with the pro-inflammatory or M1 macrophages and anti-inflammatory/repair or M2 macrophages on both ends of this spectrum. Stimuli from the environment can direct the macrophages to these different phenotypes. Cytokines and growth factors are the usual suspects here, but extracellular matrix, immune complexes, bacterial fragments, and implanted biomaterials can also alter the phenotype of macrophages. We have demonstrated that the secretome of synovium or infra-patellar fat pad tissue can influence the cartilage formation by bone marrow derived stromal cells (MSCs). When comparing the reaction of isolated tissue macrophages from osteoarthritic synovium or infra-patellar fat pad with the effect of peripheral blood monocytes that were differentiated to M1 and M2 macrophages in vitro, the effect of macrophages from osteoarthritic joint tissues was mostly comparable to the effect of M1 macrophages [1, 2]. We are investigating the ability to modulate the macrophage phenotype in the tissue, aiming to create an environment that fosters cartilage regeneration. First attempts are made in our lab to modulate macrophages residing in the synovium or infrapatellar fat pad using commonly used drugs. The results of these experiments are encouraging; Triamcinolone is capable of modulating macrophage phenotype in the tissue from pro-inflammatory to anti-inflammatory, whereas rapamycin has the opposite effect. Treatment of synovium and infrapatellar fat pad explants with triamcinolone reduced the negative effects of these tissues on MSC chondrogenesis in vitro. Taken together, targeting macrophages seems a good strategy when aiming for regenerative therapies and possibilities seem to exist to improve this regeneration via the macrophage.

REFERENCES

[1] Fahy N, Osteoarthritis Cartilage 2014; 22: 1167-1175.
[2] Wei W, Eur Cell Mater 2015; 30: 303-314.



Podium

Assessment of the neurovascular secretome of donor-matched adipose and bone marrow derived mesenchymal stem cells for intervertebral disc regenerative therapies

A.L.A. Binch[1], S.M. Richardson[2], J.A. Hoyland[2], F. Barry[1]
Presenting Author: Abbie LA Binch, abbie.binch@nuigalway.ie
[1]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]University of Manchester, Division of Cell Matrix Biology and Regenerative Medicine, Oxford Road, Manchester, England, UK

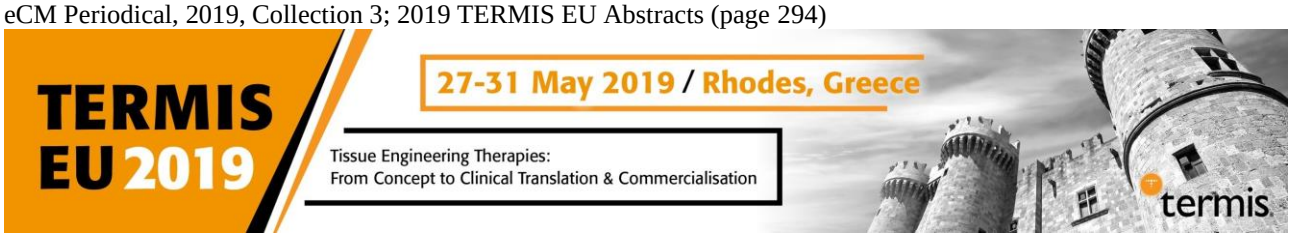
INTRODUCTION: Currently, there is a lack of knowledge surrounding the relationship between naïve mesenchymal stem cells (MSCs) and the low oxygen, pro-inflammatory niche of the degenerate intervertebral disc (IVD). The objective of this study is to identify secreted factors from donor-matched adipose (ASC) and bone-marrow (MSCs) derived stem cells that may potentiate the survival and ingrowth of sensory nerve fibres present within degenerate discs and their regulation by oxygen tensions and inflammatory cytokines.

METHODS: Donor-matched ASCs and MSCs were stimulated with either IL-1β (10ng/ml) or TNFα (10ng/ml) for 48 hours in either 21% or 5% O_2. Quantitative real-time polymerase chain reaction analysis (qRT-PCR) was performed to assess expression of trophic factors involved in the survival of nerves (NGF, BDNF & NT3), blood vessels (VEGF, FGF-2, Ang-1 & Ang-2) as well as expression of pain associated neuropeptides (Substance P & CGRP) from ASCs and MSCs. Conditioned media was collected and secreted proteins were analysed using enzyme linked immunosorbent assays and multiplex human magnetic Luminex® assay.

RESULTS & DISCUSSION: Here, we report constitutive expression and secretion of neurotrophic and angiogenic factors from donor-matched ASCs and MSCs, demonstrating that hypoxic stimulation of ASCs triggers increased secretion of NGF, NT-3, Ang-1, Ang-2 FGF-2 and VEGF-A compared to MSCs. We demonstrate transcriptional and translational upregulation of NGF, Ang-1 and FGF-2 in response to cytokines in ASCs under 21% and 5% oxygen (Figure 1).

CONCLUSIONS: To the best of our knowledge this is the first report addressing the response of human donor-matched ASCs and MSCs to a hypoxic and inflammatory environment in terms of assessing their neurovascular profiles. This work demonstrates the importance of cell-selection for tissue specific regeneration to reduce ectopic sensory nerve growth and improve patient outcomes.

ACKNOWLEDGEMENTS: Financial support provided by EU Horizon 2020 RESPINE grant (Project ID# 732163).



Single fiber surface topography regulates cell mechanotransduction

A. Omidinia-Anarkoli[1], R. Rimal[1], Y. Chandorkar[1], D. Gehlen[1], J.C. Rose[1], K. Rahimi[1], T. Haraszti[1], L. De Laporte[1,2]
Presenting Author: Abdolrahman Omidinia-Anarkoli, omidinia@dwi.rwth-aachen.de
[1]DWI-Leibniz Institute for Interactive Materials, Aachen, 52074, Germany [2]ITMC-Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Aachen, 52074, Germany

INTRODUCTION: The mechanical inputs form the surrounding microenvironment are interpreted by cells using mechanotransduction pathways. Fibers have been employed to mimic the extracellular matrix (ECM) to regulate cellular responses. [1]

METHODS: Fabrication of multi-layered 3D structured fibers with precise diameter, inter-fiber distance and angle, and specific surface topographies is demonstrated by using solvent assisted spinning (SAS). The fiber surface topography is altered by applying solvents with different solubility and volatility. The surface topography, hydrophobicity, crystallinity and stiffness of fibers are evaluated. To investigate the their mechanotransduction behavior, cells are stained for F-actin, vinculin, and two different mechanosensitive reporter proteins, Yes-associated protein (YAP) and myocardin related transcription factor A (MRTFA).

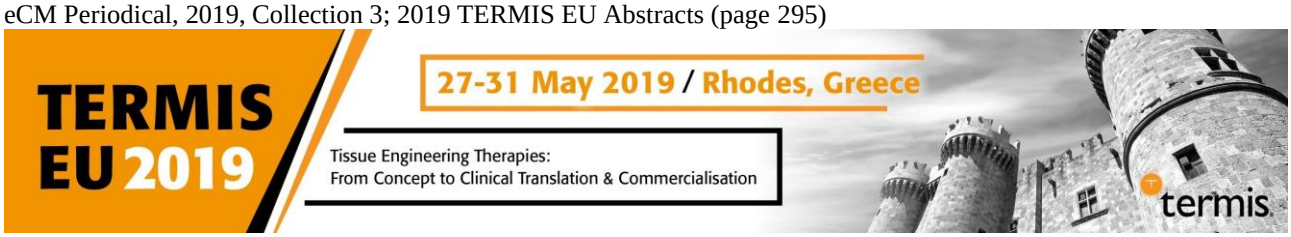
RESULTS & DISCUSSION: Depending on the solvent system, thermal or non-solvent induced phase separation results in microfibers with smooth, grooved, or porous fibers. Due to their hierarchical structures, the porous fibers are the most hydrophobic, followed by the grooved and smooth fibers, respectively. Increasing the polymer concentration or changing the nozzle size leads to a range of fiber diameters from ~2 to 10 µm. Interestingly, SAS fibers demonstrate higher degrees of crystallinity compared to polymer pellets, while AFM measurements suggest a stiffness in the MPa range without significant differences between the surface topographies. The average ratio of YAP fluorescence intensity ($F_{nucleus}/F_{cytoplasm}$) is ~ 1.91, whereas in case of smooth fibers, this ratio increases when cells are grown on porous (~ 2.91) or grooved (~ 3.37) fibers.

CONCLUSIONS: This report shows for the first time that, unlike for stiff 2D substrates (e.g. TCPS) where only stiffness matters, YAP shuttling can be influenced by the topography of substrates in case of 2.5D single microfibers. As cells undergo a forced apical/basal polarity on 2D substrates but wrap around the fibers in a more 3D manner, the internal stresses of the F-actin filaments will change, which may lead to alterations in the mechanotransduction pathways. As the composition, stiffness, and crystallinity of fibers are similar between the fibers with different surface topographies, cells seem to sense nanotopographies and spread accordingly, independent of the other parameters. SAS, therefore, provides a unique platform to study the interaction between cells and single ECM-like fibers in a precise and reproducible manner, which is of great importance for new material developments in the field of tissue engineering.

ACKNOWLEDGEMENTS: We thank the Max- Buchner Forschungsstiftung of Dechema and the European Research Council (ERC) (ANISOGEL, grant agreement No 637853) for their support.

REFERENCES

[1] A. Omidinia-Anarkoli et al. Small; 13, 2017: 1702207



Cell synchronization and a novel kidney extracellular matrix based hydrogel promote efficient derivation of renal vesicles from human pluripotent stem cells

A. Harichandan[1] S.B. Garcia[1], L. Moroni[1], C.D. Mota[1].
Presenting Author: Abhishek Harichandan, a.harichandan@maastrichtuniversity.nl
[1]Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht, The Netherlands

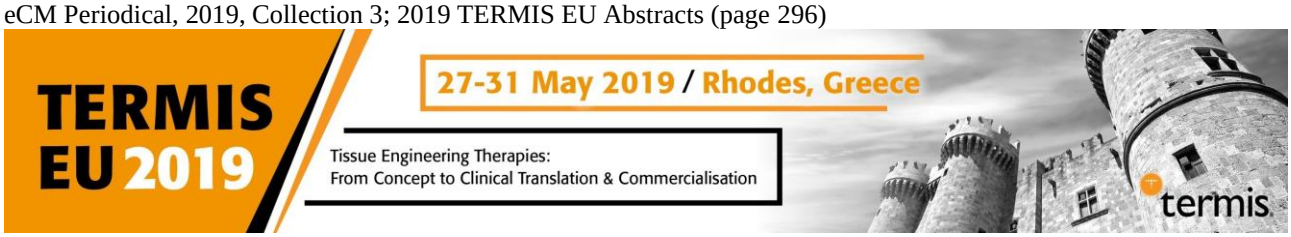
INTRODUCTION: Efficient initiation of differentiation of human pluripotent stem cells (hPSCs) is dependent on the responsiveness of stem cells to external induction cues, which is a prerequisite for deriving a homogeneous population of nephron progenitor cells (NPCs) and ureteric bud cells (UB). In this study, we improved protocols for driving efficient differentiation of hPSCs into NPCs and developed an extracellular matrix (ECM) based hydrogel for further improving the differentiation of NPCs into renal vesicles.

METHODS: We induced reversible arrest of hPSCs in G1 phase of cell cycle validated by the expression of Retinoblastoma protein-1(RB1). We compared the differentiation ability of synchronized and non-synchronized hPSCs into NPCs, and into renal vesicles. The NPCs were characterized by QPCR, immunocytochemistry, and flow cytometry. We further developed a sacrificial kidney ECM (K-ECM) based hydrogel and evaluated the morphology and architecture of the protein fibers comprising the hydrogel for long-term stability of these hydrogels in vitro.

RESULTS & DISCUSSION: RB1 expression was visualized to be homogenously over-expressed by ^{S}hPSC compared to ^{UNS}hPCS. The efficiency of derivation of NPCs and UB in ^{S}hPSC was improved (93-99%) respectively, compared to ^{UNS}hPCS (73%), which also translated to efficient renal vesicles output. The NPCs were characterized by the co-expression of SIX2, SALL1, CITED1, PAX2 and N-Cad. The UB were characterized by the co-expression of CXCR4 and CD117. K-ECM derived hydrogels not only supported the maintenance of NPCs, but also the formation of renal vesicles from NPCs upon induction with exogenous Activin-A and FGF-9, as characterized by flurochrome conjugated LTL.

CONCLUSIONS: Collectively, these data clearly illustrate the beneficial effect of cell synchronization and ECM driven efficient differentiation of hPSCs. This strategy holds the potential to provide a platform to get sufficient and homogenous number of NPCs and renal vesicles for kidney regeneration, drug screening and in vitro disease modeling of kidney disorders.

ACKNOWLEDGEMENTS: This project research was made possible by the Dutch Province of Limburg. Financial support was received from Brightlands Materials center.



Green synthesis of a synergetic structure of tellurium nanowires and metallic nanoparticles for biomedical applications

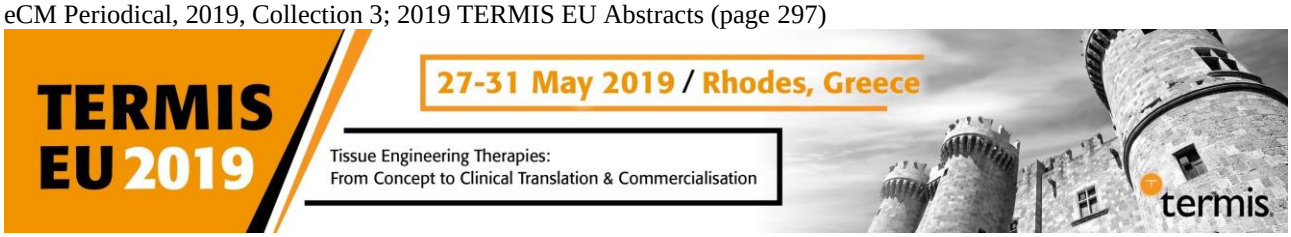
A. Vernet Crua[1]
Presenting Author: Ada Vernet Crua, vernetcrua.a@husky.neu.edu
[1]Department of Chemical Engineering, Northeastern University, Boston, MA 02115, USA

INTRODUCTION: Health care system is facing significant concerns nowadays such as antimicrobial resistance and cancer. New approaches should be considered, and nanotechnology has been found as a powerful solution to them. Current synthetic methodologies for production of nanoparticles, based on physicochemical standards are known to be easy-to-get straightforward. Nevertheless, there is a cost associated with the limitations that should be overcome from these approaches, such as the production of toxic by-products or the lack of biocompatibility of the products. Therefore, new methods are needed, and green chemistry offers itself as a suitable answer.

METHODS: In this research, tellurium nanowires were synthesized using a green synthesis methodology (TeNWs). Then, they were used as a template for the synthesis of metallic nanoparticles in an easy and straightforward method with no need of reducing agent that was completed within 1 minute of reaction. Nanoparticles were characterized, and the synthetic process was compared with the ones described in literature, with the aim to compare the methods in terms of chemical needed, reaction conditions and economic implications. Other structures containing both metallic nanoparticles and nanowires, known as synergy, were also obtained in the process of synthesis. Besides, biocompatibility and anticancer tests of both structures – the synergy and the nanowires - with human tissue were accomplished, growing human dermal fibroblast (HDF) cells and melanoma cells in media in the presence of both nanosystems. After an incubation time of 5 days, the cell growth was analyzed using MTS assay. Furthermore, antibacterial properties were tested.

RESULTS & DISCUSSION: The experiments showed that the use of green nanostructures enhanced the growth of the cells in comparison with the control. Furthermore, anticancer and antimicrobial studies showed an improved performance of the synergetic structure compared to the bare nanowires structures, causing a higher depletion of cell viability.

CONCLUSIONS: Thus, here green-synthetized nanostructures were demonstrated to overcome traditional chemistry approaches. In addition, they can be considered a suitable tool for the controllable and quick growth of metallic nanoparticles. Moreover, green synergetic nanostructures have been proved to present enhanced biocompatibility, anticancer and antibacterial properties than previous synthetized TeNWs.



Evaluation of tendon and skin fibroblasts for scaffold-free tendon tissue engineering using macromolecular crowding technology

A. Djalali-Cuevas[1, 2, 3], I. Skoufos[1], A. Tzora[1], N. Prassinos[2], N. Diakakis[2], D.I. Zeugolis[3, 4]
Presenting Author: Adrian Djalali-Cuevas, adjalali@vet.auth.gr
[1]Bioprocessing and Biotechnology Laboratory, University of Ioannina, Arta, Greece [2]School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece [3]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), NUI Galway, Galway, Ireland [4]Centre for Research in Medical Devices (CÚRAM), NUI Galway, Galway, Ireland

INTRODUCTION: Human tendon injuries exert healthcare expenditures exceeding €145 billion per year. Current repair procedures usually rely on tendon tissue grafts, which have a limited regenerative efficacy. Tissue equivalents produced in vitro by scaffold-free tissue engineering (SFTE) show promise for tissue regeneration but the long culture times that are necessary for their production hinder their clinical translation [1]. Macromolecular crowding (MMC) is a biophysical phenomenon that accelerates in vitro matrix deposition by several orders of magnitude [2], decreasing the production times of tissue equivalents in vitro. Both tendon (TFs) and dermal fibroblasts (DFs) have shown good performance in tendon regenerative settings and show promise for tendon SFTE. Herein, we sought to compare the performance of TFs and DFs in tendon SFTE by using MMC technology.

METHODS: Human TFs and DFs (25,000 cells/cm^2) were treated with MMC (10 μg/ml) and 100 μM L-ascorbic acid phosphate 24h after their seeding. Medium without MMC was used as control. Media were changed every 3 days and read-outs were performed 4, 7, and 10 days after plating. Cell morphology, proliferation, viability and metabolic activity were assessed. Collagen deposition and matrix metalloproteinases (MMPs) production were analyzed by SDS-PAGE and gelatin zymogrpahy, respectively. Different tendon markers were analyzed at the gene and protein levels by means of RT-qPCR and immunofluorescence. Additionally, the proteomic profiles of TCs and DFs treated with MMC were elucidated by means of nLC-MS/MS analysis.

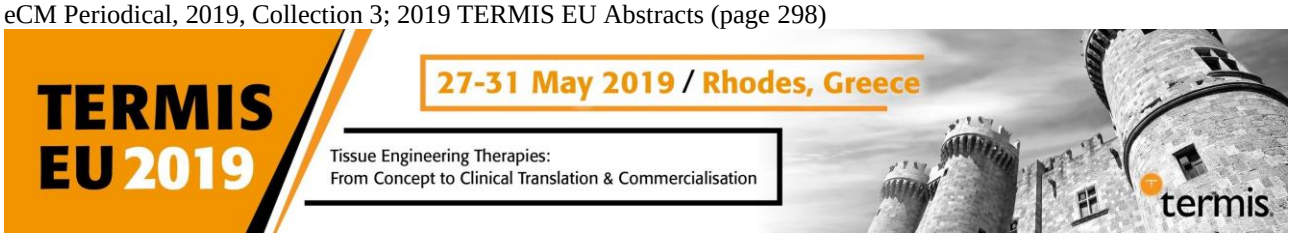
RESULTS & DISCUSSION: Cell morphology, proliferation and viability were not affected by MMC, whilst metabolic activity was slightly decreased in the presence of MMC. Collagen deposition was increased by MMC in TCs and DFs in a very similar fashion. MMPs secretion profile of TCs and DFs showed great similarity and was not affected by MMC. Also, great similarities in the expression of tendon markers by TCs and DFs were found by immunofluorescence and RT-qPCR. nLC-MS/MS provided in great detail the global protein expression profile of TCs and DFs treated with MMC.

CONCLUSIONS: TCs and DFs showed great phenotypic similarity under our experimental conditions. Collectively, these results show that DFs may be a promising alternative to TCs for tendon SFTE.

ACKNOWLEDGEMENTS: Science Foundation Ireland, (13/RC/2073) and H2020, Marie Skłodowska-Curie Actions, Innovative Training Networks, Tendon Therapy Train (676338).

REFERENCES

[1] Peck M et al. Mater Today. 2011; 5:218-24.
[2] Satyam A et al. Adv Mater. 2014; 26(19):3024-34.



Design of a microphysiological system for angiogenesis assays to optimize the design of biomaterials

A. López[1], O. Castaño[1,2,3], E. Engel López[1,2,4]
Presenting author: Adrián López Canosa, alopezc@ibecbarcelona.eu
[1]Biomaterials for Regenerative Therapies, Institute for Bioengineering of Catalonia, The Barcelona Institute of Science and Technology, Barcelona, Spain [2]CIBER in Bioengineering, Biomaterials and Nanomedicine, Madrid, Spain [3]Department of Electronics and Biomedical Engineering, University of Barcelona, Barcelona, Spain [4]Department of Materials Science and Metallurgical Engineering, Polytechnic University of Catalonia, Barcelona, Spain

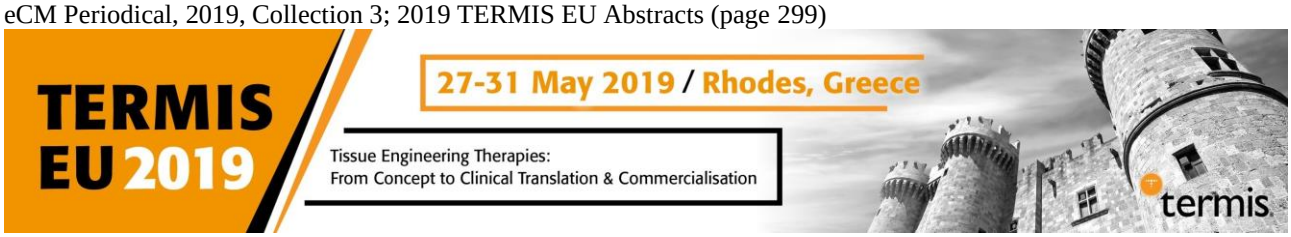
INTRODUCTION: One of the biggest challenges in the field of tissue engineering is how to achieve a functional integration of the implanted constructs with the host vasculature. This process demands the design of a new generation of smart biomaterials capable of stimulating angiogenic processes by releasing chemoattractant agents. Current methods to evaluate this process involve either the use of in vitro models that poorly represent the actual human physiology or in vivo assays which have many limitations such as their high cost or genetic interspecies differences. The goal of this work is to design a microfluidic 3D cell culture device that can better mimic human physiology to optimize the design of such biomaterials.

METHODS: Two designs of the microfluidic platform were created using CAD software. In both cases there is a main cell culture chamber flanked by two media channels. In the first design, these features are connected by an array of microchannels (2x2 μm^2), while in the second design[3], both features are in direct contact and microposts are used to confine the cells in the main chamber. After the master was obtained, PDMS replicas were created by standard soft-lithography. A finite element analysis of the device was performed to model mass transport and study the generation of biochemical gradients. Experimental validation of the transport model was performed using a fluorescent dye. Finally, the possibility to create an endothelial tissue inside the device was tested using HUVEC cells embedded in a fibrin gel. Viability and morphology were assessed by fixing the cells after 3 days of culture and performing an immunostaining with DAPI and phalloidin.

RESULTS & DISCUSSION: both microfluidic platforms were successfully fabricated, and a gradient could also be generated in both devices, being the slope for microchannel design much steeper (-30 mM/ml) than in the micropost design (-5.8 mM/ml). The HUVEC culture in 3D was also viable, while the cells didn't form an interconnected network.

CONCLUSIONS: Collectively, these data illustrate that the design based on the micropost architecture outperforms the microchannel one, as it allows to obtain a wider range of concentrations relevant to study cell migration. It was also possible to create an endothelial tissue inside the devices using HUVEC cells embedded in a fibrin gel. Future work will focus on further optimizing this platform by developing a synthetic biomaterial to support controlled endothelial network formation and apply it to test different biomaterials capable of releasing chemoattractant agents such as growth factors, ions, etc.

ACKNOWLEDGEMENTS: This work was funded by the Spanish Ministry of Economy and Competitiveness (MINECO) through the project MAT2015-68906-R and the Spanish Ministry of Education, Culture and Sports with the FPU grant (FPU17/06161).



Magnetic actuated materials modulate inflammatory cues underlying human tendinopathy

A. Vinhas[1,2], A.I. Gonçalves[1,2], M.T. Rodrigues[1,2,3], R.L. Reis[1,2,3], M.E. Gomes[1,2,3]
Presenting Author: Adriana Vinhas, adrianavinhas@i3bs.uminho.pt
[1]3B's Research Group, I3Bs–Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Tendinopathies represent half of all musculoskeletal injuries worldwide. Inflammation contributes to both tendon healing and to tendinopathy but the mechanisms leading to pathology are unknown. In previous studies, we showed that pulsed electromagnetic field (PEMF) actuation modulates tendon cell behaviour in inflammatory environments, and that PEMF actuated membranes could influence inflammation responses in a rat ectopic model [1]. Herein, we propose to investigate the synergistic action of PEMF and magnetic responsive membranes in i) tendon cells treated with IL-1β to mimic a tendinopathy environment and ii) the phenotype of macrophages cultured on the membranes.

METHODS: Human tendon cells (hTDCs) were seeded (5000 cells/cm^2) on membranes of starch and polycaprolactone impregnated with iron oxide nanoparticles before treatment with IL-1β. Afterwards, hTDCs were exposed for 1h to a 5Hz 4mT PEMF with a 50% duty cycle. The phenotype of human macrophages on PEMF actuated membranes was assessed to confirm their immunomodulatory potential.

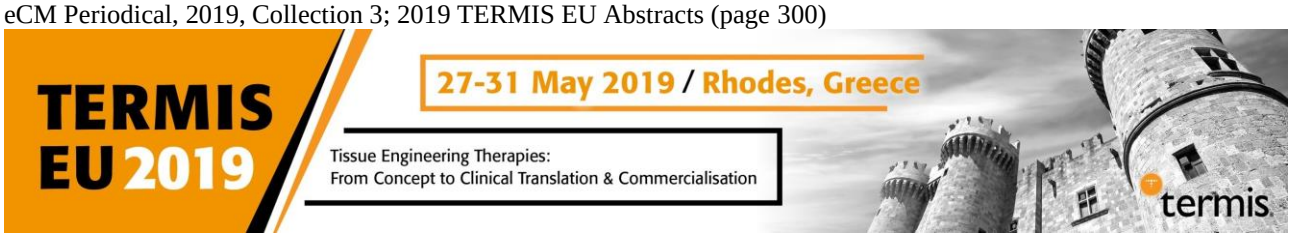
RESULTS & DISCUSSION: The expression of pro-inflammatory (TNFα, IL-6, IL-8, COX-2) and ECM remodeling (MMP-1,-2,-3 and TIMP-1) genes tend to decrease in PEMF actuated membranes, while anti-inflammatory gene expression (IL-4, IL-10) increases. Also, CD16^{++} and CD206^{+} were only detected in macrophages cultured on PEMF actuated membranes.

CONCLUSIONS: PEMF actuated membranes show a modulatory effect on the inflammatory profile of hTDCs. The expression of anti-inflammatory/repair markers in macrophages highlights the immune modulatory potential of PEMF actuated membranes envisioning tendon healing strategies.

ACKNOWLEDGEMENTS: FCT (PD/BD/ 128089/2016), Doctoral Program TERM (PD/59/ 2013), NORTE-01-0145-FEDER-000021, H2020 under the TEAMING Grant agreement No. 739572- The Discoveries CTR. Thank to Hospital da Prelada (Porto, Portugal).

REFERENCES

[1] Santos LJ et al. Nanomedicine. 2016; 11(9): 1107-1122



Development of a biomimetic, functional and vascularized peripheral nerve model

A. Malheiro, P. Wieringa, L. Moroni[1]
Presenting Author: Afonso Malheiro, a.malheiro@maastrichtuniversity.nl
[1]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands

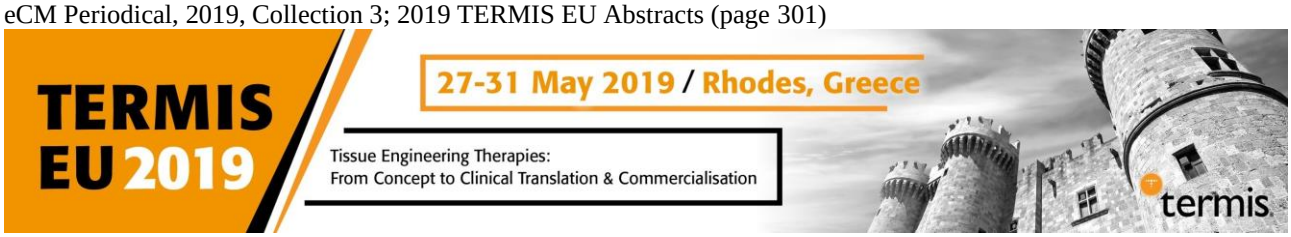
INTRODUCTION: In vitro peripheral nerve (PN) models remain underdeveloped. To overcome this, we have devised a simple and inexpensive method to fabricate a 3D biomimetic and functional peripheral nerve model. Using this model, we have shown its application for drug testing as well the ability to incorporate other target tissues, such as vasculature.

METHODS: We devised a co-culture platform composed of an aligned electrospun scaffold, containing primary Schwann cells (SCs) and PC12 cells, and a fibrin hydrogel. We carried the co-culture for 7, 21 and 28 days and at every timepoint evaluated the effect of PC12 priming and fibrin hydrogel addition, by measuring neurite network formation and neurite myelination. Through calcium imaging we assessed cell functionality. To show drug response, we applied suramin (100µM), in the presence or absence of the inhibitor Nimodipine (300µM). The following day we evaluated cell viability, apoptosis and tissue morphology. To build the neurovascular construct, we first co-cultured SCs and PC12 for at least 7 days, then added a fibrin hydrogel containing endothelial cells (hMVECs) and human fibroblasts, and carried this culture for at least 10 days longer.

RESULTS & DISCUSSION: Within 7 days of culture on aligned nanofibrous scaffolds, the SCs developed Büngner Bands-like structures, and produced robust amounts of nerve ECM proteins, such as laminin, fibronectin and collagen IV. 7 days after adding PC12, these developed aligned neurites that were longer and more numerous, when SCs were present (compared to no SCs control). Priming PC12 before seeding also improve neurite length and density in all conditions compared to non-primed cells. Fibrin gel addition led to neurite length reduction but also to an increase in the network volume. For longer co-cultures of 21 and 28 days, neurites became myelinated with evidence of nodes of Ranvier formation. Myelination area was significantly increased in conditions with primed PC12 compared to the non-primed counterparts. This area remained constant from day 21 to day 28, but the thickness increased overtime. When fibrin gel was added, 3D volumetric myelin bands were observed and the myelin thickness was slightly increase compared to bare scaffold conditions. Drug test studies revealed that PC12 neurites are sensitive to suramin (at 100µM, 24h treatment), evidencing a decrease in cell viability and neurite length/density and increase in apoptotic activity (compared to untreated samples). When simultaneously adding nimodipine (300µM), there was a partial rescue, denoted by an increased viability and neurite length/density compared suramin only conditions. Finally the neurovascular constructs showed the successful formation of a mature vessel network (vWF^+, Ve-$cadherin^+$) intertwined with the neurite network. Moreover, it was possible to observe neurovascular patterning, with neurites following the vessel direction

CONCLUSIONS: We successfully developed a 3D PN model that is biomimetic, functional and allows for drug testing. Moreover, addition of target tissues is also possible, as shown with addition of vasculature.

ACKNOWLEDGEMENTS: Financial support was provided from the Province of Limburg, Netherlands.



Development of a new vascular substitute produced by weaving threads of human amniotic membrane: Preliminary study

A. Grémare[1,2,3], S. Jean-Gilles[4], P. Musqui[1,3], L. Magnan[1,2], Y. Torres[1,2], M. Fénelon[1,2,3], S. Brun[1,3], J.-C. Fricain[1,2,3], N. L'Heureux[1,3]
Presenting Author: Agathe Grémare, agathegremare@hotmail.fr
[1]Université de Bordeaux, Bordeaux, France [2]U1026 BioTis INSERM, Bordeaux, France [3]CHU, Bordeaux, France [4]Université de Cergy-Pontoise, Cergy Pontoise, France

INTRODUCTION: Synthetic vascular grafts cause inflammation that leads to thrombosis and intimal hyperplasia. To avoid these complications, we produced a completely biological graft by weaving threads of human extracellular matrix (ECM) [1]. Currently, threads are obtained from sheets of ECM synthesized by human skin fibroblasts cultured in vitro [1]. Here, we propose the use of human amniotic membrane (HAM) sheets, which are readily available in large quantities and at minimal cost. Moreover, the HAM has low immunogenicity and proven clinical safety [2]. In a first step towards the production of vascular grafts, the objectives of this study were to 1) perform a HAM mechanical cartography to better understand the properties of this material and, 2) to produce and characterize HAM threads.

METHODS: Fetal membranes were collected after a cesarean delivery at term. HAMs were sampled using a standardized pattern in 2 areas (placental and peripheral) and 2 directions (radial and circumferential). Tensile tests were then performed at 1% length/s to record force (Fmax) and strain (Smax). T-test or one way ANOVA were considered statistically significant when $p<0.05$. HAM was cut in ribbons with a custom cutting device and twisted at different levels to produce threads.

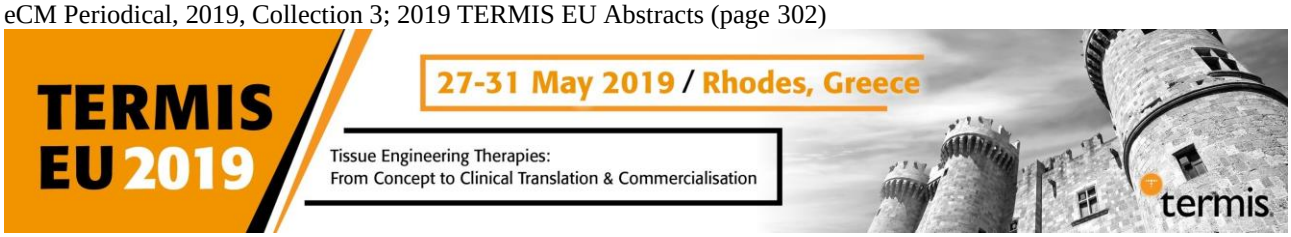
RESULTS & DISCUSSION: The 8 HAMs studied had similar mechanical properties. On average, 103 ± 10 samples were analyzed per membrane. Intra-tissue variability highlighted the fact that placental and peripheral areas could be considered as mechanically distinct. Overall, placental HAM was significantly stronger by 82 ± 45 % and more stretchable by 19 ± 6 % than its peripheral counterparts. Our results also demonstrated that placental, but not peripheral, HAM presented isotropic mechanical properties. Cryo scanning electron microscopy showed that threads surface was very smooth and transmission electron microscopy showed that collagen fiber compaction increased with twisting resulting in a decrease of thread diameter and changes in mechanical properties.

CONCLUSIONS: To our knowledge, this cartography is the most detailed analysis of HAM mechanical properties. This data is relevant to many fields since HAM is used in clinical applications and in many tissue engineering projects. Our next step will be to use HAM threads for the development of a new woven vascular substitute.

ACKNOWLEDGEMENTS: Financial support was received from the "Initiative d'excellence Université de Bordeaux". Technical Support was received from Patrick Guitton.

REFERENCES

[1] Peck M et al. J Vasc Acces. 2011;12(3):185-92
[2] Dobreva M et al. Int J Dev Biol. 2010;54(5):761-77



A vascularised tumouroid model for studying human glioblastoma invasion

A. Stavropoulou-Tatla, A.W. Justin, A.E. Markaki
Presenting Author: Agavi Stavropoulou-Tatla, as2307@cam.ac.uk
Department of Engineering, University of Cambridge, Trumpington Street, Cambridge CB2 1PZ, UK

INTRODUCTION: Glioblastoma multiforme (GBM) is the most malignant form of central nervous system. The highly lethal nature of this tumour mainly originates from its exceptionally infiltrative and dispersive nature that contributes to tumour angiogenesis and metastasis. 3D models of cancer are increasingly utilized to provide insight into how the tumour microenviroment regulates its behavior. We engineered biomimetic vascularised tumouroids to provide insight into how microenviromental cues of the tumour stroma, such as ECM density and hypoxia, regulate GBM neovascularisation and invasion along blood vessels.

METHODS: Patient-derived GBM cells and Human Umbilical Vein Endothelial Cells (HUVECs) were used to construct spheroids by the hanging drop method. These spheroids were then embedded in fibrin gels, with concentrations of 2.5, 5 or 7.5 mg mL^{-1}, containing Human Dermal Fibroblasts (HDFs). The experiments were carried out under hypoxic (1% O_2) or normoxic (20% O_2) conditions. Time-lapse images were taken using inverted fluorescent microscopy and then deconvolved using a Zeiss module. HUVECs vessel area and length were analysed using ImageJ Angiotool Software. GBM co-localization along blood vessels was quantified using the Pearson's correlation coefficient.

RESULTS & DISCUSSION: This tri-culture method triggered robust HUVECs angiogenesis creating lumenised capillaries that sprouted radially outwards into the surrounding fibrin gel. Low oxygen concentration increased GBM invasion but significantly decreased the development of endothelial networks as measured by vessel area and length. Interestingly, high fibrin gel concentration led to an increase in the percentage of GBM cells that invade along the blood vessels (see Figure 1).

CONCLUSIONS: The results demonstrate that the tissue-engineered GBM model can be used as a novel platform to study GBM invasion along blood vessels in vitro. We observe biomimetic GBM invasion on the basal surface of the blood vessels, which is reported to occur in vivo.

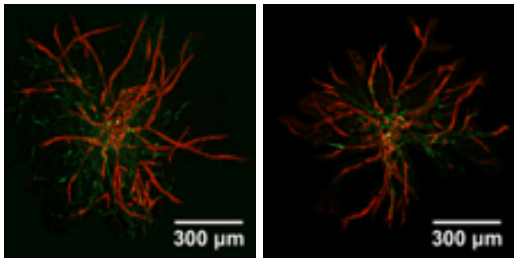
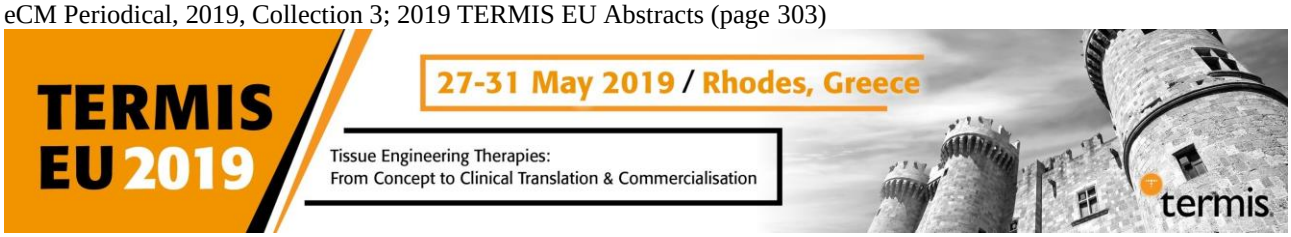


Figure 1: Typical fluorescent images of the vascularised tumoroid model taken at day 3. HUVECs (red) and GBM cells (green) are embedded in 2.5 mg mL^{-1} (left) and 7.5 mg mL^{-1} (right) fibrin gels containing HDFs under normoxia.

ACKNOWLEDGEMENTS: Financial support for AS-T has been provided via the WD Armstrong Trust Fund. AWJ acknowledges funding from EPSRC and the Isaac Newton Trust. GBM tissue samples were kindly provided by Prof. Colin Watts, Dr Rasha Rezk of Cambridge University, UK



Constructing a dynamic three dimensional in vitro model for investigation of ovarian carcinoma progression mechanisms

A. S. Baskin[1,2], R. Reich[1], B. Davidson[2], T. Tavor Re'em[3]
Presenting Author: Aharon Shmuel Baskin, aharonshmuelba@jce.ac.il
[1]Institute of Drug Research, School of Pharmacy, The Hebrew University of Jerusalem, Israel [2]The Medical Faculty, University of Oslo, Oslo, Norway [3]Department of Pharmaceutical Engineering, Azrieli College of Engineering Jerusalem, Israel

INTRODUCTION: Ovarian carcinoma (OC) presents a significant challenge in designing effective drug treatment and in understanding cellular processes during disease progression, especially during the transition from a solid form of the tumor to a detached spheroid form, in effusion. Emerging evidence shows the various functions of sphingolipids, and specifically, Sphingolipid-1-phosphate (S1P) as regulators of cancer progression [1]. Herein, we ventured to construct a 3D OC in vitro culture system using alginate macroporous-based scaffolds and dynamic perfusion bioreactor [2] to mimic the 3D tumor microenvironment.

METHODS: The basal mRNA levels of S1P receptors' expression and relative proportion were established by RT-PCR analyzing of over 250 samples from ovarian cancer patients. 433 OC cells were cultured in 4 different culture forms for X days: (a) monolayer (b) seeded into alginate porous scaffolds, under static conditions, (c) alginate scaffolds in perfusion bioreactor, (D) cell spheroids. RT-PCR analysis was conducted after 3 days' culture.

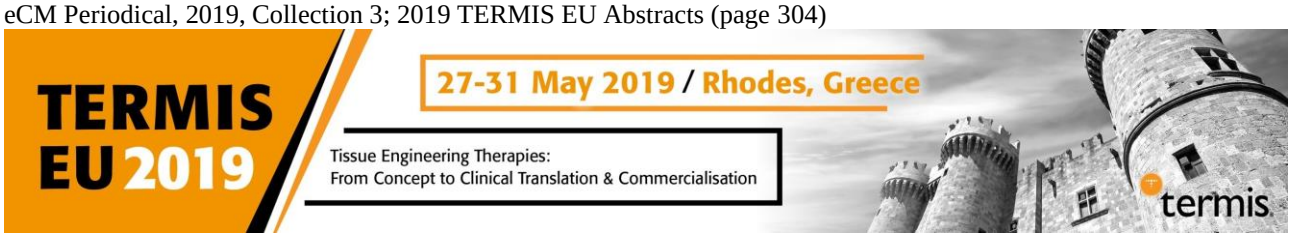
RESULTS & DISCUSSION: Cultivation of 433 cells within alginate scaffolds, cultured under flow velocity of 50 mL/h resulted in S1P receptor mRNA expression levels similar to those of primary samples from OC patients. Moreover, the relative proportions of each receptor were also similar to those of the primary tumor samples. By contrast, no such similarity was detected for monolayer and spheroid cultures. Confidence Interval comparison was performed, supporting the bio-similarity of the dynamic model results to those of the solid tumor results.

CONCLUSIONS: A novel in vitro model was designed and established for Primary OC, appropriated for experiments with a relatively large number of the samples.

ACKNOWLEDGEMENTS: Financial support was received from Azrieli college (AB).

REFERENCES

[1] Dai L et al. Oncotarget. 2017;8(43): 74947-61
[2] Dvir T et al. Tissue Eng. 2016;12(10):2843-52



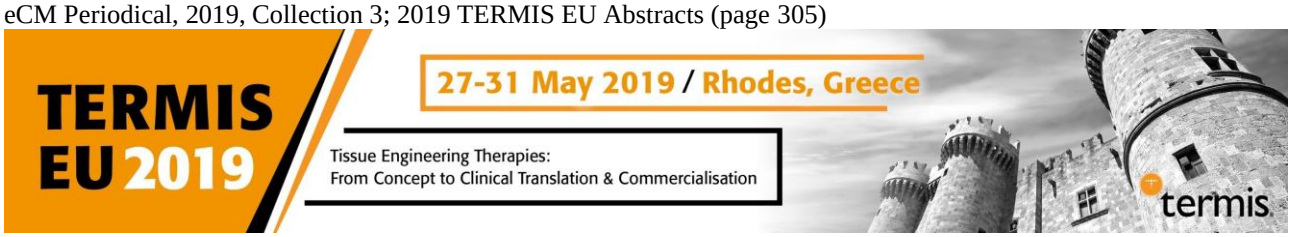
Emulsion-templated porous polymers as scaffolds for 3D cell culture and tissue engineering

A. M. Eissa
Presenting Author: Ahmed. M. Eissa, a.m.eissa@warwick.ac.uk
Department of Chemistry, University of Warwick, Coventry, U.K.

Highly porous polymeric materials can be prepared by emulsion templating technique, whereby a high internal phase emulsion (HIPE) is created in which the continuous (non-droplet) phase contains polymerisable monomer(s). Polymerisation then results in a well-defined macroporous polymer (foam), known as a polyHIPE. The nature of the formation of PolyHIPEs creates a highly porous, interconnected monolith structure, the architecture of which can be tightly controlled. These polyHIPE materials can serve as scaffolds for the culture of cells and, ultimately, tissues in 3D, allowing cells to mimic their native structure and function in vitro. Applications of these polymeric scaffolds are foreseen in 3D cell culture, tissue engineering and regenerative medicine. However, one potential limitation of these polyHIPE materials as 3D cell scaffolds is surface chemistry. Cells in vivo are typically surrounded by a complex extracellular matrix that contributes to cell anchorage and function. For example, it is well known that hepatocytes (liver cells) possess a galactose-binding cell receptor (asialoglycoprotein). The presence of galactose residues on the surface of polyHIPE scaffolds could improve hepatocyte adhesion and function while growing on the scaffold, leading to enhanced performance in liver tissue engineering applications. This presentation will describe different preparation strategies of such polyHIPE scaffolds and their subsequent functionalisation with biomolecules in order to further improve cell adhesion and function while growing on the scaffold, mimicking the native extracellular matrix (ECM). Initial cell culture experiments with primary rat hepatocytes and human endometrial stem cells will be presented. Subsequently, the application of these polymer materials as matrices for in vitro 3D cell culture and tissue engineering will be discussed. One current project involves the utilisation of such polymer scaffolds to create an advanced 3D model of human endometrium (womb in a dish) in order to study normal and pathological embryo implantation events that cannot be studied directly in humans.

REFERENCES

[1] A. S. Hayward et al., Biomacromolecules 2013, 14, 12, 4271-4277.
[2] A. M. Eissa et al., Biomacromolecules 2018, 19 (8), 3343–3350.
[3] S. A. Richardson et al., Macromolecular Bioscience 2019, 19, 1800351.



Engineering ectomesenchymal stem cell development and its application in craniofacial tissue engineering

A. Srinivasan[1], W.S. Toh[2,3], S.H. Teoh[4], T.C. Lim[5], Y.C. Toh [1-2*]
Presenting Author: Akshaya Srinivasan, bieaksr@nus.edu.sg
[1]Department of Biomedical Engineering, National University of Singapore (NUS), Singapore [2]NUS Tissue Engineering Program (NUSTEP), Singapore [3]Faculty of Dentistry, NUS, Singapore [4]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore [5]Department of Surgery, NUS, Singapore

INTRODUCTION: The regeneration of craniofacial bone tissues presents a unique challenge due to their complex structure, multiple embryonic origins and distinct molecular mechanisms of development [1]. With their developmental relevance to the craniofacial skeleton, high expandability and potential for personalized medicine, human pluripotent stem cell derived neural crest stem cells (NCSCs) are an attractive cell source for craniofacial reconstruction. However, current clinical utility is limited by a lack of understanding of the derivation of ectomesenchymal stem cells (eMSCs) from NCSCs, their phenotype, differentiation potential and osteogenic capacity.

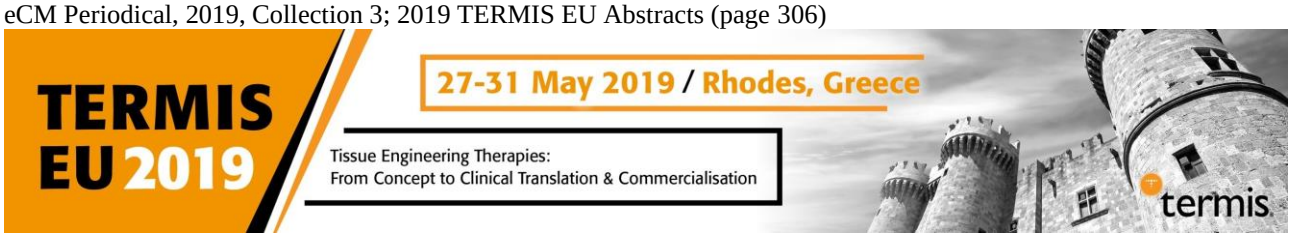
METHODS: NCSCs were derived from human embryonic stem cells following [2]. They were differentiated to eMSCs on PDMS substrates of differing stiffness for 7 days and characterized. After 21 days of osteogenic differentiation in PCL-TCP scaffolds, eMSCs were implanted in rat cranial defects to assess bone regeneration.

RESULTS & DISCUSSION: Lower substrate stiffness increased the multipotency of derived eMSCs. This process was regulated by Rho-ROCK mediated CD44 signaling, via modulation of PDGFR signaling. We benchmarked eMSCs to mesodermal bone marrow MSCs (BM-MSCs) and found them to be functionally and transcriptionally distinct, with persistent expression of cranial neural crest markers. eMSCs showed comparable osteogenic and chondrogenic ability to BM-MSCs in 2-D in vitro culture, but lower adipogenic ability. They exhibited greater proliferation than BM-MSCs and comparable construct mineralization in 3-D polycaprolactone-tricalcium phosphate (PCL-TCP) scaffolds in vitro. eMSC derived osteogenic constructs were more proliferative, less mature and showed differential levels of FGF signaling compared to BM-MSCs. Both eMSC and BM-MSC seeded scaffold constructs could enhance bone regeneration in a rat critical-sized calvarial defect model.

CONCLUSIONS: Our study sheds light on the mechanisms affecting the multipotency and osteogenesis of eMSCs and demonstrates the potential of eMSCs for future clinical applications. Our results indicate that the ontogeny of an MSC population impacts both its phenotype and functionality.

REFERENCES

[1] Tollemar, V., et al., Genes & Diseases, 2016. **3**(1): p. 56-71.
[2] Menendez, L., et al., Nat Protoc, 2013. **8**(1): p. 203-12.



Effect of matrix substrate composition on in vitro and in vivo regeneration in microporous nerve guidance conduits

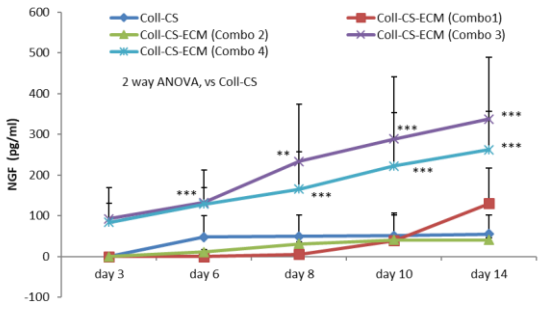
A.J. Hibbitts [1, 2, 3], Z. Koci[1, 2, 3], S. Kneafsey[1, 2, 3], L. Zilic[1,2,3], B. Cavanagh[4], C.T. Buckley[1,2,3], S.J. Archibald[5], F.J. O'Brien[1, 2, 3]
Presenting Author: Alan Hibbitts, alanhibbitts@rcsi.ie
[1]Tissue Engineering Research Group, Dept. of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Dublin, Ireland [3]Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI and TCD, Dublin, Ireland [4]Cellular and Molecular Imaging Core, RCSI, Dublin, Ireland [5]Integra LifeSciences, Plainsboro, NJ, USA

INTRODUCTION: To address current limitations in nerve guidance conduits (NGCs), a filled NGC with an internal matrix consisting of aligned microporous channels was fabricated. To allow for increased rates of Schwann cell migration, this study will investigate the incorporation of extra-cellular matrix (ECM)-derived neuro-conductive macromolecules into microporous NGCs.

METHODS: ECM molecules were incorporated into a selected matrix of collagen-chondroitin sulphate (Coll-CS). ECM combinations were incorporated into Coll-CS slurries and directionally freeze-dried. Effect of ECM incorporation on the porosity and pore alignment was assessed using SEM. Pro-regenerative effects of ECM scaffolds were assessed using primary rat isolates in 2 and 3D culture for 14 days with lead candidates progressing to in vivo testing in a rat sciatic nerve model.

RESULTS & DISCUSSION: ECM-NGCs were found to be highly porous and uniform throughout. There were significant increases in Schwann cell proliferation with the highest levels observed when multiple ECM macromolecules were present. 3D ex-vivo culture found that small alterations in the ECM composition elicited significant changes in neural regeneration potential (figure 1). Up to 50% recovery in stimulation response was observed at 4 weeks with further tests ongoing (figure 2).

CONCLUSIONS: These findings clearly demonstrate the benefit of ECM macromolecule incorporation and highlight the high impact of ECM combination selection.



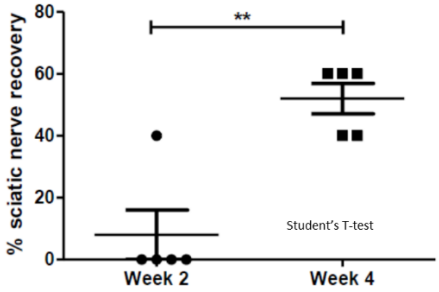
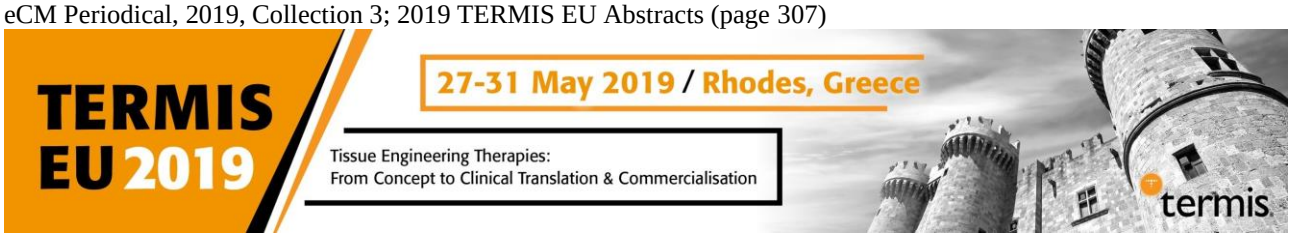


Figure 1: (Left) Incorporation of ECM molecules in the microporous matrix results in enhanced expression of neuronal growth factor **Figure 2**: (Right) Response to foot pad stimulation at week 4 in 15 mm sciatic nerve defect rats treated using Coll-CS-ECM (Combo 3) NGCs

ACKNOWLEDGEMENTS: Funded by Science Foundation Ireland and Integra LifeSciences through TP27-1846B1 as part of the AMBER Centre.



Exploring the antibacterial mechanism of action by platelet lysate patches

A.R. Franco[1,2], R. Costa-Almeida[1,2], P.S. Babo[1,2], M.E. Gomes[1,2,3], I.B. Leonor[1,2], R.L. Reis[1,2,3]
Presenting Author: Albina R. Franco, albina.franco@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [2]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

INTRODUCTION: Platelet lysate (PL) gained great interest in medical fields due to platelets' role in the immune response against infections and tissue healing[1]. The antimicrobial properties of PL patches are already known[2]; however, the main mechanisms or elicitors for its antibacterial (AB) activity are still unclear. Herein, we aimed at unraveling the AB action mechanisms of PL patches against Staphylococcus aureus.

METHODS: PL was prepared by freeze/thaw cycles[1] and concentrated by ultrafiltration. AB activity of PL patches [1,2] was evaluated against S. aureus using an overlay gel assay. PL patches were further characterized by Blue Native-2D Electrophoresis and 2D Electrophoresis under denaturing native conditions to identify AB proteins, and identified by MALDI-TOF/MS. The permeabilization of S. aureus outer and inner membranes by PL was investigated using fluorescence microscopy, after staining with PI and SYTOX Green. The expression of S. aureus genes leading to bacterial adherence and formation of biofilm, were evaluated.

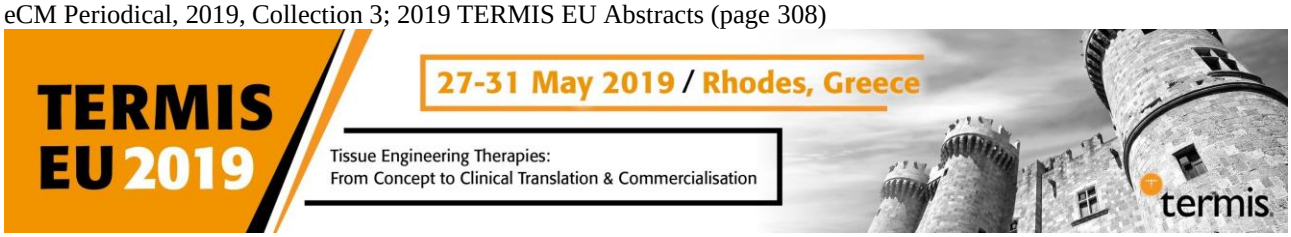
RESULTS & DISCUSSION: PL exhibited AB activity against S. aureus. Native-PAGE and 2D electrophoresis showed different proteins present in PL with similar isoelectric points (pH 3-10 and 5-8) but with different molecular weights. MALDI-TOF data showed the presence of unresolved peaks around 25-30kDa, and 70kDa. The permeabilization assays suggest that PL damages S. aureus membranes.

CONCLUSIONS: Herein, our results demonstrate AB activity of PL against S. aureus. PL is composed by different proteins or complexes with similar isoelectric points (at pH 5-8), but different molecular weights as identified through proteomics tools. Further studies will be made to unveil PL effect on microbial activity, elucidating their main mechanism of action.

ACKNOWLEDGEMENTS: We thank Serviço de Imuno-Hemoterapia do Centro Hospitalar de S. João, EPE (Porto, Portugal) for platelet concentrates; the Project NORTE-01-0145-FEDER-000021, HORIZON 2020 under the TEAMING Grant agreement No 739572 - The Discoveries CTR and FCT for ARF grant SFRH/BPD/100760/2014 and PTDC/BII-BIO/28870/2017.

REFERENCES

[1] Babo P. Inflamm Regen. 2014; 34:33-44
[2] Costa-Almeida R. Acta Biomater 2018; 68:29-40



Mesenchymal stem/progenitor cells residing in various tissue niches - Similarities and differences

A. Klimczak[1], U. Kozłowska[1], A. Krawczenko[1], T. Jurek[2], M. Rorat[2], D. Patrzałek[2]
Presenting Author: Aleksandra Klimczak, aleksandra.klimczak@hirszfeld.pl
[1]Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland [2]**Wroclaw Medical University**, Wroclaw, Poland

INTRODUCTION: Mesenchymal stem/ progenitor cells (MSCs) are applied in experimental clinical procedures as a promising tool in regenerative medicine. Although MSCs from different tissues exhibit many common characteristics, some markers and biological properties are different and depend on their tissue of origin [1]. In this study, we characterize the biological properties of MSCs during long-term culture isolated from: bone marrow (BM-MSCs), adipose tissue (AT-MSCs), skeletal muscles (SM-MSCs), and skin (SK-MSCs).

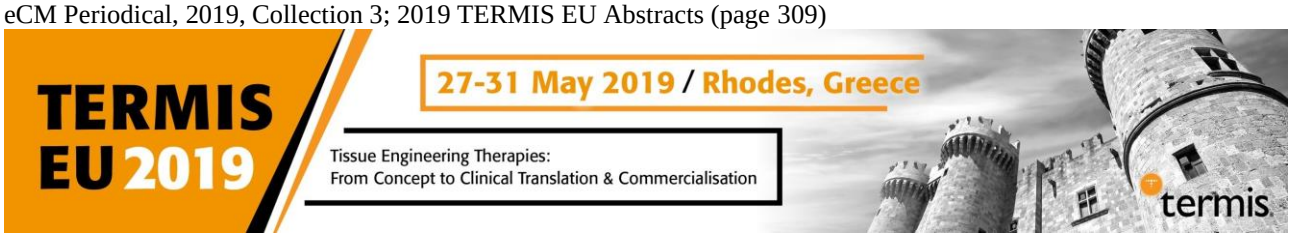
METHODS: Tissue samples were collected from deceased donors and from limbs amputated due to critical limb ischemia (study approved by a local Bioethics Committee). BM-MSC (n=8), AT-MSC (n=7), SM-MSC (n=9) and SK-MSC (n=7) were isolated and long-term cultured for 10 passages (P) in standard conditions. MSCs markers for: CD73, CD105, CD90, proangiogenic CD146, PDGFRα, CD31, and multipotency PW1 were examined by flow cytometry, and immunofluorescence. Multipotency was evaluated for osteo-, adipo- and chondrogeneic potential. Stemness markers for mRNA for Sox2 and Oct4, and genetic stability for p53 and c-Myc were assessed using qPCR. 27 bioactive factors were screened using the multiplex ELISA array. Spontaneous fusion by co-culture of SM-MSC with BM-MSC or AT-MSC stained with PKH26(red) or PKH67(green) was assessed.

RESULTS & DISCUSSION: All examined MSCs showed the basic MSC phenotype CD73, CD90, CD105 stable up to P10. However, their expression decreased with the age of culture, as confirmed by fluorescence intensity. The proangiogenic properties of MSCs were confirmed by CD146 and PDGFRα expression, but long-term culture is unfavorable for maintaining the proangiogenic function of examined MSCs, apart from BM-MSCs. All examined MSCs, except BM-MSCs, expressed PW1, a marker associated with differentiation capacity and apoptosis. Only SM-MSC express CD56, a marker characteristic for muscle progenitor cells, stable up to P5. The stemness markers Sox2 and Oct4 were detectable in all examined tissue-specific MSCs during long-term culture. All examined MSCs were stable in terms of p53 and c-Myc, however their expression differs between passages. The differentiation capacity of BM-MSCs and AT-MSCs was maintained up to P10. In contrast, SK-MSCs and SM-MSCs had a limited ability to differentiate into adipocytes. ELISA revealed secretion of IL-6, IL-8, MCP-1, MIP-1β, VEGF in different concentrations, depending on MSC niche. AT-MSC and BM-MSC were able to fuse with SM-MSC population.

CONCLUSIONS: Variability in biological properties of MSC from different tissue niche may influence on MSC quality and therapeutic potential. Long-term culture affects the biological properties of MSCs obtained from various tissues. The source of MSCs and the number of passages should be considered in the preparation of MSCs for regenerative medicine.

ACKNOWLEDGEMENTS: Supported by: National Science Center Grant No N N407 121940

REFERENCES:[1] Lv FJ et al. Stem Cells 2014; 32; 1409-19



A 3D alginate-based neuroblastoma model as in vitro platform for immunotherapies testing

A. Marrella[1], A. Dondero[2], M. Aiello[3], B. Casu[2], R. Castriconi[2], C. Bottino[2,4], S. Scaglione[1,3]
Presenting Author: Alessandra Marrella: alessandra.marrella@ieiit.cnr.it
[1]CNR – National Research Council of Italy, IEIIT Institute, Via De Marini 6, 16149 Genoa, Italy
[2]University of Genoa [3]React4life, Genoa [4]Gaslini Hospital, Genoa, Italy

INTRODUCTION: Current treatment failures of high risks Neuroblastomas (NB) may be due to the lack of in vitro models for studying, in a given patient, the efficacy of potential therapeutics, including those aimed to enhance anti-tumor immune responses. We here propose 3D alginate-based hydrogels as pre-clinical culture systems where to test recent therapeutic approaches.

METHODS: Cell-laden NB alginate spheres were prepared by using two different neuroblastoma cell lines: HTLA-230, SH-SY5Y and cultured up to 1 week. The morphology of the hydrogels, cell organization, cell viability and proliferation was monitored up to 1 week of culture. We analyzed NB cell-laden alginate spheres, cultured with and without INF-γ, for the surface expression of ligands regulating natural killer and T cell mediated immune responses. NB cells cultured in traditional 2D conditions were used as control for all the tests described above.

RESULTS & DISCUSSION: NB cell lines proliferated within our hydrogel spheres with a rate comparable with traditional 2D cultures, but with a spheroid morphology and a clustered organization, resembling in vivo phenotype. The ligands surface expression analysis highlighted a novel and clinical-relevant constitutive and IFN-γ-inducible NB immune-phenotype in the 3D hydrogels. Interestingly, we analyzed a ligand (PVR) involved in the formation of immune synapses with natural killer cells, crucial for killing of ex-vivo derived tumors. A strong downregulation of PVR, that occurs in vivo, was observed in IFN-γ-treated NB cell-laden alginate spheres (Fig.1) and was not detected in the traditional 2D culture system (data not shown).

CONCLUSIONS: This work highlighted a novel and clinical-relevant constitutive and IFN-γ-inducible NB immune-phenotype when cultured in the 3D hydrogels. In particular, the PVR trend observed in our 3D model well resemble the PVR variations occurring in vivo, which are not well appreciated in 2D standard culture conditions. Thus, 3D hydrogel-based spheres might represent a valid platform where to evaluate the efficacy of personalized interventions aimed to optimize the current and innovative immunotherapies.

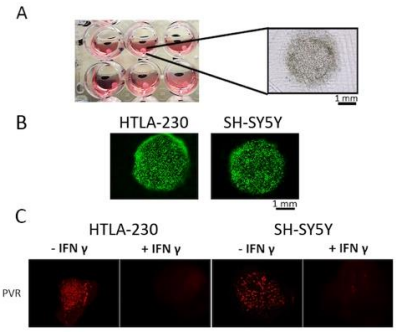
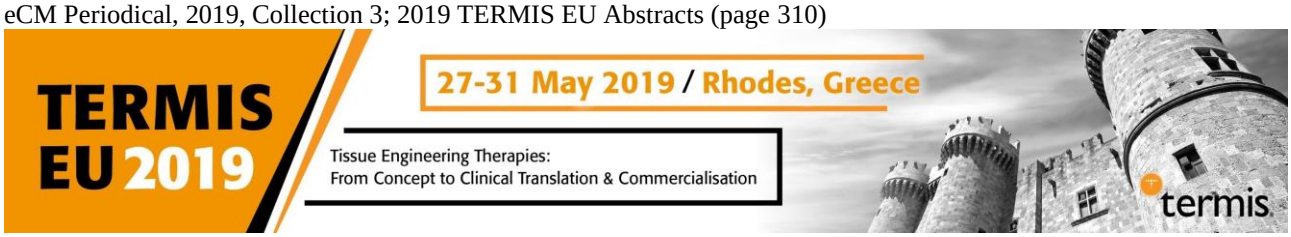


Figure 1: A) Picture and optical image of NB loaded alginate spheres. B) Cell viability represented by live/dead images of NB cell-laden alginate spheres after 24 hours of culture. C) Immunofluorescent images of PVR in NB cell-laden alginate spheres cultured a week either in the absence or in the presence of INF-γ



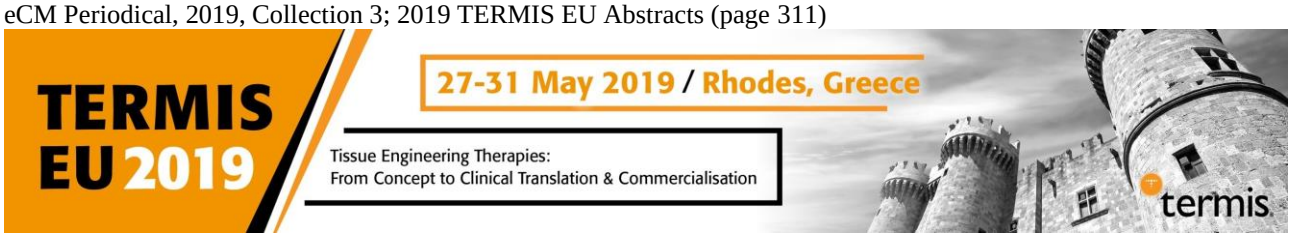
Mesenchymal and neural stem cells coculture for TERM applications in CNS

A. Speccher[1], F. Agostinacchio[2], W. Bonani[2], M.E. Castellini[1], A. Motta[2], S. Casarosa[1]
Presenting Author: Alessandra Speccher, a.speccher.1@unitn.it
[1]Lab. of Neural Development and Regeneration, CIBIO, University of Trento; Italy [2]Department of Industrial Engineering and BIOtech Research Center, University of Trento; Italy.

Disruptions of central nervous system (CNS) architecture are devastating, due to the irreversible process of neuronal death, the limited regenerative capabilities of brain and the current lack of effective treatments. Nowadays, regenerative medicine and cell replacement therapies are very promising approaches to treat the damaged brain [1]. In this context, stem cells represent an important cell source for transplantation therapies, and biomaterials can help in recapitulating the three-dimensional environment of the brain that usually gets compromised in the injured site. Among the different types of scaffolds, hydrogels are very interesting for brain tissue engineering. A few years ago, we have demonstrated that alginate (obtained through CaCl2 crosslinking) supports and enhances neural differentiation of murine Embryonic Stem Cells with respect to traditional 2D-cultures, and the encapsulated cells spread neurites and form connections among themselves [2]. Currently, we are testing alginate-based hydrogels to support human neural- and mesenchymal- SCs injection into the brain. Indeed, regarding the cells source, both the transplantation of neural stems cells (NSCs) and mesenchymal cells (MSCs) has been reported to elicit beneficial effects by regenerating neurons or secreting molecules and factors that help in the healing process [3]. The surprising ability of MSCs to regulate neural stem cell proliferation and differentiation has prompted research in understanding their interaction with NSCs and their impact on CNS regeneration. At the moment we are focusing on the identification of the best conditions for NSCs and MSCs coculture in alginate hydrogels. We are investigating: the best conditions for NSCs encapsulation, the optimal culture media and culture conditions, whether MSCs can survive in the coculture medium (maintaining their stemness) and setting up a preliminary coculture system. In the setting of the coculture, one of the major difficulties is the use of serum, as it is necessary for MSCs, while pushing NSCs toward glial differentiation. We have thus started to define the culture conditions and investigate whether we can create "compartmentalized" cocultures that would at least partially retain serum. By adding an alginate outer layer to our beads, we were able to retain the BSA (used as a "proxy" for serum) inside the biomaterial, creating a promising approach for coculture. In parallel, we positively observed that MSCs can survive, proliferate and maintain their stemness (CD44+ and CD73+) even in absence of serum, supporting the hypothesis that the use of “compartmentalized” coculture systems with low serum content would likely be exploitable for MSCs culture. In the future, the coculture of MSCs and NSCs would allow in vivo applications such as transplantation in injured animal models for CNS tissue regeneration.

REFERENCES

[1] Li X. et al. Progress in polymer science, 2012, 37.8: 1105-1129.
[2] Bozza A et al. Biomaterials, 2014, 35.16: 4636-4645.
[3] Zanier E. R., et al. Critical care medicine, 2011, 39.11: 2501-2510.



Development of serum-free culture conditions for differentiation of human bone-marrow derived mesenchymal stromal cells to tenocyte-like cells

A. Dei[1,2], A. Sampaio[3], W. Chang[1], D.I. Zeugolis[2,4]
Presenting Author: Alessandro Dei, alessandro.dei@stemcell.com
[1]STEMCELL Technologies UK Limited, Cambridge, United Kingdom [2]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway [3]STEMCELL Technologies Inc. Vancouver, Canada [4]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Adult mesenchymal stem cells (MSCs) are a promising candidate for treating tendon injuries. Recent studies have demonstrated that adult MSCs are capable of differentiating down tendon-like cell lineages and can improve tendon healing in animal models [1]. Despite critical limitations and safety concerns, fetal bovine serum (FBS) is still predominantly used for MSC expansion in clinical protocols [2]. Here we present preliminary data on a prototype serum-free medium optimized for in vitro tenogenic differentiation of human bone marrow-derived MSCs.

METHODS: Human bone marrow stem cells were seeded at 30,000 cells/cm^2 in 24-well plates. Once culture wells reached >95% confluence, cells were cultured in serum-free tenogenic differentiation medium (SF-TDM) and maintained for up to 21 days. Medium change was performed every 3 days. Tenogenic differentiation was assessed via qPCR, immunocytochemistry, flow cytometry, western blot and bilineage differentiation assays, carried out at day 0-3-8-21.

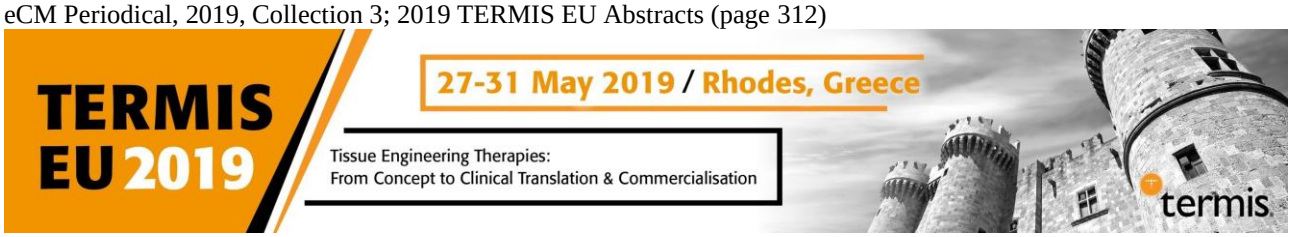
RESULTS & DISCUSSION: qPCR analysis showed that SF-TDM induces early Scx gene expression, which is followed by upregulation of Tnmd, Mkx, Col1, and Col3. Immunocytochemistry confirms Scx expression at early time points. Cells with spindle-shaped morphology and high Tnmd expression are seen in cultures after 21 days in SF-TDM. Moreover, evaluation of collagen deposition clearly indicates that cells cultured in SF-TDM first synthesize a matrix rich in collagen type III, followed by collagen type I, thereby resembling the physiological tendon repair process. Eventually, bilineage differentiation assay indicates that tenogenic lineage-committed cells exhibit decreased capacity to differentiate towards osteogenic and adipogenic lineages.

CONCLUSIONS: This defined serum-free formulation is capable of generating tenocyte-like cells derived from hBM-MSCs in vitro expressing tenogenic markers, such as Scx, Tnmd, TnC, Collagen I and Collagen III.

ACKNOWLEDGEMENTS: Financial support was received from H2020, Marie Skłodowska-Curie Actions, Innovative Training Networks (676338) and Science Foundation Ireland, (15/CDA/3629 and 13/RC/2073).

REFERENCES

[1] Kapacee et al. Matrix Biology 2010; 29(8):668-677
[2] Schnitzler et al. Biochemical Engineering Journal 2016; 108:3-13



Supramolecular hydrogels based on novel polyurethanes and cyclodextrins

A. Torchio[1], M. Boffito[1], A. Gallina[1], C. Cassino[2], G. Ciardelli[1]
Presenting author: Alessandro Torchio, alessandro.torchio@polito.it
[1]Politecnico di Torino, Department of mechanical and aerospace engineering, C.so Duca degli Abruzzi 24, 10129, Torino, Italy [2]Università degli Studi del Piemonte Orientale, Department of Science and Technological Innovation (DISIT), Viale Teresa Michel 11, Alessandria, Italy

INTRODUCTION: Linear poly(ethylene oxide) (PEO)-based polymers can be mixed with α-cyclodextrins (CDs) in water to form poly(pseudo)rotaxanes (PPRs), thus obtaining supramolecular (SM) gels with thixotropic and self-healing properties [1]. Moreover, the presence of free CDs can be exploited to encapsulate and release drugs in biological environments. Herein, novel polyurethanes (PUs) were synthesized to improve mechanical properties and stability in aqueous environment of SM hydrogels based on CDs.

METHODS: PUs were synthetized using Poloxamer P407 as diol (PEO 70%wt), 1,6-hexamethylene diisocyanate and two different chain extenders: 1,4-cyclohexane dimethanol (PU: CHP407) and N-Boc serinol (PU: NHP407). PU and CD aqueous solutions were then mixed at different ratios and the resulting SM structures were characterized by Attenuated Total Reflectance Infrared (ATR-FTIR) spectroscopy, Proton Nuclear Magnetic Resonance (^{1}H-NMR), Dynamic Light Scattering (DLS) and X-Ray Diffractometry (XRD). Self-healing and thixotropic behavior was assessed by rheological characterization. Swelling and dissolution were studied at 37°C and cytotoxicity tests were performed according to ISO10993.

RESULTS & DISCUSSION: ATR-FTIR, NMR, DLS and XRD results proved the formation of SM structures based on PPRs. SM hydrogels were obtained by mixing PU and CD solutions at final PU and CD concentrations of 1-9%w/v and 9-10%w/v, respectively. Gelation kinetics required from 20 minutes to overnight incubation at room temperature to obtain stable hydrogels. Rheological strain-sweep tests showed the ability of the gels to adapt to high strains (up to 500%) recovering the starting properties within 15 minutes. Moreover, some formulations showed an instantaneous recovery of gel state after complete rupture (Fig. 1). PU-based SM hydrogels exhibited good stability in aqueous environment and were non-cytotoxic.

CONCLUSIONS: The good mechanical and biological properties of the designed PU-based SM gels make them promising platforms for advanced drug delivery applications.

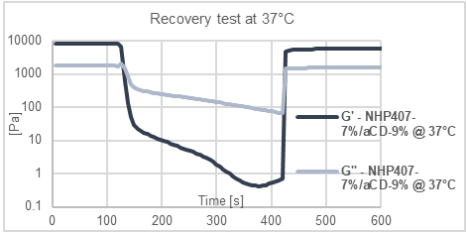
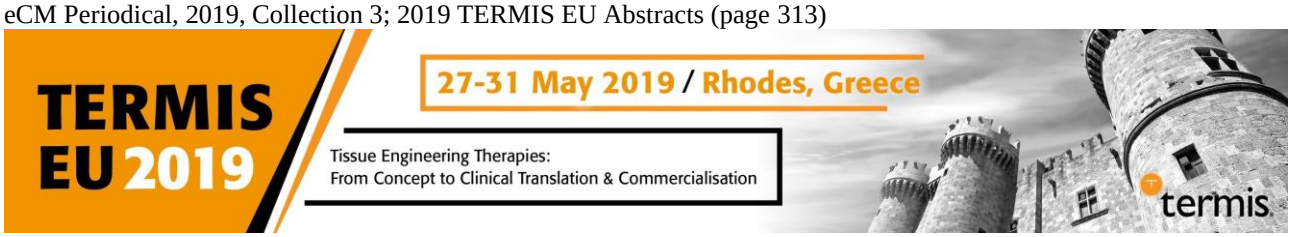


Figure 1: Self-healing test showing G' and G" trend over time. Rupture (G">G') was caused at 120s by increasing the applied shear stress. The starting stress was again applied at 420s and gel state (G'>G") was instantaneously recovered.

REFERENCES

[1] Li et al. J Biomed Mater Res A. 2003,1;65(2):196-202.



Non-autologous MSCs for endochondral bone regeneration

A. Longoni[1,2], I. Pennings[1,2], M. Cuenca Lopera[3], M.H.P. van Rijen[4], A.J.W.P Rosenberg[1], D. Gawlitta[1,2]

Presenting Author: Alessia Longoni, a.longoni@umcutrecht.nl

[1]Department of Oral and Maxillofacial Surgery & Special Dental Care, University Medical Center Utrecht, Utrecht University, The Netherlands [2]Regenerative Medicine Center Utrecht, The Netherlands [3]Laboratory for Translational Immunology, University Medical Center Utrecht, The Netherlands [4]Department of Orthopedics, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

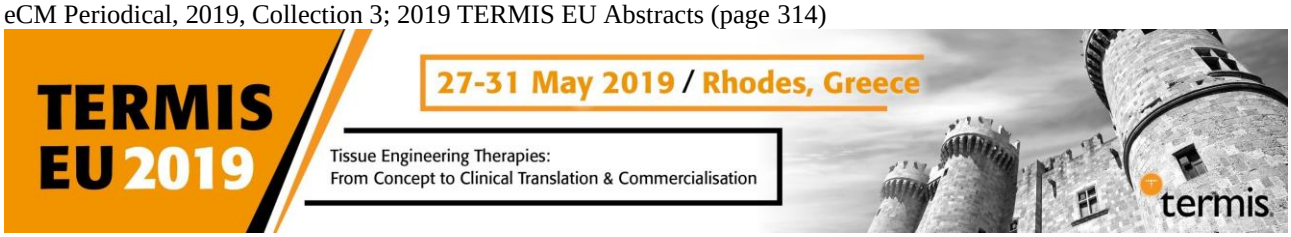
INTRODUCTION: When regenerating bone by mimicking the endochondral pathway, the cartilaginous template engineered in vitro acts as a transient substrate to trigger remodeling and new bone formation in vivo. The regenerated bone will temporarily have a mosaic composition derived of both, donor and host cells and, after remodeling it will be entirely host derived. Since the host is only transiently exposed to foreign cells, it has been postulated that non-autologous mesenchymal stromal cells (MSCs) could be potentially used for endochondral bone regeneration (EBR). However, a potential immune reaction against the non-autologous implant could impair the regenerative process. The aim of this study is to characterize endochondral bone formation and the immune response evoked when different types of non-autologous MSCs are implanted.

METHODS: Four groups were included in this study to compare the EBR of autologous and non-autologous chondrogenically stimulated MSCs. Constructs containing MSCs were cultured in chondrogenic differentiation medium for 28 days. The spheroidal constructs were embedded in a 6x3.5x3.5 mm collagen gel and implanted in a critical size femur defect, stabilized with a PEEK plate in Brown Norway (BN) rats. MSCs derived from Dark Agouti rats or humans were implanted in BN rats to obtain an allogeneic and a xenogeneic transplantation, respectively. Collagen only and constructs with embedded BN MSCs represented the negative and the syngeneic control groups. To quantify bone formation over time, μCT was performed at 4, 8 and 12 weeks. At 1 and 12 weeks, samples were explanted and decalcified to analyze the early (n=5) and late immune response and extent of bone formation on histological sections (n=8). In addition, at 12 weeks, lymphocytes were isolated from the superficial inguinal lymph node and co-cultured with the corresponding implanted MSCs as previously described.

RESULTS & DISCUSSION: After 12 weeks, bone formation was highest in the syngeneic group (89.2±11.3 mm^3), followed by 37.2±32.6 mm^3 for allogeneic, 16.4±5.9 mm^3 for xenogeneic, compared to the collagen control (5.96±5.9 mm^3). Furthermore, 7/7 defects of the syngeneic control group and 2/8 of the allogeneic group were fully bridged. No full bridges were observed in the xenogeneic or the collagen groups. Lymphocytes-MSCs co-culture showed mild donor-specific T cell proliferation only for the xenogeneic group.

CONCLUSIONS: These data show that non-autologous MSCs can be used for EBR. The closer the donor cells are related to the recipient, the more mineralized tissue formation and defect bridging was observed. Currently, further analyses are ongoing to confirm EBR on histology and to characterize the local and systemic immune responses evoked.

ACKNOWLEDGEMENTS: Financial support was received under AO grant S-16-130G.



Fracture localisation of osteogenic polymersomes and modulation of their accumulation by Kupffer cell depletion

A.R. Hailes[1,2], E. Scarpa[1], J.M. Kanczler[1], J.P. May[1], T.A. Newman[1], N.D. Evans[1,2]
Presenting Author: Alethia Hailes, ah5g13@soton.ac.uk
[1]Human Health and Development, Southampton General Hospital, UK [2]Bioengineering Sciences, Faculty of Engineering and Physical Sciences, University of Southampton, UK

INTRODUCTION: Pharmaceutical treatments to aid bone fracture repair are not yet available in clinic. Potential therapies may target signaling pathways involved in fracture repair, but are limited by off-target effects and poor bioavailability at the injury site [1]. Polymeric nanoparticles (PMs) may provide a solution enabling controlled spatio-temporal drug delivery [2]. In this study we test the hypotheses that PMs loaded with CHIR-99021, a Wnt agonist, can activate the Wnt signalling pathway in vitro and in vivo, and that PMs will passively accumulate at a fracture site, when injected at different times points post-injury. Furthermore PM accumulation at fracture sites can be modulated by depletion of Kupffer cells of the liver.

METHODS: A luciferase reporter cell line was exposed for 24 hours to a range of concentrations of free and PM-encapsulated Wnt agonists, BIO (PM-BIO) or CHIR-99021 (PM-CHIR), and luminescence was quantified. To assess activity in vivo, 200 µL of PM-BIO and PM-empty (control) at a concentration of 2 $x10^{12}$ PMs/ml were injected IV. Bone and liver tissue was then extracted and processed for qPCR to assess Wnt-related gene expression at 24 and 48 hours. For in vivo studies, a 1 mm drill-hole defect was made in the femur of male MF1 mice. PMs loaded with fluorescent dye, DiR, were injected either 1 hour or 7 days post-surgery, with or without prior Kupffer cell depletion by administration of 5 mg/ml clodronate 2 days prior to surgery. Kupffer cell depletion was confirmed by immunohistochemistry. Whole body and extracted organs were imaged using the in vivo imaging system (IVIS) and fluorescence was quantified.

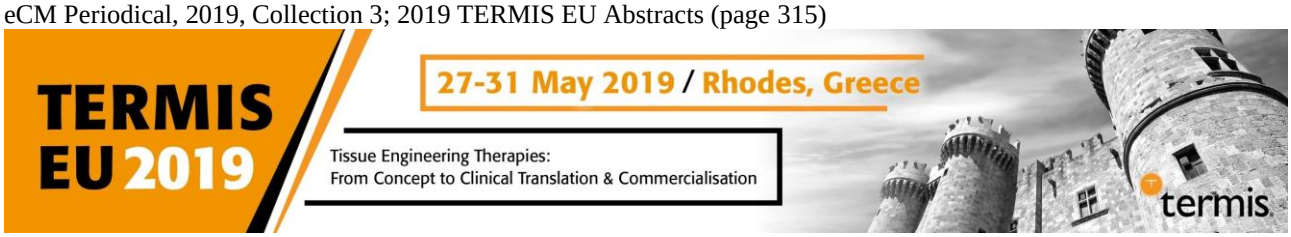
RESULTS & DISCUSSION: Free and PM-encapsulated BIO/CHIR induced luminescence in vitro, in a dose-dependent manner, indicating Wnt signaling activation. PM-BIO injected in vivo induced no significant difference in Wnt-related gene expression. PM-DiR fluorescence was observed at the site of injury. Intensity was 4-fold higher when PM-DiR were injected 1 hour compared to 7 days post-surgery. Initial quantification suggests lowered liver-specific accumulation after Kupffer depletion with no effect on fracture-specific accumulation.

CONCLUSIONS: PMs loaded with BIO/CHIR induce Wnt signaling in vitro. PMs also accumulate passively in vivo at a target fracture site, during different phases of healing. Future work will investigate the effect of increasing the dose of encapsulated BIO on in vivo Wnt-related gene expression and fracture repair outcome.

ACKNOWLEDGEMENTS: Financial support was received from the University of Southampton, DSTL, and the Grundy Educational Trust.

REFERENCES

[1] Einhorn T et al. Nature Rev. Rheum. 2014; **11**:45-54.
[2] Scarpa E et al. Nanomed: Nanotech., Bio. and Med. 2018; **14:**1267-1277.



Efficient clearance of lipoproteins from anti-coagulated and native blood-derived products to yield pure extracellular vesicle preparations

A. Otahal[1], O. Kuten[1,2], Z. Lacza[2], S. Nehrer[1], A. De Luna[1]
Presenting Author: Alexander Otahal, alexander.otahal@donau-uni.ac.at
[1]Center for Regenerative Medicine, Danube University Krems, Austria [2]IOrthoSera GmbH, Krems, Austria

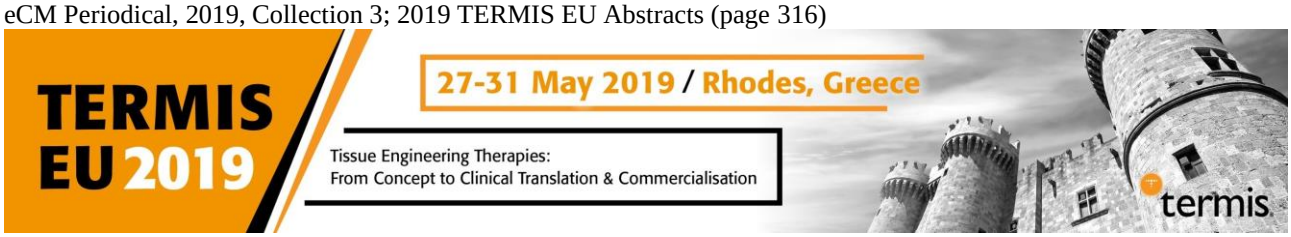
INTRODUCTION: Extracellular vesicles (EVs) increasingly gain focus in regenerative medicine for promoting tissue repair and alleviating inflammation. However, there are no standards for EV isolation from patient blood nor for quality assessment owing to lack of knowledge about active components or mechansims of action. It is known that high, low and very low density lipoproteins (HDL, LDL, VLDL) as well as chylomicrons copurify with EVs during isolation from various body fluids including blood via ultracentrifugation (UC) or size exclusion chromatography (SEC). The aim of our study was to develop an isolation strategy to purify EVs from blood derived products which are already in clinical use. Therefore, we analysed EV preparations from citrate-anticoagulated platelet-rich plasma (CPRP) and hypACT™ serum.

METHODS: Particle concentrations after UC, SEC or a combination of both were assessed via nanoparticle tracking analysis (NTA). EVs were labeled with annexin V (AnnV), CD63 as well as CD41 and analyzed by flow cytometry (FC). Low-density and high density lipoprotein (LDL and HDL) content was determined via FC in EV preperations by labeling of Apolipoprotein A1 (ApoA1) and Apolipoprotein B100/48 (ApoB-100) as well as detection via Western Blot. Presence of EVs was confirmed by cryo electron microscopy.

RESULTS & DISCUSSION: NTA revealed 100-fold higher particle concentrations after SEC than after UC or UC+SEC in both, CPRP and hypACT^{TM} serum. AnnV, CD63 as well as CD41 were detected on EVs via FC. It also revealed efficient clearance of ApoB-100 bearing particles by UC, while ApoA1-positive particles persisted. SEC alone removed ApoA1-positive particles, but failed to remove ApoB-100 bearing particles. The combination of enrichment via UC and purification via SEC enabled efficient clearance of ApoA1 as well as ApoB-100 indicative of removal of HDL and LDL. These findings are also supported by Western Blot analysis.

CONCLUSIONS: EV preparations are commonly contaminated with lipoproteins due to their similar size and density. The coupling of UC to separate EVs from lipoproteins by density and SEC to yield separation by size enabled efficient clearance of lipoproteins from CPRP or hypACT^{TM} serum and obtaining pure EV preparations. The developed isolation strategy will benefit the field of regenerative medicine by avoiding obfuscation of results when testing EV preparations from blood derived products in disease models.

ACKNOWLEDGEMENTS: Financial support was received from the European Fund for Regional Development (EFRE) and the Science Fund of Lower Austria.



The backstage of radiotherapy: Does the treatment with mesenchymal stroma cells reduce abdominal pain in patients suffering from pelvic radiation disease

C. Durand[1], A. Chapel[2], A, C. Demarquay[2], S. Pezet[3], H. Eutamène[4], F. Milliat[2], A. Sémont[2]
Presenting Author: Alexandra Sémont, alexandra.semont@irsn.fr
[1]Laboratoire de Radiotoxicologie, Radiobiologie Expérimentale (IRSN, SESAN), Fontenay-aux-Roses, France [2]Laboratoire de Radiobiologie des Expositions Médicales (IRSN, SERAMED), Fontenay-aux-Roses, France [3]Laboratoire de Neurobiologie, CNRS UMR 7637, (ESPCI), Paris, France [4]INRA, UMR 1331 Toxalim, Neuro-Gastroenterology & Nutrition Group, Toulouse, France

INTRODUCTION: Radiotherapy is an established part of treatment of both primary and recurrent pelvic malignancies. The efficacy of pelvic radiotherapy requires an optimal compromise between tumor control and toxicity to healthy, non-neoplastic tissues. Many cancer survivors, exhibit overlapping symptoms resulting from multiple visceral organ dysfunctions which have been recently recognized as a new pathology called “pelvic-radiation disease”. Up to 50% of these cancer survivors suffer from chronic visceral pain. Persistent pain considerably affects the patient’s quality of life. Stem cell-based approaches using mesenchymal stromal cells (MSC) have proved promising for the development of future therapeutic approaches (http://www.clinicaltrials.gov).

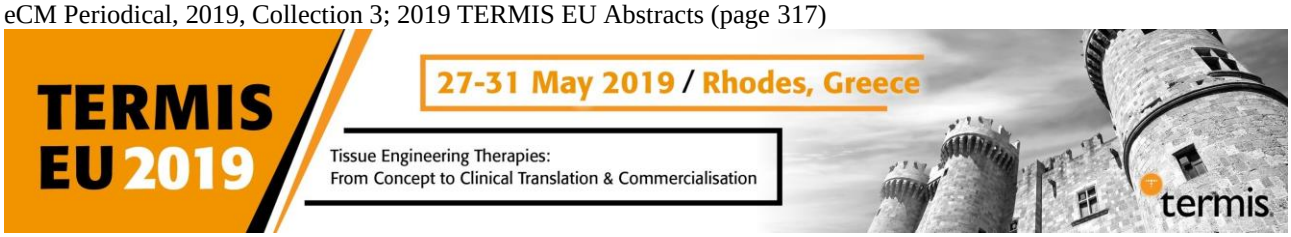
METHODS: Rats were subjected to 27 Grays colorectal irradiation. Visceral hypersensitivity (VH) and involved mechanisms were assessed over time using the visceromotor response to colorectal distention. VH was analyzed following the stabilization of mast cells (MC) by Ketotifen (twice a day for 1 week by oral gavage, 1 mg/ kg/day). Additionally, studies were performed in rats receiving MSC therapy, e.g. intravenously injection of 1.5 or 5 million MSC, 3 or 4 weeks after irradiation.

RESULTS & DISCUSSION: Radiation-induced chronic VH. We demonstrated the involvement of peripheral mechanisms where mast cells play a significant role. MSC treatment decreased the interactions between mast cells and nerve fibers and reversed mechanical VH. In patients treated by pelvic radiotherapy, we also reported increased spatial interactions between MCs and nerve fibers in the rectum [1]. Moreover, we showed that MSC compassionate treatment in patient’s refractory to regular treatment, seems to have substantial benefits on gastrointestinal complications like abdominal pain [2]. Finally, we have recently submitted a dossier to initiate a phase II clinical trial, the aim of which is to assess MSC therapeutic benefit on hemorrhage and pain in patients subjected to pelvic or abdominal radiotherapy (“PRISME”, protocol number: P130935; EUDRACT 2014-001462-99; NCT02814864).

CONCLUSIONS: Our work will provide new insights into the follow-up of the MSC efficiency in the treatment of VH and co-morbidities associated to “pelvic radiation disease”.

REFERENCES

[1] Durand et al, Pain (2015) 156:1465–76,
[2] Voswinkel J, Clin Rev Allergy Immunol (2013) 45:180–92



Flow shear stress-induced neuroinflammation to reproduce peri-electrode gliosis macro environment

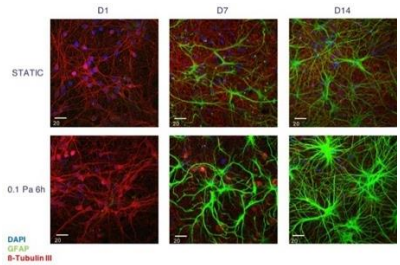
A. Trotier[1], M. Biggs[1]
Presenting Author: Alexandre Trotier, a.trotier1@nuigalway.ie
[1]Centre for Research in Medical Devices (CÚRAM), NUI Galway, Galway, Ireland

INTRODUCTION: Neuroprosthetic electrodes are routinely used in clinic to treat multiple diseases of the central nervous system including Parkinson's and Alzheimer's disease [1]. However, studies indicate that, in situ, mechanical traumas due to the insertion and micromotions of the electrode results in an adverse tissue response characterized by glial scar formation and electrode encapsulation [2]. In this study, we aim to develop an inflammatory model using fluid shear stress on neural cell populations to reproduce gliosis in vitro.

METHODS: Using a parallel-plate flow chamber system, ventral mesencephalic mixed primary cells were exposed to different level of pressure-driven fluid flow allowing to apply a defined shear stress (from 0.1 to 4 Pa) for either a 5-minute pulse to reproduce the insertion only, or up to several hours to mimic micro-motions between the implant and the tissue. The cells were then kept in culture for 14 days before being assessed for gliosis hallmarks. The morphology and protein expression of neurons and glial cells were quantified by image analysis. qPCR and Western-blots were used to detect the expression level of neuroinflammatory proteins.

RESULTS & DISCUSSION: Data have shown that the applied shear flow leads to astrocyte reactivity and inflammatory environment. Shear stresses from 0.1 to 4 Pa have all significantly increased the GFAP protein expression, number and size of astrocyte cells (Figure 1), along with the up-regulation of several neuronal pro-inflammatory markers and reactive oxygen species.

CONCLUSIONS: In conclusion, we developed an in-vitro model using parallel flow shear stress that mimics damages at the interface of the neuro-electrode. This model will certainly be a precious tool for future researchers developing anti-inflammatory and anti-gliosis biomaterial approaches.



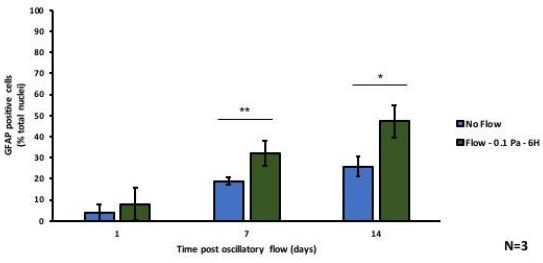
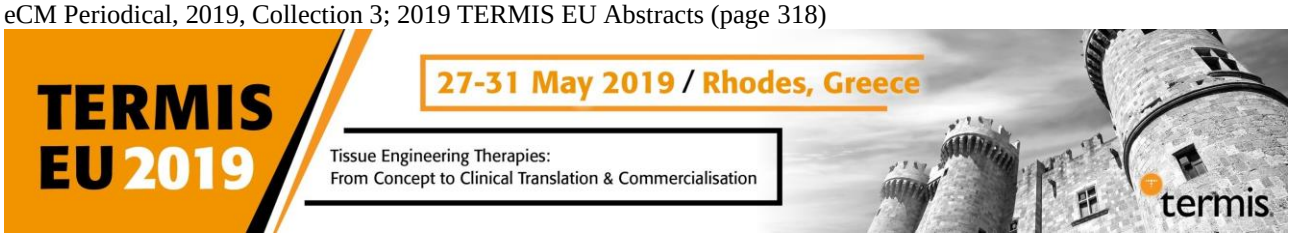


Figure 1: (Left) Immunostaining against GFAP (green) and ß-tubulin III (red) of VM cells after 1, 7 and 14 days of flow exposition at 0.1 Pa during 6h. **Figure 2**: (Right) Quantification of GFAP+ cell number after flow exposition vs no flow.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland (SFI) and the European Regional Development Fund (Grant Number 13/RC/2073).

REFERENCES

[1] Armstrong, R. A., Lantos, P. L., & Cairns. Neuropathology (2005).
[2] Durand DM, Ghovanloo M, et al. J Neural Eng. (2014).



Tissue engineering repair of peripheral nerve transsections

A. Thibodeau[1,2], C. Fauvel[1], T. Galbraith[1], H. Khuong[1,2], F. Berthod[1,2]
Presenting Author: Alexane Thibodeau, alexane.thibodeau.1@ulaval.ca
[1]Centre LOEX de l'Université Laval, centre de recherche du CHU de Québec, Québec, Canada
[2]Département de Chirurgie, Faculté de médecine, Université Laval, Québec, Canada

INTRODUCTION: Peripheral nerve transsections can lead to tactile loss and complete paralysis of the upper or lower limbs. Rapid regeneration is the key for an optimal recovery of motor functions, but so far clinicians do not have effective tools to repair wide nerve gaps when autograft is not possible [1]. Our goal is to produce a fully autologous viable nerve tube (NT) in which a network of capillaries is developed by seeding endothelial cells (ECs) for rapid vascularization and Schwann cells (SC) for axonal migration. These nerve tubes containing EC and SC's (NTECSC) could allow a faster recovery for patients with major peripheral nerve transsections by supporting better axonal migration, an essential aspect for a functional recovery.

METHODS: NT's consist of human fibroblast sheets and seeded with both EC and SC's which are rolled to form a filled tubular structure. NTECSC were implanted in immunodeficient RNU rats to repair a 15 mm sciatic nerve defect. Graft remodeling is followed by axonal migration quantification using immunofluorescent staining of neurofilament M after 2, 4 and 8 weeks of implantation.

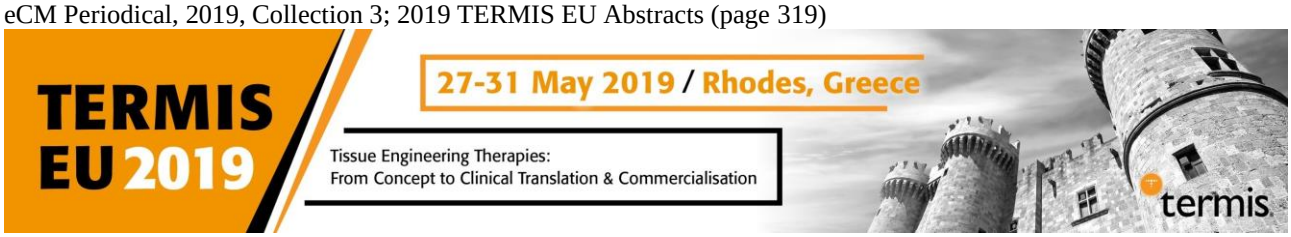
RESULTS & DISCUSSION: After NTECSC were successfully implanted in rats, their internal structure was quickly remodeled. A revascularization was observed along the entire tube length as early as the 4th week post-implantation. Red blood cells were presented in the pre-establish human capillaries showing the effectiveness of the anastomosis. Nerve fibers migrated and reached the distal nerve stump after 8 weeks. Axonal migrations over short distances were similar to the autograft control. Moreover, myelinated fibers are present in the NTECSC.

CONCLUSIONS: The development of a pre-vascularized NT with autologous ECs may greatly increase the speed of blood vessel reconnection in the graft. This fast vascularization is an essential parameter to facilitate axonal migration over long distances in a well-oxygenated environment [2]. In addition, long term SC incorporation within the NT may release neurotrophic factors and improve peripheral nerve regeneration.

ACKNOWLEDGEMENTS: Financial support was received from Canadian Institutes of Health Research (CIHR).

REFERENCES

[1] Burks SS et al. J Neurosurg. 2014;121:210-18
[2] Gao H et al. Biomed Res Int. 2013;392618



Evaluation of the cellular communication between the vascular and the sensory nervous systems

A. Leroux[1], B. Paiva dos Santos[1], H. Oliveira[1], J. Leng[2], J. Amédée[1]
Presenting Author: Alice Leroux, alice.leroux63@gmail.com
[1]Bioingénierie Tissulaire (BioTis), INSERM U1026, Université de Bordeaux, Bordeaux, France
[2]Laboratory of the Future (LOF), UMR 5258 CNRS/Solvay/Université de Bordeaux, Bordeaux, France

INTRODUCTION: Physiological and experimental data highlight the role of the sensory nervous system in angiogenesis, bone repair and regeneration [1]. Our previous work demonstrated that sensory neurons induce the osteoblastic differentiation of mesenchymal stem cells (MSCs) [2]. Here, we study the cellular communication between sensory neurons (SNs) and endothelial cells (ECs) in order to assess the influence of the sensory nervous system on angiogenesis.

METHODS: Rat primary dorsal root ganglia-derived SNs and bone marrow-derived ECs were cultivated using microfluidic platforms. This indirect co-culture approach allows us to better mimick the in vivo physiological microenvironment by physically separating the cellular bodies of both cell types. Specific immunostaining after 4 days of culture was performed for confocal microscopy and classical transmission electron microscopy in order to study the close interaction between both cell types. Expression of angiogenic markers was assessed by RT-qPCR after 4 and 7 days of mono- and co-culture, and Mmp2/Mmp9 concentration was determined using an assay kit.

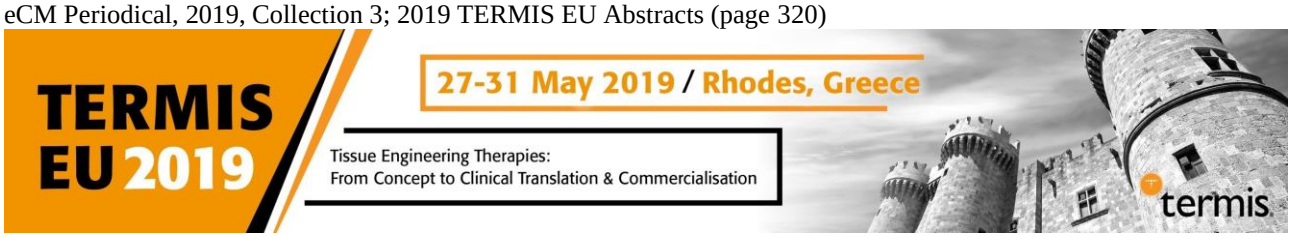
RESULTS & DISCUSSION: SNs are able to emit neurites toward the compartments containing ECs, where they closely interact. Among the angiogenic markers analyzed (Tek, Angpt1, CD31, VegfA, Col4 and Mmp2), a significant up-regulation of VegfA and Mmp2 was detected in ECs co-cultured with SNs. In particular, after 7 days of culture, ECs show a 20-fold upregulation of Mmp2 in presence of SNs. Further analysis showed that Mmp2/Mmp9 enzyme concentration was also significantly increased in ECs when they were co-cultured with SNs compared to the mono-culture of ECs.

CONCLUSIONS: Our preliminary results suggest that SNs can closely interact with ECs. SNs are able to modulate the expression of vascular endothelial genes and stimulate angiogenesis through ECM remodeling.

ACKNOWLEDGEMENTS: The authors would like to thank the Direction Générale de l'Armement (DGA), the Institut national de la santé et de la recherche médicale (Inserm) and Agence Nationale de la Recherche (ANR) for their financial support.

REFERENCES

[1] Marrella A et al. Mater Today (Kidlington). 2018; 21(4):362-76
[2] Silva DI et al. Cell Death and Dis. 2017; 8(12):3209



Electrospun elastin-like fibers for cardiovascular tissue engineering

A. Fernández-Colino[1], F. Wolf[1], S. Rütten[2], J. C. Rodríguez-Cabello[3], S. Jockenhoevel[1,4], P. Mela[1]
Presenting Author: Alicia Fernández-Colino, fernandez@ame.rwth-aachen.de
[1]Department of Biohybrid & Medical Textiles (BioTex) AME—Institute of Applied Medical Engineering Helmholtz Institute, RWTH Aachen University Forckenbeckstraße 55, 52074 Aachen, Germany [2]Electron Microscopy Facility Uniklinik RWTH Aachen Pauwelsstrasse, 30, D-52074 Aachen, Germany [3]Bioforge Lab University of Valladolid CIBER-BBN Paseo de Belen 11, 47011 Valladolid, Spain [4]Aachen-Maastricht-Institute for Biobased Materials (AMIBM) Maastricht University Urmonderbaan 22, 6167 RD, Geleen, The Netherlands

INTRODUCTION: Cardiovascular disease is the most common cause of death worldwide [1], but surgical options are restricted by the limited availability of autologous vessels segments, and the suboptimal performance of prosthetic grafts. This calls for advanced materials and fabrication schemes. Here, we describe a one-step electrospinning approach to obtain elastin-like fibrous scaffolds for cardiovascular applications.

METHODS: Chemically-modified elastin-like recombinamers (ELRs) bearing azide and cyclooctyne groups were coaxially electrospun to fabricate crosslinked click-elastin-like fibers. SEM was used to investigate fiber morphology. Cellular studies were carried out with primary human vascular smooth muscle cells (SMCs) and endothelial cells (ECs).

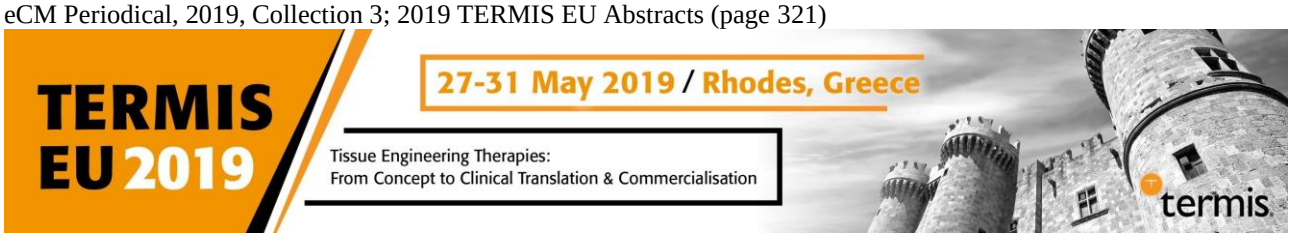
RESULTS & DISCUSSION: We fabricated, in a single step, electrospun elastin-like fibers that were crosslinked via catalyst-free click chemistry [2]. The fibers were structurally stable in aqueous environments at physiological temperature for at least 3 months, and they showed a mean width of 1.45±0.32 µm, which is in the reported range for the native elastin fibers in the vascular tunica media. No catalysts, additives, crosslinking agents or highly toxic solvents were needed, obviating the risk of compromising the cytocompatibility. SMCs and ECs interacted with the fibers, and proliferated, which demonstrates that ELRs' bioactivity was maintained after the electrospinning. These cells are relevant for cardiovascular tissue engineering as they provide the main extracellular matrix synthesis and hemocompatibility, respectively. Indeed, the potential of the system was demonstrated by fabricating fibrous tubular scaffolds for e.g. vascular bioengineering

CONCLUSIONS: Here we developed an approach that combines the advantages of the click chemistry and the innovation of the ELRs with the coaxial electrospinning for the obtaining of water stable fibrillar elastin-like structures in a single step under physiological conditions. This represents a significant step in advancing the electrospinning technology to biomedical applications.

ACKNOWLEDGEMENTS: This work was funded by the Excellence Initiative of the German federal and state governments in the framework of the i³tm Rotational Position (2014-R4-01) and START-UP (StUpPD_330-18) Programs, and by the START-Program of the Medical Faculty of RWTH Aachen University (60/17). J.C.R.-C. acknowledges the financial support from the following projects: MAT2013-42473-R, MAT2015-68901-R, MAT2016-78903-R, VA313U14, VA015U16, and PCIN-2015-010

REFERENCES

[1] Townsend N et al. European heart journal 2016;37(42):3232-45.
[2] Fernández-Colino A et al. Macromol. Biosci. 2018;18(11).



Improvement of antifungal and antibacterial properties of hydroxyapatite coatings by the Ag addition

A. Vladescu[1,2], L.R. Constantin[1], C.M. Cotrut[2,3], M. Dinu[1]
Presenting Author: Alina Vladescu, alinava@inoe.ro
[1]National Institute for Optoelectronics INOE2000, Magurele, Romania [2]National Research Tomsk Polytechnic University, Tomsk, Russia, [3]University Politehnica of Bucharest, Romania

INTRODUCTION: During many years of evaluation of the reaction of the human body to metallic implants, various studies have been made to investigate the resistence of several materials at different infections [1, 2]. The aim of the study is to investigate different Ag- containing hydroxyapatite (HA) coatings enriched with SiC obtained by RF magnetron sputtering, against the action of different microorganisms.

METHODS: The coatings were prepared at different deposition conditions, by varying the RF power fed on Ag cathode (0.6 W, 0.9 W and 1.2 W), while maintaining the power fed of HAP and SiC cathode constant (50 W and 15 W, respectively). The detailed conditions are given in Ref. [3]. Thereafter, HAP+SiC+Ag coatings were sterilized for 30 min and incubated with Candida albicans (A), Streptococcus pyogenes (B), Salmonella Typhimurium (C), Staphylococcus aureus (D), for 1 and 7 days of cultivation.

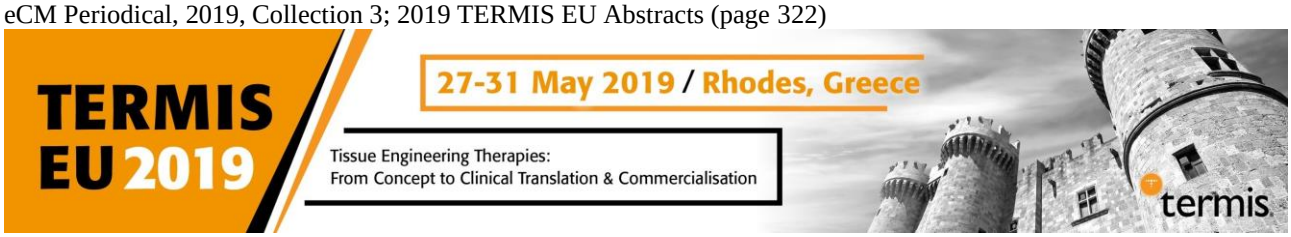
RESULTS & DISCUSSION: The negative reaction means that the growth has failed during experimented time intervals, while positive reaction shows the growth of different microorganisms. Based on the obtained results, one can observe that only sample S2 indicated an antifungal and antibacterial activity, in presence of the concentrated (Conc.) and diluted (Dil.) germ suspensions. Probably for the S1 coating, the Ag content is too low to provide effects, while for S3, the Ag is too high. A very small reaction was observed for uncoated sample in presence of concentrated germ suspension when incubated with Streptococcus pyogenes.

CONCLUSIONS: Based on the results of the study, it can be concluded that a small addition of Ag into HA coatings can improve the antifungal and antibacterial properties of HA.

ACKNOWLEDGEMENTS: The work was supported by the following grants of the Romanian National Authority for Scientific Research and Innovation, CCCDI – UEFISCDI, Project no. 60PCCDI/2018 (MedicalMetMat), no.68/2018 (CoatDegraBac), within PNCDI III, as well the Core Program 2019 and Institutional project no. PFE19/2018.

REFERENCES

[1] Ducheyne P et. al. Online Version, Elsevier Science, 2011
[2] Hashmi S et. al. Newnes, 2014
[3] Badea M et. al. Ceram. Int. 2016; 42: 1801-11



ExCeL: Combining extrusion bioprinting on cellulose scaffolds with lamination for perfusable tissue constructs

A. Shahin-Shamsabadi[1], P. R. Selvaganapathy[1,2]
Presenting Author: Alireza Shahin-Shamsabadi, shahinsa@mcmaster.ca
[1]School of Biomedical Engineering, McMaster University, Canada [2]Department of Mechanical Engineering, McMaster University, Canada

INTRODUCTION: Three-dimensional tissue constructs have considerable applications in understanding of human physiology as in vitro models. They accurately mimic physiological responses of natural tissues [1,2] and several methods such as bioprinting have been developed to fabricate them [3]. Previously developed bioprinting techniques have limitations in incorporating multi-materials and cell types and integrating perfusion channels to form thick 3D structures. Here, we report a new method termed ExCeL that combines extrusion printing, xurography, and lamination to create scalable, multi-material, multi-layer constructs.

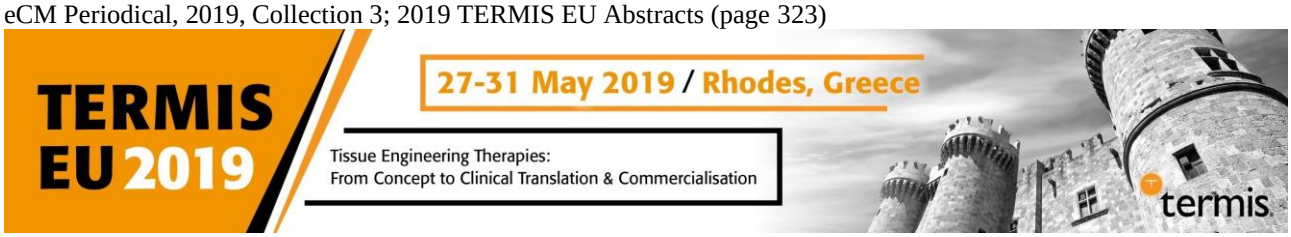
METHODS: This process starts by pretreating the paper with the hydrogel's crosslinker (calcium (Ca) for sodium alginate). Hydrogel is then extrusion printed which rapidly crosslinks as it wicks into the paper. In order to create 3D structures, the pretreated paper is cut into desired patterns that constitute channels and then bioinks (hydrogel with cells) is printed on it in predefined patterns. Finally, the individually printed layers are stacked on top of each other and allowed to bond through gel penetration and crosslinking to create the final 3D structure.

RESULTS & DISCUSSION: Initially the paper scaffold was treated with 1 M Ca (printing speed of 2000 mm/min and flow of 0.5 mL/min) solution to incorporate the cross-linking agent in it. Then paper scaffolds were cut using xurography to create 2 mm sized hollow channel region. Next, bioinks (2 wt% alginate with cells) were printed using gauge 18 needle, speed of 500 mm/min, and flow rate of 0.5 mL/min achieving a print resolution of 1.08±0.07 mm. HUVEC bioinks were printed annular to the hollow channels while 3T3 bioink was printed surrounding it. The printing allowed clear separation of different cells within a homogeneous matrix. Finally, lamination of multiple layers allowed formation of perfusable channels.

CONCLUSIONS: In summary, a new bioprinting technique has been developed that is based on printing hydrogel on paper and its in situ crosslinking. Paper provides mechanical stability that thin hydrogel layers lack and allows for patterned printing of complex structures. This bioprinting method has the capability to fabricate structures that mimic some of the biological complexity of tissues in vitro.

REFERENCES

[1] Griffith LG et al. Nat Rev Mol Cell Biol.2006. 7(3): 211-24.
[2] Caddeo S et al. Front Bioeng Biotechnol, 2017. 5: p. 40.
[3] Antoni D et al. Int J Mol Sci, 2015. 16(3): p. 5517-27.



Pro-inflammatory cytokines neutralization by intra-articular injection of biofunctionalized nanoparticles as advanced treatment for osteoarthritis

A.C. Lima[1,2], D. Amorim[2,3], I. Laranjeira[2,3], A. Almeida[2,3], H. Ferreira[1,2], F. Pinto-Ribeiro[2,3], N. M. Neves[1,2,4]
Presenting Author: Ana Cláudia Lima, claudia.lima@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal [4]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

INTRODUCTION: Pro-inflammatory cytokines, especially tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), play a crucial role in the development and progression of osteoarthritis (OA). [1] Although the beneficial effects of antibody (Ab) therapy, they present deleterious side effects and inadequate efficacy due to their short half-life. Therefore, this works proposes the immobilization of anti-TNF-α and anti-IL-6 Abs at the surface of polymeric nanoparticles (NPs) to selectively capture and neutralize those cytokines. Our system aims to extend and increase the Abs therapeutic efficacy (NPs protects them from degradation), and to reduce the systemic side effects using intra-articular (IA) delivery.

METHODS: NPs were prepared as previously described [2]. After accessing the maximum immobilization capacity, a co-culture model of inflammation was used to examine the cytokines neutralization effect in vitro. The in vivo therapeutic effect was assessed in an experimental monoarthritis rat model by measuring clinical parameters, nociceptive behaviour (mechanical allodynia - flexion/extension test, and mechanical hyperalgesia - pressure application measurement (PAM)) and histological analysis.

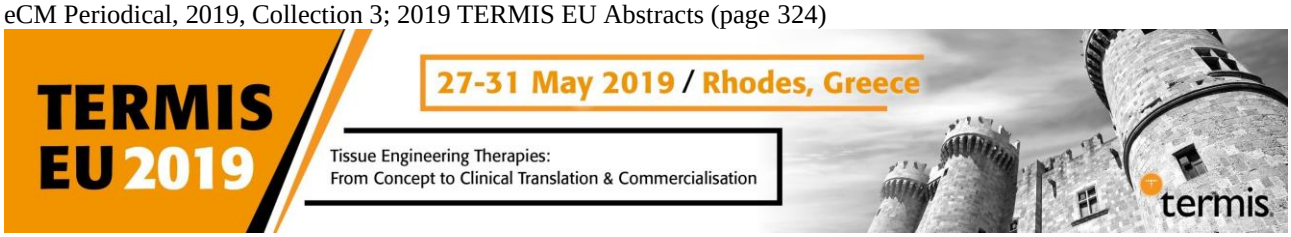
RESULTS & DISCUSSION: The maximum Ab immobilization ability was 10 μg/mL for anti-TNF-α Ab and 15 μg/mL for anti-IL-6 Ab. The biological properties as well as the synergistic effects of the two antibodies were validated in the co-culture system. Indeed, the biofunctionalized NPs with both Abs exhibited a prolonged action and stronger efficacy than free Abs. In vivo experiments confirmed the safety profile and a higher efficacy of the bio- functionalized NPs in comparison with free Abs, since they significantly decreased mechanical allodynia and hyperalgesia. Overall, it was shown the successful reverse of arthritis nociceptive impairments and inflammatory scenario.

CONCLUSIONS: These results clearly demonstrate the beneficial effects and safety profile of the biofunctionalized NPs after local delivery. In conclusion, this approach can lead to a revolution in the OA treatment.

ACKNOWLEDGEMENTS: Financial support was received from FCT (PD/59/2013 and PTDC/CTM-BIO/4388/2014 - SPARTAN).

REFERENCES

[1] Roy K et al. Int J Nanomedicine. 2015;10:5407-20
[2] Lima AC et al. ACS Appl Mater Interfaces. 2018;10:13839-13850



The sweet side of the brain: Complete characterisation of the N-glycome of human brain tissue and its alteration upon Parkinson's disease

A.L. Rebelo[1], F. Gubinelli[2], E. Brouillet[2], R. Saldova[3,4], A. Pandit[1]
Presenting Author: Ana Lúcia Rebelo, analucia.rebelo@nuigalway.ie
[1]Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway, Ireland [2]Neurodegenerative Diseases Laboratory, Université Paris-Saclay, and MIRCen, Institut François Jacob, CEA, France [3]National Institute for Bioprocessing Research and Training (NIBRT), University College Dublin, Ireland [4]UCD School of Medicine, UCD Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland.

INTRODUCTION: Parkinson's Disease (PD) is a neurodegenerative condition related with the death of dopaminergic neurons in the substantia nigra and to an increased state of neuroinflammation due to the aggregation of aberrant proteins [1]. Given that glycans may behave as regulators of protein stability [2], it is important to understand how glycosylation relates to PD pathophysiology. Furthermore, the cross-species comparison between human and rat brains may also elucidate the relevance of animal models in terms of brain N-glycosylation in the development of novel biomaterial approaches for tissue regeneration.

METHODS: Brain tissues were homogenised using RIPA buffer and the proteins entrapped in an acrylamide gel. PNGAseF was used to release the N-glycans and 2-Aminobenzamide to label them. The labelled glycans were purified and analysed on HILIC-UPLC, WAX-UPLC and Mass Spec. Further validation was performed using lectin histochemistry on tissue sections.

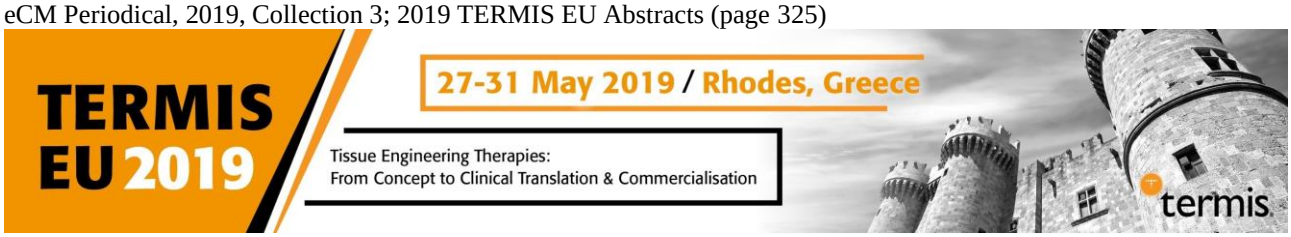
RESULTS & DISCUSSION: Reproducible chromatographic profiles of 60 peaks, containing glycan structures, for both striatum and nigra were obtained from human brain tissue. These were shown to have high prevalence of mannose and fucose residues. The abundance of 13 peaks is significantly different between the healthy brain regions. Most of the detected glycans in the striatum are neutral (78% both in healthy and PD brains), whereas in the nigra this percentage differs (85% in healthy vs 78% in PD). In the case of rat brains, sialylation differences are seen in the striatum since the binding of SNA-I and MAA lectins differs between healthy and LPS model brains.

CONCLUSIONS: This study presents an UPLC-based method for N-glycan profiling of brain glycoproteins with high reproducibility, both in rat and human tissues. The differences in sialylation in the nigra of PD patients (vs. healthy controls) highlights the involvement of sialic acids in the onset of the disease.

ACKNOWLEDGEMENTS: Financial support by EU H2020-MSCA-ITN-2015 under the Marie Skłodowska-Curie Innovative Training Network (Grant Agreement No.676408) and SFI/European Regional Development Fund (13/RC/2073); CMI at NUIG; Parkinson's UK Brain Bank.

REFERENCES

[1] Moore D.J., Annual Rev in Neurosci, 2005
[2] Kleen R., Nat Neuro Rev, March 2004



Magnetic scaffolds to promote angiogenesis

A.C. Manjua[1,2*], J.G. Crespo[1], J.M.S. Cabral[2,3], C.A.M. Portugal[1], F.C. Ferreira[2,3]
Presenting Author: Ana Carina Manjua, carina.manjua@tecnico.ulisboa.pt
[1]LAQV-Requimte, FCT-Universidade Nova de Lisboa, Caparica, Portugal [2]Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

INTRODUCTION: Cardiovascular disease represents 31% of global deaths every year [1], requiring novel options to promote adequate vascularization for tissue substitutes [2]. Herein, we assessed the influence of an external magnetic field on mesenchymal stem cells (MSCs) cultured on magnetic responsive scaffolds, specifically targeting the increased secretion of angiogenic factor VEGF-A.

METHODS: Scaffolds of gelatin (type A, Sigma Aldrich), containing 1% (V/V) Fe_3O_4 nanoparticles, were fabricated. Human bone marrow MSCs were seeded at 80,000 cells/cm^2 on top of gelatin scaffolds. A neodymium magnet was placed beneath the cell culture plate to provide low magnetic field intensity (0.08T). Controls include MSCs cultured on scaffolds in the absence of the magnet (0T) and in polystyrene culture plate (TCP), in the absence or exposed to the magnet. VEGF-A expression and respective protein were quantified by rtPCR and ELISA, respectively.

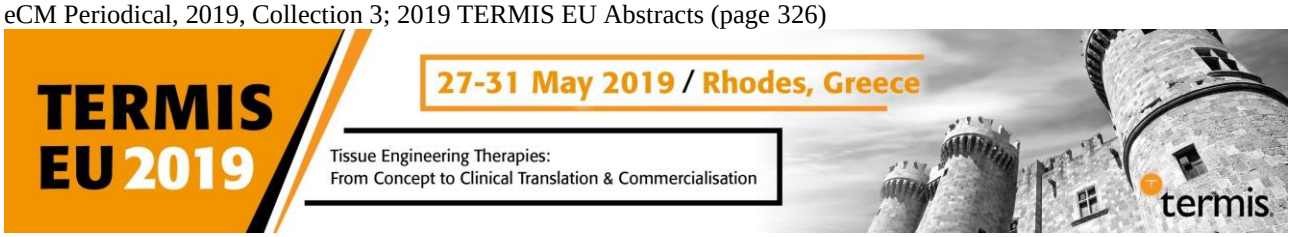
RESULTS & DISCUSSION: The MSCs in the scaffold exposed to the magnetic field expressed increased values of VEGF-A ($p<0.0001$) and respective protein (results not shown) ($p<0.0001$) in comparison with the values obtained in the absence of magnetic field. It was also observed an increase in the expression of VEGF-A by the MSCs cultured on TCP under magnetic field ($p<0.0001$) when compared with the TCP condition in the absence of magnetic stimulation. Sprouting functional assay of endothelial cells (HUVECs) revealed an improvement in the number of tubes and branch points (results not shown) when cultivated in the media from the MSCs cultured in the scaffolds by comparison with the TCP reference ($p<0.0001$).

CONCLUSIONS: These data illustrate the improvement of the MSCs capacity to secrete VEGF-A when cultured on the scaffolds under the application of a low intensity magnetic field.

ACKNOWLEDGEMENTS: Financial support was received from PORL2020, FCT (007317, UID/BIO/04565/2013); FCT/MEC (UID/QUI/50006/2013), PT2020 (POCI-01-0145-FEDER-007265); PhD scholarship (PD/BD/114043/2015); PRECISE (N.16394), BeLive (PTDC/EMD-EMD/30828/2017); EU (H2020-WIDESPREAD-2014, 664558).

REFERENCES

[1] WHO, Cardiovascular disease, accessed 30 November 2018, <http://www.who.int>
[2] Lovett M. et al. Vascularization Strategies for Tissue Engineering. Tissue Engineering. Part B, Reviews. 2009; 15(3): 353-370.



Tumor-on-a-chip for the development of novel anti-cancer therapies: An alliance between tissue engineering and nanomedicine

A.M. Martins[1,2,3,4], M.G. Barbato[1], R.L. Reis[2,3,4], P. Decuzzi[1]
Presenting Author: Ana M. Martins, ana.martins@iit.it
[1]Laboratory of Nanotechnology for Precision Medicine, Italian Institute of Technology, Via Morego 30, 16163 Genoa, Italy [2]3B's Research Group, I3Bs–Research Institute on Biomaterials, Biodegradable and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Portugal [3]ICVS/3B's–PT Government Associated Laboratory, Portugal [4]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Portugal

INTRODUCTION: In this work, we used a double channel microfluidic chip with two parallel microchannels (vascular and extravascular compartment) connected by a micropillar constituting a permeable vascular membrane [1]. The microfluidic chip can be used for studying the vascular transport and tissue permeability of nutrients, waste products and drug molecules, and the intravasation and extravasation of cancer cells.

METHODS: The extravascular channel was seeded with 18,000,000 human glioblastoma U87 GFP+ cells/mL embedded in 50:50 matrigel matrix for 72 hours (Figure 1A). The high density of cells allows one to mimic the tridimensional (3D) architecture and replicate "a piece of tumor tissue" in only 24 h. Free Docetaxel (DXTL) was injected in the vascular channel at physiologically relevant flow rates. Different concentrations of DXTL (0.01, 0.1 and 10 µM) were added into the vascular channel for 24, 48 and 72 h.

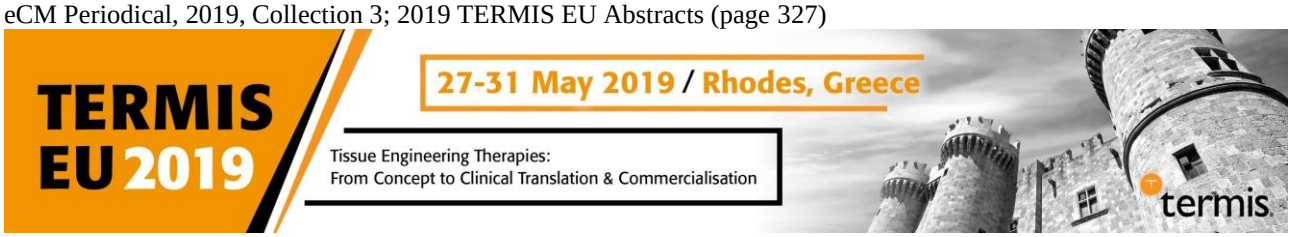
RESULTS & DISCUSSION: The highest concentration of DXTL (10 µM) has a remarkable deleterious effect on U87 cells over time corroborated also by cell viability measurements. Also, Figure 1B clearly shows the positive influence of flow in cell death after 48 and 72 h. The extravascular channel in the direction of flow exhibits less viable cells as a function of time. 3D images of U87 cells, measuring the same area in different time points, show that the size significantly decreased upon treatment with 10 µM DXTL over time. The cell viability data obtained by measuring the intensity of fluorescence shows that only 10 µM DXTL was able to kill cells after 48 and 72 h.

CONCLUSIONS: This cancer-on-chip model allows us to simulate the in vivo environment of a tumor by means of 3D culture conditions of cells allowing the spatial organization of the tumor resembling a piece of tissue and the dynamic flow of culture medium and therapeutic drugs simulates the real scenario. This 3D dynamic system can also be used to mimic several brain diseases, to test new therapeutic compounds and personalized treatments using cells from patients.

ACKNOWLEDGEMENTS: This work was supported by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement no. 754490.

REFERENCES

[1] Manneschi C et al. Microfluid Nanofluid. 2016; 20:113



Natural polyphenols reduce the toxicity of soluble Aβ42 oligomers

A.R. Araújo[1,2,3], S. Gigante[4], L. Moura[5], R. L. Reis[1,2,3], R. A. Pires[1,2,3]
Presenting Author: Ana Rita Araújo, anarita.araujo@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Portugal [4]Physical Chemistry "Rocasolano", Spanish National R&D, Spain [5]Research Institute for Medicines, Universidade de Lisboa, Portugal

INTRODUCTION: Aging and oxidative stress are the main triggers of supramolecular self-assembly of amyloidogenic proteins, such as Amyloid β 1-42 (Aβ42), one of the hallmarks of Alzheimer Disease.[1] Recent studies indicate that Aβ42 toxicity is not derived from the protein aggregates themselves but to their soluble oligomeric structures[2]. Herein, we evaluated the capacity of natural polyphenols[3] to: modulate Aβ42 aggregation; reduce the cytotoxicity of Aβ42 oligomers; and to reduce the presence of reactive oxygen species (ROS) in the biological environment.

METHODS: The polyphenols vescalagin/ castalagin were extracted from the outer bark of Cork oak tree. The capacity of these molecules to modulate the Aβ42 fibrillization was evaluated by CD, Thioflavin T, STEM and AFM. The antioxidant potential was accessed by DPPH assay and DCFH-DA dye in the presence H_2O_2. Cell studies were conducted with SHSY-5Y neuroblastoma cell line. The capacity of these colecules to decrease the cytotoxicity of Aβ42 oligomers was evaluated by Live/dead staining, alamarBlue® assay and immunocytochemistry.

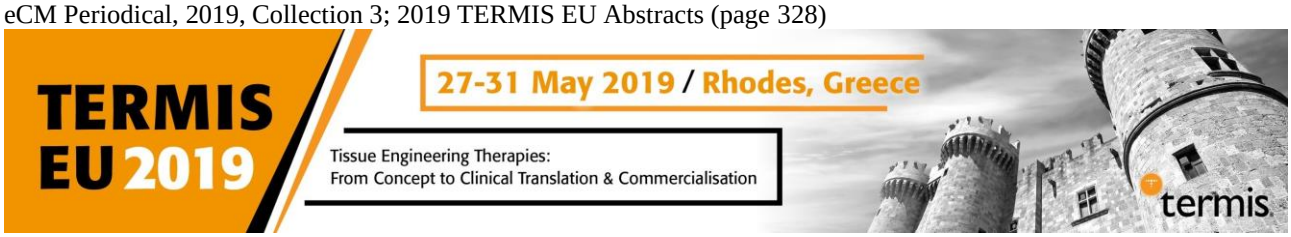
RESULTS & DISCUSSION: CD and Thioflavin T measurements revealed that vescalagin/castalagin was able to remodel the Aβ42 aggregation pathway, through the inhibition of the self-assembly of its oligomers/monomers. These polyphenols were also able to disassemble preformed fibrils/oligomers. STEM and AFM images showed a remodeling and significant decrease of Aβ42 fibril size. DCFH-DA dye results also showed the ability of these molecules to decrease the ROS under cell culture conditions, while reducing the presence of toxic oligomers/fibrils.

CONCLUSIONS: Both vescalagin and castalagin were able to remodel the aggregation process of Aβ42 and rescue cells from oxidative stress (H_2O_2) diminishing the toxicity promoted through the spreading of Aβ42 monomers/oligomers.

ACKNOWLEDGEMENTS: Financial support: Norte 2020, NORTE-08-5369-FSE-000037; EU: #692333-CHEM2NATURE,#668983-FORECAST, and #739572-THE DISCOVERIES CTR.

REFERENCES

[1] Eisele Y.S., et al., Nat Rev Drug Discov, 2015.
[2] Benilova I. et al., Nat Neuroscience. 2012
[3] Araujo A.R., et al., Rsc Advances. 2015



Acoustically-stimulated drug carriers for bone fracture repair

A.E. Polydorou[1], D. Carugo[1], N.D. Evans[1 2]
Presenting Author: Anastasia Polydorou, aep1g17@soton.ac.uk
[1]Bioengineering Science Research Group, Faculty of Engineering and Physical Sciences, University of Southampton, UK [2]Health and Developmental Sciences, Southampton General Hospital, UK.

INTRODUCTION: Impaired bone fracture healing is a major, increasing socioeconomic burden. The targeted delivery of therapeutic agents such as acoustically-stimulated microbubbles (MBs) and nanodroplets (NDs) may overcome pharmacokinetic limitations in treatment. Therefore, in this is study, we tested the hypothesis that MB and ND preparations are non-toxic to human bone cells and promote osteoblastic differentiation in human marrow stromal cells.

METHODS: MBs were fabricated using a 9:1 molar ratio of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) to polyoxyethylene(40) stearate (PEG40S), which were hydrated in PBS and sonicated in a two-step process to form a MB suspension. NDs were formed in a similar way using PFP for the core as opposed to room air. A range of volumetric concentrations of MBs (1:100 - 1:2) or NDs (1:100000 - 1:100) were applied to MG63 osteocarcinoma cells for 20 minutes, 40 minutes, 6, 24 or 72 hours. Alamar Blue® test was carried out to quantify cell metabolic activity, as an indicator of cell viability. To determine the effect of MBs on osteogenic differentiation, room air filled MBs were applied to Stro-1^+ marrow stromal cells for 14 days. Osteoinduction was tested using alkaline phosphatase (ALP) assay and gene expression studies.

RESULTS & DISCUSSION: DSPC-PEG40S MBs had a dose-dependent effect on cell metabolic activity of MG63 cells over 72 hours; this was significant only at 1:2 concentration (63% ± 6%, $p<0.0001$). Up to 24 hours, no cytotoxic effects were observed by either MB or ND exposure. MBs in the presence of osteoinductive factors induced the expression of osteoinductive markers (Runx2).

CONCLUSIONS: DSPC-PEG40S MBs and NDs are non-cytotoxic within clinically relevant time frames at suitable concentrations, proving a safe drug delivery method. These results also support in vivo studies reported in the literature to have used up to 8×10^8 MB/ml in mice [2]. Further studies are taking place to assess the extent of Stro-1^+ osteogenic differentiation induced by MBs.

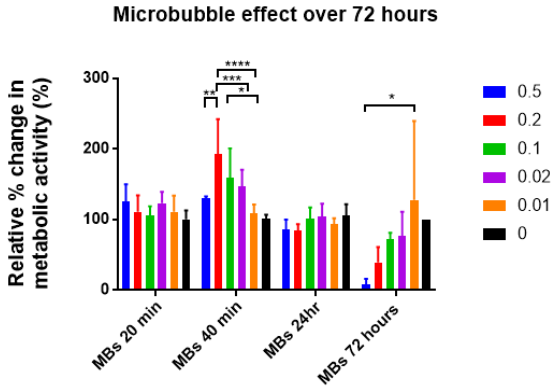
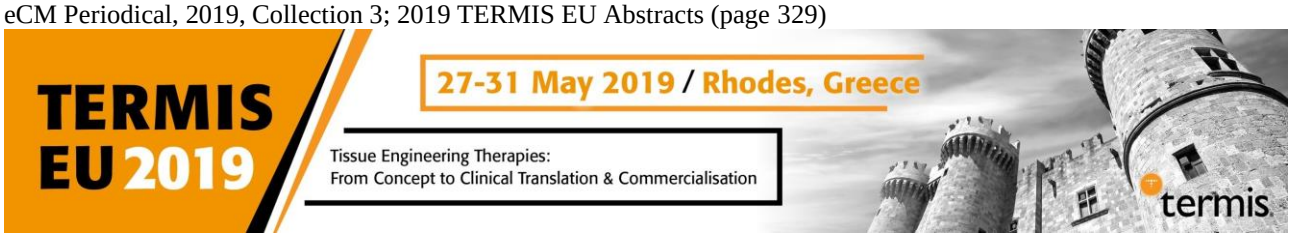


Figure 1: MB exposure to MG63 cells, up to 24 hours, had no cytotoxic effect. However, exposure of MBs for up to 72 hours significantly decreased cell metabolic activity.

ACKNOWLEDGEMENTS: EPSRC, MRC and Institute for Life Sciences, Southampton



Bottom-up study of vasculogenesis induced by microenviroment structure cues

A. Shpichka[1], A. Gorkun[1,2], A. Kosheleva[2,3], P. Timashev[1]
Presenting Author: Anastasia Shpichka ana-shpichka@yandex.ru
[1]Institute for Regenerative Medicine, Sechenov University, Moscow, Russia [2]FSBSI Institute of General Pathology and Pathophysiology, Moscow, Russia [3]Lomonosov Moscow State University, Faculty of Biology, Moscow, Russia

INTRODUCTION: To date, one of the key issues in regenerative medicine and tissue engineering is the lack of blood vessels, which ensure nutrient and oxygen transport within the engineered constructs and prevent their necrosis after transplantation. One of the approaches to fabricate the microvasculature is the use of biomaterials that induce vasculo- and angiogenesis, and fibrin is of particular interest to achieve this goal. As fibrin has rapid biodegradability and opacity, this study aimed to synthesize a non-toxic biodegradable fibrin modification which can induce vasculogenesis.

METHODS: We prepared PEG-fibrin conjugates [1] at different molar ratios and characterized their structural (scanning electron microscopy, atomic force microscopy, differential scanning calorimetry [2], small-angle x-ray scattering [2], infrared spectroscopy) and biological properties (cell viability and vasculogenesis assays).

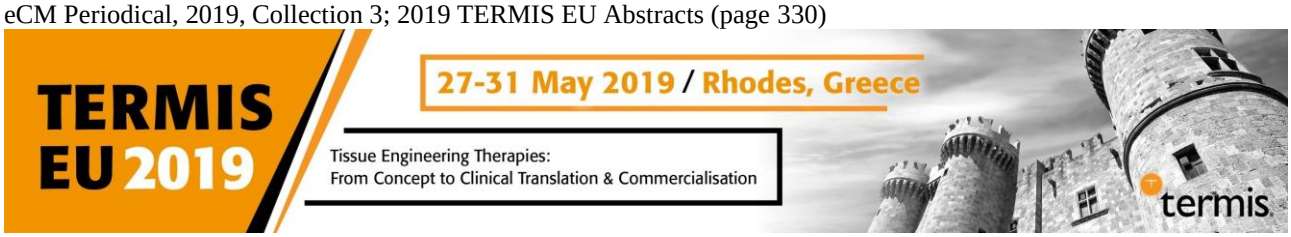
RESULTS & DISCUSSION: PEGylation of fibrinogen allowed us to overcome the mentioned drawbacks and achieve the gel transparency and preserve its biocompatibility. The use of high resolution crystallographic structures of fibrinogen allowed us to perform rigid body modelling in the case of oligomer formation and restore the mutual orientation of the oligomeric species. Atomic force and scanning electron microscopy and other methods together with small-angle X-ray scattering permitted us to achieve a clear picture of the conjugate structure and properties. We revealed that the gel prepared from PEGylated 5:1 fibrinogen (25 mg/mL) using 1:0.2 U protein to thrombin ratio provided the most favorable microenvironment for spreading, growth, and proliferation of fibroblasts and human umbilical vein endothelial cell (HUVECs) and human adipose-derived stem cell (hASC) coculture. Within this gel, hASCs and HUVECs formed cell extensions and cell-to-cell contacts and expressed specific extracellular matrix proteins and vasculogenesis inherent enzymes which were studied via immunofluorescent staining, gelatin zymography, western blot, etc. The morphological development of this coculture within a perfused hydrogel over 12 days led to the formation of interconnected HUVEC-hASC network. Moreover, this gel supported better the formation of multibranched cords than the pure fibrin gel and other modifications. Analysis of tubule growth rate, length, and branching showed that the differentiated ADSCs had higher angiogenic potential than the differentiated hUC MMSCs.

CONCLUSIONS: All data collected allowed us to reveal optimal composition of the modified fibrin gel, which is required for tailored gel formation with the needed particle sizes and homogeneity level for successful cell encapsulation and induction of vasculogenesis.

ACKNOWLEDGEMENTS: Russian Science Foundation (18-15-00407, gel characterization) and Grant of the President of the Russian Federation (МК-1297.2019.7, biological experiments)

REFERENCES

[1] Shpichka et al. Cell and Tissue Biology. 2017; 11(1):81-87
[2] Shpichka et al. Sovremennye Tehnologii v Medicine. 2018; 10(1):64-69



Coagulation influences properties of extracellular vesicles isolated from autologous blood derived products

A. De Luna[1], A. Otahal[1], O. Kuten[1,2], Z. Lacza[1, 2], S. Nehrer[1]
Presenting Author: Andrea De Luna, andrea.deluna@donau-uni.ac.at
[1]Center for Regenerative Medicine, Danube University Krems, Austria [2]OrthoSera GmbH, Krems, Austria

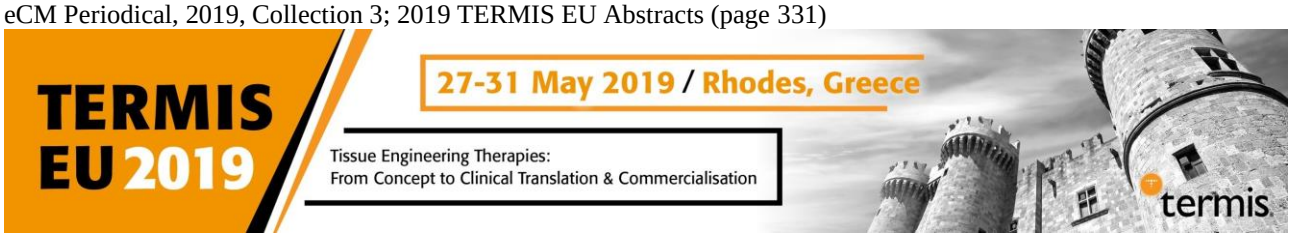
INTRODUCTION: Autologous blood derived products gain more and more interest in the field of regenerative medicine due to their ability to stimulate soft and hard tissue regeneration. Platelet rich plasma (PRP) is the most commonly used blood derivative in clinical practice due to its high concentration of platelets and the perceived high growth factor levels. Drawbacks of using PRP is high donor variability, discrepancies among preparation protocols and the presence of cells (platelets, leukocytes) which can evoke cellular processes, especially inflammation, when injected into the host. It is a possibility to isolate only the active components of blood derivatives which may overcome this problem. EVs are particles transporting bioactive molecules (proteins, miRNAS, lipids) and therefore represent novel mechanisms by which signals are communicated among different cell types. In the current study we focused on extracellular vesicles (EVs) isolated from two autologous blood derivatives, PRP and hyperacute serum and investigated whether the clotting cascade influences EV properties.

METHODS: EVs were isolated from citrate-anticoagulated PRP (CPRP) and hyperacute serum using differential ultracentrifugation followed by a size exclusion chromatography. Particle concentration and size were determined by nanoparticle tracking analysis. Cryo-electronmicrosopy was performed to visualize isolated EVs. Expression of miRNAs transported within EVs as well as in their respective input material was analysed by qPCR.

RESULTS & DISCUSSION: Nanoparticle tracking analysis revealed higher particle concentrations and bigger sized EVs within CPRP compared to hyperacute serum. These findings were confirmed by cryo-electronmicroscopy. Profound differences were detected regarding miRNA expresion between the two blood derivatives. 126 miRNAs were identified which were expressed both in input material (PRP or hyperacute serum) as well as in the corresponding EVs. Interestingly, the correlation between miRNAs in EVs and input material was higher in CPRP compared to hyperacute serum meaning that in hyperacute serum miRNAs were identified which were higher expressed in EVs than in the corresponding input material.

CONCLUSIONS: EVs from autologous blood products represent a novel and cell free approach for regenerative strategies. We observed that the clotting cascade (plasma versus serum) has an influence on concentration, size and miRNA expression patterns of EVs. These differences might have an impact on the biological mode of action of blood derived products used in clinics.

ACKNOWLEDGEMENTS: Financial support was received from the European Fund for Regional Development (EFRE) and the Science Fund of Lower Austria. miRNA expression analysis was performed by TAmiRNA GmbH. Cryo-electronmicroscopy was conducted at the Core Facility of the Vienna Bio-Center.



Engineering self-assembled multilayered tissue substitutes

A. De Pieri[1,2,3], S. Korntner[1,2], Yuri Rochev[2], D.I. Zeugolis[1,2]
Presenting Author: Andrea De Pieri, andrea.depieri@proxybiomedical.com
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway [2]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland [3]Proxy Biomedical Ltd., Coilleach, Spiddal, Galway, Ireland

INTRODUCTION: Tissue engineering by self-assembly consists of growing cells on surfaces made of thermoresponsive polymers, that allow the production of contiguous cell. In this approach cell-cell junctions and deposited extracellular matrix (ECM) remain intact, which provides a better cell localisation at the site of injury. However, these systems lack the possibility to fabricate multi-layered and three-dimensional cell sheets that would better recapitulate native tissues. Moreover, the fabrication of ECM-rich cell sheets would be highly desirable. This limitation could be overcome by inducing macromolecular crowding (MMC) conditions [1]. Herein we venture to fabricate electrospun thermoresponsive nanofibres to sustain the growth and detachment of ECM-rich tissue substitutes.

METHODS: A copolymer of 85% poly-N-isopropylacrylamide and 15% N-tert-butylacrylamide (pNIPAm/NTBA) was used to produce homogeneous fibres. Human adipose derived stem cells (hADSC) were treated with media containing macromolecular crowders to enhance matrix deposition. Immunocytochemistry was conducted in order to estimate matrix deposition and composition. Adipogenic, osteogenic and chondrogenic assays were performed both with and without the presence of MMC. Non-invasive cell detachment was enabled by decreasing the temperature of culture to 10 °C for 20 minutes.

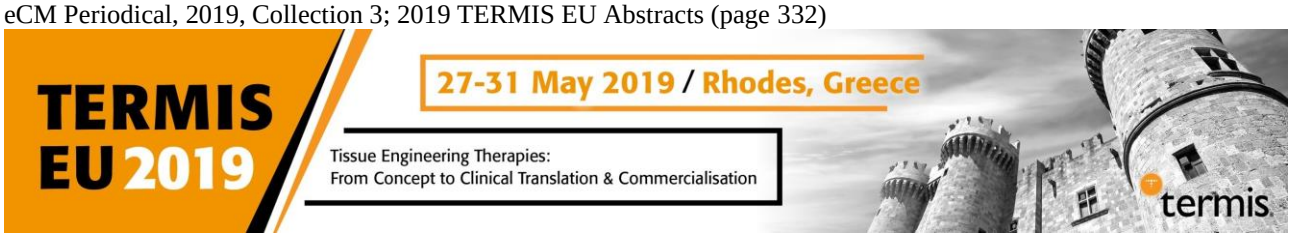
RESULTS & DISCUSSION: The electrospinning process resulted in the production of pNIPAm/NTBA fibres in the diameter range from 1 to 2 µm. Cell viability, proliferation and metabolic activity revealed that hADSCs were able to grow on the thermoresponsive scaffold. The cells were able to detach as an intact cell sheet in presence of MMC. Moreover, it was demonstrated that MMC, by a volume extrusion effect, enhances Collagen type I deposition, which is one of the main components of the ECM. Histological analysis revealed that in the presence of MMC the cells were able to self-assembled into three dimensional multi-layers. The cells were able to differentiate towards the osteogenic, adipogenic and chondrogenic lineage in the presence of MMC.

CONCLUSIONS: The pNIPAm/NTBA fibres were able to successfully sustain growth and detachment of ECM-rich tissue equivalents. We believe that replacement, repair and restoration of tissue function can be accomplished best using cells that create their own tissue-specific extracellular matrix with a precision and stoichiometric efficiency still unmatched by man-made devices.

ACKNOWLEDGEMENTS: Tendon Therapy Train-H2020-MSCA-ITN-2015-ETN (Grant no: 676338) and Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM) (Grant no: 13/RC/2073).

REFERENCES

[1] Satyam. et al., Adv Mater, 26.3024-3034,2014



Elastin-Like recombinamers functionalized with an angiogenic peptide attract vascular growth on encapsulated microparticles

A. Grosso[1], I. Gonzàlez de Torre[2], M. Herrero[2], M. Gonzàlez[2], Oliveira MB[3], JF. Mano[3], JC. Rodriguez-Cabello[2], N. Di Maggio[1], A. Banfi[1]
Presenting Author: Andrea Grosso, andrea.grosso@usb.ch
[1]Cell and Gene Therapy, Department of Biomedicine, Basel University Hospital, Basel, Switzerland [2]Bioforge, University of Valladolid, Valladolid, Spain [3]Department of Chemistry, CICECO - Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal

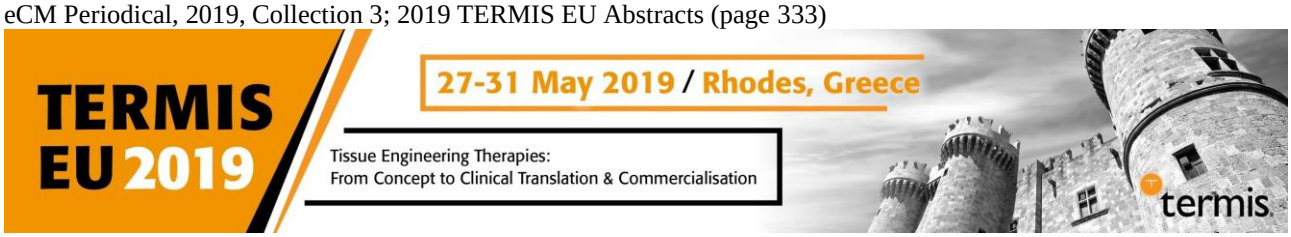
INTRODUCTION: Transplantation of donor-derived pancreatic islets or beta cells has the potential to treat diabetes, but is impaired by poor survival and immune rejection. The ELASTISLET project aims at developing a novel biocompatible encapsulation system, based on functionalized Elastin-Like Recombinamers (ELR), to both attract rapid vascularization and provide immune isolation, in order to achieve long-term viability and function of transplanted islets. Integration and survival of transplanted islets depends on the rapid vascularization of the capsule surface, while avoiding invasion with breakage of the capsule integrity. To promote vascularization, ELRs were functionalized with a pro-angiogenic peptide (QK peptide). Here, we sought at determine the optimal concentration of QK-peptide crosslinked to ELR hydrogels which ensure robust, normal and functional vascular growth.

METHODS: ELR hydrogels were functionalized with increasing concentrations of QK peptide (0%, 10%, 50% and 100%) Bulk hydrogels or ELR-coated gelatin microparticles (GMPs) were implanted into the posterior leg muscles (gastrocnemius) of SCID mice. Vascular growth was quantified after 1, 4 and 8 weeks in vivo by histological analysis.

RESULTS & DISCUSSION: Bulk ELR injections showed that both 50% and 100% concentrations of QK peptide significantly increased vascularization compared to controls, whereas the 10% dose was insufficient. Implantation of GMPs coated with 3 ELR layers confirmed that grafting with both 50% and 100% QK induced increased vascularization compared to both empty ELR and uncoated GMPs already after 1 week in vivo. Further, invasion of GMPs by mononuclear cells and blood vessels was not observed and vascular growth was limited to the GMP surrounding area. Remarkably, the vascular response induced by both 50% and 100% QK-ELR: 1) was stable over at least 8 weeks (no regression over time); and 2) did not show any tendency to penetrate the ELR coating and grow inside the coated GMPs.

CONCLUSIONS: QK-functionalized ELRs increase vascularization dose-dependently both in bulk and in the form of thin-layer capsules. This composition could be exploited as an encapsulation strategy in order to improve viability and function of transplanted pancreatic islets or other cell types.

ACKNOWLEDGEMENTS: Financial support was received from the EU H2020 grant ELASTISLET (646075).



AntimiR-221 activated hydrogels enhance cartilage repair by endogenous cells in vivo

A. Lolli[1], K. Sivasubramaniyan[1], M.L. Vainieri[1,2], N. Kops[1], A. Yayon[3], G.J.V.M. van Osch[1,4]
Presenting Author: Andrea Lolli, a.lolli@erasmusmc.nl
[1]Erasmus MC, Department of Orthopaedics, Rotterdam, The Netherlands [2]AO Research Institute, Davos, Switzerland [3]Procore Ltd., Nes Ziona, Israel [4]Erasmus MC, Department of Otorhinolaryngology, Rotterdam, The Netherlands

INTRODUCTION: Strategies for endogenous cartilage repair aim to treat cartilage injuries by targeting joint-resident progenitor cells in situ. Previously, we found that silencing microRNA (miR)-221 in human mesenchymal stromal cells (hMSCs) in vitro strongly enhanced their cartilage production in vivo [1]. We here aimed to develop a fibrin/hyaluronan (FB/HA) hydrogel delivery system to inhibit miR-221 in endogenous cells, for in situ guided cartilage repair.

METHODS: FB/HA was loaded with antimiR-221/lipofectamine complexes and the release of antimiR-221 was monitored over 14 days in vitro. FB/HA-mediated transfection of hMSCs was determined by flow cytometry and qRT-PCR. An animal cartilage defect model consisting of bovine osteochondral units implanted subcutaneously in nude mice was employed to assess endogenous cell invasion and cartilage production in vivo.

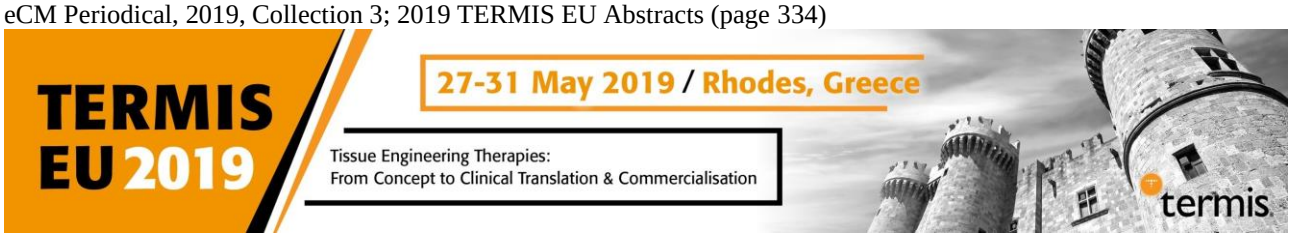
RESULTS & DISCUSSION: FB/HA functionalized with antimiR-221 strongly retained the inhibitor in vitro (>85% after 14 days). Culture of hMSCs into antimiR-221 loaded FB/HA led to highly effective cell transfection (~80%) and miR-221 knockdown (>99%), as validated by flow cytometry and qRT-PCR, respectively. When hMSCs were seeded on the surface of the constructs, hydrogel invasion by the cells led to miR-221 knockdown within 7 days. Interestingly, the use of lipofectamine was found to be not necessary for hydrogel-induced miR-221 silencing. When implanted in a cartilage defect in vivo, antimiR-221 loaded FB/HA enhanced cartilage production by endogenous cells, with a statistically significant 2-fold increase in the units displaying high cell infiltration. The newly formed tissue showed extensive production of collagen II and good integration with native cartilage and bone.

CONCLUSIONS: We developed a novel hydrogel-based system that induces miR-221 silencing in endogenous cells and enhances endogenous cartilage repair. Our study opens a new avenue for antimicroRNA therapy for cartilage injuries, providing a versatile methodology for in situ guided tissue repair.

ACKNOWLEDGEMENTS: The study was performed with the financial support of the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska Curie grant agreement No 642414.

REFERENCES

[1] Lolli A et al. Stem Cells. 2016; 34(7):1801-11



Towards the joint on a chip: A microfluidic osteochondral model

A. Mainardi[1,2], P. Occhetta[2], G.S. Ugolini[1], M. Ehrbar[3], I. Martin[2], A. Barbero[2], M. Rasponi[1]
Presenting Author: Andrea Mainardi, andrea.mainardi@polimi.it
[1]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy [2]Departments of Surgery and of Biomedicine, University Hospital Basel, Switzerland [3]Department of Obstetrics, University Hospital Zurich, Switzerland

INTRODUCTION: Osteoarthritis (OA) is the most prevalent musculoskeletal disease, but no pharmacological treatment is yet available. This lack in anti-OA drugs finds among its causes the inadequacy of present OA in vitro models. OA is a multi-factorial disease leading to pathological changes not only in the cartilage but also in the subchondral bone and in the calcified hypertrophic interzone interfacing these two tissues. Classic in vitro systems do not capture this complexity. Here, building on our recently developed microfluidic techniques [1, 2], we established a 3D microsystem capable of hosting two directly interfaced microtissues and suitable for induction of OA traits through chemical or mechanical stimulation. Specifically, we aimed at generating a cartilaginous layer in contact with a layer mimicking the hypertrophic interzone.

METHODS: Cartilaginous and hypertrophic layers were generated from human articular chondrocytes (AC) and human bone-marrow mesenchymal cells (MSCs), respectively. Cells were embedded in enzymatically formed and degradable poly-ethyleneglycol based hydrogels. Hydrogels' mechanical properties, cellular density and culture medium were optimized. Specifically, we aimed at a unique culture medium allowing for hypertrophic traits induction in the interzone layer, while maintaining a stable cartilaginous phenotype in the cartilage layer. RT-PCR analyses and immunofluorescence (IF) were conducted on separated and composite constructs at day 14.

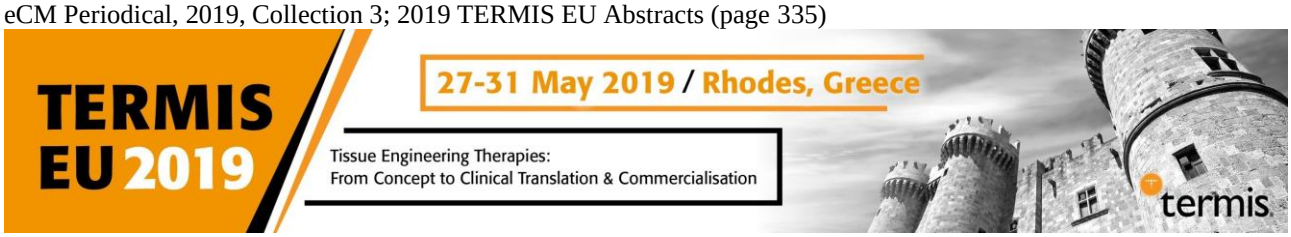
RESULTS & DISCUSSION: In single culture, supplementation of both chondrogenic (CH) and osteochondral (OCM), phosphate enriched, media resulted in MSC expressing significantly higher levels of hypertrophic markers (Col10a1, IHH, BSP2 and BMP2, Fig 1b) with respect to ACs. OCM resulted in MSC derived denser calcified tissues, while not changing the capability of ACs to deposit a matrix rich in Glycosaminoglycan and Collagen type-II. Direct coculture allowed reaching a double tissue construct with a direct interface. Present experiments aim at a deeper tissues' characterization, and investigation of changes occurring following chemical and mechanical induction of OA traits.

CONCLUSIONS: Results demonstrate the potential of the strategy to develop a microfluidic joint on a chip model with possible application in anti-OA drugs screenings.

ACKNOWLEDGEMENTS: SNF Grant project no 310030-126965.1 and 310030_149614/1. Fondazione Cariplo, grant no. 2018-0551.

REFERENCES

[1] Ugolini GS, et al. Adv. Healthc. Mater. 2017,1601170.
[2] Marsano A. et al. Lab Chip. 2016. 16: 599-610.



The effect of ageing on tenocytes cultured in the presence of xenogeneic vs allogeneic serum

A. Rampin[1,2,3], A. Tzora[1], I. Skoufos[1], N. Diakakis[2], N. Prassinos[2], D.I. Zeugolis[1,3,4]
Presenting Author: Andrea Rampin, andrearampin@vet.auth.gr
[1]Bioprocessing and Biotechnology Laboratory, University of Ioannina, Arta, Greece [2]School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece [3]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM) National University of Ireland Galway (NUI Galway), Ireland [4]Regenerative, Modular & Developmental Engineering Laboratory, NUI Galway, Ireland

INTRODUCTION: Tenocytes can be expanded in vitro to study their characteristics, to understand the mechanisms underlying tendinopathies, or else to obtain a suitable amount of cells for tissue engineering applications. However, tenocytes expansion in vitro has been associated to phenotypic drift (1). Moreover, their synthetic activity as a function of ageing in culture has not been completely assessed to date. The use of macromolecular crowding (MMC) reportedly increases the deposition rate of fibrillar collagens (2). Herein we compare xenogeneic and allogeneic sera as media supplements for the expansion of equine tenocytes (eTC) at three different sub-culture passages, in the presence or absence of MMC.

METHODS: eTC were isolated by migration method from the superficial digital flexor tendons (SDFT) of 5 donor horses. Cells were cultured in the presence of 10% fetal bovine (FBS) or equine (ES) serum. eTC were examined at P3, P6 and P9, in the presence or absence of 75 ug/mL of crowding agent carrageenan. Viability, metabolic activity and proliferation were assessed by calcein AM / ethidium homodimer staining, resazurin reduction and DAPI staining respectively. Collagen type I deposition was assessed by silver staining and immunofluorescence (IF). ECM composition was investigated by IF. MMPs synthesis was assessed by zymogaphy assay. All experiments were performed at days 3, 5 and 7 after seeding.

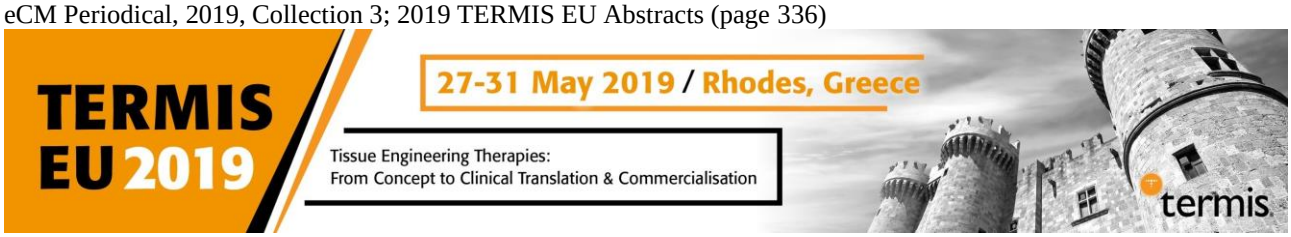
RESULTS & DISCUSSION: Silver staining and IF showed increased deposition of collagen type I in the presence of MMC and FBS. This result remained steady throughout the examined passages. In the absence of MMC the deposition of Collagen type III was constantly higher in ES, however no difference was observed in the presence of MMC. The highest deposition was observed in P6. Deposition of collagen type IV was generally higher with ES, was not affected by MMC and peaked in P6. Deposition of Collagen type V was dramatically increased by MMC, was higher with FBS and peaked at P6. Collagen type VI deposition was slightly increased by MMC, markedly increased with FBS in P6, while it dropped with ES at P9.

CONCLUSIONS: The present data suggest ES in the presence of MMC at early passages as a preferable choice for the production of extracellular matrix rich, xenogenic free cell sheets applicable to tendon tissue engineering. On the other hand, FBS is clearly preferable for eTC expansion to later subculture passages.

ACKNOWLEDGEMENTS: H2020, Marie Skłodowska-Curie Actions, Innovative Training Networks (676338), and Science Foundation Ireland, (15/CDA/3629 and 13/RC/2073).

REFERENCES

[1] Yao L et al. Tissue Eng 2006;12(7):1843-8
[2] Kumar P et al. Tissue Eng Part C Methods. 2015;21(7):660-70



Assessing cell motility on line and pillar microengineered surfaces: An automatic tool to detect and track cells in brightfield microscopy

A. Carvalho[1,2,3], T. Esteves[1,2], P. Quelhas[1,2], F.J. Monteiro[1,2,3]
Presenting Author: Angela Carvalho, angela.carvalho@ineb.up.pt
[1]Instituto de Investigação e Inovação em Saúde, U. Porto, Portugal [2]Instituto de Engenharia Biomédica, U. Porto, Portugal [3]Faculdade de Engenharia, U. Porto, Portugal

INTRODUCTION: When studying cell-surface interactions, it is of major interest to evaluate how different topographical stimuli can alter cell functions. Cell motility, essential in many biological processes, is known to be influenced by the surrounding environment. Several software's have been developed for analysis of cellular motility. However, application on periodic patterned substrates is ineffective, with interference of the patterns on the automatic tracking of cells [1-2]. In this work, we developed a MATLAB-based tool to automate the process of tracking unmarked cells on patterned surfaces.

METHODS: hMSCs were seeded on microengineered SiO_2 surfaces with line and pillar-shape patterns. A spectral Confocal Microscope Leica TCS-SP5 AOBS was used to record time-lapse images every 5 min for a period of 18h. Time-lapse videos were processed using the developed cell tracking tool to obtain information on cell migration distance, velocity, trajectory and persistence. Both automatic and manual modes were tested, as well as manual correction of incorrectly tracked cells.

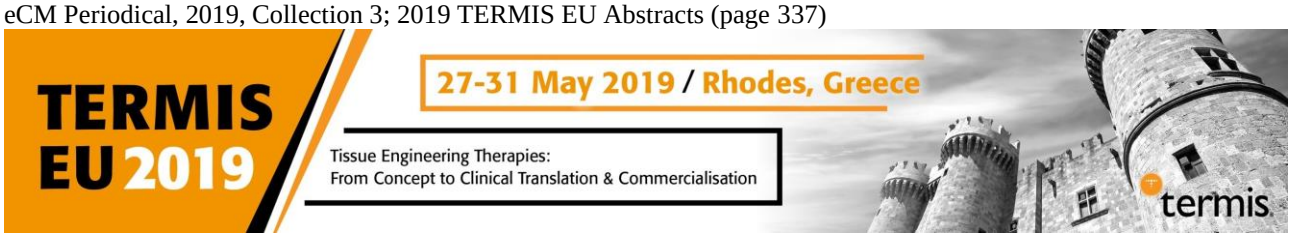
RESULTS & DISCUSSION: The software accurately detected and tracked cells on both micropatterned surfaces and a flat control. The automatic mode was significantly faster than the manual, also eliminating user subjectivity. Both micropatterned surfaces showed a significantly higher migration distance and velocity when compared to the flat surface. The trajectory and persistence results showed that cell movement was modulated by the underlying topography.

CONCLUSIONS: We proposed a novel approach to detect and track unmarked cells on patterned substrates, by removing the existing background patterns in brightfield microscopy. Cellular behaviour was evaluated in terms of trajectory, velocity, migration distance and persistence. Cells on both microstructured surfaces displayed improved motility parameters when compared with the flat control.

ACKNOWLEDGEMENTS: This works was financed by the project NORTE-01-0145-FEDER-000012, supported by NORTE 2020, under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and by Portuguese funds through FCT/MCTES in the framework of the project "Institute for Research and Innovation in Health Sciences" (POCI-01-0145-FEDER-007274) and in the framework of grant FCT/SFRH/BD/87624/2012.

REFERENCES

[1] I. Dang et al. Methods Mol Biol.2018; 1749:1-9.
[2] T. Esteves et al. Lecture Notes in Comp Sci. 2014; 8815: 123-131



3D bioprinted/electrospun bilayer biomembrane for regeneration of tendon synovial sheath

A. Imere[1], J.K.F. Wong[2], M. Domingos[3], S.H. Cartmell[1]
Presenting Author: Angela Imere, angela.imere@postgrad.manchester.ac.uk
[1]School of Materials, The University of Manchester, Manchester, UK [2]Plastic Surgery Research, Institute of Inflammation and Repair, The University of Manchester, Manchester, UK [3]School of Mechanical, Aerospace and Civil Engineering, The University of Manchester, Manchester, UK

INTRODUCTION: The clinical treatments for tendon lacerations can be compromised by adhesion formation [1,2] due to tendon synovial sheath disruption and aberrant healing. Hence, there is the need for development of novel anti-adhesion systems capable of allowing tendons to glide. One of the most promising approaches relies on the introduction of a biomembrane that acts as a physical barrier for adhesion-forming cells whilst regenerating tendon synovial sheath [3]. Here, we propose a novel hybrid approach that combines electrospinning and 3D bioprinting techniques to produce a bilayer biomembrane for the restoration of tendon synovial sheath integrity and the prevention of post-operative adhesions.

METHODS: Polymeric meshes were prepared by electrospinning, using a 10% w/v solution of poly(ε-caprolactone) (PCL) (M_n=50,000) in HFIP and setting process parameters at 1ml/h (flow rate), 20kV (voltage) and 20cm (needle-collector distance) for 1h. Mesh morphology was assessed via SEM and fibre diameter evaluated with ImageJ. Tensile testing of PCL meshes was performed using an Instron 3344 equipped with a 10N load cell at 10mm/min strain rate. 3T3 cells were encapsulated in PGD-α1 self-assembling peptide hydrogel (SAPH) (2×10^6 cells/ml) and cell-laden constructs were printed using a 3D Discovery. Cell viability was evaluated at 1h, 1, 3 and 7 days post-printing via LIVE/DEAD assay.

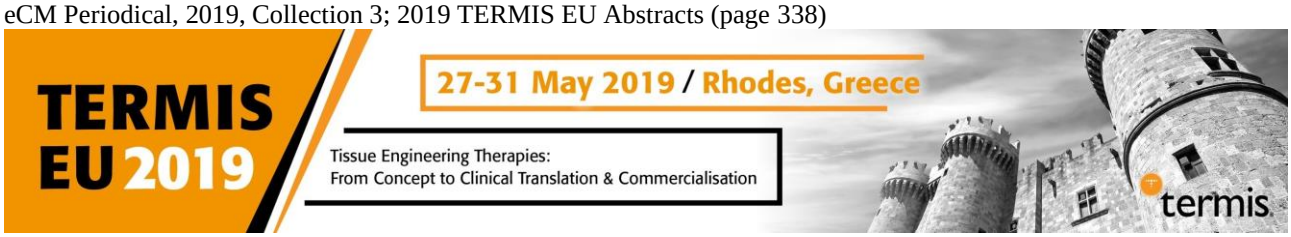
RESULTS & DISCUSSION: The spinning process produced PCL meshes with thin fibres (Mean = 0.254 µm) (Fig. 1A) and high mechanical properties (Fig. 1B). Cell-laden constructs with high structural integrity, reproducibility, geometrical and dimensional accuracy were obtained. Good cell viability and proliferation was detected at day 7 post-encapsulation (Fig. 1C). PCL mesh and cell-laden SAPH construct can be combined (Fig. 1D) to create a bilayer biomembrane for restoration of tendon lubrication and prevention of adhesions.

CONCLUSIONS: The thin fibres and high mechanical properties of electrospun mesh provide a physical barrier with good structural integrity. 3D bioprinting allows accurate spatial distribution without affecting cell viability. Moreover, in the proposed approach, ECM-mimicking SAPHs can potentially stimulate cells to produce HA for long-term lubrication.

ACKNOWLEDGEMENTS: The authors thank EPSRC & MRC for funding (EP/L014904/1).

REFERENCES

[1] Rawson S et al. MLTJ. 2013; 3:220–228.
[2] Wong JKF et al. Am. J. Pathol. 2009; 175: 1938–1951.
[3] Khanna A et al. Br. Med. Bull. 2009; 90:85–109.



Priming dental pulp stem cells from human exfoliated deciduous teeth with fibroblast growth factor-2 enhances mineralization within tissue-engineered constructs implanted in craniofacial bone defects

A. Novais[1,2], J. Lesieur[1], J. Sadoine[1], L. Slimani[1], B. Baroukh[1], B. Saubaméa[3], A. Schmitt[4], S. Vital[1,2], A. Poliard[1], C. Hélary[5], GY. Rochefort[1], C. Chaussain[1,2 Ω], C. Gorin[1,2 Ω]
Presenting Author: Anita Novais, anitarsnovais@gmail.com
[1]EA 2496 Pathologies, Imagerie et Biothérapies Orofaciales et Plateforme Imagerie du Vivant (PIV), Dental School, Université Paris Descartes Sorbonne Paris Cité, Montrouge, France [2]AP-HP Département d'Odontologie, Hôpitaux Universitaires PNVS, Charles Foix et Henri Mondor, Ile de France, France [3]Cellular and Molecular Imaging facility, Inserm US25, CNRS UMS 3612, Faculté de Pharmacie de Paris, Université Paris Descartes Sorbonne Paris Cité, Paris, France [4]Cochin Institute, Transmission Electron Microscopy Platform, INSERM U1016, CNRS UMR8104, Université Paris Descartes Sorbonne Paris Cité, Paris, France [5]Sorbonne Universités, CNRS, Collège de France, Laboratoire de Chimie de la Matière Condensée de Paris, 75005 Paris, France [Ω]Co last

INTRODUCTION: The craniofacial area is prone to trauma or pathologies often resulting in large bone damages. One potential treatment option is the grafting of a tissue-engineered construct seeded with adult mesenchymal stem cells (MSC). The dental pulp appears as a relevant source of MSC as dental pulp stem cells display strong osteogenic properties, and are efficient at bone formation and repair. FGF-2 and/or hypoxia primings were shown to boost the angiogenesis potential of dental pulp stem cells from human exfoliated deciduous teeth (SHED). Based on these findings, we hypothesized here that these primings would also improve bone formation in the context of craniofacial bone repair.

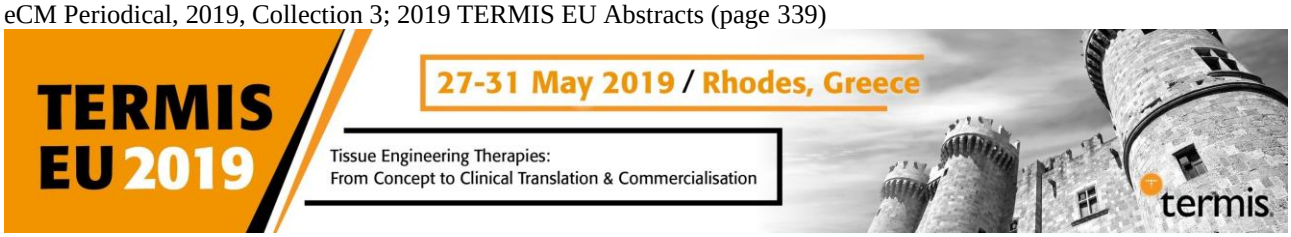
METHODS: SHED were seeded in dense collagen matrices [1] and cultured in osteogenic medium for 21 days after FGF-2 or hypoxia priming. The level of mineralized nodule formation was assessed by alizarin red and von Kossa staining, and micro-CT analysis. The osteogenic differentiation was evaluated by immunohistochemistry and Western blotting. Matrices were implanted in critical size bilateral calvarial bone defect in immunodeficient mice to assess in vivo bone formation.

RESULTS & DISCUSSION: We found that both hypoxic and FGF-2 primings enhanced SHED proliferation and osteogenic differentiation into plastically compressed collagen hydrogels, with a much stronger effect observed with the FGF-2 priming. After implantation in immunodeficient mice, the tissue-engineered constructs seeded with FGF-2 primed SHED mediated faster intramembranous bone formation into critical size calvarial defects than the other groups (no priming and hypoxia priming).

CONCLUSIONS: This study reveals that FGF-2 priming of tissue engineered constructs formed by dental pulp stem cells from human exfoliated deciduous teeth seeded within plastically compressed collagen scaffolds strongly enhances craniofacial bone regeneration.

ACKNOWLEGEMENTS: Financial support was received from University Paris Descartes, Fondation des Gueules Cassées and the National French agency for research (ANR PulpCell 2014-2018) for EA2496, and the Fondation de la Recherche Médicale for Plateforme d'Imagerie du Vivant Paris Descartes (FRM DGE20111123012).

REFERENCES: [1] Coyac BR et al. J Dent Res. 2013; 92:648-654.



3D-printed TPMS-based structures for bone regeneration

A. Diez-Escudero[1], H. Harlin[1,2], P. Isaksson[2], C. Persson[1]
Presenting Author: Anna Diez-Escudero, anna.diez@angstrom.uu.se
[1]BioMaterial Systems, Department of Engineering Sciences, Uppsala University, SE-751 21, Uppsala, Sweden [2]Solid Mechanics, Department of Engineering Sciences, Uppsala University, SE-751 21, Uppsala, Sweden

INTRODUCTION: Triply periodic minimal surfaces (TPMS) have recently been explored as promising geometries for bone replacement as they give the possibility to combine mechanical stability with high porosity and pore interconnectivity [1]. Furthermore, they have a mean curvature close to zero, just like trabecular bone. Additive manufacturing gives the possibility to produce these types of structures in a straightforward way. Here, a combination of such architectures with polylactic acid (PLA), a resorbable polymer, and fused deposition modelling was explored with the aim to provide scalable complex geometries optimized for bone tissue scaffolding.

METHODS: PLA filament of 2.85 mm was used to print the scaffolds using a 0.25 mm nozzle on a FDM Ultimaker 2+. Three porosities (35-50-65%) were generated through nodal equations for each geometry (Diamond, Gyroid, and Schwarz). Specimen dimensions were 12x24 mm^2, for mechanical testing (n=6), and 15x5 mm^2 (diameter x height) for cell cultures (n=3). Compression tests were performed at a displacement rate of 1mm/min. Osteoblast-like cells SaOS-2 (50.000 cells/ml) were seeded dropwise, and proliferation was measured after 1, 2 and 4 days. Tissue culture plastic styrene (TCPS) was included as control. Cells were examined after fixation in a scanning electron microscope (SEM).

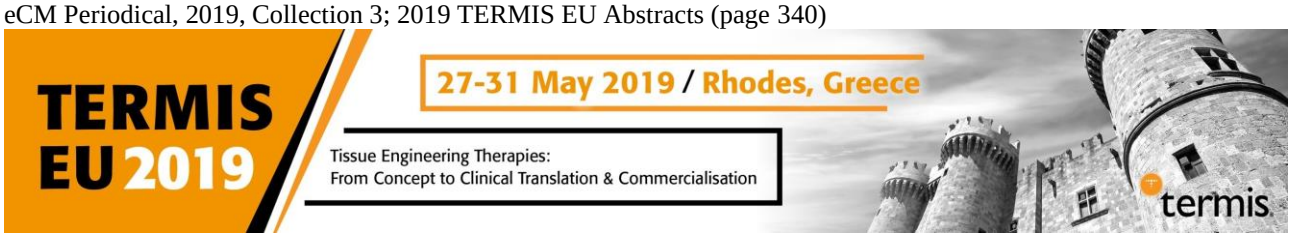
RESULTS & DISCUSSION: Young's modulus followed the following trend for 35% porosity: Schwarz (585 ± 28 MPa) > Diamond (421 ± 89 MPa) > Gyroid (278 ± 70 MPa). Scaffolds dropped 1% in modulus after increasing porosity to 50%, and 20-30% for 65% porosities. Cell proliferation was evaluated with respect to control at 1 day and expressed as percentage. Denser geometries showed higher adhesion after 1 day, however, cell proliferation was more pronounced as porosity increased. Cells were only observed inside pores regardless of the type of geometry, depicting a well spread morphology.

CONCLUSIONS: Gyroid, Diamond and Schwarz geometries gave Young's moduli in the range of cancellous bone (50-500MPa), even at a relatively high porosity (65%). Even though a lower number of adhered cells at 1 day were found on higher porosity scaffolds, the cell studies suggest that increasing the porosity favors cell proliferation at longer times (4 days) on TPMS scaffolds. These results encourage further studies on the effect of TPMS scaffold porosity as a trigger for cell differentiation.

ACKNOWLEDGEMENTS: The authors are grateful for financial support from the Göran Gustafsson Foundation.

REFERENCES

[1] Bobbert et al. Acta Bio. 2017; 53: 572-84



Fabrication and characterization of porous pectin-based scaffolds crosslinked by GPTMS for tissue engineering applications

A. Lapomarda[1], A. De Acutis[1], G. Fortunato[1], F. Biagini[1], F. Montemurro[1], C. Magliaro[1], C. De Maria[1], G. Vozzi[1]
Presenting Author: Anna Lapomarda, anna.lapomarda@gmail.commailto:
[1]Research Center E. Piaggio and Department of Information Engineering, University of Pisa, Pisa, Italy

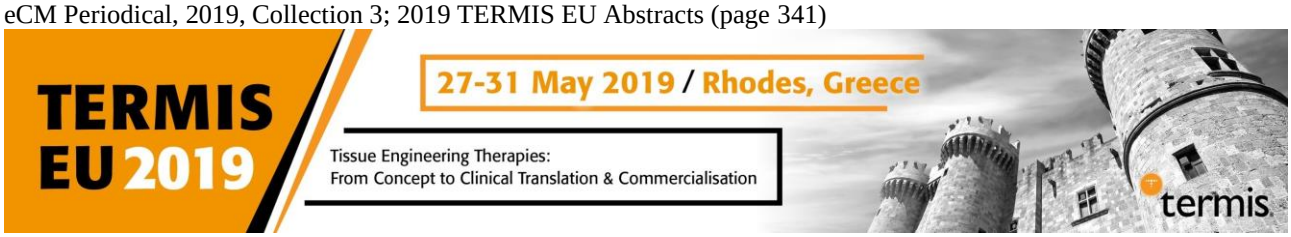
INTRODUCTION: Green and renewable biomaterials are gaining a great interest for the sustainable fabrication of biologically, physicochemically and mechanically relevant scaffolds for tissue engineering applications [1]. Pectins are heteropolysaccharides extracted from plant cell wall and represent one of the most abundant renewable polymers. Recently, pectins emerged as novel and potential candidate as tissue engineering-based biomaterials due to their biocompatibility and non-toxicity. In this work, the fabrication and characterization of porous pectin-based scaffolds chemically crosslinked by (3-Glycidyloxypropyl) trimethoxysilane (GPTMS) is presented.

METHODS: Low methoxyl pectin (from citrus peel, Herbstreith&Fox) was dissolved in Milli-Q water at 70°C to obtain a 4% (w/v) solution. GPTMS (Alfa Aesar) crosslinker was added to the pectin solution at a concentration of 3.68% v/v to improve its stability in aqueous media. The resulting solution was poured into cylindric molds (13 mm diameter x 10 mm height) and cooled at -20°C overnight to induce a phase separation. Then, the samples were freeze-dried at -60°C for 48 h to obtain porous matrices. The swelling degree (SD) of the pectin scaffolds (n = 3) was evaluated in Milli-Q water at 37°C at predefined time points for 24h. At each time point the SD was calculated as eq: $(W_s-W_0)/W_0$, where W_0 and W_s are the weight of the dry and swollen sample, respectively. All samples were subsequently dried at room conditions for 48 h, and the final dry weight (W_f) was measured to estimate the dissolution percentage (D) as eq: (W_0-W_f/W_0). Mechanical properties of the pectin samples (n = 3) were assessed by uniaxial compressive tests conducted with a Z005 Zwick/Roell testing machine equipped with a 100 N load cell at strain rate of 0.01 s^{-1}. To prevent the specimen disruption, a 30% maximum deformation was applied. The samples were fully swollen before the mechanical test in Milli-Q water at 37°C.

RESULTS & DISCUSSION: Porous scaffolds were obtained by crosslinking pectin with GPTMS. The SD of pectin scaffolds reached the plateau of 361 ± 47% after 1h. The D of the samples after swelling experiments was 5.5 ± 0.4 % The compressive modulus of pectin scaffolds was evaluated as the slope of the linear region of the stress-strain curve. The compressive modulus was 1.4 ± 0.3MPa.

CONCLUSIONS: A novel crosslinking method for obtaining porous pectin-based scaffolds is here shown. Based on the presented results, the proposed structures could be used as scaffolds with mechanical, swelling and degradation cues for tissue engineering applications. Further experiments are currently taking place to investigate the morphonology of the scaffolds. Moreover, the porosity and mechanical properties of the scaffolds will be modulated by changing pectin and GPTMS concentrations. Finally, cell experiments will be carried out in order to evaluate the cytocompatibility of the proposed chemically crosslinked scaffolds.

REFERENCES: [1] Jahangirian, H., et al. (2018) International journal of nanomedicine, 13, 5953.



The ECM niche in IPF directs cellular responses

L. Elowsson[1], A. Löfdahl[1], C. Müller[1], H. Isaksson[2], E. Åhrman[3], J. Malmström[3], G. Westergen-Thorsson[1]
Presenting author: Anna Löfdahl, anna.lofdahl@med.lu.se
[1]Lung Biology, Lund University, Lund, Sweden [2]Biomedical engineering, Lund University, Lund, Sweden [3]Infection Medicine Proteomics, Lund University, Lund, Sweden

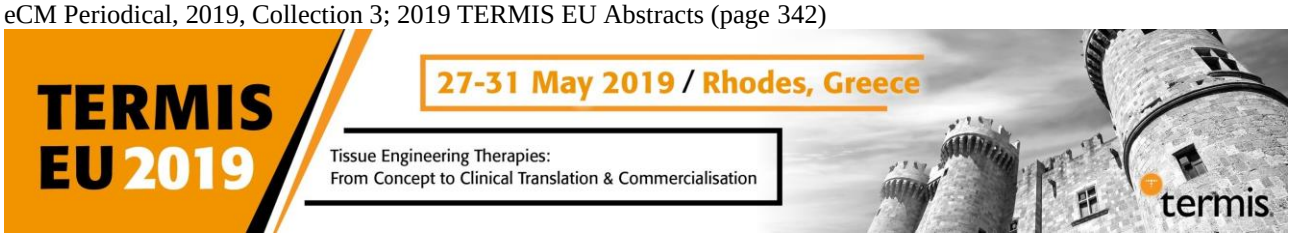
INTRODUCTION: In idiopathic pulmonary fibrosis (IPF) there is a massive build-up of extracellular matrix (ECM) causing a pathological remodeling of the lung with a progressive decline in lung function. We aim to describe how alterations in the tissues biomechanical properties and the composition of the ECM directs cellular responses in IPF.

METHODS: Human lung samples from healthy and IPF donors were decellularized to generate acellular scaffolds (350µm). The tissue was examined for density, stiffness and protein composition using mass spectrometry. Scaffolds, mounted on holders, were repopulated with healthy primary human fibroblasts and cultured up to 9 days. Using SILAC culture medium and mass spectrometry, the protein composition of newly synthesized heavy cellular proteins in relation to light scaffold proteins were analyzed. The organization and localization of proteins were examined with histology.

RESULTS & DISCUSSION: IPF scaffolds demonstrated an increased tissue density (2.6-fold increase $p<0.01$) and stiffness (2.4-fold increase, $p<0.01$) in comparison to healthy scaffolds. Several ECM proteins were differentially expressed between IPF and healthy derived scaffolds, which was also seen in newly synthesized proteins from fibroblasts cultured on IPF or on healthy scaffolds. Repopulated IPF scaffolds showed tendencies towards an increased production of collagen type VII, an anchoring fibril collagen, giving rise to structural alterations. Additionally, the expression of collagen type VII was visualized with antibody labeling. Production of agrin and Wnt-11 were decreased in IPF scaffolds, proteins that are associated with Wnt/β-catenin signaling, a pathway linked to IPF pathology.

DISCUSSION: We show that changes in composition and structural properties have strong impact on cellular responses. Collagen type VII is specifically involved in cell-ECM interaction. Agrin and Wnt-11 are involved in mechano-transduction pathways, which we are now further investigating with RNA analysis. Studying ECM-alterations in healthy and IPF lung tissue revealed important biomechanical and regenerative properties that can be used in the development of new therapeutic treatments.

ACKNOWLEDGEMENTS: Financial support was received from Swedish foundation for Strategic Research, Swedish Heart-Lung foundation, Swedish Research Council, Crafoord foundation, Royal Physiographical Society of Lund, Österlund Foundation.



Engineered co-culture strategies using chitosan-based hydrogels for facilitated articular cartilage regeneration

A. Scalzone[1], A.M. Ferreira[1], C. Tonda-Turo[2], G. Ciardelli[2], K. Dalgarno[1], P. Gentile[1]
Presenting Author: Annachiara Scalzone, A.Scalzone2@newcastle.ac.uk
[1]School of Engineering, Newcastle University, Claremont Road, NE1 7RU Newcastle Upon Tyne, UK [2]DIMEAS, Politecnico di Torino, Corso duca Degli Abruzzi 29, 10129 Turin, Italy

INTRODUCTION: Articular cartilage tissue lacks the ability to self-repair, thus there is need to explore new bio-approaches for its regeneration. This work aimed to develop a mesenchymal stromal cell (MSC) and human articular chondrocyte (hACH) co-culture for cartilage regeneration, utilizing an innovative 3D thermo-sensitive chitosan (CH)-based hydrogel, ionically cross-linked with β–glycerophosphate (BGP). The direct-contact co-culture system aimed to facilitate cell–cell interactions through surface receptors and enhance the transduction of the molecular signals coordinating chondrogenic differentiation.

METHODS: CH (3.6%w/v) was dissolved in 5 ml of 0.2 M HCl and BGP (1.1g dissolved in 2.2 ml of PBS) was added to obtain a 2.5%w/v final solution for the gelification. Hydrogels were characterised physico-chemically, morpholo-gically (ESEM) and mechanically. The cytocompatibility of an MSC-laden hydrogel was assessed with a Live/Dead assay. Immunofluorescence of collagen type II and CD44 expression, and a histological study on GAGs (AlcianBlue) and collagen (SiriusRed) production were carried out for up to 28 days on the co-culture of hACH spheroids on a MSC-laden hydrogel (A) and three controls: MSC spheroids on a MSC-laden hydrogel in chondrogenic media (B) or in chondrocyte growth media (C), and hACHs spheroid on an acellular hydrogel (D).

RESULTS & DISCUSSION: The sol/gel transition of the fabricated CH/BGP hydrogel occurred at 31-33°C within 5±1 minutes. A compressive and equilibrium Young's modulus were 37±4 kPa and 17.0±0.8 kPa respectively. ESEM images showed excellent porous structures with pore diameters < 30µm, suitable for cells attachment and nutrients diffusion. Finally, the cytocompatibility was demonstrated, as was the influence of hACH spheroids on new cartilage-like tissue formation.

CONCLUSIONS: The fabricated hydrogels presented fast sol/gel transition time that allowed to encapsulate MSCs, while their intrinsic porous structure and high uptake capability were vital for cell maintenance. This formulation offered a new and valuable platform for co-culturing cells and spheroids in close proximity, and the interactions between MSCs and hACHs resulted in enhanced chondrogenesis and cartilage production.

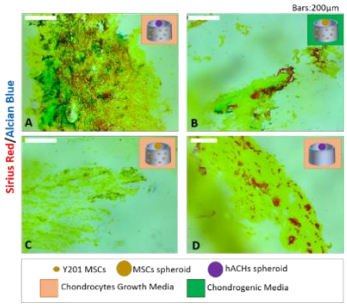
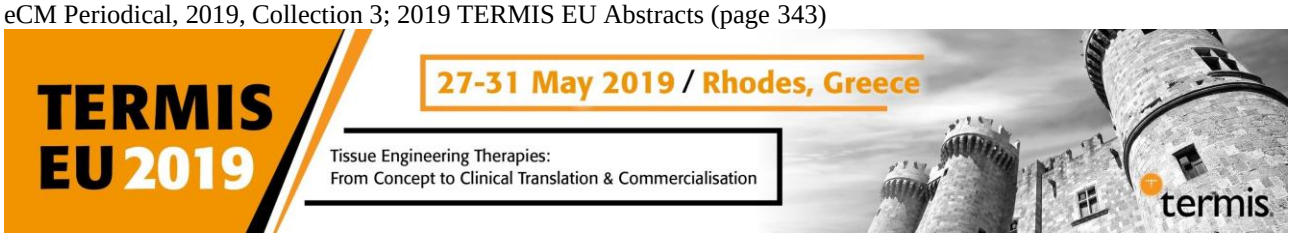


Figure 1: Hydrogel histological sections of co-culture system (A) and controls (B-D). Red (SiriusRed) indicates collagen and blue GAGs (AlcianBlue) production.



Defined media for the setup of vascularized adipose tissue

A.-C. Volz [1,2], L. Hack[1], F.B. Atzinger[1], P.J. Kluger[1,3]
Presenting Author: Ann-Cathrin Volz, ann-cathrin.volz@reutlingen-university.de
[1]Smart Biomaterials, Reutlingen University, Reutlingen, Germany [2] University of Hohenheim, Stuttgart, Germany [3]Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB), Stuttgart, Germany

INTRODUCTION: Animal derived sera are associated to high batch-to-batch variations and potential contaminations and are nevertheless frequently used in cell culture. In vitro engineered adipose tissue is still highly needed to replace lost, damaged or burned subcutaneous soft tissue. Additionally adipose tissue test systems could serve to analyze (patho)physiological processes or screen for potential drugs. In the current approach, we aimed to develop serum-free, defined media for the adipogenic differentiation of human adipose-derived stem cells (hASCs), the maintenance of mature adipocytes and the co-culture of adipocytes and microvascular endothelial cells (mvECs).

METHODS: HASCs were differentiated to mature adipocytes based on a developed defined medium for 14 days. Consecutive, adipocytes were maintained in a defined maturation medium up to day 42. Adipocyte functionality was evaluated by the release of leptin and glycerol, the expression of perilipin A and the incorporation of lipids. Additionally, adipocytes were co-cultured with mvECs in a defined co-culture medium. Next to the expression of CD31 and von Willebrand factor, the functional uptake of acetylated low density lipoprotein was analyzed for the mvECs. Vascular structure formation was evaluated quantitatively.

RESULTS & DISCUSSION: The chemically defined medium led to an efficient adipogenic differentiation of hASCs with 80% compared to serum containing controls. Even after the consecutive long-term maturation till day 42, adipocytes' cell-specific characteristics were preserved. On top, the co-culture medium facilitated the combined culture of adipocytes and mvECs until day 42 including the formation and maintenance of vascular-like structures.

CONCLUSIONS: Efficient in vitro generation of mature adipocytes out of hASCs based on defined differentiation and maturation media was implemented for the first time. This represents a fundamental achievement for the future application of in vitro adipose tissue in regenerative medicine. Additionally the transfer of the results to other approaches will be of great value for the tissue engineering branch in general.

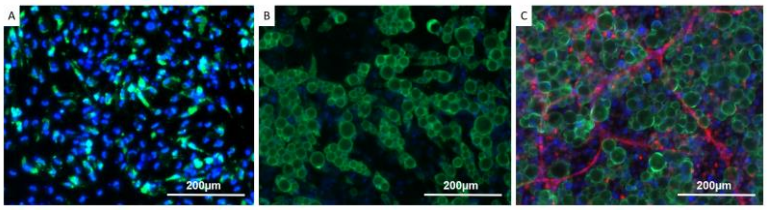
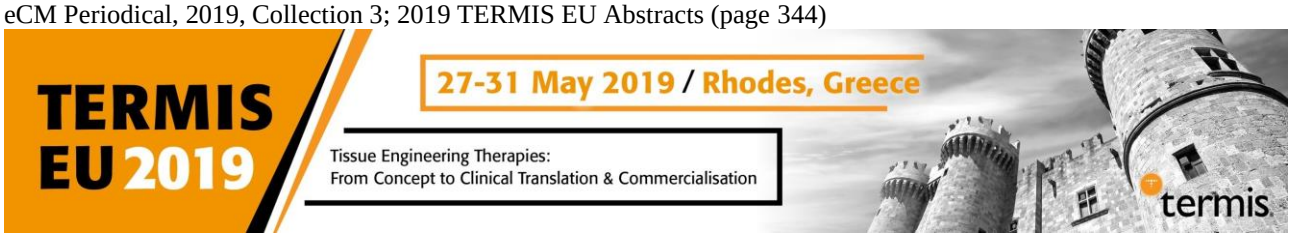


Figure 1: Immunofluorescence staining of defined differentiated adipocytes on day 14 (A) day 42 (B) and in defined co-culture with mvECs on day42 (C), perilipin A in green, CD31 in red, cell nuclei in blue (DAPI), scale bar = 200µm.

ACKNOWLEDGEMENTS: Federal Ministry of Education and Research (#03FH012PX4).



Wnt1-CRE-Rosa^{Tomato} fluorescent dental pulp stem cells to identify the cellular and molecular mechanisms underlying the repair of critical sized craniofacial defects

A.M. Collignon[1,2], J. Lesieur[1], E[1], Guilbert T[3], C. Chaussain[1,2] and A. Poliard[1]
Presenting author: Anne Poliard-Arias, anne.poliard@parisdescartes.fr
[1]EA 2496 Orofacial Pathologies, Imagery and Biotherapies, Dental School Faculty, University Paris Descartes, and Life imaging Platform (PIV), Montrouge, France [2]University Hospitals, AP-HP, Paris, France [3]Plateforme IMAG'IC, Institut Cochin, Inserm U1016-CNRS UMR8104, University Paris Descartes, Paris France

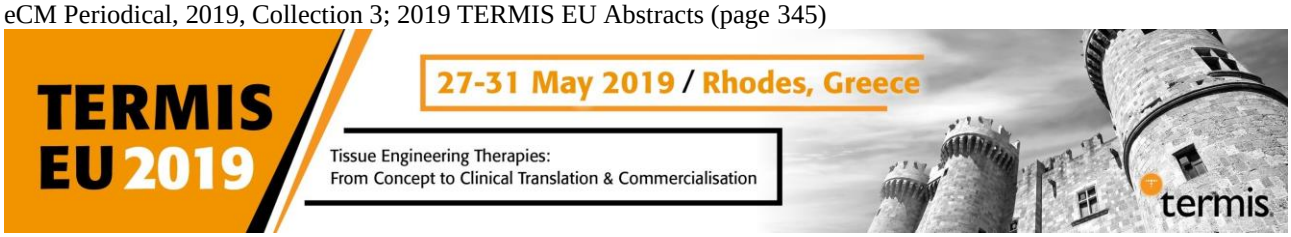
INTRODUCTION: Stem cells endowed with skeletogenic potentials seeded in specific scaffolds are considered attractive tissue engineering strategies for treating large bone defects. In the context of cranio-facial bone, mesenchymal stromal/stem cells derived from the dental pulp (DPSCs) have demonstrated significant osteogenic properties. Their neural crest embryonic origin further makes them a potential accessible therapeutic tool to repair cranio-facial bone. The stem cell's direct involvement in the repair process versus a paracrine effect is however still discussed. In addition, the best cell culture conditions for maximizing the in vivo DPSC osteogenic potential have to be established.

METHODS: To clarify these questions, we have followed and compared the fate of fluorescent murine DPSCs derived from PN3 Wnt1-CRE-Rosa^{Tomato} mouse molar (T-mDPSCs) cultured under normoxia and 5% hypoxia during the repair process of calvaria bone defects. Two symmetrical critical defects created on each parietal region were filled with either i) dense collagen scaffolds seeded with T-mDPSCs cultured under hypoxia, ii) dense collagen scaffolds seeded with T-mDPSCs cultured under normoxia iii) non cellularized scaffolds, or iv) no scaffold. Mice were imaged over a 3-month period by microcomputer tomography to evaluate the extent of repair and by bi-photonic microscopy to track T-mDPSCs. Histological and immunocytochemical analyses were performed in parallel to characterize the nature of the repaired tissue.

RESULTS & DISCUSSION: We show that depending on culture conditions (normoxia vs hypoxia): 1) T-mDPSCs are able to survive in the healing defect up to 3 months after implantation and 2) the mechanisms underlying the observed bone repair process are dependent on the DPSC culture conditions before implantation.

CONCLUSIONS: Altogether these observations are important for designing efficient therapeutic tissue engineering strategies for the treatment of large craniofacial bone defects. Further work should now focus on dissecting the signaling mechanisms underlying the mDPSCs fate choice upon implantation as well as the molecular dialogue initiated with the host cells.

ACKNOWLEDGEMENTS: Financial support was received from University Paris Descartes, Fondation des Gueules Cassées, the National French agency for research (ANR PulpCell 2014-2018) for EA2496 and Fondation pour la Recherche Médicale for Plateforme d'Imagerie du Vivant of Université Paris Descartes (FRM DGE20111123012) and DIM Île de France Biothérapies.



Unravelling the angiogenic properties of stem cells derived from the dental pulp and the apical papillae in vitro and in vivo

A. Bronckaers[1], P. Hilkens[1], J. Ratajczak[1], T. Struys, U[1]. Himmelreich[2], I. Lambrichts[1], E. Wolfs[1]
Presenting Author: Annelies Bronckaers, annelies.bronckaers@uhasselt.be
[1]Group of Morphology, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium
[2]Biomedical MRI, Department of Imaging and Pathology, University of Leuven, Leuven, Belgium

INTRODUCTION: Angiogenesis, the formation of capillaries from pre-existing blood vessels, is a fundamental physiological process in health and disease and key in tissue engineering. Insufficient blood vessel formation is implicated in a wide variety of diseases with devastating consequences such as myocardial infarction and stroke. Within the tooth, precursor cells with mesenchymal stem cell properties can be found, such as dental pulp stem cells (DPSC) and stem cells from the apical papilla (SCAP). Previously, our research group demonstrated their angiogenic potential in vitro and in the 'chicken chorioallantoic membrane (CAM) assay [1, 2]. The purpose of this study is to investigate whether DPSC and SCAP are able to establish functional blood vessel formation and blood flow in the mouse matrigel model and to identify the host-induced angiogenic pathways involved.

METHODS: Firefly luciferase (fluc) and the fluorescent protein GFP was stably overexpressed in both DPSC and SCAP by lentiviral transduction. Gelatin sponges loaded with matrigel alone or matrigel containing 1 x 10^6 $fluc^+$ DPSCs or $fluc^+$ SCAPs were subcutanously injected into nude mice. 7, 14 and 28 days xenografts were harvested for qPCR. Survival of transplanted cells was evaluated 7, 14 and 28 days after transplantation using bioluminescence imaging (BLI). Blood flow in the transplants was measured with arterial spin labeling (ASL) MRI. At the final time point of 28 days, samples were processed for ultrastructural analysis.

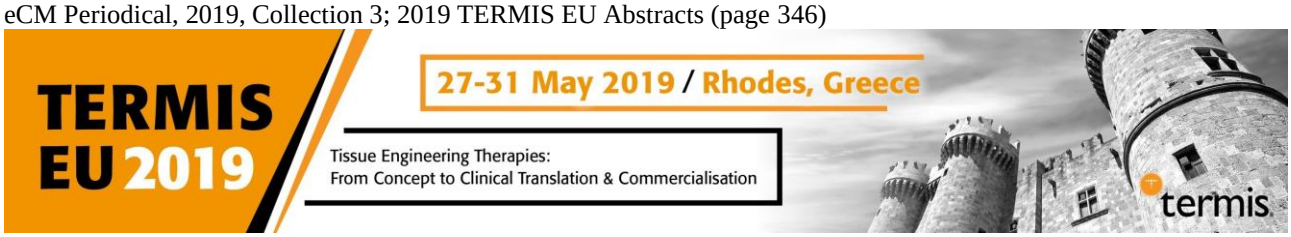
RESULTS & DISCUSSION: DPSC and SCAP showed remained viability and persistent after transplantation as confirmed with BLI. ASL MRI indicated an increased blood flow in gelatine sponges of both stem cell types compared to control sponges on 28 days but not 7 or 14 days after transplantation. This was supported by an increased number of blood vessels in the electron microscopy samples as well as increased mouse CD31 gene expression. qPCR analysis showed elevated levels of mouse angiogenic genes such as Ang-1 and VEGF on 14 and 28 days after transplantation in all constructs loaded with stem cells compared to controls. Increased levels of human CD31 was not observed, indicating DPSC and SCAP do not transform into endothelial cells but attract host endothelial cells to induce functional blood vessel formation.

CONCLUSIONS: Both DPSC as well as SCAP induce angiogenesis in a paracrine fashion, suggesting that they have a high therapeutic potential in pathologies correlated with inadequate angiogenesis such as stroke, myocardial infarction and chronic wounds.

ACKNOWLEDGEMENTS: This research was supported by research grants of the FWO (Research Foundation – Flanders- grant number 1266015N, 1522518N and G0A7514N)

REFERENCES

[1] Bronckaers A et al. PLoS One 2013;8(8):e71104
[2] Hilkens P et al. Stem Cell Res 2014;12(3):778-90



Protease-resistant peptide-grafted bioceramics for bone tissue engineering

A. Zamuner[1], P. Brun[2], H. Elsayed[1], E. Bernardo[1], M. Dettin[1]
Presenting Author: Annj Zamuner, annj.zamuner@phd.unipd.it
[1]Department of Industrial Engineering, University of Padova, Padova, Italy [2]Department of Molecular Medicine, University of Padova, Padova, Italy

INTRODUCTION: Bio-ceramic foams, as wollastonite/diopside (W/D), are obtained from preceramic polymers containing micro-sized filler powders. This process presents limited processing temperature and microstructural homogeneity, resulting in foams that mimic porous internal structure of human cancellous bone [1]. Recent discovery of short peptides able to communicate with the biological environment are opening new avenues toward the manufacture of osteoinductive materials. We demonstrated that a nona-peptide (HVP) derived from Vitronectin sequence is able to enhance human osteoblast adhesion through an osteoblast-specific mechanism [2]. In this study, polymer-derived silicate foams were covalently and selectively functionalized with a retro-inverted dimeric analogue of HVP (D2HVP) designed to increase ionic interactions with cellular Glico-Amino-Glycans (GAGs) avoiding enzymatic degradation of HVP peptide in serum-containing media [3]. Bioceramic scaffolds were then embedded with an ionic-complementary self-assembly peptide (SAP), EAK, to promote cell viability on foam-scaffolds' surface. Moreover, W/D scaffolds were functionalized with EAK bonded to a pro-angiogenic sequence G7TIM (mapped on Timosine-ß4). In vitro and in vivo bioassays were carried out on differently functionalized W/D foams.

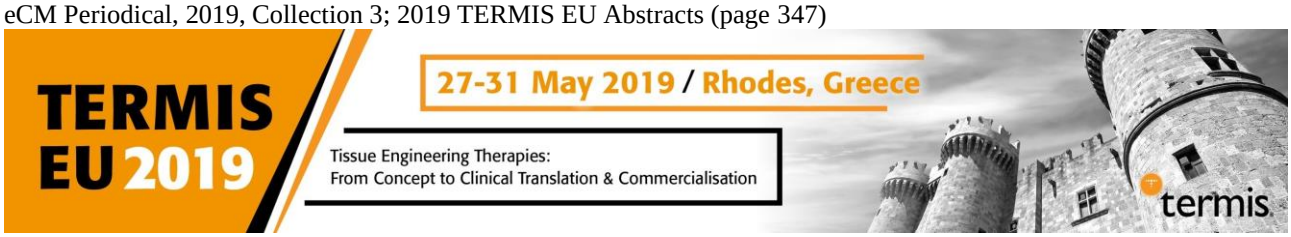
METHODS: W/D foams were prepared as reported in Fiocco et al. [1]. W/D was selectively and covalently functionalized with D2HVP, and treated with 0.15% w/v EAK solution, or 0.15% w/v EAK enriched with 5×10^{-5}M of EAKG7TIM. Primary h-osteoblasts were used for in vitro bioassays. In vivo assays were performed inserting samples/controls under-skin in a murine model.

RESULTS & DISCUSSION: A compressive strength test revealed no significant decrease in foams' mechanical properties after functionalization treatment. In vitro results confirmed the absence of cytotoxicity at 4 and 6 days. D2HVP functionalized foams gave a dramatic proliferation increase (more than 15 times) at 6 days in respect to the controls (silanized foams and W/D functionalized with a non-adhesive peptide) and enhanced mRNA specific transcript levels coding IBSP, VTN, RUNX2 and SPP1 at 2 days. In vivo bioassays proved that all functionalized foams were able to stimulate the process of ectopic mineralization, whilst silanized foams (control) didn't develop any degree of mineralization. W/D functionalized with D2HVP, EAK and EAKG7TIM showed higher Md.S/Po.S (mineralized surface/pores surface) and greater vascularization, with a higher number of blood vessels within the individual pores.

CONCLUSIONS: The synergistic effect between bioceramic structure/composition and specific covalent conjugation of a protease-resistant osteoblast-specific adhesive peptide, combined with the properties of SAPs and their ability to deliver bioactive molecules as pro-angiogenic sequences, represents a potentially useful approach for the production of a next generation scaffold for bone tissue engineering.

REFERENCES

[1] Fiocco L. et al., Materials. 2015;8:2480-2494.
[2] Brun P. et al., Acta Biomater. 2013;9:6105-6115.
[3] Zamuner A. et al., Bioact. Mater. 2017;2:121-130.



Multi-scale architectured scaffold for the regeneration of large bone defects via endochondral ossification

A Petersen[1], H Leemhuis[2], C Gayer[3], M Tortorici[1], A Herrera[1], I Heschel[2], GN Duda[1]
Presenting author: Ansgar Petersen, ansgar.petersen@charite.de
[1]Julius Wolff Institute and Berlin-Brandenburg Center for Regenerative Therapies, Charité – Universitätsmedizin Berlin, Germany [2]Matricel GmbH, Herzogenrath, Germany [3]Fraunhofer Institute for Laser Technology ILT, Aachen, Germany

INTRODUCTION: Endochondral ossification (EO) has been discussed as a promising strategy for bone defect regeneration [1]. We have shown previously that a soft collagen-based biomaterial with a channel-like pore architecture was able to induce EO in vivo [2]. Here, we present the development of a mechano-hybrid scaffold (MHS) [3] that overcomes contradictory requirements for mechanical stiffness on the cell vs. the tissue length-scale by the incorporation of a 3D-printed support structure.

METHODS: MHS were produced by: 1. Design of the support structure; 2. Production by selective laser sintering; 3. Immersion of the support structure into the collagen dispersion, unidirectional freezing and freeze-drying; 4. Crosslinking and sterilization of the MHS. Mechanical compression tests and structural characterization (SEM and second harmonic imaging [SHI]) were performed. Migration of mesenchymal stromal cells (MSCs) into the material was analyzed and the formation of extracellular matrix (fibronectin collagen-I) by fibroblasts was studied time-dependently.

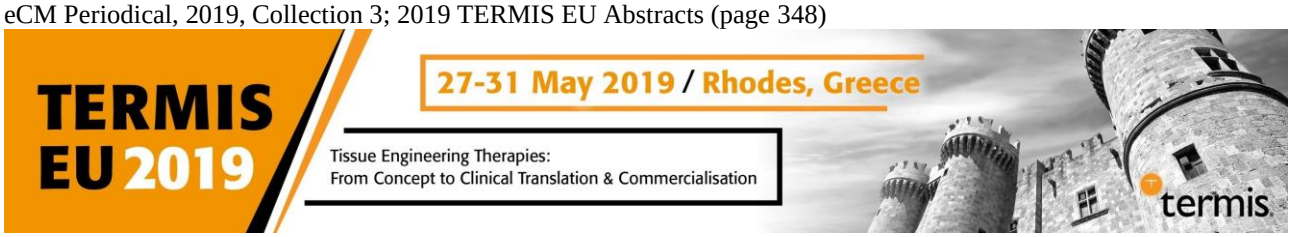
RESULTS & DISCUSSION: SEM and SHI showed that the incorporation of the support structure had no effect on pore size distribution and pore alignment of the collagen guiding structure. The stiffness of the MHS was controlled by the support structure that could be varied between 1.8±0.2MPa and 12.5±1.1MPa by design modification at constant porosity. By incorporating the support structure the scaffold stiffness increased by more than 3 orders of magnitude (from low kPa for collagen to MPa-range for MHS). While no differences in MSC migration into the materials were observed, in vitro culture revealed that the support structure stabilized the collagen pore walls against cell-induced deformation leading to an aligned cell and ECM-network.

CONCLUSIONS: MHS are a novel multi-scale approach in which mechanical characteristics can be tuned independently at the micro-scale (cell-level) and the macro-scale (tissue-level). Furthermore, MHS allow to extend the principle of scaffold-guided endochondral ossification towards large animal models and thus represent a promising strategy for a translation into the clinical settings.

ACKNOWLEDGEMENTS: The authors acknowledge financial support by the German Federal Ministry of Education and Research (grants no. 13XP5048A-D).

REFERENCES

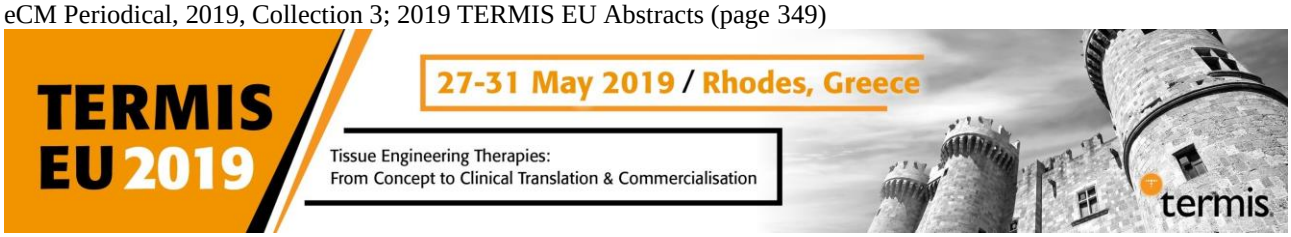
[1] Thompson et al. (2015), TERM 9(8): 889-902.
[2] Petersen et al. (2018), Nat. Commun. 9:4430.
[3] Patent pending: DE102016007931.2; PCT/DE2017/000183



Tissue Engineering: The impact, growth, and future of the field. Highlighting the 25th anniversary of the journal

A.G. Mikos[1], J.P. Fisher[1], K. Schenke-Layland[1], H. Shin[1], J.A. Jansen[1], and X. Wang[1]
Presenting Authors: Antonios G. Mikos, mikos@rice.edu and Katja Schenke-Layland, kschenkelayland@me.com
[1]Tissue Engineering, Mary Ann Liebert, Inc., publishers, New Rochelle, New York, USA

The field of tissue engineering and regenerative medicine has grown exponentially over the past three decades. Through developments in bioprinting, optogenetics, and the encouraging advancements in biomaterials and various other new technologies, the progressions in the field continue to make a positive impact in clinical applications and patient outcomes, as well as the overall reshaping of modern healthcare. Tissue Engineering is proudly celebrating its 25th year in publication in 2019 and would like to highlight and celebrate the many advancements and achievements of the field and the journal during this talk. With every major milestone publication year, the Journal Editorial Office strives to publish the most significant research in the field as a tribute to the TERMIS and tissue engineering community. We will present the vast achievements the field has successfully executed, as well as talk to new and exciting advances including the importance of specific research that will be highlighted in upcoming special issues. Please join us in celebrating the impact, growth, and future of the field. Tissue Engineering is the official journal of TERMIS and is proud to share this milestone anniversary with all TERMIS members and leadership.



Screening extracellular matrix environments by quantifying matrix deformations and cellular forces around angiogenic sprouts

A. Shapeti[1], M. Vaeyens[1], J. Barrasa Fano[1], A. Jorge-Peñas[1], C. Steuwe[2], M. Roeffaers[2], A. Ranga[1], H. Van Oosterwyck[1,3]
Presenting Author: Apeksha Shapeti, apeksha.shapeti@kuleuven.be
[1]Biomechanics Section, Department of Mechanical Engineering, KU Leuven, Leuven, Belgium [2]Center for Surface Chemistry and Catalysis, KU Leuven, Leuven, Belgium [3]Prometheus, div. Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium

INTRODUCTION: Cellular force generation is underlying angiogenesis, the process of growing new blood vessels, and is mediated by both chemical and physical (mechanical) cues. We report here on a three-dimensional (3D) live invasion assay utilizing natural collagen and synthetic polyethylene glycol (PEG) matrices to characterize the relationship between cellular traction-induced deformations and extracellular matrix (ECM)-properties during angiogenic invasion. These workflows allow for a mechanical characterization of the angiogenic response to different extracellular microenvironments as a tool for biomaterial screening and disease modeling.

METHODS: Human umbilical vein endothelial cells (HUVECs) were cultured in a monolayer on substrates containing 200 nm fluorescent fiducial markers for live imaging of matrix deformations in 3D. Up to 2.5 hour time lapse confocal fluorescence microscopy imaging was performed after 24 hours of invasion and image stacks were registered to quantify 3D deformations. Angiogenic invasion was quantified in 3D across substrates with varying adhesion ligand binding, stiffness and degradability.

RESULTS & DISCUSSION: Maximum collagen deformations were of the order of 2 to 10 microns. Spatial analyses of displacement fields around in vitro sprouts show displacement maxima near sprout tips and bases. Computer simulations allow estimating sprout forces, based on calculated displacements and elastic properties. Sprout protrusions exert local forces of the order of 20 nN. Displacements vary depending on substrate properties. Stiffness dependent invasion in PEG is found to be further modulated by ligand binding and degradability.

CONCLUSIONS: Our methods quantify displacements and cellular forces around angiogenic sprouts. Our analyses can help in characterizing the mechanical component of the angiogenic response to varying microenvironments allowing for a material screening technique.

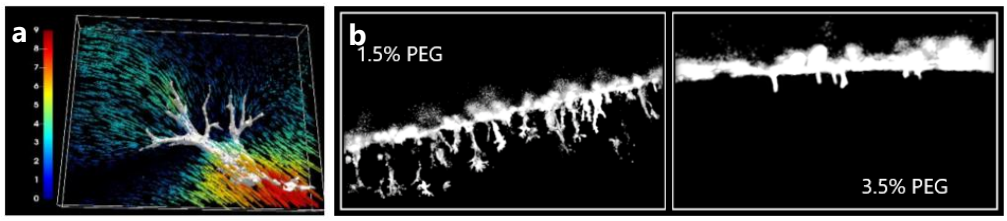
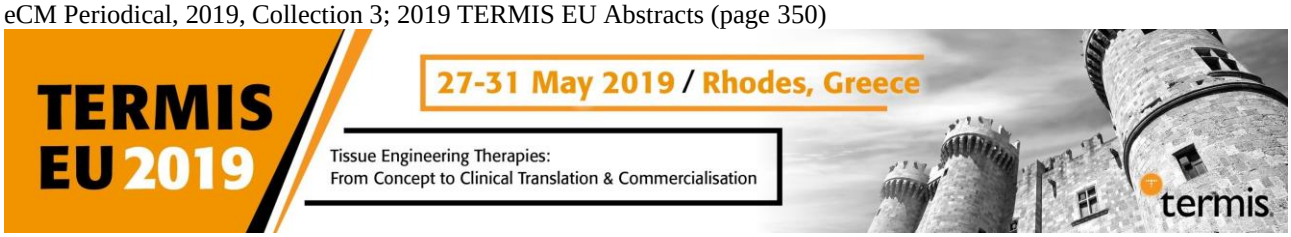


Figure 1: a. Displacement field around an angiogenic sprout b. Angiogenic invasion in PEG is stiffness dependent

ACKNOWLEDGEMENTS: FP7/2007-2013/ERC Grant n° 308223, FWO-Vlaanderen (grant n° G.0821.13), FWO and EU Horizon 2020 (MSCA n° 665501), and FWO SB fellowship (Apeksha Shapeti, application n° 1S68818N).



Computer assisted analysis for quantification of macrophages response to biomaterials

A. Uka[1], A. Halili[1, 2], Xh. Polisi[1], N. E. Vrana[3]
Presenting Author: Arban Uka, auka@epoka.edu.al
[1]Computer Engineering Department, Epoka University, Tirana, Albania [2]Department of Information Technology, AleksandërMoisiu University, Durrës, Albania [3]Protip Medical, Fundamental Research Unit, Strasbourg, France

INTRODUCTION: Noninvasive imaging techniques have taken precedence over other analysis techniques and this has increased the amount of data considerably. Macrophage response to biomaterial surface morphology and surface chemistry can be better analyzed from images, since other measurable biochemical tests might show a smaller response to the macrophage stress conditions. Under prolonged interaction of a macrophage with a given biomaterial, an analysis of macrophages conducted using imaging techniques may exhibit stressful condition associated with shape change at a faster rate (in time), whereas the chemical response that is acquired using other sensors may continue to be the same [1]. The quantifiable chemical response such as MTT tests, or electrochemical measurements may be less sensitive to the amount of the cytotoxicity of the biomaterial.

METHODS: Medical image analyses rely on tedious preprocessing, image enhancement, and morphological segmentation. In this work, band pass filters, Notch filters, Gabor filters and texture analysis were used and their performance was evaluated based on the type of the input image. Different conditions included: i) patterned surfaces with different groove sizes (2□m, 20 □m) comparable to the macrophage size, ii) unpatterned surface, iii) clustered macrophages, iv) non-clustered macrophages, v) macrophages with high variance in size and shape, and vi) different macrophage density per unit area. When the macrophages have parameters with a small variance a single algorithm can be employed, whereas in cases where the macrophages have high variance more steps are needed.

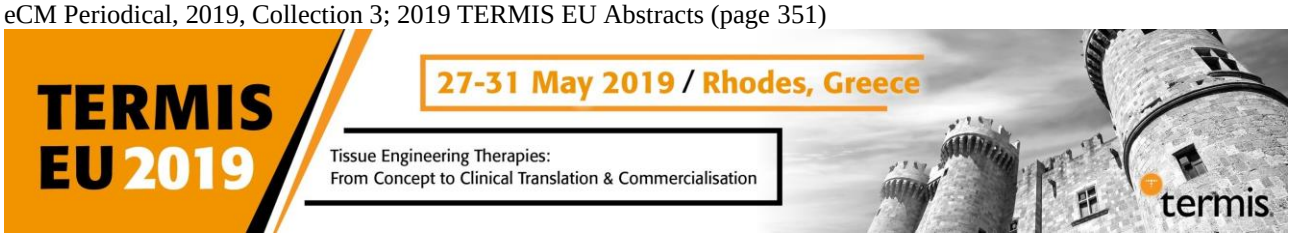
RESULTS & DISCUSSION: Different parameters of macrophages were analyzed using the techniques mentioned above. Notch filters proved to be very effective to remove the effect of the micropatterned surfaces on the images. Gabor filters were carefully adjusted to select macrophages with different spatial extents, and the selectivity in the orientation allowed to neglect features that resulted from spurious noise and objects that represented cellular debris. The texture analysis could distinguish macrophages that were affected by the cytotoxicity of the environment (where cytoplasm collapsed around the nucleus) from the unaffected ones (cytoplasm was distinct).

CONCLUSIONS: Different macrophage conditions such as size, shape, macrophage density and host surface morphology were analyzed. Due to different parameters used on the algorithm, further analysis is required in order to make the algorithm fully automated.

ACKNOWLEDGEMENTS: Financial support was received from H2020 under Grant Agreement No 760921.

REFERENCES

[1] Uka A et al. IEEE EUROCON 2017 – 17th Int. Conf. on Smart Techn.Ohrid, Macedonia.10.1109/EUROCON.2017.8011080.



Evaluating the effect of acidic microenvironment on human induced pluripotent stem cells-derived cardiomyocytes

A. Ferrini [1,4], L. Zwi-Dantsis [1], N. Rosenthal [4,5], M.M. Stevens [1,2,3]
Presenting Author: Arianna Ferrini, a.ferrini14@imperial.ac.uk
[1]Department of Materials, Imperial College London, London, UK [2]Department of Bioengineering, Imperial College London, London, UK. [3]Institute of Biomedical Engineering, Imperial College London, London, UK [4]National Heart and Lung Institute and BHF Centre for Research Excellence, Imperial College London, London, UK [5]The Jackson Laboratory, Bar Harbor, ME USA.

INTRODUCTION: One of major events occurring after a myocardial infarction is an acidification of the tissue, with the pH dropping to 6.5-6.8, due to the switch from aerobic metabolism to anaerobic glycolysis. It has been reported that cardiac differentiation of murine embryonic stem cells is strongly inhibited by extracellular acidic pH [1]; however, the effects of pH on cardiac differentiation of human induced pluripotent stem cells (hiPSCs) remain to be investigated. The aim of this study was to assess the effects of low pH on cardiac differentiation of hiPSCs and on differentiated hiPSC-Cardiomyocytes (CMs) in terms of cell viability, CMs yield and gene expression profile. Four pH values were tested: control (pH 7.7), pH 6.8 (value of the ischemic myocardium), pH 7.1 (intermediate between acidic and physiologic) and pH 7.4 (standard recommendation for cell culture).

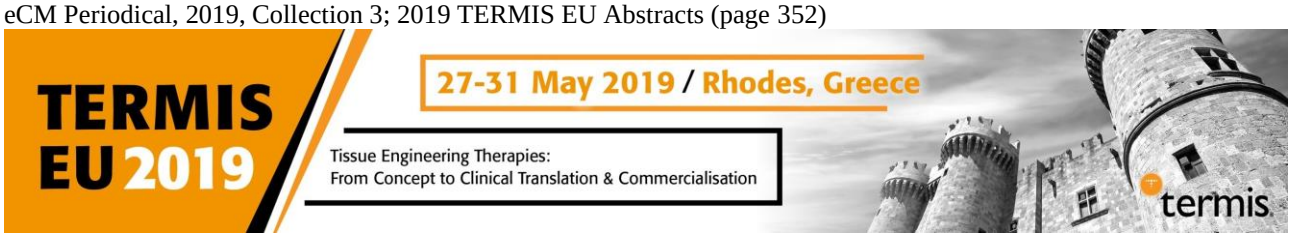
RESULTS & DISCUSSION: Results from MTT assay and LIVE/DEAD® staining showed that pH 6.8 significantly reduces hiPSC-CMs metabolic activity and viability during differentiation, respectively. The CMs yield quantified by flow cytometry analysis at day 21 was 83.8 ± 1.3% in the control, while it was halved at pH 6.8. Next, gene expression analysis showed a significant down-regulation of cardiac-specific markers in the cells cultured in acidic pH. The same results were confirmed when the pH was lowered at later timepoints, on beating hiPSC-CMs. Finally, we demonstrated that Insulin like Growth Factor-1 (IGF-1) is able to rescue the low pH phenotype, and by adding IGF-1 at concentrations of 10 and 50 ng/mL the yield and viability of the CMs cultured in pH 6.8 were increased back to the control values.

CONCLUSIONS: Taken together, these findings demonstrate that acidic pH significantly affects cardiac differentiation of hiPSCs and already differentiated hiPSC-CMs. A better understanding of the sensitivity of these cells to the acidic ischemic microenvironment is crucial when considering their potential delivery for cardiac repair purposes. Moreover, we showed that the pro-survival growth factor IGF-1 is able to protect the cells from the acidic pH, making it a good candidate for the encapsulation in biomaterial strategies for cell therapy.

ACKNOWLEDGEMENTS: Arianna Ferrini is supported by a British Heart Foundation Centre of Research Excellence PhD Studentship (RE/13/4/30184).

REFERENCES

[1] Teo, A., Mantalaris, A. & Lim, M. Influence of culture pH on proliferation and cardiac differentiation of murine embryonic stem cells. Biochemical Engineering Journal **90**, 8-15, doi:10.1016/j.bej.2014.05.005 (2014).



Engineering scaffold-based advanced therapies that accelerate bone repair by recapitulating juvenile-derived stem cell mechanoresponse in adult

A. Gonzalez-Vazquez[1,2,3], R. Raftery[1,2,3], G. Chen[4], DJ. Murray[5], FJ. O'Brien[1,2,3]
Presenting Author: A. Gonzalez-Vazquez, agonzalez@rcsi.ie
[1]Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland [2]Trinity Centre for Bioengineering, Trinity College Dublin, Ireland [3]Advanced Materials and BioEngineering Research Centre, Ireland [4]Department of Physiology and Medical Physics, Centre for the Study of Neurological Disorders, RCSI, Ireland [5]National Paediatric Craniofacial Centre, Temple Street Children's University Hospital, Ireland.

INTRODUCTION: Biomimetic collagen and nanohydroxyapatite (coll-nHA) scaffolds enhance bone repair in small defects[1]. However, large defects require the incorporation of additional biomolecules. Having previously identified c-Jun N-terminal kinase 3 (JNK3) as a mechanically-activated modulator of the superior osteogenic potential of MSCs derived from children in comparison to adults[2]. Our aim was to design a therapeutic nanomedicine able to facilitate the intracellular activation of JNK3, and incorporate this into a coll-nHA (coll-JNK3*free) scaffold to recapitulate the extraordinary regenerative potential of children in adults.

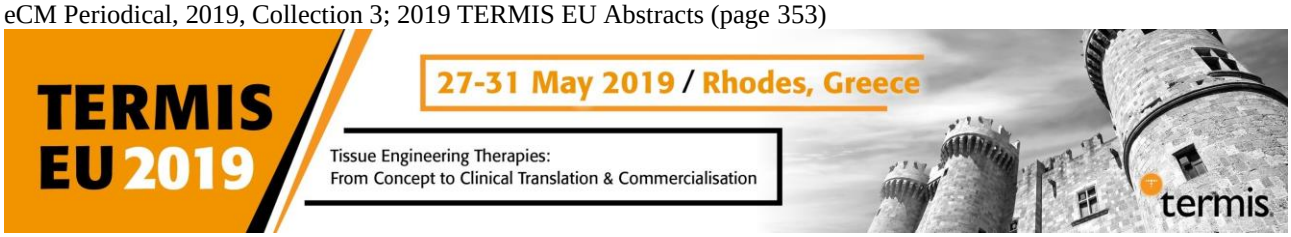
METHODS: A JNK3 activator (JNK3*) was made by complexing a JNK3 activator mini-peptide with in-house nHA nanoparticles (NPs), previously used for miRNA delivery. Effects of JNK3* on the metabolic activity and the osteogenic potential of C-MSCs (10-11y) and A-MSCs (20-30y) were evaluated. JNK3* was then, incorporated into the 3D collagen-nHA delivery platform (coll-JNK3* scaffold) and effects were assessed in vitro and in vivo.

RESULTS AND DISCUSSION: no toxic effects of the JNK3* doses (0-5 µM) were observed in either C-MSCs or A-MSCs. When treated with the 5 µM dose, JNK3* uptake reached 90% in C-MSCs and 88% in A-MSCs, and promoted osteogenesis in A-MSCs toward the superior levels previously observed in non-treated C-MSCs. When JNK3* was incorporated into the coll-nHA -coll-JNK3*- the nanomedicine was taken up by A-MSCs as early as day 3 and their osteogenic capacity was significantly increased. When this coll-JNK3* activated scaffold was implanted for two weeks in critical-sized rat calvarial defects, the infiltration of host cells positive for MSC markers CD44, CD105, and CD90 was observed, as was the upregulation of pathways associated with osteogenesis and angiogenesis. Furthermore, significantly enhanced bone volume fraction and histological analysis proved accelerated bone healing in coll-JNK3* treated defects compared to JNK3*free scaffolds or untreated empty defects as early as 4 weeks post-implantation.

CONCLUSIONS: we have successfully developed an off-the-shelf 3D-scaffold-based therapeutic delivery platform, which enhances the osteogenic capacity of MSCs by facilitating JNK3 activation, ultimately showing we can recapitulate children's regenerative capacity and accelerate bone repair in adults.

ACKNOWLEDGEMENTS: Funded by the HRB (HRA_POR/2014/569), Children's fund TSCUH (RPAC-2013-06).

REFERENCES[1] Curtin C et al. Adv.Mat. 2012; 24:749-54. [2] Gonzalez-Vazquez A et al. Acta Biomaterialia. 2017; 53:59-69.



Osteogenic differentiation of the human placenta derived amniotic membrane using Simvastatin and BMP9

A. Ahmadi[1,2†], S.S. Ebadi[1,2†], N Halvaei[1,2], R. Mazloomnejad-Meybodi[1,2], H. Niknejad[1]
Presenting Author: Armin Ahmadi, arminahmadi@sbmu.ac.ir
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

INTRODUCTION: The amniotic membrane (AM) is considered to be an ideal means for clinical applications due to providing a substantial source of stem cells alongside with having great scaffold-like characteristics including biocompatibility, low immunogenicity, anti-inflammatory and anti-microbial effects [1],[2]. Simvastatin, a previously well-known in vitro osteogenic factor, has been recently shown to induce osteogenesis and vasculogenesis in vivo concurrently [3]. In the present study, we conducted an in vitro experiment investigating the in situ osteogenic differentiation of the amnion cells cultured in an osteogenic medium and compared the results with a control medium.

METHODS: Human placentas were collected after ceasarian sections and the AM was removed by blunt dissection. After being washed by PBS, biopsies were made and placed in 12 well plates containing Control Medium or Osteogenic Medium. The Control Medium comprised of a combination of traditionally used osteogenic regulators consisting of Dexamethasone, vitD3, Ascorbic acid and beta-Glycerophosphate. In the Osteogenic Medium, BMP9 and/or Simvastatin, two molecules which we previously hypothesized to have synergistic osteogenic properties, were added as treatment group. The medium was changed every other day. In order to study the effects of the medium, we measured the amounts of osteogenic specific genes and proteins as well as cell viability. We also evaluated Calcium content as an indicator of late phase mineralization.

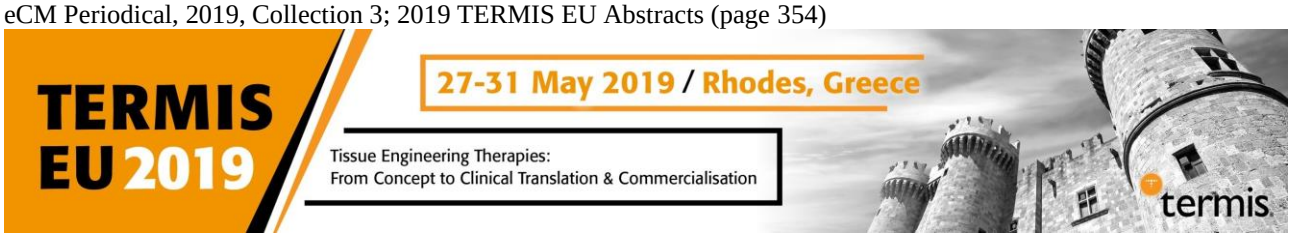
RESULTS & DISCUSSION: The results showed that simvastatin and BMP-9 have a noticeable impact on expression of osteogenic specific genes and proteins. The synergistically effects of simvastatin and BMP-9 make them an appropriate candidate for differentiation of stem cells.

CONCLUSIONS: We presented here promising results of a new protocol of in situ osteogenic differentiation of amniotic cells by simvastatin and BMP-9 with possible applications in the clinic.

ACKNOWLEDGEMENTS: The authors would like to thank the operation room personnel of Erfan Hospital for their assistance in this research. Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran. We would also like to thank Dr. Tahereh Tayebi for her many assists.

REFERENCES

[1] Niknejad H et al. Eur Cell Mater. 2008 Apr 29;15:88-99
[2] Lindenmair, A et al. Biomaterials, 2010 Nov;31(33):8659-65
[3] WL Yu et al. Sci. Rep. 2017;7:4412



Skeletal muscle healing mediated by ELR-based hydrogels

A. Ibáñez-Fonseca[1], D. Gorbenko del Blanco[1], S. Santiago-Maniega[2], B. Catalán-Bernardos[3], A.J. Álvarez-Barcia[4], M. Alonso[1], H.J. Aguado[2], J.C. Rodríguez-Cabello[1]
Presenting Author: Arturo Ibáñez-Fonseca, aibanez@bioforge.uva.es
[1]BIOFORGE Lab, University of Valladolid – CIBER-BBN, Valladolid, Spain [2]Servicio de Traumatología y cirugía ortopédica, Hospital Clínico Universitario de Valladolid (HCUV), Valladolid, Spain [3]Servicio de Neurofisiología clínica, HCUV, Valladolid, Spain [4]Servicio de Investigación y Bienestar Animal, University of Valladolid, Valladolid, Spain

INTRODUCTION: Skeletal muscle injuries often result in an incomplete regeneration due to the formation of a fibrotic scar tissue [1]. In this work, we evaluated the suitability of different ELR-based hydrogels for the healing of a volumetric muscle loss (VML) defect in rats.

METHODS: ELRs were recombinantly biosynthesized and chemically modified (if needed) as previously described [2]. A VML rat model was used to study the healing of tibialis anterior (TA) skeletal muscles with ELR-based hydrogels. A total of 19 adult male Wistar rats (n = 38 TAs) were randomized in four groups: untreated control (n = 11), biodegradable chemically crosslinked ELR hydrogel (n = 11), biodegradable physically crosslinked SELR hydrogel (n = 11) and no injury control (n = 5). TA muscles were harvested after 2 and 5 weeks and studied through histology (H&E, Picrosirius Red for collagen) and immunofluorescence (anti-M1 (CCR7) and M2 (CD206) macrophages).

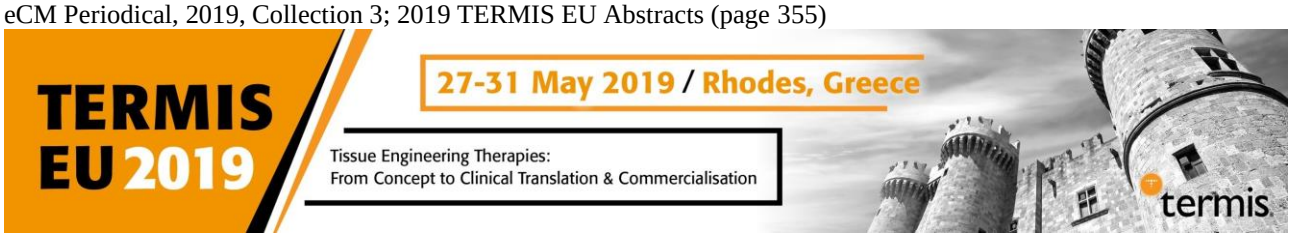
RESULTS & DISCUSSION: H&E stained slices showed no significant differences among groups in a gross view, with all the samples showing almost full muscle repair. However, when studied thoroughly, we found a difference in the density and size of the newly formed myofibers, being the ELR-treated samples more similar to the healthy ones, suggesting a better muscle healing. Furthermore, collagen content increased from 2 to 5 weeks in the non-treated samples and those treated with the physical ELR hydrogel ($p < 0.05$), which is characteristic of fibrotic non-functional tissue. Finally, the samples obtained from animals treated with both ELR-based hydrogels showed a higher content of M2 macrophages than the non-treated control at 2 weeks ($p > 0.001$), which indicates that the initial inflammatory response may resolve more beneficially, also suggesting a better muscle healing with the use of the ELR-based hydrogels.

CONCLUSIONS: Overall, these results suggest the potential beneficial effect of biodegradable ELR-based hydrogels in muscle healing, which implies a great advance in the field. Further studies should focus in the assessment of the regeneration of skeletal muscle in larger animal models, where a more critical defect can be created and other methods can be used to evaluate the functional recovery of skeletal muscle.

ACKNOWLEDGEMENTS: The authors are grateful for the funding from the European Commission (NMP-2014-646075, MSCA-ITN-2012-317304), the Spanish Government (PCIN-2015-010, MAT2015-68901-R, MAT2016-78903-R, MAT2016-79435-R), Junta de Castilla y León (VA015U16) and Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León

REFERENCES

[1] Li Y et al. Curr Opin Orthop. 2001; 12:409-415.
[2] Rodríguez-Cabello JC et al. Methods Mol Biol. 2012; 811, 17



Therapeutic roles of SDF-1α gene-activated collagen scaffolds for wound healing applications

A.L. Laiva[1], F.J. O'Brien[1-3], M.B. Keogh[1,4]
Presenting Author: Ashang L. Laiva, luwanglaiva@rcsi.ie
[1]Tissue Engineering Research Group, Dept of Anatomy, Royal College of Surgeons in Ireland, Ireland [2]Trinity Centre for Bioengineering, Trinity College Dublin, Ireland [3]Advanced Materials and Bioengineering Research Centre, Royal College of Surgeons in Ireland and Trinity College Dublin [4]Dept of Biomedical Science, Royal College of Surgeons in Ireland, Bahrain

INTRODUCTION: Gene-activated scaffolds (GAS) offer promising wound healing therapeutic capabilities by functional activation of infiltrated cells at the implant site [1]. Using our self-derived pro-angiogenic GAS composed of the gene stromal-derived factor-1α (SDF-1α) loaded onto collagen scaffolds (SDF-1α GAS), we sought to investigate the therapeutic effects on wound healing cells (endothelial, Schwann and adipose-derived stem cells; healthy and diabetic).

METHODS: A collagen-GAG scaffold was soak-loaded with polyethyleneimine-SDF-1α plasmid polyplex (N/P 10) to develop SDF-1α GAS. Experiments were performed by seeding either 1) human umbilical vein endothelial cells (HUVECs) 2) human Schwann cells (SCs) 3) human adipose-derived stem cells (ADSCs). qRT-PCR and immunofluorescence was conducted at days 7 and 14. Specifically, a VE-cadherin (soluble) ELISA and an angiogenesis proteome profiler were employed to monitor endothelial growth and secretion of angiogenic factors in HUVECs and ADSCs respectively. Cultures on gene-free scaffolds were used as control.

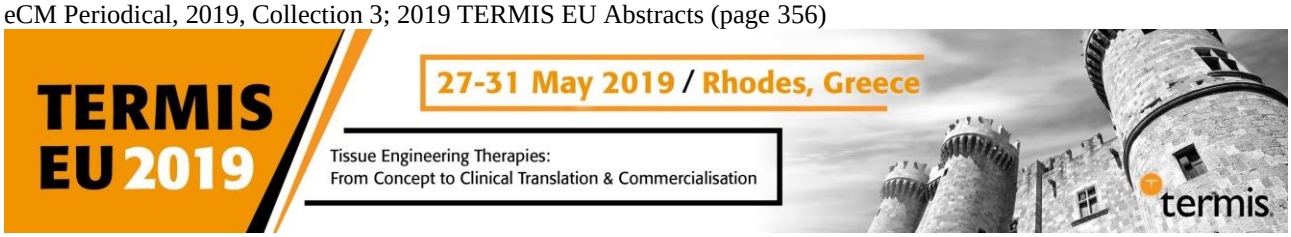
RESULTS & DISCUSSION: In HUVECs, SDF-1α GAS significantly suppressed the release of soluble junctional adhesion molecule, VE-cadherin at day 7 ($p<0.05$) and 10 ($p<0.0005$). Immunoreactivity also showed a significantly ($p<0.05$) high expression for VE-cadherin at day 14. In SCs, SDF-1α GAS induced transient activation of SDF-1α, VEGF-A and CXCR7 at both mRNA and protein level. SCs on SDF-1α GAS displayed significantly high, early expression of functional receptor p75NGFR ($p<0.0005$) and pro-neurogenic laminin ($p<0.005$). ADSCs (healthy and diabetic) also showed similar activation of SDF-1α, VEGF-A and CXCR7 as that of SCs. ADSCs on SDF-1α GAS displayed strong anti-fibrotic response (~2x) with significantly ($p<0.005$) low deposition of collagen VI by day 7.

CONCLUSIONS: Overall, SDF-1α GAS displayed enhanced therapeutic effects by (1) preserving endothelial integrity in morphogenic HUVECs, by (2) enhancing the differentiation of SCs towards a functional phenotype and by (3) inducing an early anti-fibrotic response in both healthy and diabetic ADSCs.

ACKNOWLEDGEMENTS: RCSI Dilmun PhD scholarship

REFERENCES

[1] Raftery et al. Biomaterials. 2017; 149:116-27



Fabrication of thick cell sheet constructs via nanofibrous membrane interlayers

A. Tevlek[1], H.M. Aydin[1,2]
Presenting Author: Atakan Tevlek, atakantevlek@gmail.com
[1]Bioengineering Division, Institute of Science, Hacettepe University, Ankara, Turkey [2]Centre for Bioengineering, Hacettepe University, Turkey

INTRODUCTION: Thick tissue engineering constructs produced by layering of cell sheets suffer from poor gas and nutrient exchange and consequently fail[1]. In this study, we report a facile method to produce viable thick cell sheet constructs using poly (L-lactic acid) (PLLA) fibrous membrane interlayer between MC3T3-E1 cell sheets.

METHODS: PLLA membranes approximately in 150±20 µm thickness were obtained by using an electro spinning set-up. Then, MC3T3-E1 cell sheets were obtained via Nunc UpCell™. The multilayered structures were formed by laminating two cell sheets onto one PLLA membrane and a final constructs were composed of a total of 3 PLLA membranes and 4 cell sheets. There was no PLLA membrane implemented in the construction of control group. All constructs were characterized in terms of cell viability and cell death while ALP activity, collagen synthesis and sGAG quantity were examined only in PLLA/cell sheet construct.

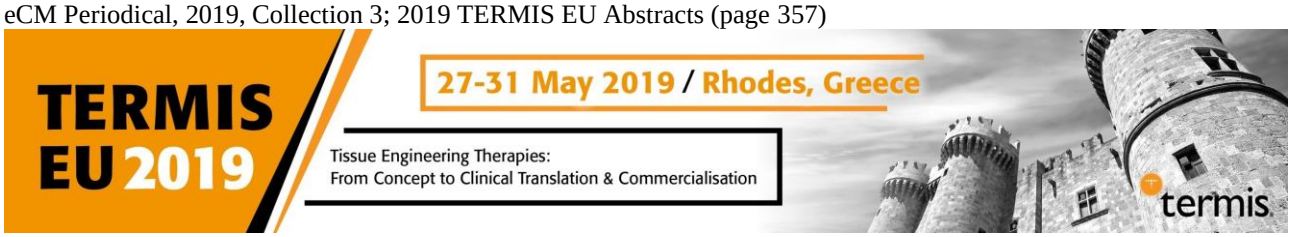
RESULTS & DISCUSSION: The superior viability of multilayered structures containing PLLA membranes was demonstrated during the culture period. Three-dimensional tissue conjugates consisting of cell-biomaterial combinations, which can be transferred in a practical manner and have a wet state thickness of 1 mm, were achieved. According to the results of cell viability by Alamar blue assay, the PLLA/cell sheet structures were observed to survive and proliferate during culture. However, the control group exhibited a decreased cell viability during the same culture period. Lactate Dehydrogenase (LDH) analysis confirmed the acute cell death in the control group. Increased sulfated glycosaminoglycan (sGAG) and collagen synthesis was recorded in the multilayered constructs. In addition, the success of the survival rate of the constructs was verified with live/dead staining.

CONCLUSIONS: A porous nanofiber membrane interlayer used in the fabrication of cell sheet constructs of this study yielded a thick and viable product with enhanced transport, mechanical and handling characteristics.

ACKNOWLEDGEMENTS: The authors would like to thank TUBITAK for 2211-C Domestic Priority Areas PhD scholarship program and BMT Calsis Co.

REFERENCES

[1] Sekine et al., J Biochip Tissue Chip, S1, 2011.



3D bioprinting of hydrogel constructs with microchannels and vasculature

L. Ouyang[1,2,3], A. Moore[3], M.M. Stevens[1,2,3]
Presenting Author: Axel Moore, axel.moore@imperial.ac.uk
[1]Department of Materials, UK [2]Department of Bioengineering, UK [3]Institute of Biomedical Engineering, Imperial College, South Kensington, SW7 2AZ, London, UK

INTRODUCTION: Despite the recent progress in 3D bioprinting custom cell-laden structures, it is still challenging to engineer well-defined and interconnected vasculature into hydrogels. Conventional bioprinting processes (e.g., printing lattice structure) usually compromise the printability because of the inadequate gelation of bioinks during layer-by-layer deposition. Here we introduce a non-porous approach to fabricate3D hydrogel constructs with tubular porosity to potentially construct vasculature.

MATERIALS & METHODS: Gelatin is used as printable and sacrificial bioink due to its reversible thermo-sensitivity. Gelatin methacryloyl (GelMA) is used as a matrix bioink after photo-crosslinking. Gelatin and GelMA are simultaneously printed side-by-side for each layer, creating a relatively flat surface for the next layer deposition. We hypothesize that this method enhances the printability of the matrix bioink and enables the pre-loading of endothelial cells during printing.

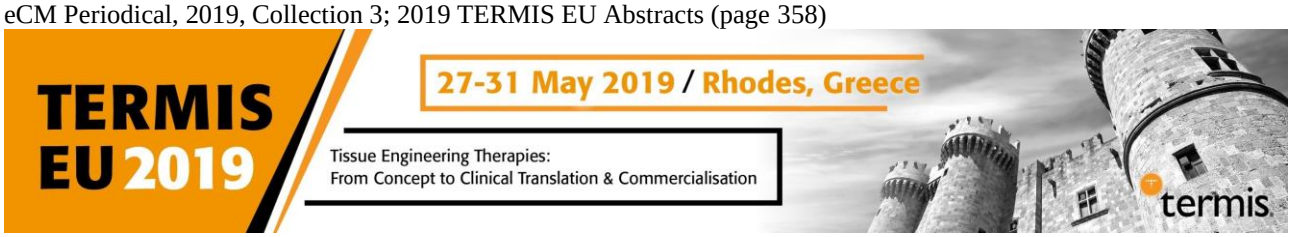
RESULTS & DISCUSSION: Though viscous bioinks are usually used, in the conventional bioprinting process it is still easy to encounter a fusion of layers and thus poor porosity features on the side. Our approach benefits from the structural support obtained when printing two (potentially more) bioinks side-by-side. There is no concern for layer fusion and the tubular lumen can be generated after removing one of the bioinks. To engineer vascular tissues, endothelial cells are commonly post-seeded into the lumen area which is very likely to result in poor seeding efficiency and homogeneity, especially when dealing with complicated 3D channel networks. The presented approach herein enables the encapsulation and migration of viable endothelial cells in the sacrificial phase and thus the formation of an endothelial monolayer during culturing. Furthermore, this approach could be used to fabricate perfusable hydrogel constructs, with the potential for engineering thick tissue.

CONCLUSIONS: We developed a versatile 3D bioprinting approach to fabricate cellularized vascular constructs in one-pot without the need of post cell seeding. The method enables good printability of constructs with tubular porosity and in situ endothelization.

ACKNOWLEDGEMENTS: The authors acknowledge the financial support from EPSRC Programme Grant (EP/P001114/1).

REFERENCES

[1] Ouyang L et al. Adv Mater. 2017, 29(8):1604983
[2] Place ES et al. Nat Mater. 2009, 8(6): 457-470



The physiochemical property and cell types of cancer stroma affect therapeutic response of anticancer drugs

B. Kundu[1,2], V. Brancato[1,2], J.M. Oliveira[1,2,3], V.M. Correlo[1,2,3], R.L. Reis[1,2,3], S.C. Kundu[1,2]
Presenting Author: Banani Kundu, bkundu@i3bs.uminho.pt
[1]I3B's, [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães 4805-017, Portugal
[3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Chemotherapeutic resistance is a topical question in longstanding treatment of cancer. Therefore, deciphering the basis of chemotherapeutic resistance of cancer cells has evident significance. The cellular response of cancer cells is pre-dominantly modulated by different cell types present in tumor-associated stroma and extra-cellular matrix [1, 2]. At the time of metastasis, cancer cells involve in remodeling the extra-cellular matrix; results in change in pore sizes, interconnectivity and stiffness of cancer stroma [3]. Hence, the critical role of individual cells and extra-cellular matrix in resistance of chemotherapeutics needs to be unwound. Mesenchymal stem cells are actively recruited by tumor-associated stroma [4]. Herein, we venture to explore the influence of human adipose derived stem cells (hASCs) on therapeutic response of human osteosarcoma (Saos 2) within 3D extra cellular environment created using silk fibroin and gellan gum.

METHODS: Silk fibroin – gellan gum based hydrogels with tunable mechanical properties were prepared by varying the blending ratio of gellan gum (GG) to silk to regulate the formation of spheroids of Saos 2 in presence of hASCs in an engineered 3D osteosarcoma model. The proliferation of cells within the spheroids was assessed by Alamar blue assay. For chemotherapeutic response studies, the spheroids were exposed to different dilutions of doxorubicin for 24 h and the viability of cells after treatment was assessed by MTT assay. The morphologies of both cells were examined under confocal and scanning electron microscopies.

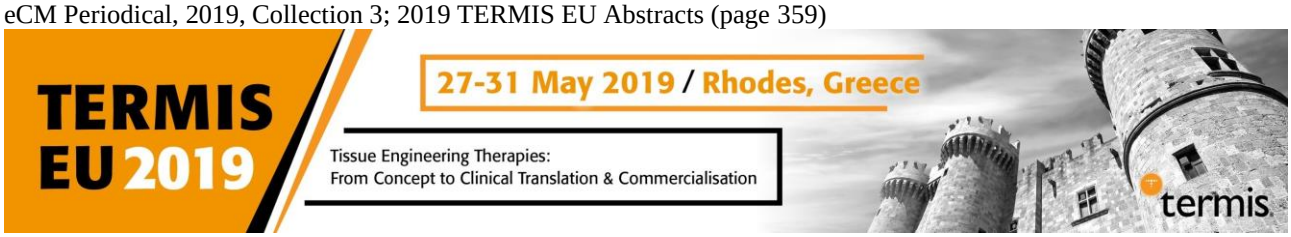
RESULTS & DISCUSSION: The highest rates of cell proliferation, spheroid formation and collagen secretion were observed in less stiff hydrogels. It was also identified that the blending of silk with gellan gum enhanced cell survival and functionality possibly owing to biocompatibility of natural silk fibroin. When cultured in equal seeding ratio and treated with the model chemotherapeutic agent such as doxorubicin, the heterotypic culture exhibited different sensitivity compared to homotypic spheroid

CONCLUSIONS: The heterogeneity of the present model at structural and cellular level, recapitulates more closely the dynamic characteristics of physiological tumor stroma. This is anticipated to be useful in predicting the efficacy of cancer chemotherapeutics.

ACKNOWLEDGEMENTS: European Union Framework Programme for Research and Innovation Horizon 2020 (nº 668983 —FoReCaST), Investigator FCT program IF/01214/2014 and IF/01285/2015

REFERENCES

[1] Rodrigues T et al., Pharmacology and Therapeutics 2018;184:201-11.
[2] Kundu B et al., Biomaterials 2013;34(37):9462-74.
[3] Guzman A et al., Biomaterials 2014:35(25), 6954-63
[4] Roodhart JML et al., Cancer Cell 2011; 20(3):370–83.



Innovation in amniotic epithelial cell-based tendon regenerative protocols

A. Mauro, V. Russo, A. Canciello, A. Muttini, E. Mazzotti, M. Turriani, O. Di Giacinto, P. Berardinelli, M Mattioli, B. Barboni
Presenting Author: Barbara Barboni, bbarboni@unite.it
Unit of Basic and Applied Bioscience, Faculty of Bioscience and Agro-Food and Environmental Technology, University of Teramo, Via Renato Balzarini 1, 64100 Teramo, Italy

INTRODUCTION: Amniotic epithelial cells (AEC) have demonstrated to stimulate healing after transplantation in experimental and spontaneous tendon defects [1]. The present research has been designed to assess whether the amplification protocols are able to enhance AEC -based tendon regenerative potential. To this aim, AEC amplified under standardized (CTR) [2], progesterone (P4) [2], and teno-inductive conditions (PRE-DIFF) [3] were compared (Tab. 1). Tendon regeneration was analyzed at d14 and d28 by evaluating AEC ability to promote damage resolution by sparing inflammation-induced healing moieties and encouraging ECM repair.

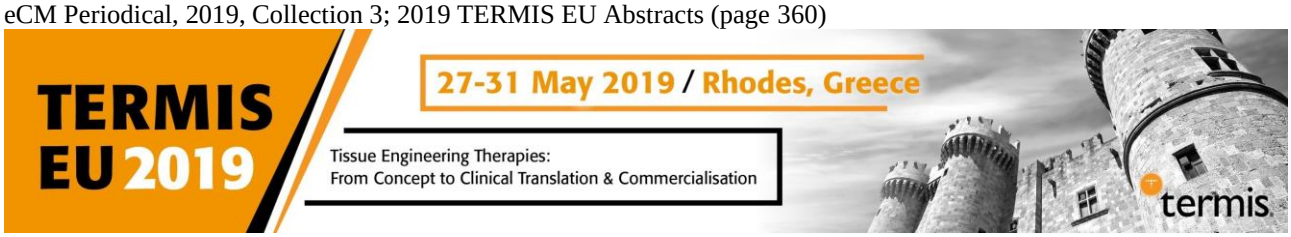
METHODS: A tendon defect of $5mm^3$ in diameter was induced in rams to avoid any endogenous P4 influence. Lesions were filled with fibrin glue in SHAM (n=20) or with fibrin glue plus 5×10^6 CTR (n=20), P4 (n=20) and PRE-DIFF (n=20) amplified AEC (passage III). Tendon microarchitecture was analyzed in explants with hematoxylin-eosin and Herovici and ECM remodeling using immunohistochemistry (IHC) in order to detect von Willebrand Factor and COL 3 and 1. Tendon inflammation was assessed through the IL 12/IL 10 ratio by RT-qPCR.

RESULTS & DISCUSSION: The injured SHAM tendons showed a still disorganized ECM at d14 displaying scattered COL 3 fibers, hypercellularity and a high vascular density. On the contrary, AEC transplanted tendon revealed clear signs of resolution. In particular, transplantation of P4 and PRE-DIFF cells lead to a mature and organized ECM displaying aligned COL 1 fibers and blood microvessels sustained by a lower IL12/IL10 ratio ($p<0.05$ vs CTR and $p<0.001$ vs SHAM). Tissue regeneration further improved in AEC groups at d28. However, the more advanced tissue recovery was observed in PRE-DIFF group. In this case, a significant increase in COL 1 fiber density, a complete alignment of ECM fibers and vessels were recorded in combination with the lowest IL12/IL10 ratio ($p<0.05$ vs both CTR and P4).

CONCLUSIONS: For the first time, it has been demonstrated that phenotype has to be considered in order to potentiate AEC tendon regenerative influences. P4 or tenogenic-pre-commitment AEC have showed, indeed, to better encourage early ECM deposition (d14), both by blunting tendon pro-inflammatory phase. PRE-DIFF maintained this anti-inflammatory mediated regenerative ability even at d28 when a complete ECM remodeling was surprisingly observed in the defect area. The present results confirmed the powerful tendon regenerative potential of AEC by pointing towards advanced in vitro protocols insights as a good strategy to improve and standardize AEC-tendon based therapy.

REFERENCES

[1] Barboni B. et al. Cell Transplant. 2018;27(1):93-116.
[2] Canciello A et al. Sci Rep. 2017; 7:3761
[3] Barboni B et al. PLoS One. 2012;7(2):e30974



Adhesion and growth of MC3T3 cells on surface-modified polymeric microspheres in different cell culture media

B. Mielan[1], D.M. Sousa[2], M. Krok-Borkowicz[1], M. Lamghari[2], E. Pamuła[1]
Presenting Author: Bartosz Mielan, barmie@agh.edu.pl
[1]AGH University of Science and Technology, Faculty of Science and Ceramics, Department of Biomaterials and Composites, Kraków, Poland [2]Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal

INTRODUCTION: Polymeric microspheres (MS) thanks to their high surface area can be used as substrates for cell culture both in static and dynamic conditions [1]. Moreover, they can be assembled to produce cell-material constructs according to "bottom-up" tissue engineering approach. The drawback of MS is their hydrophobicity and lack of cell-adhesive molecules on the surface. In this study, we propose to develop poly(L-lactide-co-glycolide) (PLGA) MS and modified their surface by adsorption of collagen type I (COL) or poly(L-lysine) (PLL) in order to promote cell adhesion and growth.

METHODS: MS were obtained with emulsification oil-in-water method. 7.5% PLGA (85:15, M_n=100 kDa, M_w=210 kDa) solution in DCM was added to 1% PVA, stirred (1000 rpm) and left for 24 h for DCM evaporation. Formed MS were vacuum filtered, washed, dried and sieved to collect particles with diameter 50-100 µm. 20 mg MS were incubated in 0.1 M NaOH for 30 min, washed 3x in UHQ-water to activate PLGA surface and coated with COL (40 µg/ml, 10 h) or PLL (0.01%, 10 h). MC3T3 osteoblast-like cells were cultured with MS for 14 days in three media: α-MEM, osteogenic medium (α-MEM with 0.05% ascorbic acid and 0.05% β-glycero-phosphate) and osteoclast conditioned medium. Cell adhesion, proliferation, viability and morphology were tested.

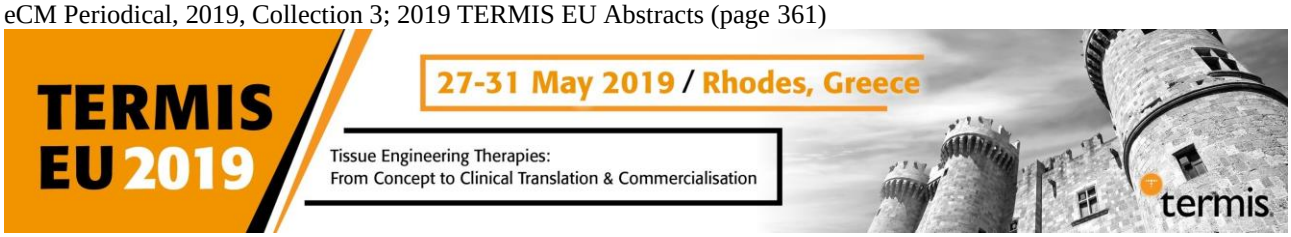
RESULTS & DISCUSSION: Emulsification method allowed us to obtain MS with regular shape and size for cell culture. The presence of PLL and COL coatings improved cell adhesion on day 1 in all studied media. The cells were better spread on PLL- and COL-modified MS when compared to non-modified PLGA MS.

CONCLUSIONS: The obtained results show that PLL and COL coatings on PLGA MS improved cell adhesion and growth of MC3T3 cells in all studied media.

ACKNOWLEDGEMENTS: This work was supported by the National Science Centre, Poland (Grant No. UMO-2016/21/D/ST8/0185) and Polish National Agency for Academic Exchange (PPI/PRO/2018/1/00026/U/001).

REFERENCES

[1] Ahmed I. Development of microspheres for biomedical applications : a review. 2015;1–19.



StemCellFactory III: Concept towards automated generation of CRISPR/Cas engineered iPSC lines

B. Nießing[1], P. Wanek[2], A. Elanzew[3], M. A. S. Toledo[2], S. Jung[1], N. König[1], M. Peitz[3], S. Haupt[4], M. Zenke[2], O. Brüstle[3,4], R. Schmitt[1,5]
Presenting Author: Bastian Nießing, bastian.niessing@ipt.fraunhofer.de
[1]Fraunhofer Institute for Production Technology IPT, Aachen, Germany [2]Institute for Biomedical Engineering – Cell Biology -, RWTH Aachen, Germany [3]Institute of Reconstructive Neurobiology, University Bonn, Germany[4] Life & Brain GmbH, Bonn, Germany [5]Laboratory for Machine Tools and Production Engineering (WZL), RWTH Aachen, Germany

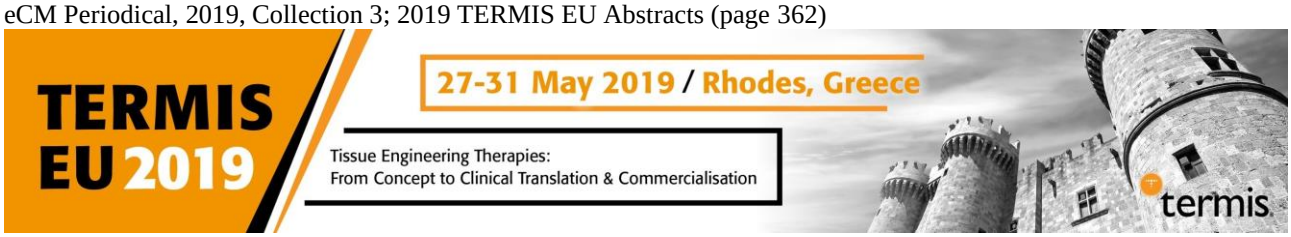
INTRODUCTION: The use of patient-derived induced pluripotent stem cells (iPSCs) for disease modelling increased our knowledge of disease processes and offers fascinating perspectives for personalized therapy. The globally increased demand for patient-derived iPSCs has created an urgent need for standardized and automated production processes. To that end, we developed the StemCellFactory, an integrated system for automated reprogramming and expansion of iPSCs for disease modeling and drug screening.

RESULTS & DISCUSSION: On the StemCellFactory platform, we automated reprogramming of human dermal fibroblasts, clonal selection and expansion of primary iPSC clones, and scaled enzyme-free sub-cultivation of iPSC lines. The platform contains various devices, including incubators, liquid handling units, storage places, analytical devices and robots for transportation and handling of materials. For process control, we developed a novel software tool that controls all processes executed by the StemCellFactory and computes on-line acquired metrology data of iPSC cultures for automated decision making, like confluency cell morphology, and turbidity. Management for every device is implemented, comprising data tracking and two-stage error handling. Automated management of resources, disposables and liquids avoids support shortages and guarantees constant production conditions.

OUTLOOK: Within the scope of further enhancing the system towards the generation of state-of-the-art iPSC models, we have devised an implementation plan for the automated genome editing by CRISPR/Cas technology. Furthermore, hardware and software extensions for the generation of iPSC from blood samples were implemented.

CONCLUSIONS: We developed an automated platform and novel software tools that address the technological challenges for automation of complex stem cell culture processes. This includes automated CRISPR/Cas-based genome editing of iPSCs. We expect the StemCellFactory platform to meet the challenges of the increasing demand for patient-derived iPSCs and their derivatives.

ACKNOWLEDGEMENTS: The project "StemCellFactory III" is funded by the European Regional Development Fund (ERDF) under grant number EFRE-0800972.



Microfibrillated silk to make strong yet degradable biomaterials

B.J. Allardyce[1], J. Zhang[2], Y. Liang[1], S. Kalita[1], S. Redmond[3], S. Shafei[1], R.J. Dilley[3], X. Wang[1], R. Rajkhowa[1]
Presenting Author: Ben Allardyce, ben.allardyce@deakin.edu.au
[1]Deakin University, Institute for Frontier Materials, Geelong, Australia [2]Deakin University, School of Engineering, Geelong, Australia [3]Ear Science Institute Australia, School of Surgery, University of Western Australia, Australia

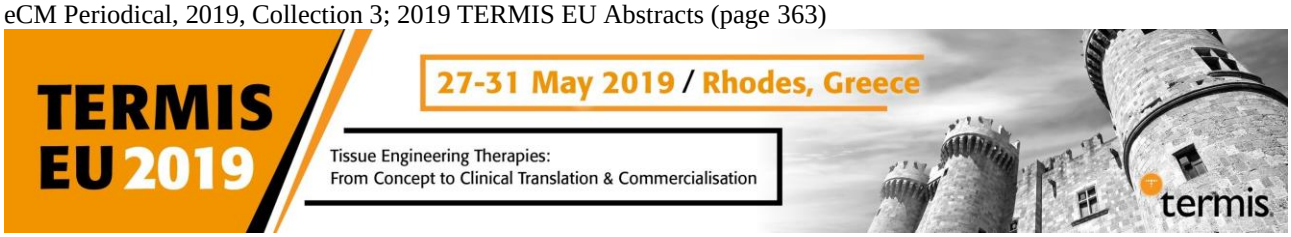
INTRODUCTION: The production of silk nanofibers using traditional methods (eg. electrospinning) requires the silk to be dissolved before forming nanofibers. This process destroys the hierarchical structure of silk fibroin that imparts its exceptional mechanical properties. Regenerated nanofibers also have much faster biodegradation rate. We have pioneered the production of silk nanofibers, referred to here as microfibrillated silk (MFS), directly from silk fibres using mild chemicals and mechanical exfoliation. The process is easily scalable and can be manipulated to control fibre aspect ratio. Here we report the use of MFS as free standing scaffolds or as reinforcing fillers for hydrogel 3D printing.

METHODS: Degummed silk fibres from Phiosomia ricini silkworms were fibrillated using a combination of milling and homogenisation under alkaline conditions. MFS suspensions were either cast directly to form nonwoven scaffolds or combined with chitosan solution and printed to form MFS reinforced hydrogel scaffolds. The resulting materials were characterised (chemical and mechanical properties, morphology) and tested for cytocompatibility using human derived fibroblasts and HaCaT keratinocytes.

RESULTS & DISCUSSION: The addition of MFS significantly improved the shape fidelity and compressive strength of 3D printed chitosan hydrogel scaffolds without affecting fibroblast attachment and proliferation. By controlling the silk degumming, milling and homogenisation steps, MFS with fibre diameters under 500 nm were produced. These fibre based constructs were able to support the growth and proliferation of fibroblasts and HaCaT keratinocytes. Scaffold degradation testing is ongoing and will be discussed.

CONCLUSIONS: The development of MFS enabled the production of silk biomaterials with superior mechanical properties, as the structural component of the material and as a filler/bulking agent. The flexibility of the process will enable the development of a range of fibre reinforced composite biomaterials.

ACKNOWLEDGEMENTS: Financial support was received from the Australian Research Council (IH140100018).



Preparation of optic nerve grafts: Optimization of decellularization method

B. Topuz[1], H.M. Aydin[1,2]
Presenting Author: Bengisu Topuz, bengisutopuz@gmail.com
[1]Bioengineering Division, Institute of Science, Hacettepe University, Ankara, Turkey [2]Centre for Bioengineering, Hacettepe University, Ankara, Turkey

INTRODUCTION: Glaucoma, vascular and ischemic malfunctions, and trauma result in severe damage to optical nerves and may lead to blindness. Currently there is no treatment option for such conditions. In this study, we aimed to develop a novel decellularization protocol to produce optical nerve grafts. The ECM composition and in vitro cytotoxicity characterizations are reported.

METHODS: The optic nerve segments were isolated from bovine eyes. A decellularization protocol, modified version of a previously suggested method for sciatic nerves, was established and conducted [1]. Several protocols were compared. Tissues were agitated in distilled water for 7 h and rinsed in PBS for 72 h in all groups.

In order to investigate the effectiveness of the proposed protocols and to understand the extent of matrix protection levels, collagen and glycosaminoglycan (GAG) content were analyzed. in vitro degradation and swelling tests were performed. The cell removal capacity of each protocol was studied by routine histology and transmission electron microscopy (SEM).

RESULTS & DISCUSSION: The H&E staining studies showed enhanced preservation of scaffold integrity in all groups (Figure 1 A-G). There were no cell nuclei observed in tissues after DAPI stainings (Figure 1 H-N). Analyses revealed a 28.11 mg/mg collagen content in native optic nerves while these values were calculated as 17.72, 10.32, 9.28, 14.21, 15.85, and 9.27 for decellularization groups of 1 to 6, respectively. There was a significant difference ($p < 0.001$) in collagen content per mg tissue among the control and decellularization groups. Additionally, decellularized tissues showed semi-transparent appearance.

CONCLUSIONS: A suitable method was developed as a result of thorough comparisons. The proposed decellularization protocol may pave the way for prospective studies towards novel approaches in the treatment of optic nerves.

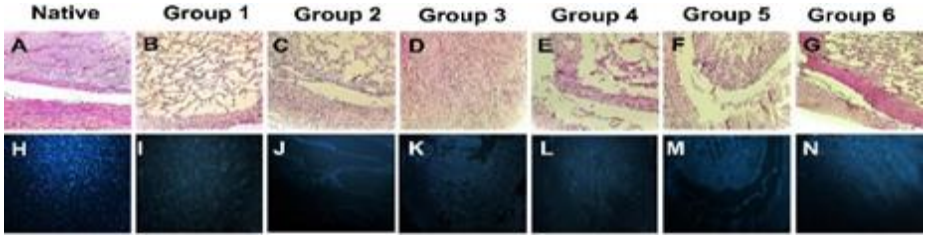
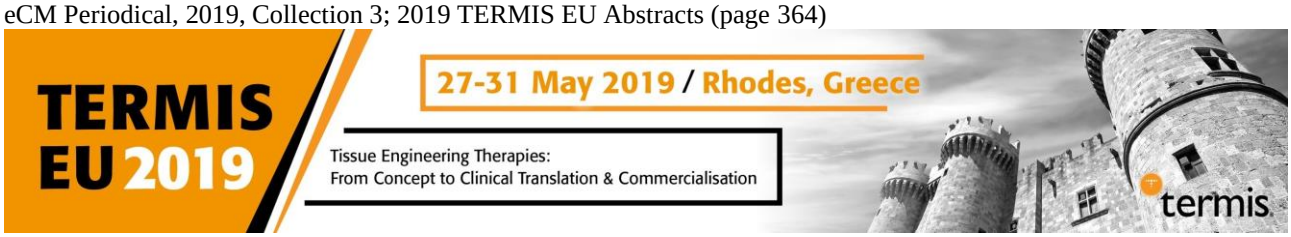


Figure 1: (right) H&E stainings of optic nerves: (A) Native, (B-G) Decellularization groups; and DAPI fluorescence staining of optic nerve: (H) Native, (I-N) Decellularized groups.

ACKNOWLEDGEMENTS: Financial support was received from Hacettepe University (No: FYL-2018-17399). Equipment were kindly donated by BMT Calsis Co., Turkey.

REFERENCES

[1] Sondell M et al. Brain Research 1998,795 44–54.



Microfluidic platforms for the future of preclinical studies: Patient-on-a-chip

B. Atac[1], E.M. Dehne[1], U. Marx[1]
Presenting Author: Beren Atac, beren.atac@tissuse.com
[1]TissUse GmbH, Oudenarder Str. 16, 13347 Berlin, Germany

INTRODUCTION: In the recent years, microfluidic systems have shown to be a powerful tool for recreating tissue- and organ-like functions by different approaches, providing basis for developing preclinical assays with improved predictive power [1]. The development of new drugs is increasingly expensive, time-consuming while only a few compounds can reach the market fulfilling all safety and efficacy criteria. Evaluating potential drug candidates at early stages of the preclinical process became essential by finding new technologies. Microfluidic culture platforms conjoining human microtissues in a physiological-like arrangement could provide a translational solution [2]. Starting with assay relevant a few organs, Human-on-a-chip platforms aim to combine more than ten organs to mimic the healthy and diseased physiology with patient specific engineered tissues to create the Patient-on-a-chip. This allows to create personalized medicine solutions for diseases and evaluate drug responses with the patient's specific genetic background [3].

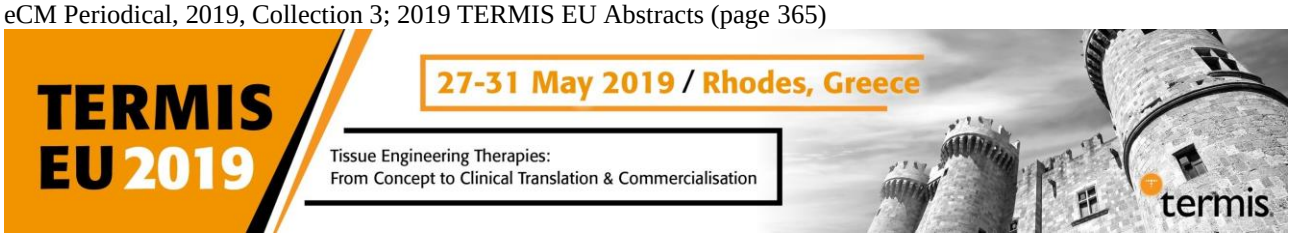
METHODS: In multi-organ-chip (MOC) platform integrated with an on-chip micro-pump, we established thirteen different organ equivalents combined into twelve different co-culture platforms interconnecting two to four organs.

RESULTS & DISCUSSION: We showed that in MOC we can study physiologically relevant organ crosstalk, induce and study diseases such as Diabetes type II, lung cancer and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) of compounds [4-6] and will present our latest advancements on the MOC platform.

CONCLUSIONS: The microphysiological systems create new opportunities and solutions to drug discovery dilemma at the preclinical phase as well as bringing new challenges up [7]. Advancements in tissue engineering field to create patient specific physiologically relevant combination of organ models with advanced microfluidic platforms to mimic the responses of different genetic backgrounds and emulate human biology at the preclinical phase would substantially improve prediction of drug safety and efficacy.

REFERENCES

[1] Dehne EM et al. Future Sci OA. 2017 Mar 31;3(2):FSO185.
[2] Marx U et al. ALTEX. 2016;33(3):272-321.
[3] Rebelo SP et al. Adv Exp Med Biol. 2016;856:299-316.
[4] Bauer S. et al. Sci Rep. 2017 Nov 6;7(1):14620.
[5] Hübner J et al. Sci Rep. 2018 Oct 9;8(1):15010.
[6] Maschmeyer I et al. Lab Chip. 2015 Jun 21;15(12):2688-99.
[7] Beilmann M et al. ALTEX. 2018 Dec 20.



Engineering 3D hiPSC-based cardiac tissues for preclinical research

B. Abecasis[1,2], P. Costa[1,2], H.V. Almeida[1,2,3], S. Rosa[3], P.J. Gouveia[3], P. Gomes-Alves[1,2], L. Ferreira[3,4], M. Serra[1,2], P.M. Alves[1,2]
Presenting Author: Bernardo Abecasis, babecasis@ibet.pt
[1]Animal Cell Technology Unit, iBET, Oeiras, Portugal [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal [3]CNC - Centre for Neurosciences and Cell Biology, Universidade de Coimbra, Coimbra, Portugal [4]Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal

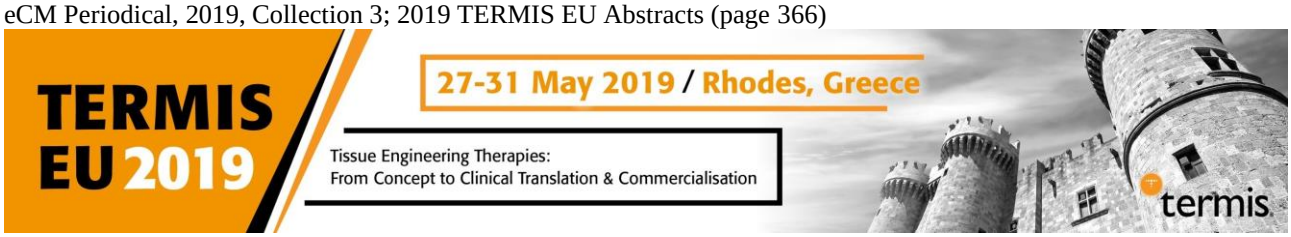
INTRODUCTION: The development of complex in vitro cell-based models and advanced tools that enable their characterization are of utmost importance for drug screening and disease modeling. Current in vitro cell-based models for cardiotoxicity assessment rely on homotypical cardiomyocyte cultures, which do not resemble the complexity of the human cardiac environment. The aim of this study was to develop a physiologically relevant in vitro cardiac microtissue, using human-induced pluripotent stem cell (hiPSC) that better recapitulates the human heart microenvironment features and allows the prediction of potential cardiotoxic effects.

METHODS: A novel 3D hiPSC-derived tri-culture cardiac microtissue was developed by combining aggregates of hiPSC-derived cardiomyocytes (hiPSC-CM) with hiPSC-derived endothelial cells and mesenchymal cells (hiPSC-EC+MC) inside alginate microcapsules that were cultured in dynamic conditions for up to 2 weeks. A toolbox of analytical techniques, including quantitative MS-based proteomics, was implemented to unveil the molecular crosstalk involved in the communication between the different cell types and their impact on hiPSC-CM maturation.

RESULTS & DISCUSSION: The tri-culture microencapsulation strategy was successfully implemented showing maintenance of cell viability, metabolic activity and cellular phenotype up to 15 days of culture. Particularly, characterization of the cardiac microtissue showed evidences of structural maturation of hiPSC-CM when compared to hiPSC-CM mono-cultures. For further depiction of the communication between the different cell types co-cultures of hiPSC-CM and hiPSC-EC were established also showing evidences of structural maturation of the hiPSC-CM in co-culture. Evaluation of hiPSC-CM functionality by calcium imaging showed similar calcium handling properties of hiPSC-CM in both mono and co-culture, although some differences were observed in response to drugs, namely to norepinephrine. More importantly, quantitative whole proteomic analysis revealed increased expression of: (i) ratios of morphological cardiac maturation-related proteins (MYH7/MYH6, MYL2/MYL7, TNNI3/TNNI1); and (ii) extracellular matrix proteins, namely collagens I, III, fibronectin and other soluble proteins, which may be responsible for the communication between both cell types.

CONCLUSIONS: Overall, this study provides insights towards the establishment of biologically-relevant in vitro cardiac tissue models. Additionally, the characterization methods herein implemented were demonstrated to have an essential role in in vitro cardiac preclinical research for the understanding of in vivo microenvironment recapitulation.

ACKNOWLEDGEMENTS: This work was supported by FCT-funded projects MetaCardio (PTDC/BTM-SAL/32566/2017), NETDIAMOND (SAICTPAC/0047/2015) and NeoCoronary (029590); and iNOVA4Health UID/Multi/04462/2013.



Boosting biological performance of multiscale porous scaffolds by in vitro generated extracellular matrix decoration

B. Aldemir Dikici[1], G.C. Reilly[2], F. Claeyssens[1]
Presenting Author: Betül Aldemir, baldemirdikici1@sheffield.ac.uk
[1]University of Sheffield, The Kroto Research Institute, Department of Materials Science and Engineering, Sheffield, UK [2]University of Sheffield, INSIGNEO Institute for in silico medicine, Department of Materials Science and Engineering, Sheffield, UK

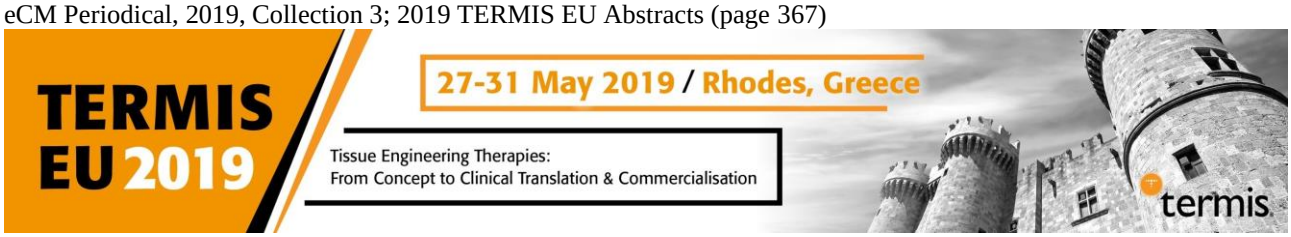
INTRODUCTION: Polymers are widely used biomaterials for tissue engineering applications due to having tailorable properties, however, they need modifications to improve their limited interaction with biological tissues. Two key points were aimed in this study. First, manufacturing of multiscale porous scaffolds, made of photocurable polymer, by combining techniques of emulsion templating and 3D printing. Secondly, decorating these scaffolds with in vitro generated ECM to increase their biological performances.

METHODS: Scaffolds, made of high internal phase emulsion (HIPE) of methacrylated PCL, were created via additive manufacturing. MLO-A5s were cultured on scaffolds for 4-weeks. Cellular activity, mineral and collagen deposition assays were performed. Cell penetration depth was assessed by histology images. Microarchitecture of scaffolds and cell attachment were observed under SEM. After decellularisation, to confirm the efficiency of the process, DNA content was measured. Both plain and hybrid scaffolds were recellularisated by using human embryonic stem cells-derived mesenchymal progenitors (hES-MPs) and compared. Finally, chick allantoic membrane (CAM) assay was used to explore the angiogenic potential of the scaffolds.

RESULTS & DISCUSSION: Multiscale porous scaffolds were obtained successfully. ECM matrix composed of mineral and collagen was deposited on scaffolds and then 95% of DNA was removed (decellularisation). In recellularisation process, cells not only showed higher attachment on ECM decorated scaffolds but also exhibited significantly higher increase in cellular activity and cell infiltration. Additionally, CAM assay showed that the presence of ECM on scaffolds resulted in higher angiogenic potential in comparison with plain scaffolds.

CONCLUSIONS: 3D printed PCL polymerized HIPE scaffolds support cell adhesion, proliferation and migration and it is a promising candidate to fulfill the requirements of defect-matching bone grafts. Additionally, our in-vitro generated ECM decorated polymer system proposed an applicable approach to improving bioactivity and angiogenesis of polymer scaffolds to encourage precursors to differentiate mature bone.

ACKNOWLEDGEMENTS: The author BA was funded by Turkish Ministry of Education.



Bioreactor derived human fetal mesenchymal stem cell secretome promote diabetic skin wound healing

B. Wang[1,2,3], Q. Pan[1,2,3], H.X. Wang[1,2,3], Y. Li[1,2,3], S. Lin[1,2,3], WYW. Lee[1,2,3], G. Li[1,2,3]
Presenting Author: Bin Wang, yew123et@gmail.com
[1]Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, PR China [2]Stem Cells and Regenerative Medicine Laboratory, Lui Che Woo Institute of Innovative Medicine, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, PR China [3]The CUHK-ACC Space Medicine Centre on Health Maintenance of Musculoskeletal System, The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, PR China.

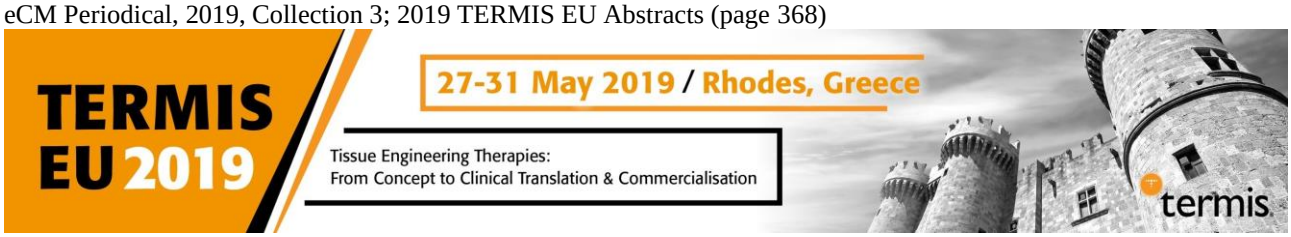
INTRODUCTION: Previous studies have elucidated the possible role of fetal tissue in scar-less healing. Our study takes one-step forward and makes use of bioreactor expended human fetal mesenchymal stem cell (FMSC) to produce MSC secretion (HFS). We employ poly (lactic-co-glycolic acid) (PLGA) particle as a slow release capsule and prove the promoting effect of HFS encapsulated PLGA (HEP) particle on the healing of diabetic skin wound which is considered as refractory skin defect in clinical scenario.

METHOD: FMSC were seeded on collagen-coated polystyrene microcarrier in the ratio of one million cells/30g carriers in a commercially available bioreactor (PBS, USA). Upon collection of HFS, cultures were incubated with serum-free medium for 24hrs. Collected condition medium was filtered with 70μm cell strainer (Falcon) and removed the cell debris by centrifugation. The supernatant was subjected to lyophilization. HFS was reconstituted in the concentration of 100ng/mL. Alamar blue (Invitrogen) was used to test cell viability. Scratch assay and co-culture insertion (BD Bioscience) was used to test cell migration ability. Furthermore, fibroblast populated collagen lattice (FPCL) model and organotypic keratinocyte-fibroblast co-culture (OKC) system were used to test the effect of HEP in vitro. For in vivo study, we employed streptozotocin-induced diabetes model in rat and created a full thickness defect on rat back skin.

RESULTS & DISCUSSION: FMSCs were mixed with microcarriers and cultured under stirring within bioreactor for 14 days. Alamar blue test showed that 50ng/mL HFS maintained human fibroblast and keratinocyte viability ($n=3$, $p<0.05$). HFS also promoted cell migration of the two types of cells after treatment for 3 days ($n=3$, $p<0.05$). Simultaneously, HFS prohibited FPCL contraction and promoted keratinocyte differentiation in OKC model after 7 days treatment ($n=3$, $p<0.05$). Then HFS was encapsulated in PLGA particle by double emulation method. BCA test showed that in vitro release of proteins of HEP could sustain at lease for 1 week. ELSA data showed that HFS contains PDGF-BB. The promoting effect has also been observed on the diabetic rat model ($n=6$, $p<0.05$). HFS also contains long non-coding RNA H19 that may contribute to regulate skin healing.

CONCLUSIONS: In summary, HEP may slow release encapsulated HFS to promote skin defect healing in the diabetic rat model. PDGF-BB and H19 may contribute to the promoting effects of HFS on skin healing.

ACKNOWLEDGEMENTS: Hong Kong Government Research Grant Council, Themed-based Research Scheme (T13-402/17-N); General Research Fund (14119115, 14160917, 14120118, 9054014 N_CityU102/15) and National Natural Science Foundation of China (81430049 and 81772322); Hong Kong Innovation Technology Commission Funds (ITS/UIM-305).



Identification of adhesive proteins in marine invertebrates

B. Lengerer[1], J. Wunderer[2], R. Pjeta[2], M. Algrain[1], P. Ladurner[2], P. Flammang[1]
Presenting Author: Birgit Lengerer, birgit.lengerer@umons.ac.be
[1]University of Mons, Research Institute for Biosciences, Biology of Marine Organisms and Biomimetics Unit, 23 Place du Parc, 7000 Mons, Belgium [2]University of Innsbruck, Institute of Zoology and Center of Molecular Bioscience Innsbruck, Technikerstr. 25, 6020 Innsbruck, Austria

INTRODUCTION: Biological glues are a promising source of inspiration for biomedical applications. Many temporary attaching animals, like flatworms and sea stars, rely on a duo-gland adhesive system, where adhesive glands secrete the proteinaceous glue and a different gland type produces a de-adhesive substance [1]. We aim to identify and characterize these secretions, in order to provide templates for biomimetic approaches.

METHODS: To understand the molecular and morphological basis of biological adhesion, we used a large toolbox combining molecular biology and histological methods. We combined proteomics, RNA-seq, in situ hybridization, RNAi, lectins, immunohistochemistry, histological staining, transmission- and scanning electron microscopy.

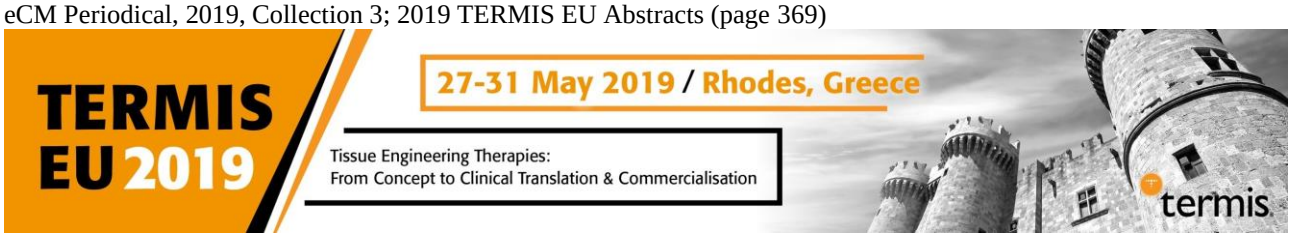
RESULTS & DISCUSSION: The simplest temporary adhesive system can be found in the marine flatworm Macrostomum lignano. We showed that the attachment of M. lignano relies on the secretion of two large adhesive proteins, Mlig-ap1 (5,407 aa) and Mlig-ap2 (14,794 aa) [2]. In Mlig-ap1 several known protein-carbohydrate and protein-protein interaction domains are present. Furthermore, Mlig-ap1 is characterized by lysine- and arginine-rich repeats, rendering regions of this protein extremely positively-charged. Mlig-2 is glycosylated and contains two von Willebrand factor domains, two trypsin inhibitor-like domains, a low density-lipoprotein receptor domain, and multiple thrombospondin type-1 repeats. Based on RNAi knockdown experiments, we propose that Mlig-ap2 attaches to the substrate and Mlig-ap1 exhibits a cohesive function [2].
The adhesive secretion of sea stars shares many characteristics to that of flatworms. In Asterias rubens, one protein was characterized and named sea star footprint protein-1 (Sfp1) [3]. Sfp1 resembles Mlig-ap1 in many aspects including the unusual large size, similar protein domains and carbohydrate binding sites. In A. rubens, 22 additional proteins were identified in adhesive secretions and at least two were found to be glycosylated.

CONCLUSIONS: Our findings indicate that temporary bioadhesion is based on the interaction of adhesive and cohesive proteins. Cohesive proteins share a large size and certain functional domains, while glycosylation is prevalent in adhesive proteins. Characterization of the adhesive proteins in flatworms and sea stars may inspire the development of new biomimetic adhesives.

ACKNOWLEDGEMENTS: The work is supported by the Austrian Science Fund (FWF): [J 4071P, 25404-B25, P 30347] and the COST Action CA15216 (European Network of Bioadhesion Expertise, ENBA).

REFERENCES

[1] Lengerer B and Ladurner P. J Exp Biol. 2018; 221(16)
[2] Wunderer J, Lengerer B, Pjeta R, et al. Proc Natl Acad Sci. in revision.
[3] Hennebert E et al. Proc Natl Acad Sci. 2014; 111: 6317-6322



Lipid nanocapsules for the sustained release of therapeutic miRNA: New perspective in regenerative medicine of intervertebral disc

B. Le Moal[1,2], E. Lepeltier[2], V. Geoffroy[1], A. Galvani[1], C. Le Visage[1], C. Passirani[2], J. Guicheux[1], J. Clouet[1]
Presenting Author: Le Moal Brian, brian.lemoal@univ-nantes.fr
[1]Inserm, UMR 1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS, Nantes, F-44042, France [2]MINT, UNIV Angers, INSERM 1066, CNRS 6021, Université Bretagne Loire; Angers – France

INTRODUCTION: Dysregulation of miRNAs, notably miR155 [1], has been associated with disc degenerative disease (DDD) [2]. The relevance of unprotected miRNAs for therapeutic applications suffers from their fast in vivo degradation. Thus, the development of nanocarriers is a prerequisite for miRNA therapeutic use. Lipid nanocapsules (LNCs) offer a suitable strategy thanks to their ability to encapsulate nucleic acid [3]. The purpose of this work was to formulate and fully characterize innovative miR155-LNC for a potential use in DDD treatment.

METHODS: miR-155-LNCs were formulated by phase inversion process. After purification, miR155-LNCs were fully characterized (size, polydispersity index (PDI), zeta potential). Encapsulation efficiency (EE) and drug loading (DL) were assessed by Quant-IT-dye® quantification. miRNA-155 release and enzymatic protection were investigated by dialysis and gel electrophoresis. miR-155-LNCs internalization and impact on cell viability in human adipose stromal cells (hASC) were assessed by confocal/FACS analysis and MTT assay, respectively.

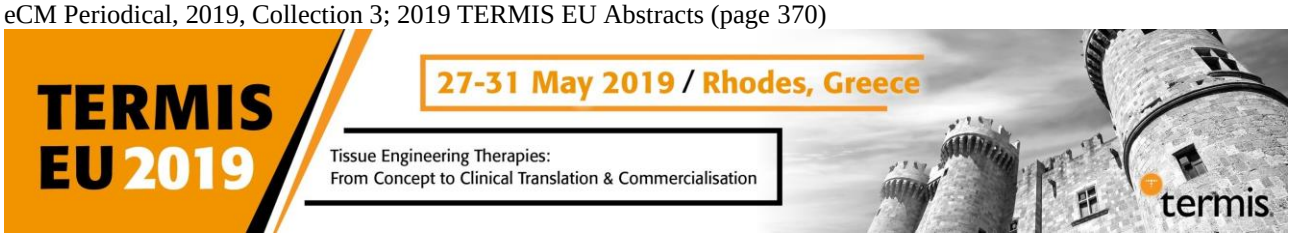
RESULTS & DISCUSSION: miR155-LNCs exhibit a diameter of 75.0 ± 1.3 nm, a PDI of 0.06 ± 0.03 and a positive zeta potential. EE and DL were estimated to 75.2 ± 1.2% and 590 ± 9.3µg/g of LNC respectively. miR155 sustained release from LNCs was observed (23.3 ± 7.1% compared to 94.8 ± 5.4% for unencapsulated miR-155, after 4 h). miRNA-endonuclease protection by LNC was confirmed by electrophoresis. After 24h of incubation with fluorescent miR155-LNCs, hASC viability was of 71.66% ± 4.23% for 5,9 ng/mL of miRNA, a recommended concentration for efficient cell transfection. Internalization of miR-155-LNCs in hASC cells was also demonstrated.

CONCLUSIONS: LNCs could be a promising approach to protect, release and transfect therapeutic miRNA. Further experiments (in vitro and in vivo) will be needed to confirm the interest of this nanoplatform to vectorize new therapeutic in order to counteract DDD.

ACKNOWLEDGEMENTS: This study was supported by grants from the Région des Pays de la Loire, ANR JCJC 2016 (STIMUDISC project).

REFERENCES

[1] Ye D et al. Dis Markers. 2016; 6984270:1-7
[2] Clouet J et al. Adv Drug Deliv Rev. 2018; ADR-13298:1-18
[3] Passirani C et al. Patent. INPI. 2014; Fr3026009



Effect of sintering on in vivo biological performance of bovine hydroxyapatite

B. De Carvalho[1], E. Rompen[1], G. Lecloux[1], P. Schupbach[2], E. Dory[3], F. Lambert[1].
Presenting Author: Bruno De Carvalho, bcarvalhomd@gmail.com
[1]Department of Periodontology and Oral Surgery, University of Liège, Belgium [2]Adjunt Professor Augusta University, USA [3]Project Manager Wishbone SA, USA

INTRODUCTION: The influence of the manufacturing process of xenogenic biomaterials on their biological performance remains incompletely understood in terms of bone regeneration [1]. Calcium-phosphate-based (CaP) materials have received a lot of attention due to their chemical similarity to bone, but also due to their excellent biocompatibility [2-3]. The primary objective of this study was to analyze the in vivo biological performance (new bone formation and osteoconductivity) of experimental bovine hydroxyapatites (BHA) sintered at different temperature. The secondary objective was to correlate the in vivo results with the physicochemical and topographical characteristics of the studied biomaterials.

METHODS: Protein-free hydroxyapatite from bovine origin was produced under sub-critical conditions and then either briefly hardened at mid-range temperature (820ºC), or sintered at 1200°C. The physicochemical properties were assessed by scanning electronic microscopy (SEM), measurement of surface specific area (BET) and X-ray diffractometry (XRD). The materials were then implanted in standardized alveolar bone defects in minipigs. Histological and histomorphometric evaluations (% of newly formed bone and % of bone-to-material contact) were performed using non-decalcified sections.

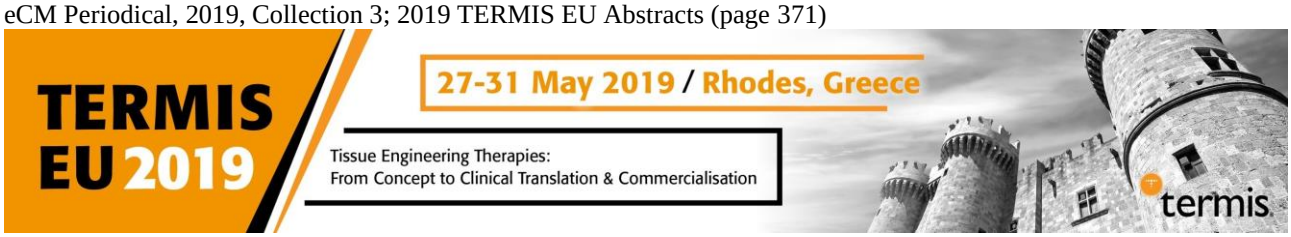
RESULTS & DISCUSSION: Marked topographical differences were observed by SEM analysis, showing highly structured surfaces on the unheated and hardened BHAs, while the highly sintered one was characterized by a much smoother surface and very few remaining porosities. In vivo samples showed a similar regenerated area observed in the 3 groups, while the highly sintered BHA presented a significantly lower percentage of newly formed bone than the unheated one (p=0,009). In addition, the percentage of bone-to-material contact (osteoconductivity) was significantly lowered by sintering when compared to the unheated (p=0,01;) and to the hardened (p=0,02) groups.

CONCLUSIONS: Sintering of BHA at 1200°C has a marked effect on its physicochemical characteristics as described already on the literature. However, its biological performance is significantly lowered in the present study. By contrast, mid-range sintering temperature such as 820°C preserves surface roughness and microporosity, and allows good bone regeneration and osteoconductivity with no statistically significant differences compared with the non-sintered BHA. Clinicians must take into account that the process of biomaterial manufacturing can significantly influence the quality of bone regeneration, when choosing the biomaterial for their regenerative procedures.

ACKNOWLEDGEMENTS: The co-authors France Lambert, Eric Rompen and Geoffrey Lecloux are integrating members of the Wishbone SA board.

REFERENCES

[1] A. Khoshzaban, Mater. Sci. Eng. C. 77 (2017) 142–150
[2] L.L. Hench, J. Am. Ceram. Soc. 74 (1991) 1487–1510
[3] M. Epple, J. Mater. Chem. 20 (2010) 18–23



Constitutive and conditional reporter genes in primary human cells

B.G.H. Filippi, J. Pally, B. Keller Abu Seda, J. Rohrer
Presenting & corresponding author: Bruno Filippi, bruno.filippi@zhaw.ch
Group of Cell Physiology and Cellular Engineering, Zürich University of Applied Sciences, Wädenswil, Switzerland

INTRODUCTION: Tissue engineering aims at the generation of biological constructs to heal or replace damaged tissues and organs. Tissue engineering also includes the use of tissues to study complex 3D biological models, and to test the pharmacology and the toxicity of experimental medicines. In this work, we show that primary human cells, which are essential building blocks of functional biological tissues, can be modified efficiently and stably with constitutive or conditional reporter genes to measure precisely specific cellular conditions (e.g. vitality, cellular stress, activity of intra-cellular pathways).

METHODS: Various reporter gene architectures sharing a common human secreted embryonic alkaline phosphase (hSEAP) cDNA under the transcriptional control of different human promotors were tested in primary human fibroblasts, primary human keratinocytes and HaCaT. In addition to the hSEAP cDNA and the promotor sequence, some reporter gene architectures were made conditional by including an antidoxidant responsive element (ARE) motif that is a target of the Nrf2/KEAP1 pathway [1]. The activity of the reporter gene is determined by measuring the concentration of hSEAP secreted in the cell culture medium.

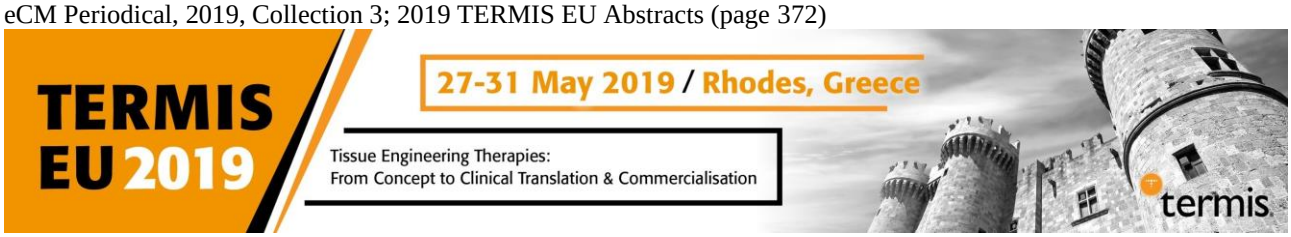
RESULTS & DISCUSSION: First, promotors play a pivotal role in the activity of reporter genes and the activity of the promotors strongly depend on the cellular environment. Interestingly, the ARE motif does not function with all promotors and has no effect in specific conditional reporter gene architectures. Finally, the ability of the ARE motif to modulate the activity of conditional reporter gene architecture is strongly dependent on the cell type.

CONCLUSIONS: Collectively, these data show that the choice of regulatory elements is critical for the proper function of reporter genes, especially when working with primary cells. Nevertheless, specific constitutive or conditional reporter gene architectures can work efficiently in primary human cells. Primary human cells containing such reporter gene architectures could be used to generate innovative reporter tissues or organs.

ACKNOWLEDGEMENTS: Financial support was received from the Zürich University of Applied Sciences.

REFERENCES

[1] Kobayashi A et al. Methods Enzymol. 2004;378:273-86



Development of elastin-like polypeptide-based hydrogel capable to promote innervation and angiogenesis

B. P. dos Santos[1], B. Garbay[2], E. Garanger[2], S. Lecommandoux[2], H. Oliveira[1], J. Amédée[1]
Presenting Author: Bruno Paiva dos Santos, bruno.paiva-dos-santos@inserm.fr
[1]Bioingénierie Tissulaire (BioTis), Inserm U1026, Université de Bordeaux, Bordeaux, France
[2]Laboratoire de Chimie des Polymères Organiques (LCPO), UMR 5629 CNRS, Bordeaux INP, Université de Bordeaux, Pessac, France

INTRODUCTION: Accumulating evidence has been revealing the importance of the sensory nervous system in the orchestration of bone turnover and repair [1]. The aim of this work is to develop a cell-free and growth-factor free hydrogel capable to promote neurotization and angiogenesis in a bone regeneration context.

METHODS: Hydrogels were produced using Elastin-like polypeptides (ELP) and different ratios of poly(ethylene glycol) (PEG), and the laminin-derived adhesion peptide IKVAV (or its scrambled VKAIV): ELP + (PEG); ELP + [low adhesion peptide] and ELP + [high adhesion peptide] and characterized by rheology, porosity and enzymatic degradation. Primary rat cell cultures: mesenchymal stromal cells (MSCs), endothelial cells (ECs) and sensory neurons (SNs) had metabolic activity, cellular behaviour and/or gene expression evaluated. Best in vitro performance composition was implanted subcutaneously in mice and the vascularization and innervation potential were assessed by histology and immunohistochemistry.

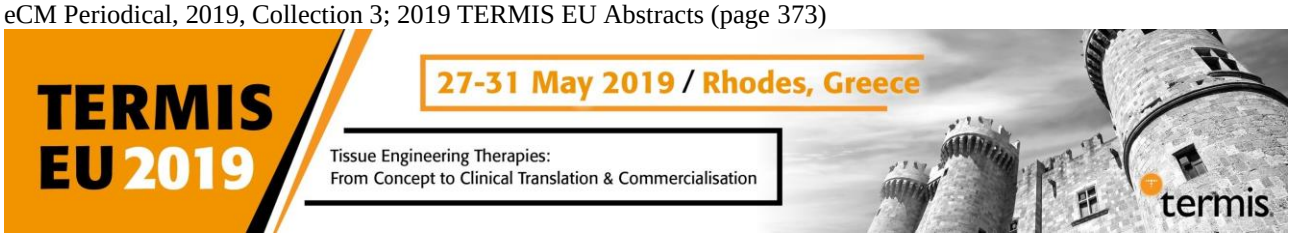
RESULTS & DISCUSSION: The [high IKVAV] composition had an important performance in vitro inducing: (i) upregulation of a panel of osteogenic markers in MSCs; (ii) upregulation of important molecules that trigger angiogenesis process in ECs; and (iii) the formation of the longest neurites in SNs. In vivo, hydrogels induced no signals of major inflammation. The surrounding region of the [high IKVAV] composition had higher vessel density relative to the scrambled after 26 days and the innervation process was detected only in IKVAV composition implantation for both time points. The density and surface of neuronal structures seem to increase over time.

CONCLUSIONS: We produced ELP-based hydrogels with fine-tunable rheological properties, porous structure and biocompatible in vitro. [high IKVAV] composition showed higher osteogenic, angiogenic and neurotization potential in vitro. In vivo, hydrogels were not inflammatory and [high IKVAV] composition induced higher vascularization and neurotization subcutaneously. [High IKVAV] composition has important features for biomedical applications, being a cell- and growth factors-free composite able to support vascularization and neurotization in vitro and in vivo with a great potential to induce osteogenesis.

ACKNOWLEDGEMENTS: The authors would like to thank the Fédération de Recherche Technologies pour la Santé (TecSan), Bordeaux Consortium for Regenerative Medicine (BxCRM), Université de Bordeaux, and Conseil Régional de Nouvelle Aquitaine.

REFERENCES

[1] Marrella A et al. Mater Today (Kidlington). 2018; 21(4):362-76



Size exclusion chromatography is an efficient method of isolating extracellular vesicles from a novel human umbilical cord MSC population

C. Conroy[1,2], L. Deedigan[2], J. Cabral[1], E. Horan[2], L. O'Flynn[2], M. Griffin[1], S. Elliman[2]
Presenting Author: Callum Conroy, callum.conroy@orbsentherapeutics.com
[1]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]Orbsen Therapeutics LTD, Galway, Ireland

INTRODUCTION: Mesenchymal stromal cells (MSCs) exert numerous therapeutic effects including immunomodulatory and anti-inflammatory effects in vitro and in vivo. These therapeutic effects can be attributed in part to the paracrine action and soluble factors released, including extracellular vesicles (EVs). Size exclusion chromatography (SEC) has recently been demonstrated as an effective alternative to differential ultracentrifugation (UC) for the isolation of EVs [1-3]. Herein, we assessed the isolation of EVs by SEC from MSCs that were culture-expanded from primary CD362+ human umbilical cord stromal cells.

METHODS: EVs were isolated from human umbilical cord CD362+ MSC conditioned media by UF and SEC. Size and particle count of eluted SEC fractions were determined using nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). Identification and characterisation of EVs was achieved by SDS-Page and using flow cytometric analysis (MACSPlex human exosome detection kit, Miltenyi).

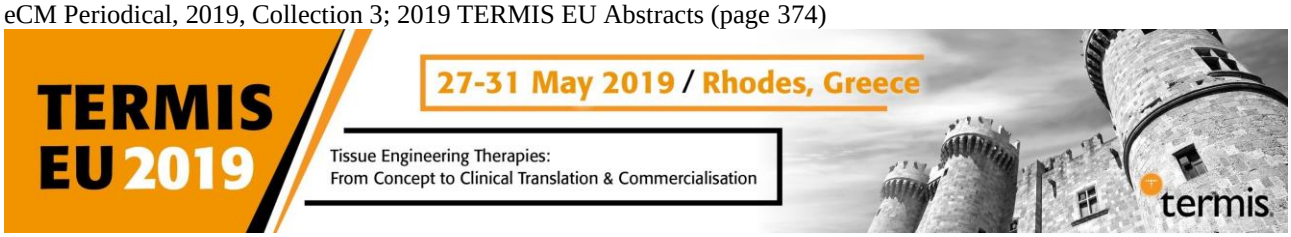
RESULTS & DISCUSSION: EVs were identified primarily within the first four SEC eluted fractions. Within these, mode and mean particle size were 87.7nm and 142.7nm respectively ± 68.5nm. Average particle yield was 6.89 x 10^3/cell. Detection of the EV marker CD63 in elution fractions corresponded with low protein concentration. Additional EV markers CD9, CD63 and Alix were detected by western blotting. Flow cytometric analysis determined the surface expression phenotype of MSC derived EVs shared several phenotypic MSC markers, specifically CD29, CD44, CD73, CD105, CD142, CD146 and MCSP were all detected.

CONCLUSIONS: The results demonstrate SEC to be an appropriate and efficient method of isolating EVs from human umbilical MSC. Furthermore, the preliminary flow cytometric surface phenotyping of EVs from CD362+ human umbilical cord MSC is a novel finding.

ACKNOWLEDGEMENTS: Financial support was received from the Irish Research Council and Orbsen Therapeutics LTD.

REFERENCES

[1] Lozano-Ramos I, et al. J Extracell Vesicles. 2015;4:27369
[2] Boing AN, et al. J Extracell Vesicles. 2014;3;23430
[3] Hikita T, et al. Sci Rep. 2018;8;14035



Mesenchymal stromal cell secretory activity: The role of secretome fractions in angiogenesis

C. Gorgun[1,2], F. Villa[2], R. Quarto[1,2], R. Tasso[1,2]
Presenting Author: Cansu Gorgun, cansu.gorgun@edu.unige.it
[1]Department of Experimental Medicine, University of Genova, Genova, Italy [2]U.O. Cellular Oncology, IRCCS Ospedale Policlinico San Martino, Genova, Italy

INTRODUCTION: MSC paracrine activity, including both soluble molecules and factors released within extracellular vesicles (EVs), can be modulated by cues from the microenvironment, mimicking the milieu established during an injury event as the one generated by a bone fracture [1]. Although several cell-secreted factors have been identified, their exact role in some aspects of the fracture healing process, i.e. in angiogenesis, is currently unknown. Given that the presence of areas of hypoxia is a prominent feature of various inflamed and diseased tissues, we here aim to determine the role of the injury microenvironment in modulating the angiogenic potential of MSC secretome fractions.

METHODS: Human MSCs were obtained from liposuction aspirates of subcutaneous adipose tissue. When MSCs reached 80% confluence, they were incubated for 24 hours with the inflammatory factors TNF-α (50 ng/ml) and IL1-α (50 ng/ml), either under normoxic or hypoxic conditions. The angiogenic potential of total conditioned media (CM), corresponding EVs, and EV-free CM were evaluated in vitro by analyzing their effect on HUVEC migration, proliferation and differentiation. Additional ex vivo metatarsal assay was performed to confirm in vitro results.

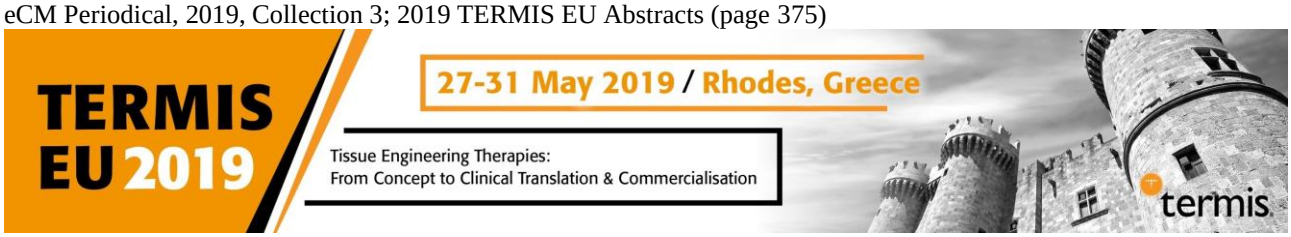
RESULTS & DISCUSSION: Preconditioning of MSCs under mentioned stimuli triggered the cell secretory activity and these stimulated factors affected the angiogenic process in different ways. We observed that while the secretomes stimulated under hypoxic conditions enhanced in vitro endothelial cell migration, the combinatorial effect of inflammatory factors and hypoxia significantly increased their differentiation capacity without affecting their proliferative potential. In order to confirm in vitro results, we investigated the sprouting of endothelial cells by ex vivo metatarsal assay and a widespread sprouting of blood vessels from fetal bone was induced by secretomes derived under hypoxic conditions and combination of inflammatory factors.

CONCLUSIONS: Our preliminary results confirm the importance of a proper pre-conditioning to positively influence the cell paracrine activity. Additional analysis of the secretomes and a deep characterization of the role exerted by EVs would be crucial in the generation of successful clinical cell-free therapies.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 721432.

REFERENCES

[1] Kusuma, Gina D., et al. Stem Cells and Development 2017; 26 (9): 617-631.



DAT coated 3D melt-electrowritten fiber scaffolds as an inductive substrate for adipogenesis in human mesenchymal stromal cells

C. Blum[1], K. Schlegelmilch[1], T. Schilling[1], P. D. Dalton[1], T. Blunk[2], L. E. Flynn[3], J. Groll[1]
Presenting Author: Carina Blum, carina.blum@fmz.uni-wuerzburg.de
[1]Department for functional materials in medicine and dentistry, University of Würzburg, Germany [2]Department of Trauma, Hand, Plastic and Reconstructive Surgery, University of Würzburg, Germany [3]Department of Biomedical and Molecular Sciences, Queen´s University, Ontario, Canada

INTRODUCTION: Scaffold geometry and architecture are key attributes to affect cell adhesion, tissue ingrowth and cell differentiation processes of human mesenchymal stromal cells (hMSCs). A defined scaffold geometry, fiber thickness well below 10 µm and mesh size can be achieved through melt electrowriting (MEW) that combines electrospinning of a polymer melt with computer-assisted moving of the collector [1]. Due to the low fiber diameter, such scaffolds, even when produced from PCL, remain soft and flexible. Additionally, the complex micro-environment of the extracellular matrix (ECM) is crucial to control stem cell differentiation. Individual ECM components like laminin (LN) and fibronectin (FN) are commercially available but cannot reproduce the positive effects of the complex native ECM without a proper 3D arrangement. The aim of this project is to generate a functionalized 3D fiber scaffold to enhance the remodeling and repair of adipose tissue in vitro by combining the 3D construct design with the adipose-inductive DAT (decellularized adipose tissue). Thereby, the DAT-functionality should reduce the need for artificial differentiation reagents in the cell culture medium.

METHODS: Box-structured poly(ε-caprolactone) (PCL) fiber scaffolds were fabricated with MEW and afterwards functionalized by the adsorption of DAT, FN or LN. The adipogenic differentiation behavior of hMSCs cultivated on these scaffolds and induced by the short-time application with differentiation factors in the culture medium was examined by quantitative RT-PCR and ELISA. Intracellular lipid droplets were visualized via immunofluorescence staining and quantified via trigylceride assay.

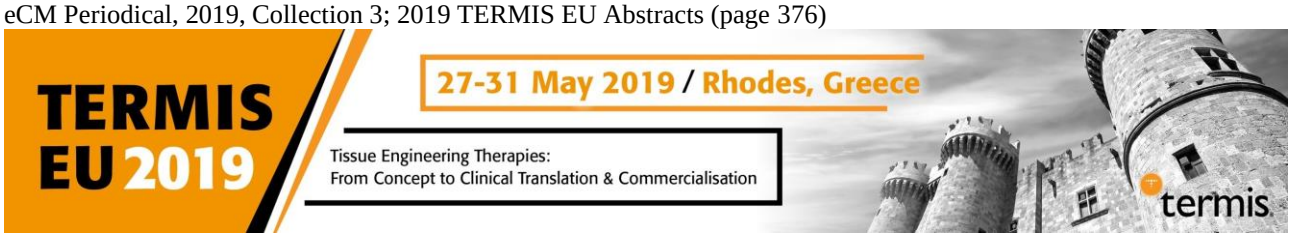
RESULTS & DISCUSSION: Adipogenic differentiation of hMSCs on functionalized (DAT, LN, FN) as well as non-functionalized scaffolds differed depending on the pre-differentiation time as detected by adipogenic mRNA, intracellular FABP4 protein, and leptin secretion. Especially, the DAT-functionalization enhanced the adipogenic outcome of short-time pre-differentiated hMSCs. Moreover, the accumulation of adipocyte-specific lipid droplets increased with extended incubation in adipogenic media and higher complexity of the fiber functionalization.

CONCLUSIONS: The beneficial impact of fiber functionality on the differentiation of hMSCs into the adipogenic lineage was proven. Even the short-time pre-differentiation of hMSCs was sufficient to achieve adipogenesis on the 3D scaffolds while the adipogenic outcome was further enhanced by the DAT-functionality. Scaffolds with a defined architecture produced by MEW combined with DAT are a promising tool to further investigate the influence on adipogenic hMSC differentiation.

ACKNOWLEDGEMENT: European Research Council (ERC) (consolidator grant Design2Heal, contract # 617989) and the German Research Foundation (DFG) (INST 105022/58-1 FUGG).

REFERENCES

[1] Brown T. D. et al., Adv. Mater. 2011, 23:5651



Effect of EpSCs conditioned medium on dermal papilla cells

C. Abreu[1,2,3] M. Cerqueira[1,2] R.Pirraco [1,2] R. Reis[1,2,3], F. Watt [4], A. Marques[1,2,3]
Presenting Author: Carla Abreu, carla.abreu@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal [4] Centre for Stem Cells & Regenerative Medicine, King's College London, UK

INTRODUCTION: Dermal papilla (DP) cells are key hair inductive cells. However, the isolation of inductive human DP cells is still very challenging. Our previous studies demonstrated that their indirect culture with epidermal cells with stem-like characteristics (EpSCs) improved DP cells phenotype, and their combined injection recreated hair follicle-like structures in the nude mice [1]. Nonetheless, this approach is limited by the required large numbers of cells. To surpass that, this work hypothesizes that EpSCs conditioned medium (CM) promotes similar beneficial effects in DP cells.

METHODS: EpSCs were selected out of a mixture of human skin-derived keratinocytes by culturing them in a 3T3-feeder layer. After being removed from the feeders, EpSCs were culture in KSFM and 48h-CM was collected and used on DP cells. After 5 days of culture in CM, DP cells were characterized regarding their proliferation (DNA quantification), glycosaminoglycans and protein (GAGs) production (Bradford, Sirius Red and DMB assays), inductivity (ALP activity) and secretome (ELISA). Moreover, cells cultured with CM were used do prepare spheroids, a 3D-system known to partially recover DP phenotype [2], and further cultured in standard DMEM to confirm the stability of the phenotype achieved in 2D-culture.

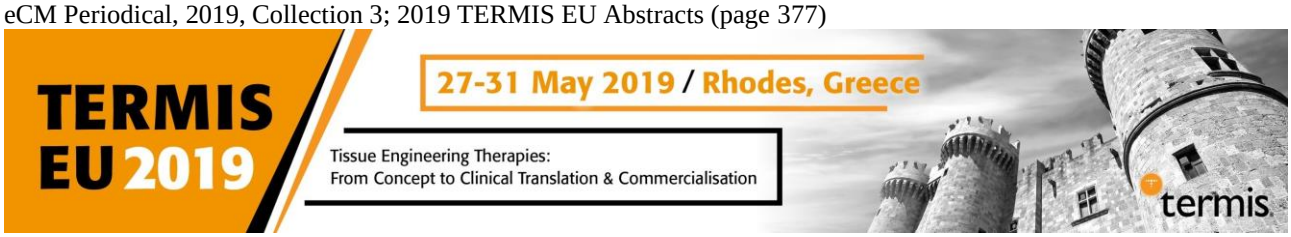
RESULTS & DISCUSSION: DP cells proliferation and ALP activity were significantly higher when DP cells were cultured with EpSCs CM, however the production of protein and GAGs decreased. Regarding the release of growth factors, DP cells cultured in CM secreted higher levels of VEGF, BMP2, IL-6 and lower levels of PDFG-AA. When aggregated in spheroids, DP cells from the CM culture behaved more closely to the native ones, proliferating less and producing higher quantity of proteins, while sustaining higher ALP activity. These cells also showed increased ALP and sustained α-SMA and versican V1 expression.

CONCLUSIONS: Our results suggest that EpSCs CM can beneficially modulate DP cellular behavior and phenotype. Further studies are being performed to confirm these results in vivo.

ACKNOWLEDGEMENTS: Financial support was received from FCT/MCTES (IF/00945/2014 and IF/00347/2015 grants), Gene2Skin Project (H2020-TWINN-2015-692221), NORTE-01-0145-FEDER-000021, FSE/POCH (PD/59/2013 and PD/BD/113800/2015 scholarships) and THE DISCOVERIES CTR (739572).

REFERENCES

[1] Abreu CM. et al. Gene2Skin Summer School. Porto, Portugal (2018).
[2] Higgins CA et al. PNAS (2013); 10(49):19679-88



Novel water-based, detergent-free decellularization to produce bioactive ECM-based scaffolds for pancreatic islets transplantation

R. Tamburrini[1], A. Asthana[1], C. Gazia[1], S. Muir[1], D.I. Marino[1], D. Chaimov[1], A. Gallego[2], A. Migliorini[3], C. Nostro[3], P. Cravedi[4], A. Tomei[5], G. Orlando[1]
Presenting Author: Carlo Gazia, cgazia@wakehealth.edu
[1]Department of Surgery, Wake Forest University School of Medicine, Winston Salem, USA [2]Nanoimmunotech, Vigo, Spain [3]McEwen Centre for Regenerative Medicine, University Health Network, Toronto, Canada [4]Icahn School of Medicine at Mount Sinai, New York, USA [5]Diabetes Research Institute, University of Miami, Miami, USA

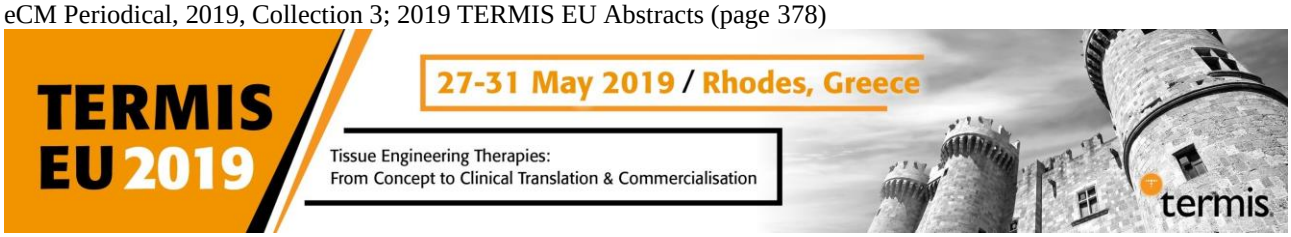
INTRODUCTION: Acellular ECM-based biomaterials are obtained at the cost of a significant loss of critical components of the innate matrisome, which will impair the bioactivity of the end product. In the frame of the BIOCAPAN project (www.biocapan.eu), a novel water-based, detergent-free method was developed to generate ECM-based biomaterials from the human pancreas, to be used to enhance islet function and prolong their lifespan.

METHODS: Human pancreases were benched to obtain 1cm^3 cubes pancreatic tissue. Samples were placed in 1-liter jars containing deionized water and shaken at 200rpm for 24 hours at 4°C. Then, they were rinsed with DNAse and a TRIS-EDTA buffer solution containing MgCl before a final wash with deionized water for 24 hours. The so-obtained biomaterial was then frozen, lyophilized and cryomilled, to produce a fine ECM powder that was eventually solubilized with HCl-pepsin solution. The powder's matrisome was analyzed using mass spectrometry and an ELISA-based multiplex assay. The effects of the powder on cell viability through MTT assays and cell functionality through glucose-stimulated insulin secretion were tested with multiple cell lines including human MSC and murine insulinoma cells (MIN6), and with hESC-derived pancreatic progenitors to determine their capacity to drive cell differentiation. The ECM was also tested with Jurkat Cell line for apoptosis assay, A549 cell for ROS production, hemolysis, coagulation, platelets, complement and lymphocyte activation to assess the safety profile. The immunogenicity of ECM was tested in a Treg induction assay where naïve human CD4$^+$ T cells were cultured with aCD3/aCD28 Ab, IL-2, TGFβ +/- ECM.

RESULTS & DISCUSSION: Human pancreas ECM-based soluble powder can be successfully and consistently obtained with our method. The powder shows the presence of several critical components of the innate matrisome, including multiple types of collagen, which was the most abundant ECM component. A large number of ECM regulators, ECM glycoproteins, ECM affiliated proteins, proteoglycans and secreted factors were also identified. Viability of MIN6 cells cultured as a monolayer was not affected by powder addition independently of its concentration in the culture media, suggesting that the ECM powder was not toxic. MSCs proliferated and grew well in presence of our ECM which was also able to induce higher yields of hESC-derived β-cells. The pancreatic ECM did not show activation of hemolysis, coagulation, platelets or lymphocyte. Finally, ECM did not impair in vitro Treg induction, a major mechanism of immune tolerance.

CONCLUSIONS: We have developed a novel, gentler detergent-free method to produce ECM scaffolds from the human pancreas whose composition recapitulates the innate islet niche. Extensive in vitro experiments are undergoing to test the ability of these ECM-based scaffolds to enhance islet function and lifespan in vitro and in vivo.

ACKNOWLEDGEMENTS: Horizon 2020, through Grant Agreement ID # 646272.



Microfluidic fabrication of 3D biomaterial libraries for high-throughput screening stem cell responses

C. F. Guimarães[1,2,3], L. Gasperini[1,2], R. S. Ribeiro[1,2,3], A. C. Pinto[1,2], A. F. Carvalho[1,2], A. P. Marques[1,2,3], R. L. Reis[1,2,3]
Presenting Author: Carlos Guimarães, carlos.guimaraes@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Within the field of Tissue Engineering, there is great need for platforms that allow high-throughput (HT) screening of cell responses to wide ranges of biomaterial formulations and environments (libraries).

METHODS: A custom microfluidic system [1] was used to produce 3D hydrogel fibers with concentration gradient of gelling polymer, ECM molecules and bioactive components. Human adipose stem cells (hASCs) were encapsulated and exposed to wide ranges of conditions within one single gradient and the most adequate formulations could be identified by measuring the best cellular outcome (Fig. 1).

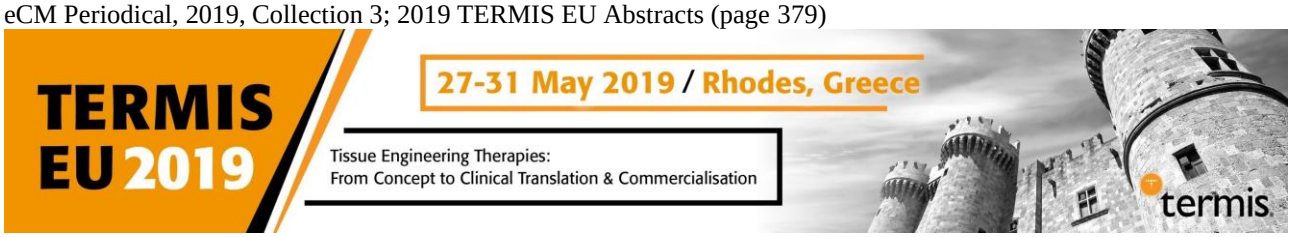
RESULTS & DISCUSSION: Gellan Gum (GG) hydrogels within 0.5-1 wt.% revealed a storage modulus range of 5-20 kPa. hASCs cultured for 1 week in chondrogenic medium showed significantly increased expression of Sox9 (chondrogenic regulator) on the stiffer hydrogels as assessed by immunocytochemistry, validated through western blot. In parallel, cell-laden GG microfibers of 0.5-1 wt.% gradient in composition were produced and subjected to similar culture conditions. The Sox9 expression in each cell was tracked along the gradient position and its gradual increase was observed with the increasing GG concentration. Reversely, the adipogenic triggering (PPAR-y expression) was favored on the softer regions, as previously validated [2]. We further employed our system to detect different stem cell responses (viability, adhesion, morphology), as function of bioactive matrixes such as hyaluronic acid and gelatin.

CONCLUSIONS: We have employed a novel microfluidic system to obtain 3D gradients for HT analysis of stem cell responses, not only recapitulating those taking place in normal hydrogels but also capable of discriminating different formulations for distinct responses.

ACKNOWLEDGEMENTS: Advanced ERC-2012-AdG-20120216-321266 project ComplexiTE. FCT PD/BD/135253/2017 (CFG), PhD Program TERM&SC (FCT), FCT SFRH/BPD/109595/2015 (AFC). The Discoveries Centre for Regenerative and Precision Med.

REFERENCES

[1] Gasperini L et al. WO2018078562A1 (Patent)
[2] Lago M et al. Bioengineering. 2018; 5(3):52



A new non-invasive optical sensor foil based technique for measuring the 3D-oxygen gradient formation during mammalian cell culture

C.J. Peniche Silva[1], G. Liebsch[2], R.J. Meier[2], M. Gutbrod[2], E.R. Balmayor[1], M. van Griensven[1]
Presenting Author: Carlos Peniche, carlos.peniche@tum.de
[1]Experimental Trauma Surgery, Klinikum Rechts der Isar, Technical University of Munich, Germany
[2]PreSens Precision Sensing GmbH, Regensburg, Germany.

INTRODUCTION: Although oxygen levels may remain apparently stable inside a cell culture incubator, we do not know much about the relationship between the oxygen consumption rate of the cells and the diffusive delivery of oxygen through the overlying medium layer [1,2]. In the present study, we tested a new "in situ" non-invasive optical sensor foil based technique for real time monitoring of the vertical oxygen gradient formed within cell culture wells with different cell concentrations.

METHODS: Human adenocarcinomic alveolar epithelial cells (A549) were seeded at 5.0×10^4, 1.0×10^5, and 1.5×10^5 cells/cm^2 in 48-well plates containing 1 ml of high glucose Dulbeccos's modified Eagle Medium (DMEM, Sigma-Aldrich), supplemented with 10% fetal bovine serum and 1% antibiotics. For the "in vitro" monitoring of oxygen levels, a camera-supported optical sensor foil-based technique VisiSens (PreSens, Regensburg, Germany) was employed. Sensor foils were attached to small, 3D-printed ramps with a 25 degrees angle and a maximum height of 3.3 mm. Oxygen levels were optically measured using a pixel profiling tool (48 pixels across the sensor) every hour for a period of 96 hours.

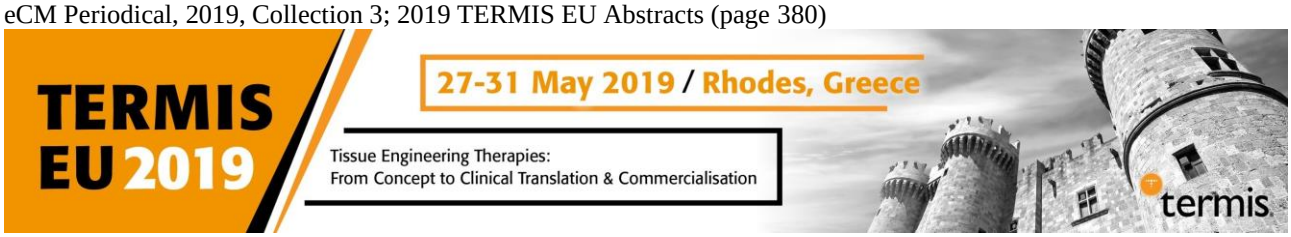
RESULTS & DISCUSSION: The experimental set up reveals that a confluent cell culture may be exposed to oxygen levels lower than required to sustain standard physiological conditions. Oxygen concentrations in high density cell cultures (1.5×10^5 cells/cm^2) dropped from 8% at 48h of culture to 2-3% after 96h, compared to a drop from 13% to 9% when cultivating 5.0×10^4 cells/cm^2 during the same time frame. In both cases, oxygen concentrations near the well surface remained constant (Figure 1).

CONCLUSIONS: With this new technique, it is possible to determine how the cell density can affect the oxygen supply during cell culture. Moreover, oxygen gradients can be measured non-invasively in a kinetic way.

ACKNOWLEDGEMENTS: Financial and material support was received from PreSens. The project was support by a grant from the Bayerische Forschungsstiftung ("Happihypo").

REFERENCES

[1] Pettersen E et al. Cell Proliferation. 2005; 38(4):257-67
[2] Wolff P et al. Material Sci & Eng C. 2018; 95(2019):422-427



"Clickable" ELR-based biomaterials

C. García-Arévalo[1], M. González-Pérez[1], M.H. Gutierrez[1], J.C. Rodríguez-Cabello
Presenting Author: Carmel García-Arévalo, cgarcia@bioforge.uva.es
[1]GIR BIOFORGE, Universidad de Valladolid CIBER BBN, Edificio LUCIA, Paseo de Belén 11, 47011, Valladolid, Spain

INTRODUCTION: Many clinical situations that require the use of biomaterials and cells combined with them hold immense potential for reversing injuries, illness or degenerative situations as they have shown promising in-vitro. However, there are many hurdles that must be drawn for their success in clinical practice (1). As far as the biomaterial itself is concerned, the specific medical application determines the design and specific parameters demanded but, in general terms, universal requirements for successful usage in the body without immune rejection could be: biocompatibility, modulable mechanical properties according to the needs or the tissue to which they are intended, stable physical and chemical properties with the biological systems (or modulable when needed), able to be functionalized and with high wear resistance or with integration capacity. About the inclusion of cells in biomaterials, they should guarantee cell survival and/or integration into the host tissue as major challenges (2). "Clickable" ELRs meet these essential requirements and have proven to be useful both in-vitro and in-vivo for many clinical situations.

METHODS: ELRs were biosynthesized and purified with the advantage of their Inverse Temperature Cycling. All the bioproduced ELRs were systematically and fully characterized for their composition, purity, thermos-responsiveness, structure and endotoxin content. The ε-amine group in the side chain of the lysine residues were modified to bear cyclooctyne and azide groups for click-chemistry crosslinking. This chemical modification has been previously reported (3). The modified ELRs were characterized by NMR and FTIR to assess the degree of modification.

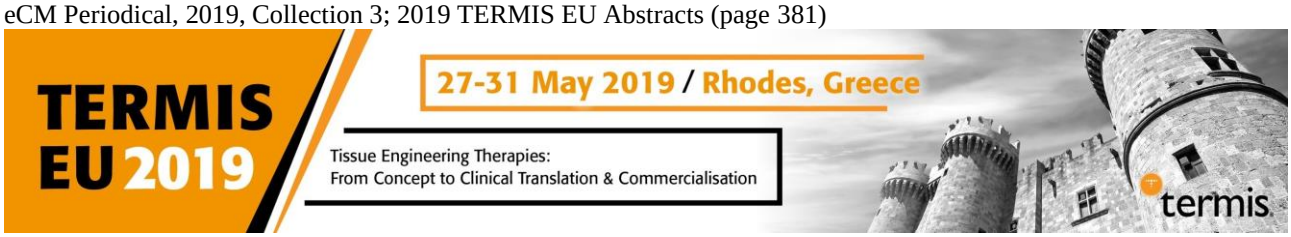
RESULTS & DISCUSSION: Structural and a biofunctionalized ELRs have been prepared and modifyed to bear the reactive groups needed to crosslink via "click chemistry". The initial dynamic covalent crosslinking of this stereospecific and fast reaction combined with thermoresponsive behavior of these engineered proteins allows to obtain different "in situ" or pre-formed supports (injectable hydrogels or fibers), that can be easily modulated for their mechanical, topological and chemical properties. These ELR-based supports can preserve cell growth both in-vitro and in-vivo.

CONCLUSIONS: Our group has a series of clickable and biocompatible ELR, suitable to support or imbibe cells for various applications in tissue engineering and regenerative medicine.

ACKNOWLEDGEMENTS: This project has received funding form the European Union´s Horizon 2020 research and innovation program under grant agreement No 646075.

REFERENCES

[1] Mitrousis N et al. Nat Rev Mater. 2018; 3:441-56
[2] Ibañez-Fonseca A et al. J Tissue Eng Regen Med. 2018; 12(3): e1450-60
[3] González de Torre I et al. Acta Biomater. 2014; 10:2495-2505.



Hydroxyapatite/MgO spherical granules: An innovative bone substitute to prevent dental and orthopaedic infections

C.C. Coelho[1,2,3,4], R. Araújo[1,2], P. A. Quadros[4], S. R. Sousa[1,2,5], F. J. Monteiro[1,2,3]
Presenting Author: Catarina Coelho, catarina.coelho@i3s.up.pt
[1]Instituto de Investigação e Inovação em Saúde, U.Porto, Portugal [2]Instituto de Engenharia Biomédica, U.Porto, Portugal [3]Faculdade de Engenharia, U.Porto, Portugal [4]FLUIDINOVA, S.A., Maia, Portugal [5]Instituto Superior de Engenharia, Politécnico do Porto, Porto, Portugal

INTRODUCTION: Nowadays, there is a great demand for bone regenerative therapies [1]. However, most of the bone substitutes present in the market are not capable of preventing bacterial colonization, leading to the development of serious infections that are a major concern in dentistry and orthopaedics [2]. For that reason, this work is focused on the development of a granular bone substitute of hydroxyapatite (HAp) and magnesium oxide (MgO) to prevent implant-associated infections.

METHODS: HAp/MgO granules were produced [3] and characterized using SEM with X-ray mapping and FTIR. In vitro antibacterial activity of the granules was accessed in terms of planktonic bacteria and biofilm formation towards Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli). Cytotoxicity tests were carried out in accordance to ISO 10993-5:2009 and with live/dead cell staining. For that purpose, ISO 10993-12:2012 was used as a guide to prepare granules extracts. The production of reactive oxygen species (ROS) was also evaluated.

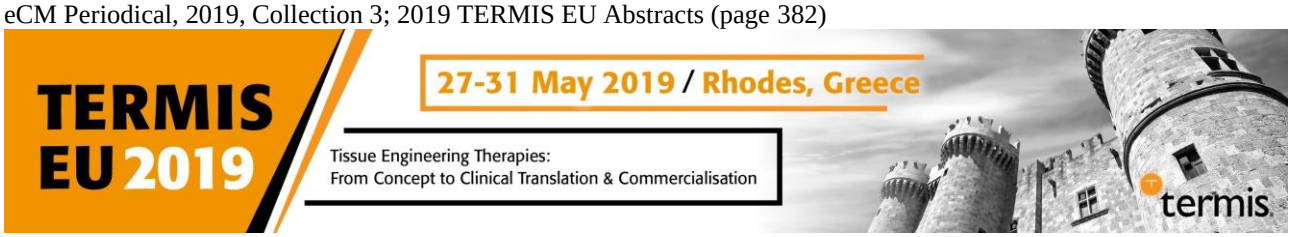
RESULTS & DISCUSSION: HAp/MgO spherical granules were successfully produced with diameters between 0.5 and 1.0 mm. SEM images showed MgO distribution in the surface of the granules and the FTIR presented the characteristic peaks for this composite. Granules containing concentrations of MgO equal or higher than 3 wt% were able to significantly reduce S. aureus and E. coli planktonic growth and biofilm formation. Cytotoxicity assays revealed that none of the produced materials were cytotoxic to L929 fibroblasts. Additionally, no significant cell death was observed with live/dead cell staining after 3 days of incubation with granules extracts. Finally, none of the materials induced ROS formation by cells.

CONCLUSIONS: HAp/MgO granules significantly reduce both S. aureus and E. coli planktonic growth and biofilm formation, being a promising antibacterial bone substitute.

ACKNOWLEDGEMENTS: Financial support was received from FEDER funds through the COMPETE 2020– POCI, Portugal 2020, Portuguese funds through FCT/MCTES in the framework of the project Institute for Research and Innovation in Health Sciences (POCI-01-0145-FEDER-007274), by projects Biotherapies (NORTE-01-0145FEDER-000012) and NoMic2Bone (NORTE-01-0247-FEDER-017905) and by the PhD grant (SFRH/BDE/108971/2015).

REFERENCES

[1] García-Garreta E et al. Bone. 2015; 81:112-21
[2] Grenho L et al. JBMR A. 2014; 102(10):3726-33
[3] Coelho et al. Mat. Sci. Eng. C. 2019; 97:529-38



Fucoidan impairs the formation of tubular-like structures in vitro and inhibits tumor growth in vivo

C. Oliveira[1,2], S. Granja[2,3], N. M. Neves[1,2,4], R. L. Reis[1,2,4], F. Baltazar[2,3], T. H. Silva[1,2], A. Martins[1,2]
Presenting Author: Catarina Oliveira, catarinasalazaroliveira@gmail.com
[1]3B´s Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho; Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine; AvePark, Parque de Ciência e Tecnologia, 4805-017, Barco, Guimarães, Portugal [2]ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal [4]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Angiogenesis is essential for cancer cells survival and cancer progression [1]. Therefore, blocking this phenomenon is one possible route to develop more effective anticancer agents. Fucoidan seems to be a promising natural-origin alternative to current cancer chemotherapeutics, with both pro- and anti-angiogenic behavior reported [2]. To clarify this biological behavior, the angiogenic potential of an effective anticancer fucoidan extract [3] was assessed.

METHODS: Two tube formation assays (TBA) were conducted: one where fucoidan (0.5 mg ml $^{-1}$) was added at the time of human endothelial cells (HPMEC-ST1.6R) seeding on top of matrigel (i.e. TBA I); and another where fucoidan was only added 4h after endothelial cells adherence (i.e. TBA II). The secretion of the angiogenic factors VEGF and PDGF was quantified by ELISA. Two different Chick Embryo Chorioallantoic membrane (CAM) assays were performed: one with only fucoidan injection (i.e. CAM I) and the other with fucoidan injection after a tumor mass (MDA-MB-231) onplantation (i.e. CAM II).

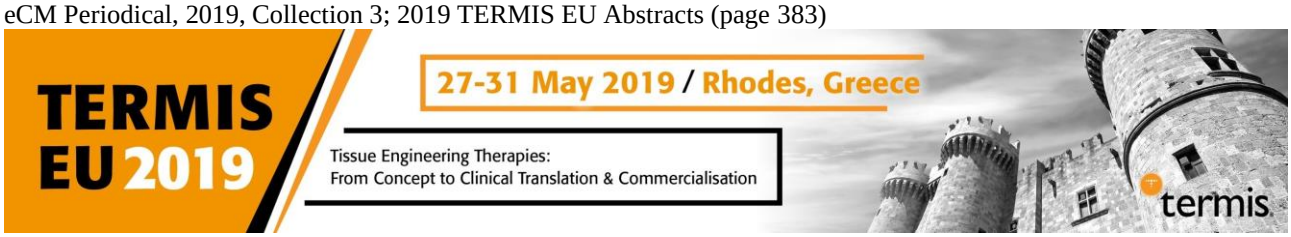
RESULTS & DISCUSSION: Tube formation assays demonstrated that fucoidan inhibited (TBA I) or disrupted (TBA II) the formation of tubular-like structures. Fucoidan, although not affecting VEGF secretion, significantly reduced the expression of PDGF (70% in TBA I and 40% in TBA II). Fucoidan injection reduces the number of blood vessels (CAM I) and significantly decreased tumor size by 25% (CAM II).

CONCLUSIONS: These results demonstrate the anti-angiogenic potential of this fucoidan extract both in vitro and in vivo. Fucoidan has a two-fold anticancer therapeutic potential: having toxic effect over cancer cells and hampering tumor growth by exerting a negative effect on tumor vascularization.

ACKNOWLEDGEMENTS: Structured projects for R&D&I NORTE-01-0145-FEDER-000013, NORTE-01-0145-FEDER-000021 and NORTE-01-0145-FEDER-000023, supported by NORTE 2020 and through FCT, under the scope of the project POCI-01-0145-FEDER-007038. The authors also thank Norte 2020, for financing the PhD scholarship of C.O. "Norte-08-5369-000037" and FCT for the grants of S.G. (SFRH/BPD/117858/2016) and A.M. (IF/00376/2014).

REFERENCES

[1] Carmeliet, P., et al 2011; Nature, 473(7347).
[2] Fitton, J. H., Stringer, et al 2015; Mar Drugs, 13(9).
[3] Oliveira, C., et al 2017; Macromol. Biosci. 17(1600340)



Human platelet lysate-based hydrogels – A humanized 3D platform for disease modelling guided by tissue engineering

C. F. Monteiro[1], C. A. Custódio[1], J. F. Mano[1]
Presenting Author: Cátia Monteiro, cfrmonteiro@ua.pt
[1]Department of Chemistry, CICECO, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

INTRODUCTION: Drug discovery and development, namely anticancer therapies, including for osteosarcoma (OS), has been hindered by the shortage of complex and robust drug screening platforms. [1,2] Human methacryloyl platelet lysates (PLMA) hydrogels are herein proposed as humanized 3D platforms for tumor invasiveness modelling and drug screening. [3]

METHODS: Spheroids of human bone-marrow mesenchymal stem cells (BM-MSC) and three tumor cell lines (MG-63, SaOS-2 and A549) were formed in ultra-low attachment plates and then encapsulated into PLMA hydrogels at 10, 15 and 20% (w/v), PEG and Matrigel for 14 days. For OS model development, MG-63 spheroids encapsulated into PLMA hydrogels at 15% (w/v) were cultured alone or surrounded by BM-MSC and human osteoblasts (OB). At 14 days of culture, a 3-day doxorubicin treatment was performed. Spheroid viability, morphology, area and invasiveness were assessed.

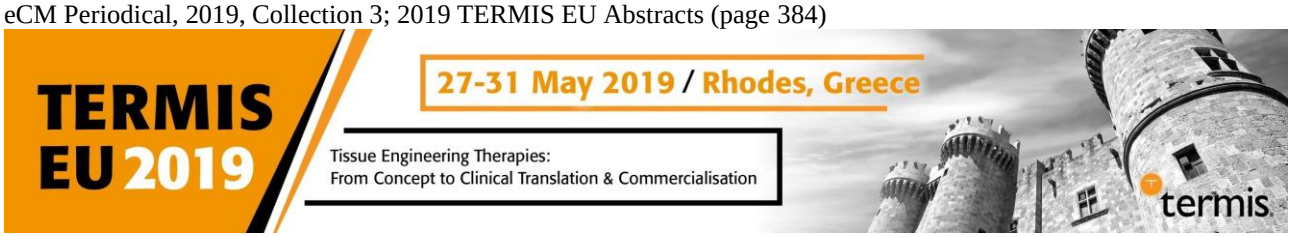
RESULTS & DISCUSSION: PLMA hydrogels supported cell spheroid viability, sustaining an in vivo-like cell polarity and invasion of all cell types. PLMA enhanced the invasiveness ability of the different cell spheroids comparing with Matrigel, providing tunable mechanical properties that allowed the control of invasion speed. In the OS co-culture model, long tumor invasive branches and a complex network of BM-MSC were formed, and OB maintained their phenotype. Comparing to OS mono-culture model, the tumor cells in the co-culture model were less sensitive to doxorubicin treatment, reflecting the protective role of stromal cells in chemoresistance. A higher cell viability in invasive branches and non-significant changes in spheroid area also demonstrated the pivotal role of stromal cell in cell behavior and drug resistance.

CONCLUSIONS: Overall, this study provides a proof-of-concept that the proposed 3D invasion models can be used to study early tumor metastatic events and as a platform for screening and validation of therapeutic agents, as clearly demonstrated in the case-of-study herein presented based on OS modelling.

ACKNOWLEDGEMENTS: This work was supported by European Research Council grant agreement ERC-2017-PoC-789760 for project MicroBone.

REFERENCES

[1] Monteiro C F et al. Adv. Ther. 2018; DOI 10.1002/adtp.201800108.
[2] Breslin S et al. Drug Discov. Today 2013; 18(5-6):240-9.
[3] Santos S C et al. Adv. Healthc. Mater. 2018; 7(23):1800849.



Harnessing the mesenchymal stem cell secretome as a therapy for ischaemic stroke

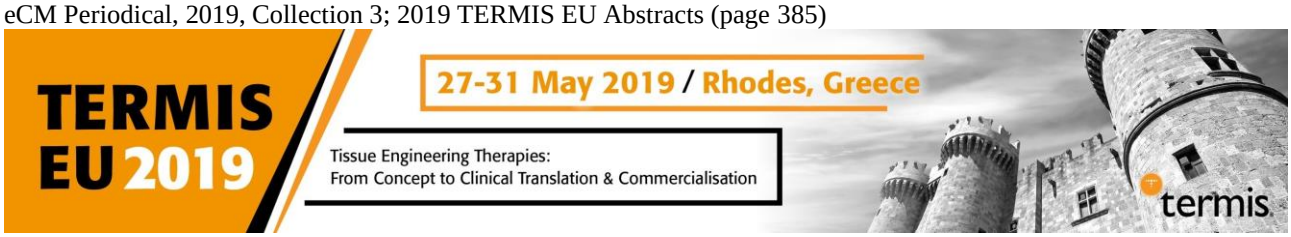
C.J. Cunningham, R. Wong, E. Redondo-Castro, J. Barrington, S. Tamburrano, E. Pinteaux, S.M. Allan
Presenting Author: Catriona Cunningham, catriona.cunningham@manchester.ac.uk
Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

INTRODUCTION: Stroke is a major global health problem with limited treatment options. Mesenchymal stem cells (MSCs) hold great potential as a regenerative therapy for stroke having previously been shown to promote repair and functional recovery in rodent models of cerebral ischaemia. As this occurs independently of cell migration to the ischaemic brain, engraftment and differentiation, focus has shifted to the paracrine actions of MSCs. MSCs secrete a vast array of chemokines, cytokines and growth factors, collectively termed the secretome. This secretome has been hypothesised to promote repair through several mechanisms including decreasing inflammation and promoting endogenous angiogenesis. The aims of our research were to assess the efficacy of the MSC-derived secretome (conditioned medium) on recovery, neuroinflammation and lesion volume in a mouse model of ischaemic stroke.

METHODS: Human bone marrow-derived MSCs were primed with 10 ng/ml human recombinant IL-1α for 5 minutes then at 24 h, conditioned medium was collected and 10x concentrated. Stroke was induced in 12-16 week old male C57BL/6 mice (Charles River Laboratories, UK) using the intraluminal filament model of middle cerebral artery occlusion. Either 400 µl 10x concentrated conditioned medium or MesenPRO (vehicle) was administered at the time of reperfusion by subcutaneous injection. Mice were then recovered for 14 days undergoing MRI scans at 48 h and a battery of behavioural tests to assess recovery. In a follow up study, treatment with conditioned medium was delayed to 24 h post-stroke.

RESULTS & DISCUSSION: MSC-derived conditioned medium at the time of stroke had a neuroprotective effect leading to ~30% reduction in lesion volume at 48 h, which was associated with modest improvements in recovery. When conditioned medium treatment was delayed to 24 h post-stroke, as expected, there were no differences in lesion volume between groups but significant improvements in recovery were observed. More specifically from day 7 onwards, conditioned medium treated mice had significantly lower 28-point neurological scores than the vehicle group and at day 8 performed better in a nest building task.

CONCLUSIONS: Our results suggest administration of MSC-derived conditioned medium at the time of stroke had a neuroprotective effect and led to modest behavioural improvements after ischaemic stroke. Delaying administration to 24 h also led to improvements in recovery and this was independent of neuroprotection. Therefore, the MSC secretome has great potential as a therapy for stroke and further work to elucidate the mechanisms of action is warranted.



Sympathetic nervous system and inflammation interplay in periprosthetic inflammation

M. Ribeiro-da-Silva [1,2], D.M. Vaconcelos[1,2], I.S. Alencastre[1,2], M.J. Oliveira[1,2], R. Henrique[3], D. Linhares[4], N. Neves[4], M. Lamghari[1,2], C.J. Alves[1,2]
Presenting Author: C.J. Alves, cjmalves@ineb.up.pt
[1]Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal [2]Instituto de Engenharia Biomédica (INEB), Universidade do Porto, Porto, Portugal [3]Serviço de Anatomia Patológica & Grupo de Epigenética e Biologia do Cancro, Instituto Português de Oncologia do Porto, Porto, Portugal [4]Serviço de Ortopedia, Hospital São João, Porto, Portugal

INTRODUCTION: The chronic inflammatory response to implant-derived wear particles is the hallmark of aseptic loosening and subsequent implant failure. Periprosthetic joint inflammation is a complex local biological response that takes place on synovial membrane-like interface tissues, triggered by the implant degradation products (polymeric, ceramic, metallic particles and ions) that shed and accumulate in this tissue. Over the past decades, accumulated evidence has clearly attributed a pivotal role to the sympathetic nervous system and its neurotransmitters in regulating chronic inflammatory conditions. In rheumatoid arthritis (patients and animal models), a deprivation of neuronal derived neurotransmitters in synovium tissue, due to the loss of sympathetic innervation, has been observed [1,2,3]. In implant aseptic loosening (AL) where the chronic inflammatory response to implant-derived wear particles results in implant failure, the involvement of the sympathetic nervous system is not yet elucidated.

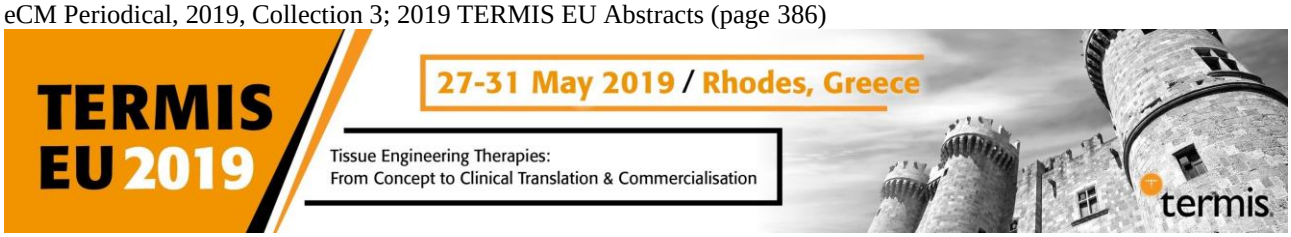
METHODS: In this study we evaluated the systemic and local profile of neuroimmune molecules involved in the interplay between the sympathetic nervous system and the periprosthetic inflammation in hip AL.

RESULTS & DISCUSSION: Our results showed that periprosthetic inflammation does not trigger a systemic response of the sympathetic nervous system, but is mirrored rather by the impairment of the sympathetic activity locally in the hip joint. Moreover, macrophages were identified as key players in the local regulation of inflammation by the sympathetic nervous system in a process that is implant debris-dependent and entails the reduction of both adrenergic and Neuropetide Y-ergic activity. Additionally, our results showed a downregulation of semaphorin 3A that may be part of the mechanism sustaining the periprosthetic inflammation. Overall, the local sympathetic nervous system emerges as a putative target to mitigate the inflammatory response to debris release and extending the lifespan of orthopedic implants.

ACKNOWLEDGEMENTS: Financial support was received form the FCT project Ref. PTDC/BIM-MED/4041/2014. CJA was supported by the project NORTE-01-0145-FEDER-000008 financed by NORTE 2020, under the PORTUGAL 2020 Partnership Agreement, through the ERDF. DMV and ISA were supported by Ph.D. and postdoc fellowships SFRH/BD/87516/2012 and SFRH/BPD/75285/2010, respectively.

REFERENCES

[1] Pongratz, G. et al. 2014; Arthritis Res Ther 16 (504): 1–12.
[2] Miller, L. E. et al. 2000; FASEB 14 (13): 2097–107.
[3] Jenei-Lanzl, Z. et al. 2015; Ann Rheum Dis 74 (2): 444–451.



TGF-β-laden polysaccharide material to modulate fibroblast-to-myofibroblast transition for scarring control

C.A. Mandon[1,2], L.P. da Silva[1,2], R.L. Reis[1,2,3], A.P. Marques[1,2,3]
Presenting Author: Céline A. Mandon, mandon@live.fr
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters, Portugal

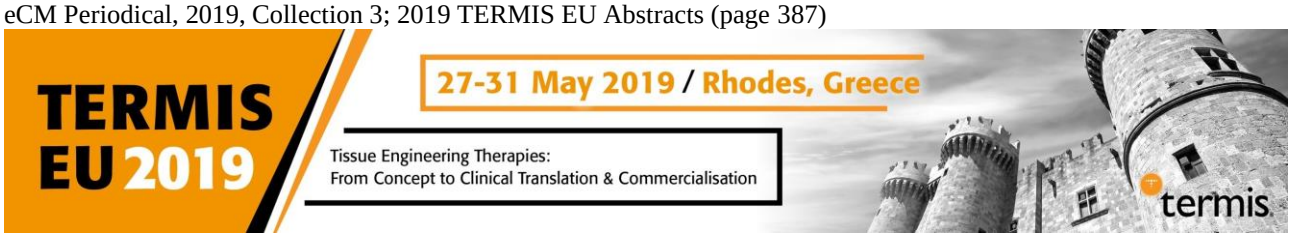
INTRODUCTION: During the reconstitution, and restoration of the integrity of injured skin, scar formation is frequently observed. A way to study and understand mechanisms underlying scaring and in turn, prevent it, is to focus on the fibroblast-to-myofibroblast transition. This work proposes the development of an ECM like material, linked with TGF-β1 and TGF-β3 growth factors, to generate a 3D platform in which their role and overlapping functions in the fibroblast-to-myofibroblast transition, and consequently on healing and scaring, can be unraveled.

METHODS: Several covalent or non-covalent chemical approaches have been considered, in order to graft gellan gum hydrogel (GG) with TGF-β1 and TGF-β3, implicated in the fibrotic scarring response and the scarless healing process. The obtained modified-GG was analyzed by 1H NMR and intrinsic viscosity tests. In addition, morphological and biological characterization of human dermal fibroblast phenotype after seeding within modified-GG will be performed, coupled to the expression pattern analysis of TGF-β-related proteins.

RESULTS & DISCUSSION: The incorporation of the growth factors within the hydrogel without any chemical bonding showed that although the proteins are retained in the higher concentration polymer network, they are rapidly released into the reacting medium from less concentrated polymeric networks. The first chemical strategy based on carbodiimide chemistry, bonded the growth factors via a non-reversible reaction. The BSA-FITC protein was linked to the GG, avoiding its release, independently of the GG concentration used.

CONCLUSIONS: TGF-βs-laden hydrogels show potential to work as 3D platforms to further investigate the implication of these growth factors in the scar formation during wound healing by modulating the TGF-β1/TGF-β3 ratio.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement N° ERC-2016-CoG-726061).



A bioartificial rat heart tissue: Perfusion decellularization and characterization

B. Ozlu[1], M. Ergin[1], S. Budak[1], S. Tunali[2], N. Yildirim[3], C. Erisken[1,4]
Presenting Author: Cevat Erisken, cevat.erisken@nu.edu.kz
[1]Department of Biomedical Engineering, TOBB University of Economics and Technology, Ankara, Turkey [2]School of Medicine, TOBB University of Economics and Technology, Ankara, Turkey [3]Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey [4]Department of Chemical and Materials Engineering, Nazarbayev University, Astana, Kazakhstan

INTRODUCTION: Heart-related defects are still prone to progress irreversibly, and can eventually lead to heart failure. A personalized extracellular matrix (ECM) based bioartificial heart created by allografts/xenografts can retain the original 3D architecture, in addition to preserved heart ECM. This study aimed at developing a procedure for decellularizing heart tissue harvested from rats, and evaluating decellularization efficiency in terms of residual nuclear content and structural properties.

METHODS: A modified version of a previously published [1] decellularization protocol was followed in this study. A total of 4 rat hearts (7 week-old, female Wistar Rats) were harvested, three of them were perfused as described below, and the forth served as a positive control. Briefly, rats were euthanized, and hearts were removed from the chest. A blunt needle was inserted into the ascending aorta and sutured to allow for retrograde coronary perfusion using chemical and biological agents (surfactants, acids, bases, and enzymes) combined with physical stimulation.

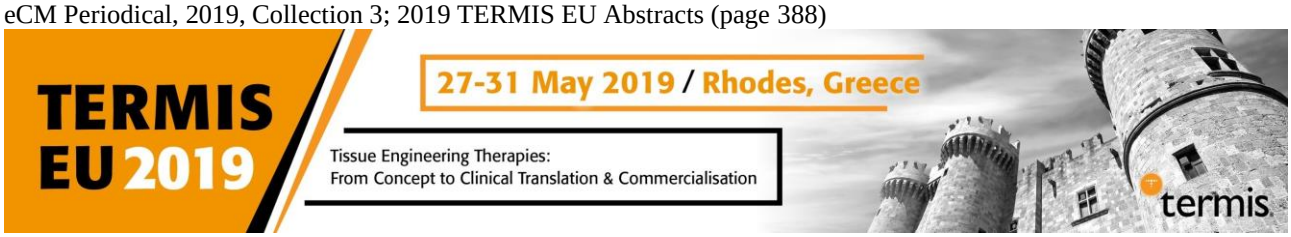
RESULTS & DISCUSSION: Removal of nuclear material (92.4%) was confirmed by histological examination. Tissue sections showed no or little visible cell nuclei in decellularized heart, whereas the native heart showed dense cellularity. Intact structure of decellularized heart was verified by the structure of whole heart, as well as similarity between decellularized and native cardiac muscles in terms of fiber alignment. Furthermore, no significant difference was detected between native and decellularized heart in terms of fiber diameter.

CONCLUSIONS: Our findings demonstrate that a heart bioartificial scaffold can be efficiently decellularized with chemical and biological agents, coupled with aortic perfusion as a physical stimulation. Technique described here could be used for other species upon necessary modifications. Previous studies commonly provided evidence for the presence of intact endocardium, myocardium and epicardium.[1,2] We recommend that fiber alignment and diameter should be included in the characterization of biological heart scaffolds as these could serves as valuable tools for evaluating structural preservation of decellularized heart. The bioartificial scaffold formed here can be functionalized with patient's own material, and utilized in regenerative engineering.

ACKNOWLEDGEMENTS: No funding was received for this study.

REFERENCES

[1] Lu, TY et al. Nat Comm. 2013; 4: 2307.
[2] Wainwright JM et al. Tissue Eng C Methods. 2010, 16(3):525-532.



Biodegradable microparticles with hierarchical topographical features influence VEGF secretion from mesenchymal stromal cells

C. Simitzi[1] R. Day[1]
Presenting Author: Chara Simitzi, c.simitzi@ucl.ac.uk
[1]Division of Medicine, University College London, London, UK

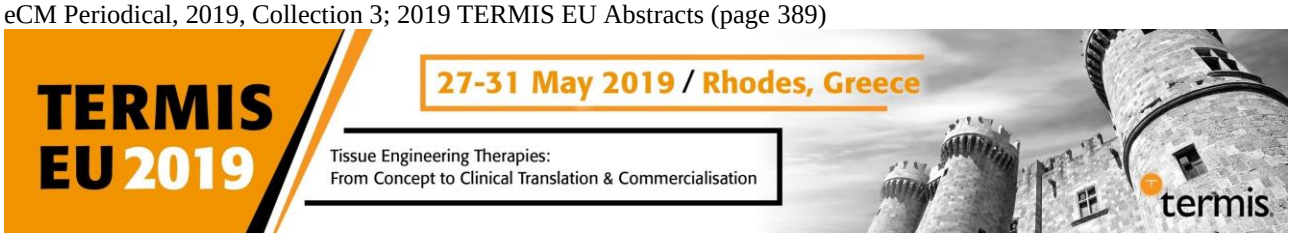
INTRODUCTION: Mesenchymal Stromal Cells (MSCs) are becoming increasingly important as therapeutic products due to the broad spectrum of trophic and immunomodulatory factors they secrete. The MSC secretome plays a role in angiogenesis and revascularization, immune modulation and tissue repair; however, there is a lack of methods suitable for controlling this effect. Evidence exists to show cell substrates with different topographical features can influence MSC behaviour. Therefore, manipulating the cell substrate could provide improved methods for controlling the secretome for new therapies; however, there is currently a lack of cell substrates suitable for implantation.

METHODS: The effect of implantable substrates consisting of highly porous biodegradable microparticles with hierarchical topographical features was investigated on MSC behaviour and secretome. Poly(DL-lactide-co-glycolide) microparticles were fabricated via the thermally-induced phase separation technique (TIPS). Three different polymer compositions of lactide/glycolide were studied. Microparticles were characterized in terms of surface topography and porosity. Human adipose-derived MSCs (ADMSCs) were incubated on the surface of the microparticles and cultured for 11 days in xeno-free medium. Cell growth on the microparticles was evaluated at six different time-points and compared with cells cultured on tissue culture plastic. The angiogenic activity of the ADMSC secretome was evaluated by ELISA and in vitro angiogenesis assays.

RESULTS & DISCUSSION: ADMSCs adhered and proliferated on all types of the microparticles. Vascular endothelial growth factor (VEGF) secretion was increased from cells cultured on the microparticles compared with cells cultured on tissue culture plastic. MSCs cultured on the microparticles remained viable after 12 days, were capable of migrating from the microparticles, and retained their lineage plasticity.

CONCLUSIONS: Our results show that attaching MSCs to biodegradable TIPS microparticles can influence the secretion of pro-angiogenic growth factors. This finding may provide a new method for re-vascularisation of tissue for regenerative medicine.

ACKNOWLEDGEMENTS: This research was funded by the British Heart Foundation (BHF Grant Number: PG/16/56/32246).



Engineering nerve tissue with silk: Graphene oxide fibres

C. Phamornnak[1], A. Magaz[1,2], X. Li[2], J.J. Blaker[1]*, S.H. Cartmell[1]*
Presenting Author: Chinnawich Phamornnak, chinnawich.phamornnak@manchester.ac.uk
[1]School of Materials, The University of Manchester, United Kingdom [2]Institute of Materials, Research and Engineering, A*STAR, Singapore

INTRODUCTION: Peripheral nerve injury (PNI) is a cause of medical consultation in more than 1 million patients worldwide, of which around 40% cases occur in Europe [1]. Graphene oxide (GO)-filled silk composite fibres hold great potential in tissue engineering due to their unique physicochemical and mechanical properties [2]. Herein, we assess the influence of GO incorporation within silk fibers as scaffolds for nerve tissue engineering.

METHODS: Regenerated silk fibroin (SF) in formic acid (FA) or hexafluoro-2-propanol (HFIP) at 10% w/v was electrospun into fibers with incorporation of GO at increasing concentrations (1%, 5% and 10% w/w GO/SF). Fibres were characterized by using field emission scanning electron microscopy (FESEM), Fourier-transform infrared (FTIR) and Raman spectroscopy. Antioxidant activity was assessed with a 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. NG108-15 neuroblastoma/glioma hybrid cells (5,000 cells/cm^2) at passage number P20 – P24 were seeded onto laminin-coated fibre scaffolds. Protein adsorption, metabolic activity, cell proliferation and viability were quantified; immunofluorescence of neuronal cell markers was investigated via confocal microscopy.

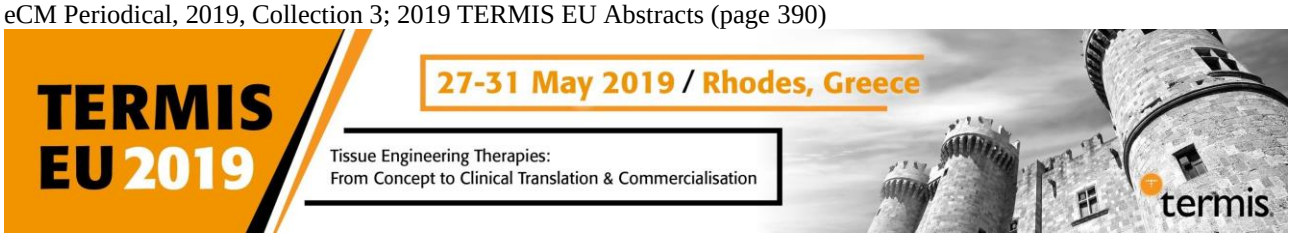
RESULTS & DISCUSSION: It is possible to control the silk fibre diameter by using FA (150 nm dia.) or HFIP (1.2 μm dia.) as solvent. Mean fibre diameter decreased ($p< 0.01$) with GO incorporation in FA fibres, with a flake lateral width distribution of around 170 nm. Fibre morphology was smooth and consistent for SF/HFIP with GO incorporation compared to SF/FA fibres. The overall chemical structure of silk remained unchanged as determined by FTIR, and Raman spectroscopy confirmed the presence of GO. Protein adsorption increased ($p<0.01$) with incorporation of GO in the fibres, as did the antioxidant properties of the scaffolds as a function of incubation time. In vitro analysis showed that the presence of GO enhanced the overall biological response of the scaffolds compared to SF alone ($p<0.01$), but this trend was non-significant for the highest GO inclusion investigated here.

CONCLUSIONS: These data illustrate the beneficial effects of incorporating GO in silk-based scaffolds for neuronal-like cell culture for cell growth and neurite extension. Further work is ongoing to develop conductive scaffolds that can enhance electrical signal propagation.

ACKNOWLEDGEMENTS: The Royal Thai Government, The University of Manchester EPSRC DTP (EP/N509565/1, 1786315) and A*STAR. C. Phamornnak and A. Magaz contributed equally to this work.

REFERENCES

[1] Magaz A et al. Adv Healthc Mater. 2018; 7, 1800308 (pp1-20)
[2] Magaz A et al. Biomacromolecules 2018; 19(12):4542-53



Investigation of endothelial cell viability and growth on 3D printed GelMa vascular networks

C. Chappell[1], F. Zheng [1], C. Deans[1,] O. Tsigkou[1,] B. Derby
Presenting Author: Chrisoula Chappell, Chrisoula.chappell@postgrad.manchester.ac.uk
[1]School of Materials, University of Manchester, Oxford Road, Manchester, M13 9PL, UK

INTRODUCTION: A major limitation for the development of 3D engineered tissues is the absence of viable and perfusable vasculature [1-3]. As a precursor to vascularized adipose tissue, cylindrical channels were formed in a cast gelatin methacrylate (GelMA) construct by printings sacrificial networks of Pluronic F127. Human umbilical vein endothelial cells (HUVECs) were seeded and cultured within the 3D printed channels, while Adipose derived stem cells (ADSCs) were cultured in the GelMa prior to casting the 3D printed channels.

METHODS: GelMa was synthesized using the one pot synthesis method [2]. The hydrogel was characterized by NMR, surface tension, contact angle and DMA. Pluronic filaments were printed onto glass slides using a robotic printer I&J 7300-LF (Fishnar, UK). HUVECs (PromoCell, UK) were cultured on GelMa substrate, whilst ADSCs (ThermoFisher) were embedded within the GelMa. Live/Dead and Alamar Blue assays were used to assess the cells' viability and proliferation respectively. Phalloidin staining was used to assess actin cytoskeleton organization.

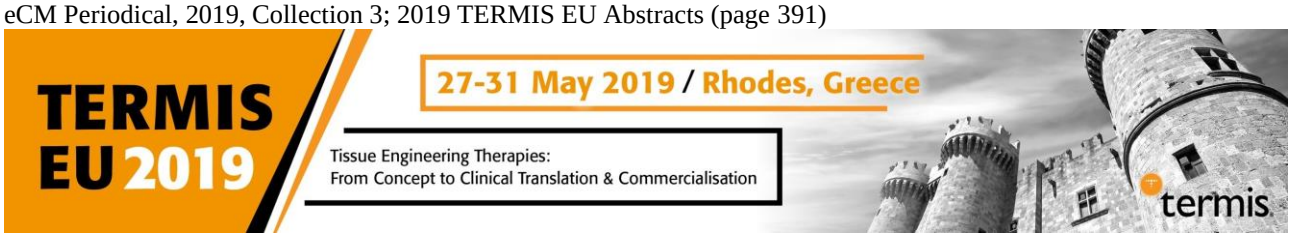
RESULTS & DISCUSSION: Once methacrylation has occurred NMR peaks are seen at 6ppm and 2ppm corresponding to lysine and methacrylated grafts of hydroxyl groups. Viability assays confirmed that HUVECs and ADSCs were viable after 48 hours. Alamar Blue data indicated an increase in cell metabolism over a 7-day period. Phalloidin staining demonstrated good organization of the actin cytoskeleton of HUVECs on GelMa. Data on HUVECs injected within the printed 2D networks and 3D culture of ADSCs within the GelMa matrix will also be presented.

DISCUSSION &CONCLUSIONS: Collectively, our data illustrate that HUVECs could potentially grow and fully line the printed networks.

ACKNOWLEDGEMENTS: CC and FZ were supported by studentships from EPSRC and CSC.

REFERENCES

[1] Loessner D et al. A Nat Protoc. 2016; 4:727-46
[2] Shirahama H et al. 2016; 6:31036
[3] Kolensky D et al. Adv Mater. 2014; 26:3124-3130



Exosomes from bench to bedside: 3D-culture and tangential flow filtration for scalable clinical grade exosome isolation

Christina M.A.P. Schuh[1,2], Aliosha Figueroa[3], Rafael Tapia[1], Maroun Khoury[1,2,3]
Presenting Author: Christina M.A.P. Schuh, cschuh@udd.cl
[1]Cells for Cells, Santiago, Chile [2] Laboratory of Nano-Regenerative Medicine, Faculty of Medicine, Universidad de Los Andes, Santiago, Chile [3]Consorcio Regenero, Chilean Consortium for Regenerative Medicine, Santiago, Chile

INTRODUCTION: Over the last years, microvesicles and exosomes have become increasingly interesting and revealed a high potential to augment treatments for several clinical conditions. The bottleneck for pre-clinical and clinical testing re-mains the reliable production of exosomes with consistent quality and isolation under GMP conditions. Existing processes such as ultra-centrifugation (UC), gradient centrifugation, etc are unreliable regarding purity and limited to small supernatant volumes (<200ml), and further-more difficult to transfer into GMP conditions. The aim of our study was to design a process and evaluation system for optimized production of exosomes using a scalable wave bioreactor system and tangential flow filtration (TFF), that can be transferred into a GMP environment.

METHODS: Mesenchymal stem cells (source: mens-trual fluid) were cultivated on BioNocII carriers in a 10-liter wave bioreactor system (both cGMP-ready) and evaluated for survival / proliferation. Exosomes were harvested using particle-free culture medium for 3 days. Supernatants were transferred into the TFF system or UC respectively. Exosome yield and composition were assessed using Nanoparticle Tracking Analysis (NTA; NanoSight), Western Blot and ELISA. Functional testing included cellular uptake, immuno-suppression and angiogenesis migration assays.

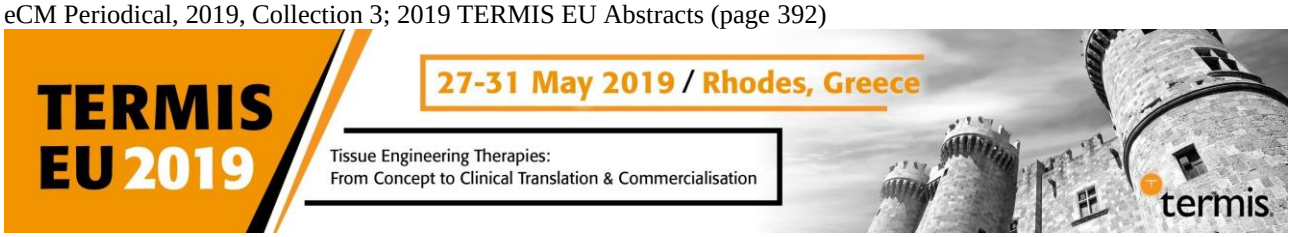
RESULTS & DISCUSSION: MSCs cultivated on BioNoc II carriers secreted a higher amount of exosomes than 2D culture. Supernatants processed with TFF dis-played a 1.5x higher particle yield as well as 7.3-9x higher exosome yield (UC:15%, TFF:90% purity). Cargo analysis revealed a higher amount of regeneration-associated proteins (FGF, HGF) in TFF exosomes. In all functional assays, TFF exosomes demonstrated a significantly higher potency (e.g. 2x higher autocrine uptake, 2.5x higher suppression of T-lymphocyte proliferation)

CONCLUSIONS: One of the prerequisites for translation of exosomes into the clinics is their efficient isolation without loss of activity, reflecting the characteristics of their cell of origin. Exosomes isolated with TFF displayed a higher activity in all functional assays, additionally to being present in higher quantity and purity. Given the decreased uptake, we speculate that high forces applied on exosomes during UC damage the integrity of the vesicle, leading to altered cellular uptake and possibly changes in their in vivo homing. Concluding, 3D culture and TFF display a scalable option for exosome production with increased biological activity, suitable for clinical application.

ACKNOWLEDGEMENTS: Financial support of COR-FO project 17CH-83954 gratefully acknowledged.

REFERNCES

[1] Cuenca et al Front.Ph. 2018; 9,464



Conductive hydrogel for bioelectronic interfaces

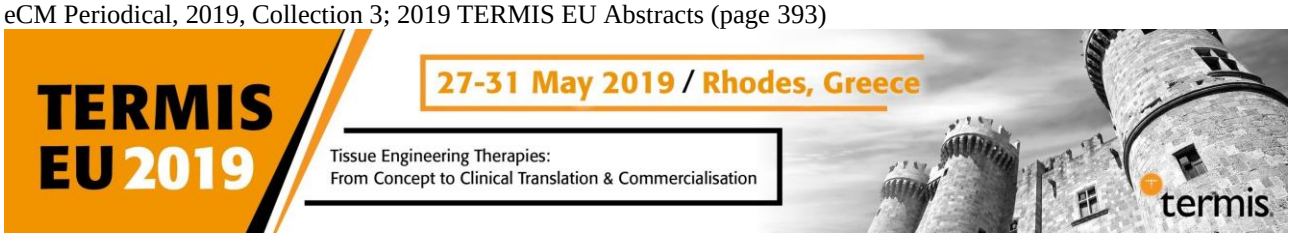
C. Tondera[1,2*], T. F. Akbar[1,2*], A. K. Thomas[3], C. Werner[2], Y. Zhang[3], V. Busskamp[4], I. R. Minev[1]
Presenting author: Christoph Tondera, christoph.tondera@tu-dresden.de
[1]Biotechnology Center (BIOTEC), Technische Universität Dresden, Germany [2]Leibniz Institute of Polymer Research Dresden (IPF), Dresden, Germany [3]B CUBE Center for Molecular Bioengineering, Technische Universität Dresden, Germany [4]DFG-Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, Germany

INTRODUCTION: The mechanical mismatch between stiff state of the art neuroprosthetic devices and soft neural tissue can lead to rejection or loss of function of the implants in chronic applications. To achieve seamless brain-machine interfaces novel tissue like conducting materials are needed.

METHODS: Here we report a multi-network hydrogel combining a non-covalent network consisting of the nanoclay Laponite with a covalent polyacrylamide network. To make the hydrogel scaffold electronically conductive poly(ethylene-3,4-diethoxy thiophene) (PEDOT) is polymerized around the polymer struts using an interfacial polymerization method. Therefore the gels are first soaked in 0.4 M ammonium persulfate in 1 M hydrochloric acid. In a second step the gels were incubated in a 0.4 M EDOT solution to polymerize PEDOT at the gel - EDOT interphase. An artificial extracellular matrix consisting of a starPEG (polyethylene glycol) layer containing a bone sialoprotein peptide sequence and the polysaccharide dextran sulfate is covalently bound to the hydrogel surface to enable cell adhesion.

RESULTS & DISCUSSION: The obtained multi-network hydrogel is combining electrical conductivity of 12 S/m, stretchability of 800% and tissue-like elastic modulus of 15 kPa. Laponite dramatically increases the conductivity of in-scaffold polymerized PEDOT in the absence of other dopants, while preserving excellent stretchability. Laponite further enables the immobilization of bioactive molecules. The artificial extracellular matrix coating allows for the adhesion, proliferation and neuronal differentiation of human induced pluripotent stem cells on the surface of the conductive hydrogels. Untreated gels on the other hand are cell repelling.

CONCLUSIONS: Due to its compatibility with simple extrusion printing and the afore mentioned mechanical, electrical and biological properties, our material promises to enable tissue-mimetic neuro-stimulating electrodes.



Automated 3D cell culture platform for investigating chemoresistance and efficacy of antibody-based therapeutics

A. Kletzmayr[1], F. Clement-Frey[1], D. Eberli[2], C.A. Millan[1,2]
Presenting Author: Dr. Christopher Millan, christopher.millan@usz.ch
[1]CellSpring AG, Zürich, Switzerland [2]Laboratory for Tissue Engineering and Stem Cell Therapy, University Hospital Zurich, Zürich, Switzerland

INTRODUCTION: Utilizing chemically modified chitosan and alginate, we developed a 3D culture platform for modeling acquired chemoresistance in ovarian, breast, lung, and prostate cancer cells. Cell seeding, media change, drug dosing, and endpoint assay were all performed on a commercially popular automated liquid handling robot suitable for large-scale screening.

METHODS: SKOV-3, A2780, SK-BR3, A549, and LNCaP cells were cultured in RPMI media supplemented with 10% FBS and 1% Pen/Strep. Cells were seeded in engineered microtissues (EMTs) as described elsewhere[1] with slight modifications. A Tecan Freedom EVO 100 was used to pipette 20ul oxidized alginate (oxAlg) per well of a U-bottom 96 well plate. Cells were resuspended in N-succinyl chitosan (sChi) at 106 cells/ml, and 5ul were pipetted into each well containing oxAlg followed by 30-minute incubation at room temperature to allow for crosslinking. Cells were then treated with a range of concentration of doxorubicin or paclitaxel for 72h. CellTiter Glo 3D was then used to determine viability following manufacturer's protocol. Cells cultured in 3D were compared to standard 2D cultures via Western blot, proteomics (LC-MS/MS), and immunostaining.

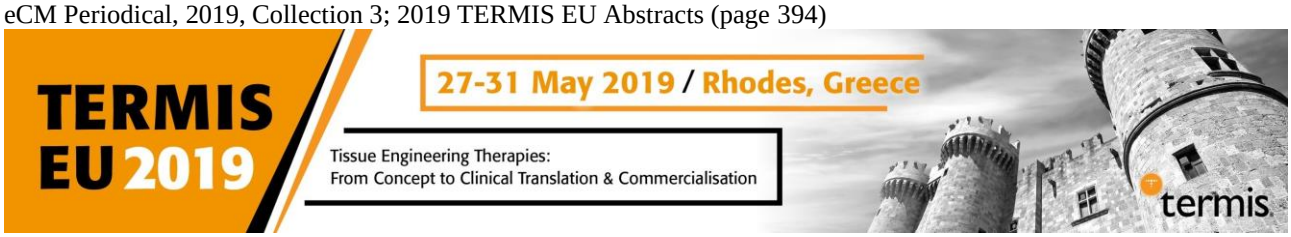
RESULTS & DISCUSSION: Strikingly, after just 4 days in culture more than 10% of proteins identified by LC-MS/MS exhibited significantly altered expression in 3D including chemoresistance-associated proteins involved in stress response (e.g. mitochondrial dysfunction), metabolism (e.g. fatty acid beta oxidation and oxidative phosphorylation), and protein biosynthesis. Chemoresistance was confirmed by comparing their response to treatment with Taxol, a routinely employed first-line small molecule drug. Resistance indices (calculated by dividing IC50 values of cells cultured in 3D by those in 2D) ranged from ±10 in ovarian cancer cells to over 5,000 for breast cancer cells. In stark contrast, antibodies developed against targets known to be overexpressed in several cancer lines were shown to be more effective in suspending growth of cancer cells in the 3D model. In situ immunostaining confirmed antibody-target binding in 3D and increased expression of targets, including HER2 and Integrin α5β1 by cells cultured in 3D compared to 2D.

CONCLUSIONS: We present a highly scalable approach for culture of cancer cells in a chemically defined hydrogel that confers advanced stage phenotypes to encapsulated cells.

ACKNOWLEDGEMENTS: Financial support was received from the ETH Zürich Foundation, and the Swiss Commission for Technology and Innovation (18391.1 PFLS-LS)

REFERENCES

[1] Millan C et al. Adv Healthc Mater. 2015; 26(19):3024-34



Force-based engineering of osteochondral gradients

C. Li[1], J.P.K. Armstrong[1], L. Ouyang[1], I.J. Pence[1], A.C. Moore[1], Y. Lin[1], C.W. Winter[1], M.M. Stevens[1]
Presenting Author: Chunching Li, c.li15@imperial.ac.uk
[1]Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London, Prince Consort Road, London, SW7 2AZ, UK

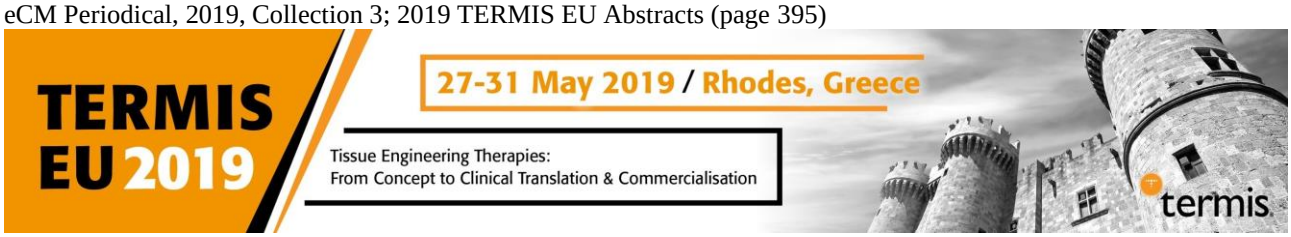
INTRODUCTION: Many tissues, such as bone, tendon and cartilage, possess an inhomogeneous structure due, in part, to biochemical gradients present during development. It is important to recapture these gradients in order to engineer tissues with native structure and function. To this end, we have developed different platforms that utilize fundamental physical forces to rapidly produce smooth biochemical gradients across different biomaterial systems.

METHODS: Bone morphogenetic protein 2 (BMP-2) gradients were presented across hydrogels laden with human mesenchymal stem cells (hMSCs). This was achieved using several configurations, including the use of magnetic fields to attract glycosylated superparamagnetic iron oxide nanoparticles sequestered with BMP-2. The gradient constructs were engineered for 28 d in osteochondral differentiation medium and characterized using immunohistochemistry, histology, and gene expression analysis.

RESULTS & DISCUSSION: We were able to generate a wide range of gradients across various hydrogel systems. Using hydrogels with BMP-2 gradients and hMSCs we were able to engineer robust osteochondral tissue constructs of cartilage tissue bearing a distinct mineralized cap. The tissue construct exhibited distinct cell types (chondrocyte and osteoblast), protein expression (distribution of collagen type II and osteopontin) and tissue mineralization (presence of HAP/β-TCP mineral). Interestingly, we observed the formation of a sharp mineral transition from a smooth BMP-2 gradient, an emergent property that mimics the formation of a tidemark at the native osteochondral interface.

CONCLUSIONS: Our approaches to engineer osteochondral tissue offer great versatility and could be readily tailored to other systems, offering new opportunities in interfacial tissue engineering.

ACKNOWLEDGEMENTS: C.L. was supported by a Top University Strategic Alliance PhD Scholarship from Taiwan. M.M.S. acknowledges support from the European Research Council Seventh Framework Programme Consolidator grant “Naturale CG” (616417), UK Regenerative Medicine Platform “Acellular / Smart Materials – 3D Architecture” (MR/R015651/1) and the Wellcome Trust Senior Investigator Award (098411/Z/12/Z).



Investigating the role of interleukin 16 in osteoarthritis progression using CRISPR/Cas9

C. Dooley[1], C. O'Flatharta[1], B. Ranera[1], M. Murphy[1]
Presenting Author: Claire Dooley, c.dooley9@nuigalway.ie
[1]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Osteoarthritis (OA) is the most common degenerative diseases of the joint. Several factors contribute to the disease and it is a major cause of pain and discomfort in the elderly population [1]. Currently the only treatment strategies available are symptomatic and the mechanisms of the progression of the disease are still poorly understood. There is a need to identify early markers of the disease and potential therapeutic targets. CRISPR/Cas9 technology is a useful molecular tool to investigate these targets. DNA methylation screening identified Interleukin 16 (IL-16) as a hypomethylated marker in MSC chondrogenesis with high levels of the protein detectable at early and late stages of the disease. The aim of this study is to elucidate the role of IL-16 in OA progression

METHODS: Bone marrow derived mesenchymal stem cells (MSCs), ethically obtained from donors, were differentiated in chondrogenic medium for 14 days. Changes in chondrogenic and hypertrophic markers were investigated by qPCR and immunofluorescence (IFC). A CRISPR/Cas9 system was designed to target domains of interest in IL-16 and the effects of its knock out were investigated in the MSC chondrogenic model.

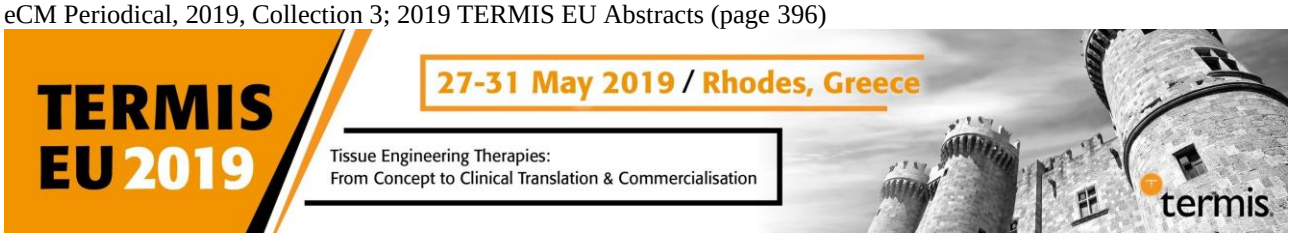
RESULTS & DISCUSSION: qPCR and western blotting showed that it was the neuronal isoform (nIL-16) of this protein that was significantly upregulated in late stage OA patient samples. Preliminary IFC performed identified co-localization of nIL-16 with the calcium ion channel Transient Receptor Potential cation ion-channel sub-Family-V-member-4 (TRPV4) in the pericellular matrix of human OA chondrocytes (Figure 1). A CRISPR/ Cas9 strategy was designed to investigate whether nIL-16 and TRPV4 work together in a pathway contributing to chondrocyte hypertrophy and calcification that is seen in late OA. Guide RNAs (gRNAs) were designed to target the nIl-16 PDZ binding domains [2].

DISUSSION & CONCLUSION: Data suggests IL-16 plays a role in OA progression. CRISPR is a useful tool to determine what it's role potentially is.

ACKNOWLEDGEMENTS: This research was funded by Irish Research Council postgraduate scholarship (GOIPG/2017/633)

REFERENCES

[1] Chen et al, Bone Research (2017) 5, 16044
[2] Kurschner et al, J Neurosci. 1999 Sep 15;19(18):7770-80.



Matrix stiffness regulates dental pulp stem cell migration

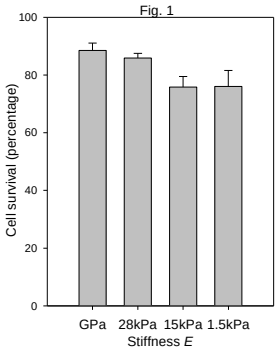
C. Ehlinger[1,2,3], Y. Haikel[1,2,3], D. Vautier[1,2,3], L. Kocgozlu[1,2,3]
Presenting Author: Claire Ehlinger, claire.ehlinger@hotmail.fr
[1]Inserm UMR-S1121, Université de Strasbourg (UdS), France [2]Faculté de Chirurgie Dentaire (UdS), France. [3]Fédération de Médecine Translationnelle, Strasbourg, France

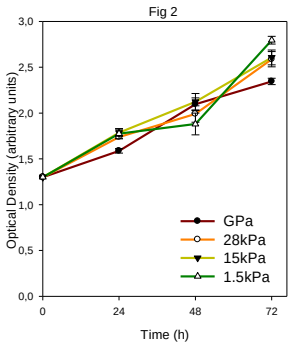
INTRODUCTION: Bacterial invasion or dental trauma may cause irreversible pulpitis and necrosis of the dental pulp. Currently, the treatment of these pathologies is the cleaning, shaping and filling of the root canal system by a biomaterial. Recently, regenerative endodontics appeared, proposing new possibilities to restore a functional pulp tissue by tissue engineering and particularly cell homing. Cell-homing approach is based on the recruitment of resident stem cells by growth factors, which induce cell migration into a custom-made scaffold. Here, we study the influence of matrix stiffness in dental pulp stem cells (DPSCs) survival, proliferation and migration to define the best mechanical properties of a scaffold for dental pulp regeneration.

METHODS: The behavior of DPSCs was assayed on 1.5 kPa, 15 kPa, 28 kPa and MPa polydimethylsiloxane (PDMS) substrates coated with laminin. Cell migration was studied by time lapse sequences during 24h on a Nikon Ti-E microscope equipped with a 10x objective.

RESULTS & DISCUSSION: Immunofluorescence analysis revealed that extracted cells were positive for CD105, CD90 and CD73, which confirmed that they were mesenchymal stem cells. Soft substrates supported DPCSs viability, proliferation and formation of focal adhesion. Furthermore, the Yes-associated proteins (YAP) were predominantly localized within the nucleus. Interestingly, progressive softening PDMS substrates increased DPSCs migration. Myosin II and Arp 2/3 inhibitions didn't prevent DPSCs migration but caused important change in the focal adhesion of these cells. Importantly, on 1.5 kPa PDMS substrates, in the presence of blebbistatin (Myosin II inhibitor) YAP was predominantly localized in the cytoplasm.

CONCLUSIONS: Studying the influence of matrix stiffness on DPSC behaviors is an important step to create more suitable scaffolds for pulp regeneration through cell homing. Here, we show that soft substrate might be suitable for cell homing as it doesn't cause increased cell apoptosis and allows normal cell proliferation. The increased cell migration speed on 1.5kPa PDMS substrate make this stiffness particularly appropriate for pulp regeneration.





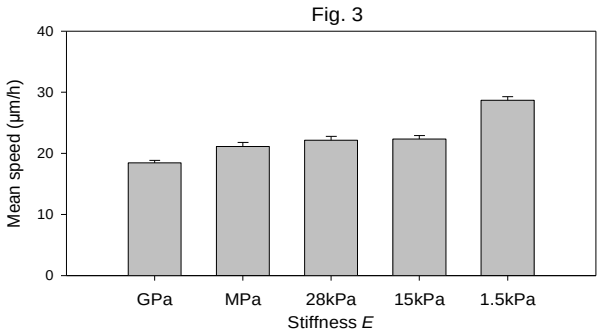
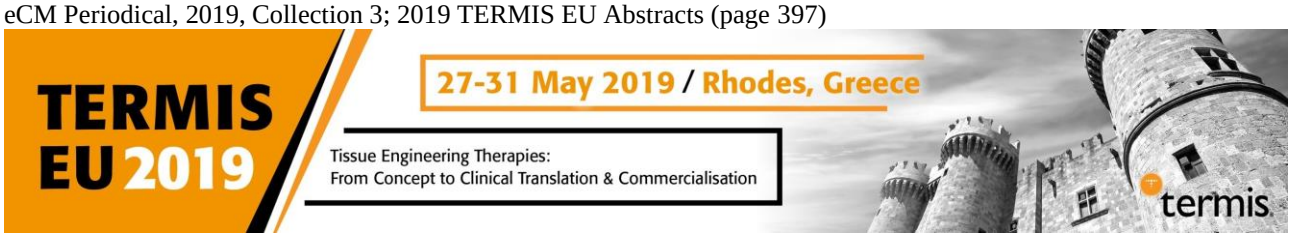


Figure 1: (left) Cell survival on PDMS gels of varying stiffness. **Figure 2**: (middle) Cell proliferation on PDMS gels of varying stiffness. **Figure 3:** (right) Cells mean speed on PDMS gels of varying stiffness.



Liquefied capsules as 3D co-culture static and dynamic systems for bone regeneration

C.R. Correia[1], S. Nadine, João F. Mano[1]
Presenting Author: Clara R. Correia, claracorreia@ua.pt
[1]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

INTRODUCTION: We developed a 3D liquified bioencapsulation system composed by the co-encapsulation of cells with surface-modified microparticles [1-3]. Here, we propose such capsules as an indirect triple co-culture system or as co-culture system for dynamic 3D culture.

METHODS: Alginate hydrogels were produced by electrospraying. Hydrogels encapsulating MC3T3-E1 or human adipose-derived stem cells (ASCs) and osteoblasts (hOBs) were coated with poly(L-lysine), alginate, and chitosan through layer-by-layer (LbL) assembly (n=12layers). The last layer was functionalized with alginate-RGD to promote HUVECs/fibroblast adhesion. Osteogenesis was evaluated up to 21 days. The angiogenic potential of capsules was evaluated by a chick chrorioallantoic membrane (CAM) assay after 13 of inoculation. The dynamic system was performed using a rotary cell culture system.

CONCLUSIONS: A bone-like tissue was developed in vitro within the core of 3D liquefied bioencapsulation systems. Additionally, capsules allowed the adhesion of an external co-culture of HUVECs and fibroblasts, thus acting also as a cellular supportive on the outside. This may represent a great advantage for a proper tissue integration after in vivo implantation. As demonstrated by the CAM assay, capsules were able to recruit new vessels at a similar level compared to the positive control. Moreover, capsules cultured in a 3D dynamic culture system demonstrated that osteogenesis was enhanced compared to static cultures, and remarkably occurred in basal medium, thus in the absence of osteogenic differentiation supplements.

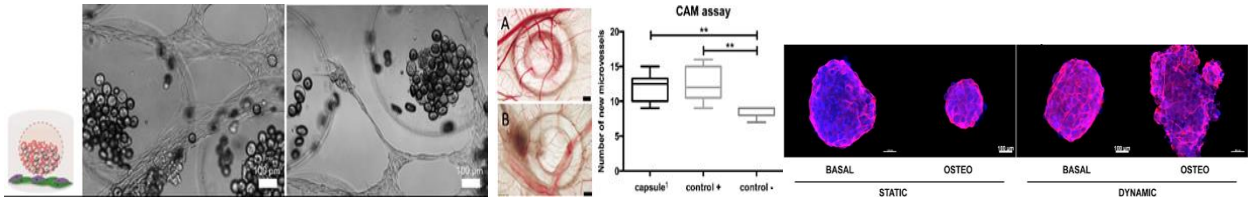
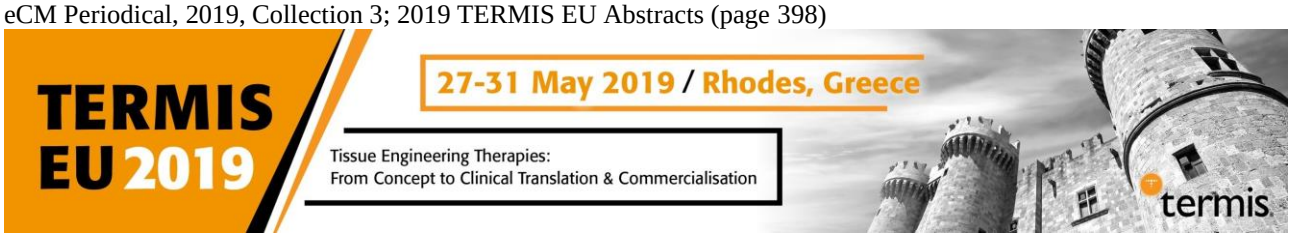


Figure 1: (left) Triple co-culture of MC3T3-E1 encapsulated within liquefied and multilayered capsules, and human fibroblasts and HUVECs on the outside environment adhered to the membrane. **Figure 2:** (middle) Optical microscopy of CAMs with (A) capsules or (B) with basic fibroblast growth factor-2 (control+). (C) New vessels recruitment quantification. Control- stands for CAM with α-MEM/1% penicillin/streptavidin. Scale bars are 50 μm (**p<0.01). **Figure 3:** (right) Osteopontin (pink) counterstained with DAPI (blue) of capsules encapsulating ASCs and hOBs in static and dynamic cultures.

ACKNOWLEDGEMENTS: Financial support was received from ERC-2014-669858 for project ATLAS and PTDC/BTM-MAT/31064/2017 for project CIRCUS.

REFERENCES

[1] Correia CR et al. Sci. Rep. 2016; 21883.
[2] Correia CR et al. Acta Biomater 2017; 53:483-494.



Improving bone regeneration by site-directed immobilized BMP2 variant onto collagen beads

C. Siverino[1], J. Nickel [1,2]
Presenting Author: Claudia Siverino, claudia.siverino@uni-wuerzburg.de
[1]Department of Tissue Engineering and Regenerative Medicine, University Hospital Wuerzburg Germany [2]Fraunhofer Translational Center for Regenerative Therapies TLZ-RT, part of the Fraunhofer ISC, Wuerzburg Germany

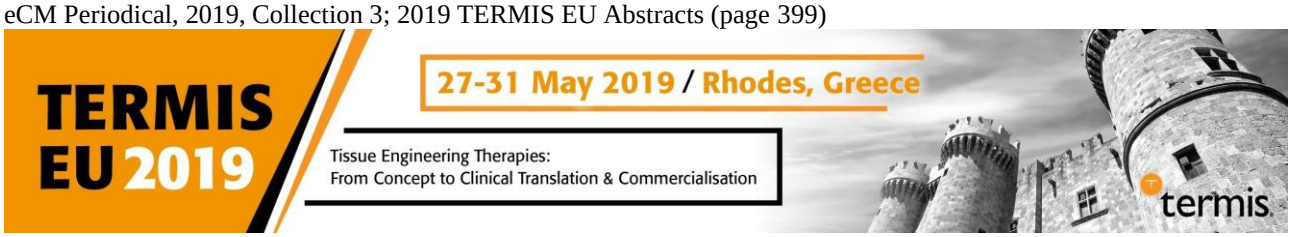
INTRODUCTION: Are biomaterials and growth factors alone sufficient for the treatment of non-healing long bone defects? The question can be answered with yes, while the safety for the patients is not guaranteed. Current approaches, such as surface absorption and encapsulation are based on the incorporation into the scaffold of the osteogenic growth factor bone morphogenetic protein 2 (BMP2). However, these methods require high doses of protein that are associated to several severe side effects. In order to overcome these problems we aim to immobilize a newly produced BMP2 variant in a site directed manner. The new BMP2 variant harbors a non-natural amino acid in each chain of the mature polypeptide allowing a site-specific coupling by “click chemistry”. With this method lower doses of the covalently coupled protein would be required to induce bone formation and thus eliminating the adverse events for patients.

METHODS: The BMP2 variant (BMP2 Azide) was expressed in E.coli and analyzed in terms of purity and biological activity in vitro. Using copper-free click chemistry reaction the protein was coupled to collagen microspheres. The efficiency of the coupling reaction was validated with different analytical methods. Finally, BMP2-functionalized microspheres were tested in vivo in a subcutaneous rat model. Bone volume and density were evaluated by micro-computed tomography (µCT) during the animal experiment. Newly formed bone was histologically analyzed.

RESULTS & DISCUSSION: BMP2 Azide showed the same bioactivity as the wild type BMP2 (BMP2 WT) and maintained its bioactivity also after the coupling reaction to microspheres. Moreover, the BMP2-functionalized microspheres induced ectopic bone formation in vivo. Histological examination of the formed ossicles revealed clear morphological differences between covalently coupled BMP2 Azide and adsorbed BMP2 WT used as control. The diffusion mechanism of BMP2 WT towards the subcutaneous environment induced a shell like structure, while the non-released BMP2 Azide induced a more uniform bone formation.

CONCLUSIONS: The covalently coupled BMP2 approach defines a new strategy to improve safety by avoiding severe side effects. This interesting method could result in the replacement of the currently used clinical devices.

ACKNOWLEDGEMENTS: This research was funded by the European Union Seventh Framework Programme FP7-PEOPLE-2013-ITN under grant agreement n° 607051.



Miniaturized imaging window for intravital nonlinear microscopy

C. Conci[1], E. Jacchetti[1], T. Zandrini[1], L. Sironi[2], M. Collini[2], G. Chirico[2], G. Cerullo[3], R. Osellame[3], M.T. Raimondi[1]
Presenting Author: Claudio Conci, claudio.conci@polimi.it
[1]Department of Chemistry, Materials and Chemical Engineering Giulio Natta, Politecnico di Milano, Milan, Italy [2]Univ. di Milano-Bicocca, Dipartimento di Fisica, Milan, Italy [3]Istituto di Fotonica e Nanotecnologie (IFN)-CNR and Department of Physics, Politecnico di Milano, Milan, Italy

INTRODUCTION: Intravital microscopy techniques are currently performed using highly invasive window chambers [1]. Moreover, there exist no device providing a specific tracking geometry to reposition the field of view of the microscope for repeated analyses. We developed a miniaturized imaging window, the Microatlas, which can be implanted subcutaneously and allows repeated observation in vivo of foreign body reactions, for example to the implantation of a biomaterial. The device hosts a miniaturized scaffold able to guide cell migration. By applying two-photon fluorescence microscopy to the Microatlas, once implanted in vivo and repopulated by cells and blood vessels, is possible to observe and quantify reactions in the same animal and tissue district, at different time points. Here, we grafted the Microatlas in living chicken embryos to conduct in vivo validation assays.

METHODS: The Microatlas scaffolds were fabricated by two-photon laser polymerization on circular glass coverslips (Ø:5-12 mm), with a biocompatible photoresist, SZ2080 [2]. The Microatlas is composed by several micro grids (500x500x100 μm^3). Reference structures were integrated in the grid to allow the microscope field-of-view repositioning at different time-points. The chicken embryo ex ovo culture was defined and optimized. Optimal implantation time points were selected. CD31 antibody staining was employed as a marker of inflammatory and endothelial reactions and DRAQ5^{TM} was employed as a nuclei marker. Then, the device was implanted, and it was inspected by two-photon fluorescence and confocal microscopy.

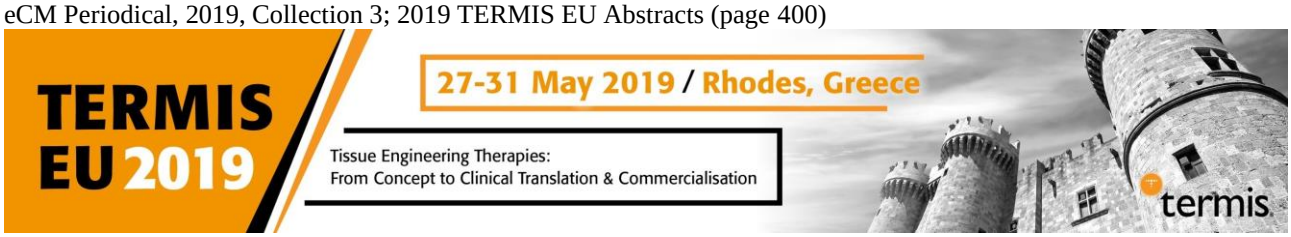
RESULTS & DISCUSSION: Confocal inspections at Microatlas implantation sites highlighted the propensity of the recipient tissue to grow inside the micro grids. Two-photon fluorescence acquisitions of label-free specimens showed the presence of a layer of collagen type I, placed mainly around or nearby the implanted Microatlas. Confocal microscope images of immunofluorescence assays revealed a strong presence of CD31 signal as a higher presence of inflammation-related cells inside the Microatlas, compared to control (i.e. glass coverslip) samples. Confocal microscope images allowed cell quantification inside the Microatlas, providing a cell density estimation.

CONCLUSIONS: The Microatlas induced a quantifiable localized reaction inside its microscaffolds, both in terms of cell repopulation, immune response and collagen generation: all signs of a probable foreign body reaction. Thus, our device can be used as a powerful imaging window for intravital fluorescence microscopy.

ACKNOWLEDGEMENTS: This project is funded by the Italian Ministry of University and Research (call MIUR-FARE 2016, project BEYOND Cod. R16ZNN2R9K).

REFERENCES

[1] Prunier, C. et al. Methods. 2017; 128, 52–64.
[2] Nava, M. T. Eng. Reg. Med. 2016; 4, 524-531.



The incorporation of collagen type II into collagen hyaluronate scaffolds as potential off the shelf approach to long term stable cartilage tissue repair

C. Intini[1,2,3], J.P. Gleeson[1,2], F.J. O'Brien[1,3]
Presenting Author: Claudio Intini, claudiointini@rcsi.ie
[1]Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland [2]SurgaColl Technologies Ltd, Invent, Dublin City University, Dublin 9, Ireland. [3]Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI & TCD, Ireland

INTRODUCTION: Cartilage-mimicking collagen hyaluronate scaffolds have demonstrated significant regenerative capacity in directing de novo chondrogenesis preclinically[1] and clinically[1]. However, the ability of these materials in supporting long term cartilage regeneration still needs to be assessed and represents an area of significant scientific interest. Therefore, the aim of this study was to investigate the incorporation of collagen type II (a potential chondro-inductive component[2]), within collagen hyaluronate scaffolds to assess its role in modulating stable cartilage regeneration.

METHODS: A series of porous collagen (0.5% w/v) hyaluronate (0.05% w/v) scaffolds were manufactured with different ratios of collagen type I:type II (1:1; 1:3; 1:5) by using a freeze-drying technique as previously described[3]. Scaffolds were subsequently cultured with $5x10^5$ rat-mesenchymal stem cells (MSCs) in vitro under chondrogenic conditions for 28 days. Then, they were analysed histologically, by means of sulphated GAG and DNA quantification and by performing a uniaxial compression test (Zwick Z005, GmbH, Germany).

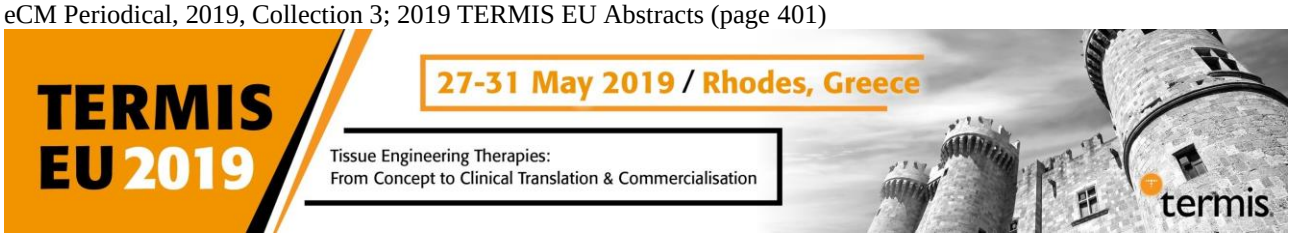
RESULTS & DISCUSSION: The incorporation of collagen type II in the ratio 1:1 to collagen hyaluronate scaffolds significantly improved ($p < 0.001$) both cell viability and cartilage matrix production without altering the overall mechanical properties. These scaffolds incorporating collagen type II resulted in 4-fold higher level of DNA and 3-fold of sulphated GAG. Furthermore, histology revealed a more homogenous cell infiltration and sulphated GAG distribution inside the matrix.

CONCLUSIONS: The addition of collagen type II to collagen hyaluronate scaffolds demonstrated an added chondrogenic benefit, suggesting them as potential cartilage-mimicking scaffolds capable of generating more stable cartilage. This simple, off the shelf approach could offer the possibility of long term stable cartilage tissue repair for localised cartilage lesions of the knee.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 721432.

REFERENCES

[1] Levingstone T et al., Biomaterials. 2016; 87:69-81.
[2] Bosnakovski D et al., Biotechnol Bioeng. 2006; 93:1152–63.
[3] Haugh MG et al., Tissue Eng Part C Methods. 2010; 16, pp. 887-894.



Peptide/graphene oxide hydrogel nanocomposites for tissue engineering of intervertebral disc

C. Ligorio[1,2], M. Zhou[2], A. Vijayaraghavan[1], J. Hoyland[3], A. Saiani[1,2]
Presenting Author: Cosimo Ligorio, cosimo.ligorio@postgrad.manchester.ac.uk
[1]School of Materials, The University of Manchester, Manchester, UK [2]Manchester Institute of Biotechnology, The University of Manchester, Manchester, UK [3]Division of Cell Matrix Biology and Regenerative Medicine, The University of Manchester, Manchester, UK

INTRODUCTION: Low back pain, caused by intervertebral disc (IVD) degeneration, represents a global disability [1]. Current treatments are highly invasive and inefficient in the long-term, therefore minimally-invasive therapies are emerging. Self-assembling peptide hydrogels (SAPHs) represent potential candidates as injectable cell carriers, due to their tissue biomimicry that supports cell viability and differentiation [2]. Moreover, the advent of graphene has made graphene-containing materials appealing to direct cell fate [3]. Here, we incorporated graphene oxide (GO) wi
thin a SAPH to develop novel peptide-GO hydrogels as potential scaffolds for degenerate IVDs.

METHODS: Peptide-GO hydrogels were prepared by incorporating 0.5 mg/ml of GO (mean size <5μm) within 10, 15 and 20 mg/ml of FEFKFEFK (F8) peptide hydrogels. Hydrogel structure at micro and nanoscale was assessed via FTIR, AFM and TEM. Mechanical properties and gel injectability were studied via oscillatory rheometry. Bovine nucleus pulposus cells (BNPCs) were encapsulated in peptide/GO and cultured in 3D for 7 days, while alamarBlue® and LIVE/DEAD assays were used to measure metabolic activity, cell morphology and viability at 1, 4 and 7 days after encapsulation.

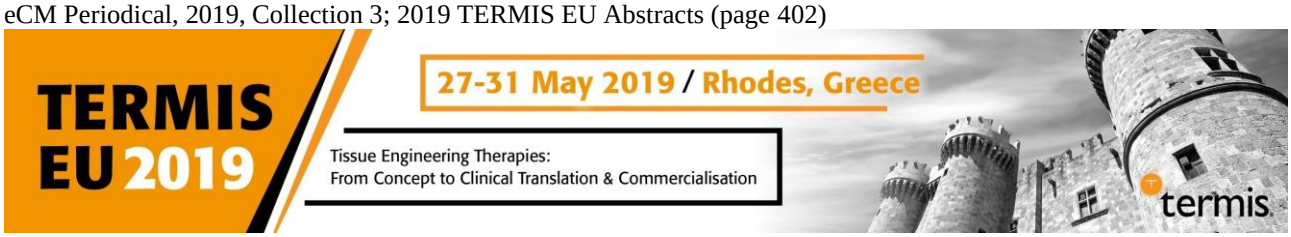
RESULTS & DISCUSSION: GO was successfully incorporated in F8 hydrogels, showing different levels of interactions with the peptide network. The presence of GO enhanced the mechanical properties of native peptide hydrogels, achieving storage moduli (~12.8 kPa) comparable with the human nucleus pulposus tissue (~10 kPa). Peptide-GO hydrogels showed excellent biocompatibility, preserving cell morphology, high viability and metabolic activity over time. Moreover, nanocomposites showed clear ease of injectability, making them suitable for minimally-invasive applications.

CONCLUSIONS: Collectively, these data showed that GO can be incorporated within SAPHs to create potential mechanically-reinforced scaffolds for novel IVD tissue engineering approaches.

ACKNOWLEDGEMENTS: The authors thank EPSRC&MRC (EP/L014904/1) BioAFM facility for financial and technical support.

REFERENCES

[1] Hoy D et al. Ann. Rheum. Dis. 2014;73(6):968–974
[2] Castillo-Diaz, L et al. J. Tissue Eng. 7:1-15
[3] Duran M et al. Curr Mol Med. 2017;17(9):619-626



Elastomeric composite scaffolds for bone tissue engineering

C. Langueh[1], S. Frasca[2], S. Changotade[1], S. Ramtani[1], J.M. Collombet[2], H. Guibourt[3], D. Lutomski[1], G. Rohman[1]
Presenting Author: Credson-yannick LANGUEH, credson-yannick.langueh@univ-paris13.fr
[1]Laboratoire Chimie, Structures, Propriétés de Biomatériaux et d'Agents Thérapeutiques CSPBAT UMR7244 CNRS, Université Paris 13, Villetaneuse, France [2]Département Médico-Chirurgical des Forces, Institut de Recherche Biomédicale des Armées IRBA, Bretigny-sur-Orge, France, [3]OST-Développement, Clermont-Ferrand, France

INTRODUCTION: Due to their unique properties, composites materials for Bone Tissue Engineering based on the combination of a biocompatible, biodegradable and highly porous polymer reinforced by various filling substances gained increasing interest [1]. The aim of our study was to develop a hybrid material bonding an elastomeric matrix to bioceramic particles while serving as a matrix for bone regeneration.

METHODS: PolyHIPE technique had been used to synthetize the composites based on ref [2]. Mechanical behaviour, morphological, chemical and elemental analyses were determined by several techniques such as SEM, EDX, compression test, volumetric absorption, colorimetric assay and infrared spectroscopy. Density measurements were carried out by pycnometry. In vitro assays were conducted in order to determine cells viability and migration within the scaffolds up to 30 days while in vivo tests were performed on segmental-like defect on rats models.

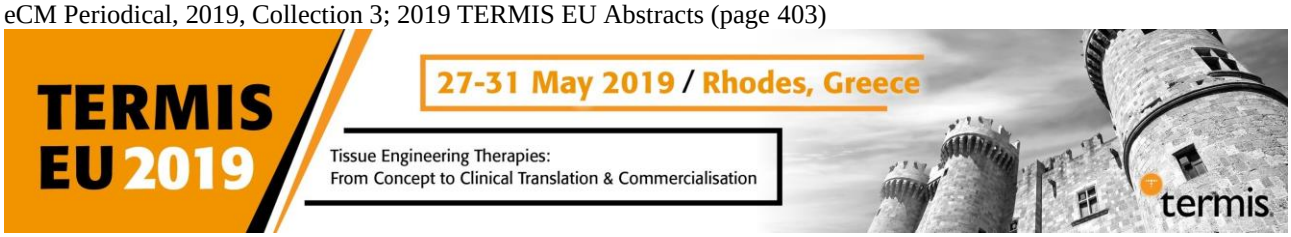
RESULTS & DISCUSSION: SEM, morphological analyses and volumetric absorption revealed the interconnected porosity of composites scaffolds. Infrared spectroscopy and elemental analysis revealed the presence of PO_4^{3-} groups, phosphorus and calcium. Alizarin Red staining showed the homogeneous distribution of the bioceramic particles within the elastomeric matrix. Density measurements allowed to determine the amount of filling particles. Compression test revealed the elastomeric behaviour of the composites. In vitro test demonstrated that cells were able to migrate, proliferate, differentiate into the whole volume of the composite. During in vivo assays, neither complication, nor sign of inflammatory response or scaffold rejection was noticed. A bone repair was observed.

CONCLUSIONS: The collected data showed the reinforcing-effect of bioceramic granules while maintaining the elastomeric properties. In vitro tests revealed the osteoconductive properties of biocomposites. In vivo assays confirmed the biocompatibility, osteoinductive properties and bone regeneration potential of the hybrid materials. Degradation studies and bioactivity analyses through the evaluation of apatite formation in simulated body fluids are currently investigated.

ACKNOWLEDGEMENTS: The authors would like to thank Les Gueules Cassées, Institut de Recherche Biomédicale des Armées, OST-Développement and ANR Labcom Proteobios for their support and the allocated grants.

REFERENCES

[1] Turnball G et al. Bioact Mater. 2018; 3(3):278-314
[2] Changotade S et al. Stem Cells Int. 2015; Vol2015: 283796



Optimal loading regime to promote cartilage and bone development in vitro

C. Parisi[1], N.C. Nowlan[1]
Presenting Author: Cristian Parisi, c.parisi@imperial.ac.uk
[1]Department of Bioengineering, Imperial College London, London, UK

INTRODUCTION: Mechanical stimulation is essential for normal cartilage and bone prenatal development [1,2]. A better understanding of what mechanical stimulation is optimal for skeletal development would provide valuable insights for recapitulation of these processes for tissue engineering of replacement cartilage and bone [3]. The aim of this study is to determine the optimal regime of mechanical stimulation (frequency and duration) for cartilage and bone development, using embryonic chick limb explants in an in vitro mechanostimulation bioreactor system [2].

METHODS: Embryonic chick hindlimb explants were cultured in vitro on polyurethane foam supports in a mechanostimulation bioreactor (Ebers TC3) for six days. A flexion regime (14±4°) induced by a 3-mm uniaxial compressive deformation was applied to all explants for two hours [2], one (1TD), two (2TD) or three times per day (3TD), in a sinusoidal waveform at frequencies of 0.33Hz (low frequency, n=6) and 0.67Hz (high frequency, n=6). Static cultures were performed as controls (n=12). Cultured explants, stained for cartilage and mineralised tissue using Alcian Blue and Alizarin Red, were scanned in 3D using optical projection tomography and assessed for 3D joint shape and extent of diaphyseal mineralisation. One-Way ANOVA and Tukey's post-hoc test were performed to verify if significant differences existed between groups ($p<0.05$).

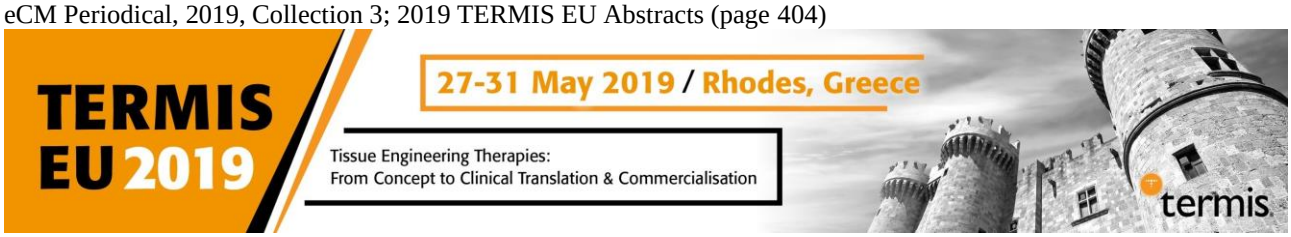
RESULTS & DISCUSSION: Higher frequency and longer duration of loading increased both mineralization and cartilage growth. Frequency effects were dominant, with most of the significant differences being between the high and low frequency groups. However, there were consistent trends due to increased duration of loading for both bone and cartilage development.

CONCLUSIONS: We found that a longer duration of loading at a higher frequency led to the most cartilage growth and mineralisation in the developing chick limb in vitro. The next steps for this work are to investigate the effects of this regime in cartilage and bone tissue engineering applications.

ACKNOWLEDGEMENTS: European Research Council (ERC Starting Grant no. 336306).

REFERENCES

[1] Henstock JR et al. Bone. 2013;53(2):468-477.
[2] Chandaria VV et al. J Biomech. 2016;49(15): 3635-3642. [3] Marcucio RS et al. J Orth Res. 2017;35(3):2356-68.



Neurotrophic factor loaded in enzymatically-crosslinked silk fibroin conduits for peripheral nerve regeneration

C.R. Carvalho[1,2,3], W. Chang[4], R.L. Reis[1,2,3], J.M. Oliveira[1,2,3], J. Kohn[4]
Presenting Author: Cristiana Carvalho, cristiana.carvalho@dep.uminho.pt
[1]3Bs Research group, I3Bs, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Portugal [4]New Jersey Center for Biomaterials, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854, USA

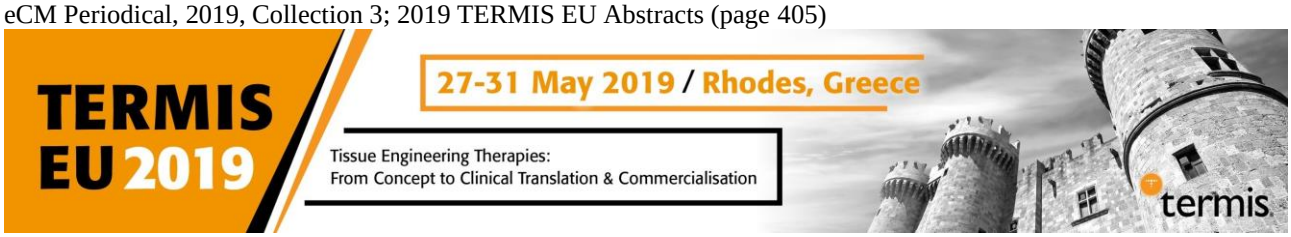
INTRODUCTION: The lack of neurotrophic factors (NTFs) following peripheral nerve injury leads to incomplete nerve regeneration and partial functional recovery. In this work, a new methodology developed by our group to produce silk fibroin conduits was used, allowing to incorporate and release bioactive Nerve Growth Factor (NGF) or Glial cell-derived neurotrophic factor (GDNF).

METHODS: Enzymatically-crosslinked silk fibroin (SF) conduits were produced by taking advantage of the tyrosine groups present in the structure of silk fibroin. In this system, hydrogen peroxide was used as the substrate and horseradish peroxidase as the enzyme. NTFs were incorporated in the silk fibroin conduits using two different methods: i) mixing the NTFs prior to silk crosslinking and ii) soaking the produced conduits in NTF solution. The release profiles were studied by means of performing ELISA. The bioactivity of the released NTFs was confirmed by in vitro assays, using neonatal rat pup dorsal root ganglion (DRGs) explants. The selected formulation was implanted in a 10 mm sciatic nerve defect in rats for 6 weeks and compared to autografts and plain SF conduits (5 rats per formulation).

RESULTS & DISCUSSION: The soaked formulations were found to release significant higher amounts of NTFs as compared to the crosslinked ones. Biological assays with DRGs allowed us to select soaked GDNF 4 µg/mL as the most promising formulation, with longer and denser axon sprouting. In the in vivo study, 80% of the animals transplanted with soaked GDNF 4 µg/mL responded positively to the nerve pinch test, as compared to 20% of animals receiving the plain SF conduit. The treatment with soaked GDNF 4 µg/mL significantly reduced muscle weight loss compared to the plain SF conduit. Furthermore, regeneration across the 10 mm nerve gap was detected in 100% of the autografts, 100% of the silk + GDNF and 80% in the SF conduits.

CONCLUSIONS: The previously described method for SF conduits production revealed to be very useful and versatile for NTFs incorporation. The incorporation was successful and preserved the bioactivity of the NTFs when released, as seen by the in vitro assays. Overall, the hypothesis that the addition of GDNF to the SF nerve conduit would yield positive results as compared to plain silk conduit was demonstrated and the nerve regeneration was found to be robust and comparable to autograft, when using the soaked GDNF at 4 µg/mL conduit.

ACKNOWLEDGEMENTS: EU-FP7-Health-2011-collaborative project 278612, Biohybrid - Templates for peripheral nerve regeneration and PhD Scholarship (Norte-08-5369-FSE-000037). J. M. Oliveira also thanks the FCT for the funds provided under the program Investigador FCT 2012 and 2015 (IF/00423/2012 and IF/01285/2015). The New Jersey Center for Biomaterials at Rutgers University provided additional support for this study.



Silicon-hydroxyapatites scaffolds decorated with VEGF for in vivo bone regeneration in osteoporotic sheep

L. Casarrubios[1], N. Gómez-Cerezo[2,3], S. Sánchez-Salcedo[2,3], M. J. Feito[1], M. Saiz-Pardo[4], L. Ortega[4], D. de Pablo[4], I. Díaz-Güemes[5], B. Fernández-Tomé[5], S. Enciso[5], F. M. Sánchez-Margallo[5], M.C. Serrano[6], M. Vallet-Regí[2,3], M. T. Portolés[1], D. Arcos[2,3]
Presenting Author: Daniel Arcos, arcosd@ucm.es
[1]Dpto de Bioquímica y Biología Molecular, Universidad Complutense de Madrid, IdISSC, Spain [2]Dpto de Química en Ciencias Farmacéuticas, Universidad Complutense de Madrid, Hospital 12 de Octubre i+12 Spain [3]CIBER de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain [4]Servicio de Anatomía Patológica. Hospital Clínico San Carlos, IdISSC, Madrid, Spain [5]Centro de Cirugía de Mínima Invasión Jesús Usón, Cáceres, Spain [6]Instituto de Ciencia de Materiales de Madrid, CSIC, 28049 Madrid, Spain

INTRODUCTION: Bone regeneration in osteoporotic patients is one of the most challenging topics of tissue engineering [1]. In this work, we have designed different silicon-hydroxypatite 3D scaffolds by means of controlling the crystalline degree of the bioceramics and associating it with VEGF, thus fostering bone regeneration in an osteoporotic environment.

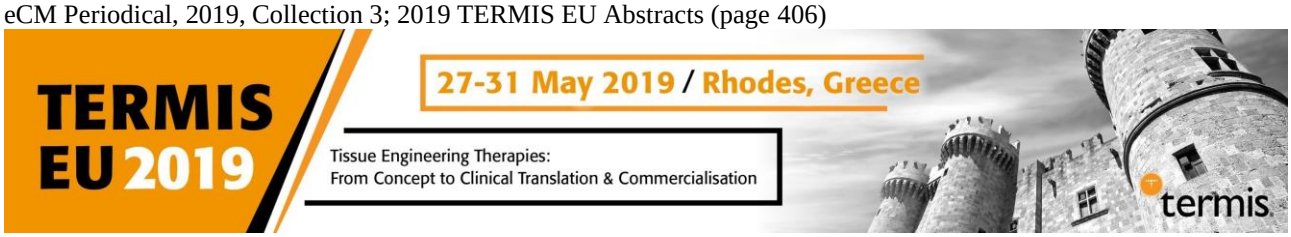
METHODS: Macroporous scaffolds were prepared by robocasting from SiHA as precipitated powders. After printing, the specimens were treated at 700ºC and 1150º C to obtain nanocrystalline (NanoSiHA) and highly crystalline (SiHA) scaffolds, respectively. VEGF was immobilized on their surface. Mature endothelial EC2 and pre-osteoblastic MC3T3-E1 cells were cultured on the scaffolds and proliferation and differentiation were determined. Six months before the implantation, six 4-year-old female Merino sheep underwent a laparoscopic bilateral ovariectomy, a low-calcium diet (0.5%) and corticosteroids administration to reproduce similar conditions as osteoporosis in humans [2]. The scaffolds were implanted in cylindrical defects (10x13mm), created in the proximal tibia epiphysis. After 12 weeks, sheep were sacrificed, and the bone segments were histologically analyzed

RESULTS & DISCUSSION: In vitro studies evidenced that endothelial EC2 cell proliferation was fostered in those scaffolds with immobilized VEGF, whereas proliferation and differentiation of pre-osteoblastic MC3T3-E1 cells were significantly enhanced in SiHA scaffolds. The in vivo study evidenced that highly crystalline SiHA associated to VEGF promoted significantly higher bone ingrowth (with 20 ± 6 % of the scaffolds volume colonized by new bone), thicker trabeculae (0.16 ± 0.04 mm), higher presence of osteoblasts and enhanced blood vessel formation. On the contrary, NanoSiHA with and without VEGF promoted a slightly higher inflammatory response and a significantly higher presence of osteoclasts.

CONCLUSIONS: Scaffolds made of bioceramics with highly crystalline microstructures facilitate adhesion and proliferation of osteoblasts, whereas the presence of VEGF promotes angiogenesis. The combination of both factors stimulates bone regeneration even in osteoporotic environments.

REFERENCES

[1] Arcos D et al. Acta Biomater 2014; 10:1793-1805
[2] Zarrinkalam MR et al. Eur. Spine J 2009; 18:244-553



Next generation magnetic bioprinting of multi-layered 3D tissues

D. Duarte Campos[1], A. Blaeser[2], C. Cristian[1], M. Vogt[3], H. Fisher[1], S. Aveic[1,4], M. Betsch[5]
Presenting Author: Daniela Duarte Campos, daniela.campos@rwth-aachen.de
[1]Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany [2]Medical Textiles and Biofabrication, Institut fuer Textiltechnik (ITA), RWTH Aachen, Aachen, Germany [3]Interdisciplinary Center for Clinical Research (IZKF), RWTH Aachen University Hospital, Aachen, Germany [4]Pedriatic Research Institute – Città della Speranza, Neuroblastoma Laboratory, Padua, Italy, [5]Department of Orthopedics, RWTH Aachen University Hospital, Aachen, Germany

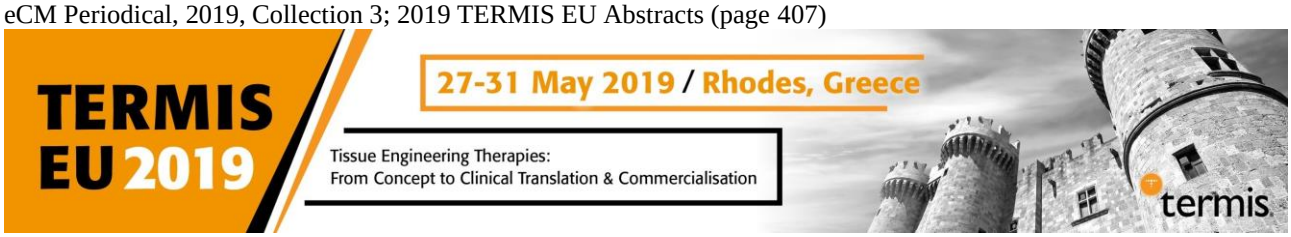
INTRODUCTION: Next generation magnetic bioprinting (NextGMB) is a promising strategy for creating geometrically complex human tissues grown in the lab. The combination of drop-on-demand bioprinting with a magnetic field enables the fabrication of native-like, functional tissues with controlled biochemical and mechanical signals and multiscale architecture.

METHODS: Magnetic 0.5 % agarose – 0.2 % collagen bioinks were loaded with 10 v/v % streptavidin-coated iron nanoparticles and added to the bioprinter head. A custom-designed 3D-bioprinter was mounted with a micro-valve for drop-on-demand bioprinting. Collagen fiber alignment was triggered in magnetic bioinks during the bioprinting process in the presence of a 2 mT magnetic field that forced iron nanoparticles to travel through the constructs. Human primary chondrocytes were isolated using standardized protocols and loaded in the magnetic bioink. Multi-layer constructs were fabricated with a gradient architecture like a native tissue. The constructs were incubated statically and dynamically for 21 days. Collagen fiber alignment was visualized by the non-linear optical effect of second harmonic generation (SHG) using a two-photon microscope at 840 nm excitation wavelength and scanning electron microscopy. Cell viability and distribution and ECM production were analyzed by histology, immunocytochemistry and qPCR.

RESULTS & DISCUSSION: Collagen fiber alignment in magnetic bioinks was confirmed after bioprinting exposed to a 2 mT magnetic field, contrarily to control samples that showed random fiber orientation. Fiber alignment additionally impacted the mechanical stiffness of the constructs, which was found significantly higher in bioprinted hydrogels with aligned collagen fibers compared to samples with random fibers. Histological evaluation showed homogeneously distributed cells in bioprinted constructs with aligned collagen fibers. Over 99 % of the cells were viable in the presence of the magnetic particles, indicating that these were non-cytotoxic. Interestingly, COL II expression was statistically higher in bioprinted bilayer constructs compared to single layer constructs with either random or aligned fibers, as shown by qPCR.

CONCLUSIONS: This study demonstrated that it is possible to align collagen fibers in magnetic bioinks in real-time whilst bioprinting. It was shown that unidirectional fiber alignment in bioprinted hydrogels can improve their mechanical stiffness. Bioprinted native-like 3D tissues showed increased remodeling properties when fabricated as multi-layered constructs compared to single layered samples. This study holds promise for the fabrication of native-like tissues with mimicking architectures and controlled mechanical signals.

ACKNOWLEDGEMENTS: This project was supported by a START Grant from the Medical Faculty, RWTH Aachen University Hospital and by the Core Facility "Two-Photon Imaging", a core Facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen.



Blood flow and O_2-transport assessment in biohybrid oxygenators

D. Dipresa[1], M. Pflaum[1], A. Haverich[1,2], S. Korossis[1,2,3]
Presenting Author: Daniele Dipresa, dipresa.daniele@mh-hannover.de
[1]Lower Saxony Centre for Biomedical Engineering Implant Research and Development, Hannover Medical School, Hannover, Germany [2]Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany [3]Cardiopulmonary Regenerative Engineering (CARE) Group, Centre for Biological Engineering, Loughborough University, United Kingdom

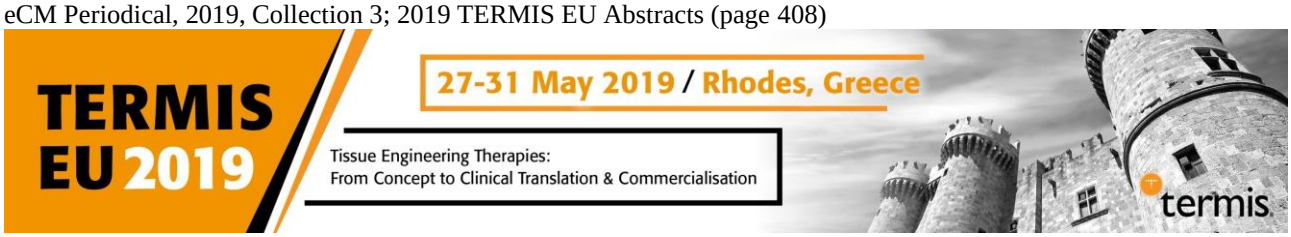
INTRODUCTION: Extracorporeal hollow-fiber (HF) membrane oxygenators (ECMOs) are the main ones used clinically. Contact of the blood with the ECMO causes thrombus formation and short-term use (2-4 weeks). Endothelialization of the HF gas-exchange membranes is a solution to thrombogenicity. However, abnormal shear stresses and inhomogeneous blood flow in the ECMO can affect O_2-transport and physical environment and thus, the function and status of the seeded endothelial cells (ECs). The study developed computational fluid dynamics (CFD) models of the iLA® (Novalung) and a rodent HF oxygenator, to assess the effect of their haemo-dynamics and O_2-transport on seeded ECs.

METHODS: Coupled O_2-transport and blood-flow 3D CFD models were developed in Ansys Fluent to predict pO_2, O_2 saturation, blood velocity, and flow-induced shear stresses on the HF walls (and thus on seeded ECs). The heterogeneous models included the full HF architecture and blood regions. The HF wall thickness defined the first diffusion region, whilst the haemoglobin-bound-O_2 contribution was modelled using the effective diffusivity of O_2. The convection-diffusion equation was used to predict the pO_2 distribution and O_2 saturation in the interstitial and fiber regions. The blood was considered as a shear dependent fluid (non-Newtonian, Carreau model).

RESULTS & DISCUSSION: The simulation results suggested that the resistance to O_2 diffusion across the HF wall, void fraction and HF configuration and diameter played a significant role in the O_2 transport efficiency and the magnitude and distribution of the HF wall shear stress (WSS). WSS was between 0.3-2.5 Pa for clinically-relevant blood flow rates (Figure 1). Predicted regions of low blood velocity were associated with very low WSS (<0.1 Pa) and areas of recirculating flow. These regions would match regions with higher incidence of thrombus formation and clotting.

DISCUSSION & CONCLUSION: This study developed important CFD and O_2-transport models for assessing ECMO performance. The models predicted low WSS and recirculating flow regions, suggesting modifications in the design of these ECMOs in order to support effective endothelialization.

ACKNOWLEDGEMENTS: This work was supported by the German Research Foundation through the SPP2014 (348028075) and the Cluster of Excellence REBIRTH (EXC 62).



Priming and exploring human mesenchymal stem cells quiescence

D. Boaventura Gomes[1], S. Camarero-Espinosa[1], L. Moroni[1]
Presenting Author: David Baoventura Gomes, david.gomes@maastrichtuniversity.nl
[1]MERLN institute, Maastricht University, Maastricht, The Netherlands

INTRODUCTION: There is a lot of controversy on how human mesenchymal stromal cells (hMSCs) are defined. This is mainly because hMSCs, when injected in vivo neither divide into new daughter cells and retain their stem cell potential, nor are they able to become quiescent. We hypothesized that cells need a more physiologically relevant micro-environment that allows them to maintain their phenotype. To do so, we tested a low RGD functionalized alginate hydrogel cell culture system, which mimics the bone marrow's physical property, allowing hMSCs to remain quiescent and maintain their multipotency.

METHODS: hMSCs were in alginate (3D) or on TCPS (2D) and cultured with 10% FBS (Normal) or without (Starving). Cell cycle was analyzed by propidum iodide cell staining. Proliferation was assessed by EdU staining. Cell viability was assessed with live/dead assay with calcein/ethidium homo-dimer and metabolic activity was monitored with PrestoBlue assay. mRNA expressing was assessed by qPCR analysis and protein expression was analyzed by western blot.

RESULTS & DISCUSSION: hMSCs cultured in hydrogels survived while they lacked the ability to proliferate. When the encapsulated hMSCs were retrieved after 7 days, they retained their multipotency and could differentiation into the three classical linages. Moreover, the encapsulated hMSCs presented a significant upregulation of quiescent markers FOXO3, EZH1, and p27 when compared to 2D. These encapsulated hMSCs expressed nuclear envelope statin (NES), a novel quiescence marker, and their cell-cycle analysis showed a decrease in the S and G2 phase when compared to 2D hMSCs culture. Also, encapsulated hMSCs showed an upregulation of Retinoblastoma 1 (RB1) at the protein level. mTOR and mTOR are currently being investigated.

CONCLUSIONS: In conclusion, we demonstrate that low RGD functionalized alginate hydrogel maintained hMSCs phenotype. We think that in long-time in vitro culture, their multipotency and viability was not compromised due to the cells retaining their quiescent state. This state was further characterized by an upregulation of enzymes that enhance and promote RB1 activity which is a cell cycle regulator that blocks G1 to S transition.

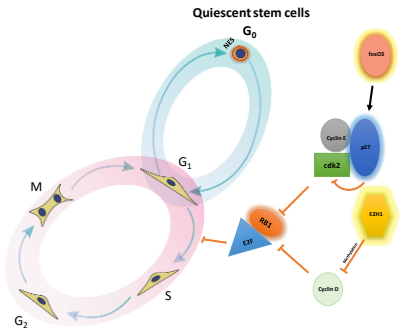
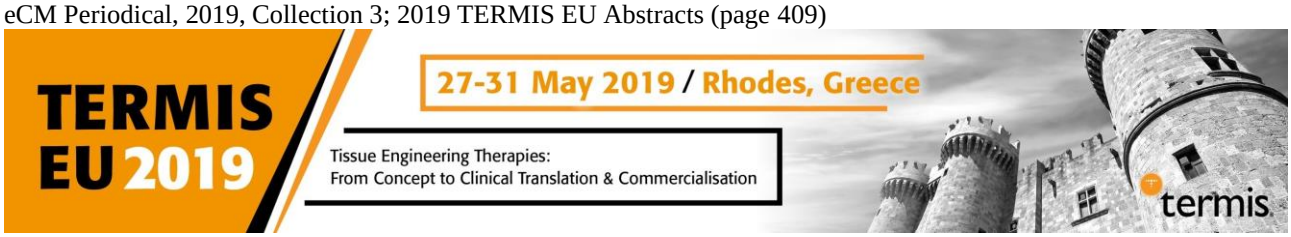


Figure 1: Proposed hMSC quiescence pathway induced by alginate 3D culture.

ACKNOWLEDGEMENTS: This work was supported by the European Research Council starting grant 637308 under the Horizon2020 framework program.



Enhancing endogenous articular cartilage repair using extracellular matrix derived scaffolds: Evaluation in a caprine model

D.C. Browe[1,2,3], R. Burdis[1,2,3], F.E. Freeman[1,2,3], P.J. Diaz Payno[1,2,3], J.M. Nulty[1,2,3], C.T. Buckley[1,2,3], P.A.J. Brama[4], D.J. Kelly [1,2,3]
Presenting Author: David Browe, browed@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity College Dublin, Ireland [2]Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin [3]Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Ireland [4] Section of Veterinary Clinical Sciences, School of Veterinary Medicine, University College Dublin, Ireland

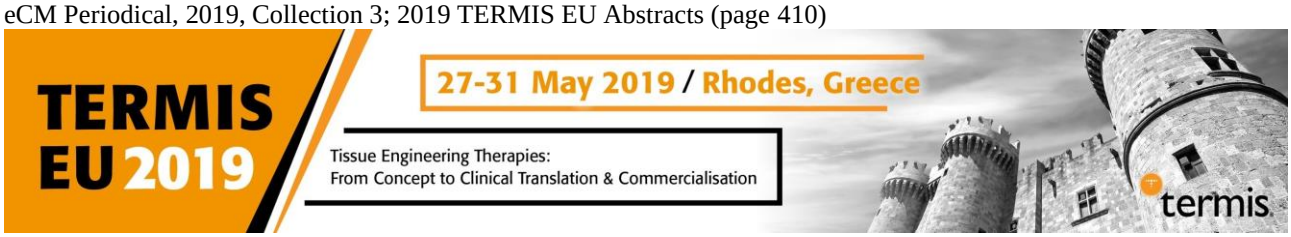
INTRODUCTION: Current treatments options for articular cartilage repair such as microfracture remain sub-optimal. While microfracture (MFX) is a well-established and well-accepted treatment for chondral defects, the repair tissue generally consists of bio-mechanically inferior fibro-cartilage. The overall goal of this study was to develop and evaluate an articular cartilage (AC)-ECM derived scaffold as a single-stage, cell-free implant to enhance articular cartilage regeneration following microfracture. To this end, we sought to: (1) evaluate if AC-ECM is indeed more chondro-inductive compared to type I collagen scaffolds typically used in cartilage repair; (2) develop highly porous and elastic scaffolds from AC-ECM and assess their capacity to support chondrogenesis in vitro; and (3) test the hypothesis that such AC-ECM derived scaffolds can enhance cartilage regeneration following microfracture in a caprine model of chondral defect healing.

METHODS: Solubilized AC and ligament was lyophilized to form 3D porous scaffolds. The in vitro chondrogenic potential of the scaffolds was assessed by seeding the scaffolds with mesenchymal stromal cells (MSCs), while the immunogenicity was assessed by seeding with human macrophages. In the pre-clinical model, bi-lateral surgery was performed on all goats with intra-animal MFX only controls in all animals. 6mm diameter by 1mm deep chondral defects were created in the medial condyles. Tissue repair was evaluated at 6 months post-surgery.

RESULTS & DISCUSSION: Solubilized AC-ECM derived scaffolds were found to be highly porous and elastic, and in vitro supported superior chondrogenesis to scaffolds derived from ligament ECM. Furthermore, the AC-ECM scaffolds did not elicit a negative immune response when seeded with macrophages. An improved level of cartilage repair was observed in animals treated with the AC-ECM scaffolds in conjunction with MFX when compared to animals treated with MFX alone.

CONCLUSIONS: This study demonstrates that MFX can be positively augmented with the use of an AC-ECM derived scaffold. To further enhance the quality of AC repair, we are currently evaluating the combination of an AC-ECM scaffold, coupled with either transforming growth factor-β3, or rapidly isolated infrapatellar fat pad stromal cells, in a caprine cartilage defect model.

ACKNOWLEDGEMENTS: This research is funded by Enterprise Ireland (CF/2014/4325) and the European Research Council (ERC-PoC-2017-779909)



Tumor protrusion fluctuations as a signature of 3D cancer invasiveness

D. Caballero[1,2], V. Brancato[1,2], VM. Correlo[1,2,3], JM. Oliveira[1,2,3], RL. Reis[1,2,3], SC. Kundu[1,2]
Presenting Author: David Caballero, dcaballero@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of EXPERTISSUES, AvePark, Barco, Guimarães, Portugal [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, AvePark, Barco, Guimarães, Portugal

INTRODUCTION: Tumor progression follows a complex cascade of events, including changes in the morphodynamics of invading protrusions. Recent evidences have shown that tumor cells modulate protrusion type to optimize their invasion in 3D [1]. However, the role of protru-sion fluctuations in this mechanism is unclear [2]. Herein, we analyze their role and define novel parameters (signature) of tumor invasiveness.

METHODS: Human A549 lung adenocarcinoma and MCF7 breast cancer cells (ATCC) were encapsulated for 3 days into a collagen I (Gibco) 3D matrix at low density (10^5 cells·ml^{-1}) to form μ-spheroids. For drug experiments, cells were incubated with 0.1, 1.0 and 10 μg·ml^{-1} of doxorubicin (DOX; Sigma) and 1.0 μg·ml^{-1} C3 Rho inhibitor (Cytoskeleton, Inc). Tumor protrusion fluctuations were monitored by time-lapse. Biophysical parameters: frequency of probing (ν_p) – # of protrusions that elongate and retract over time; stabilization lifetime (τ_s) – dwelling time of an extended tumor protrusion.

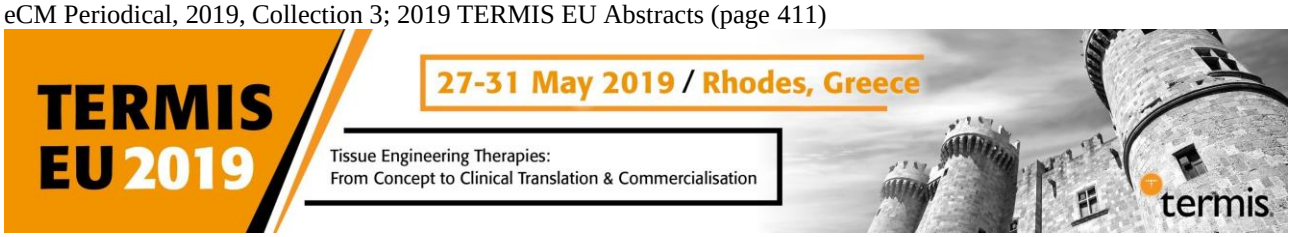
RESULTS & DISCUSSION: Protrusion fluctuations displayed a distinct morphodynamics, which depended on tumor metastatic capability. Non-metastatic A549 μ-spheroids showed a lower ν_p and larger τ_s compared to more-metastatic MCF7. The addition of DOX perturbed protrusion fluctuations, increasing (decreasing) ν_p (τ_s) in both cell types, resulting in an enhanced tumor invasiveness. Interestingly, we found a linear correlation between both parameters, which correlated with the invasive potential of cells. We also found that Rho inhibition modulated ν_p and τ_s, which abolished the invasive capacity of tumors. Next, we used a tumor-on-chip model integrating our μ-spheroids and endothelial cells to mimic the native scenario. The endothelial cells affected protrusion dynamics and orientation, suggesting a crosstalk between both cell types. Finally, all these observations were encoded into a phase diagram, which provided a novel landscape capable to ´predict´ the invasiveness of tumors based on their fluctuations.

CONCLUSIONS: These data illustrate that protrusion fluctuations are key players in the physicochemical mechanism of tumor invasion, and are governed by ν_p and τ_s.

ACKNOWLEDGEMENTS: H2020 (FoReCaST – nº 668983); Portuguese FCT (PTDC/BTM-ORG /28070/2017 and PTDC/BTM-ORG/28168/2017) funded by Norte2020 supported by FEDER.

REFERENCES

[1] Friedl P et al. J Cell Biol, 2010. 188(1):11-19
[2] Caballero D et al. Biophys J. 2014; 107:34-42



Biogenic metallic nanoparticles. A nanometric trojan horse approach

D.M. Cruz[1]
Presenting Author: David Medina Cruz, medina.dav@husky.neu.edu
[1]Chemical Engineering Department. Northeastern University, Boston, MA, 02115, USA

Antimicrobial resistance to antibiotics (AMR) and cancer and two of the main concerns that the healthcare system should face nowadays. Current drugs and antibiotic treatments are becoming ineffective or have plenty of drawbacks. Therefore, new alternatives are needed. Despite the increase in the use of nanostructures in the biomedical field, traditional synthesis of such materials is subjected to several disadvantages, such as the production of toxic-by-products and harsh conditions. Green nanotechnology is presented as a suitable answer, allowing the generation of nanostructures in a quick, cost-effective and environmentally-friendly approach. Pathogenic bacteria and human cells -both cancer and healthy ones- were used for the synthesis of metallic nanoparticles similarly. Bacteria and cells are cultured in the presence of metallic salts under standard conditions until the generation of nanoparticles, that is followed using microscopy and spectrophotometric techniques. After purification, nanoparticles are used as antimicrobial and anticancer agents using colony counting unit assays and MTS assays, respectively, as well as characterized. Pathogenic bacteria are used for the synthesis of bacteriogenic metallic selenium nanoparticles that were characterized in term of size, morphology, and composition. These agents were employed as suitable agents with antimicrobial activity against the same bacteria that synthesized them, showing low cytotoxicity for human dermal fibroblasts (HDF) cells. The synthesis of metallic nanomaterials using cancer and healthy cells -HDF and HFOB- is reported. Pure metal nanoparticles –palladium or platinum- and bimetallic structures –such as gold-platinum- are readily synthesized using the cells, and after purification, they are used as anticancer agents. Microbiological agents are successfully used as a synthetic machine for the generation of metallic nanoparticles of different compositions with biomedical properties. Therefore, they are presented as a suitable approach for the synthesis of nanomaterials in a green fashion, overcoming the limitations of traditional nanotechnology.

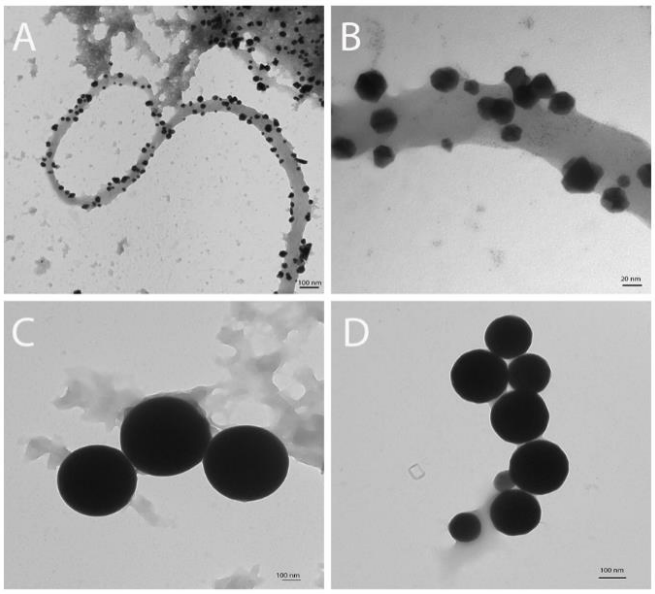
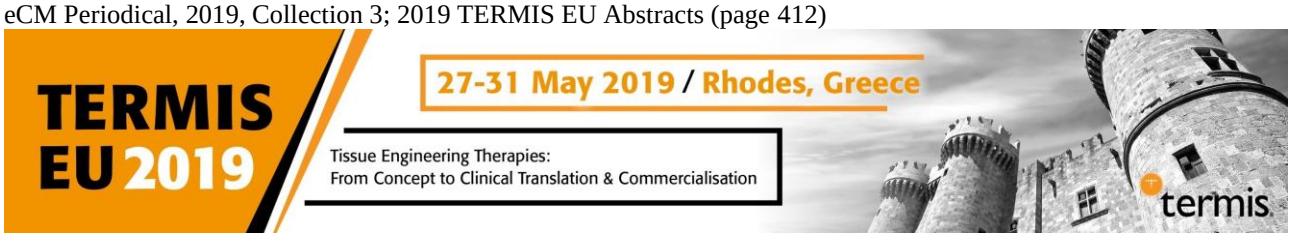


Figure 1: Metallic nanoparticles made by different microorganisms. Gold-platinum (AuPt) nanoparticles made by Melanoma cells (A, B) and selenium (Se) nanoparticles made by Staphylococcus aureus (C, D).



Evaluation of the influence of wall shear stress features on endothelial cell phenotypes in-vitro with correlation to ex-vivo bovine arteriovenous tissue

D.T. O'Connor[1], M. Franzoni[1], D. Power[2], M.T. Walsh[1]
Presenting Author: David O'Connor, david.t.oconnor@ul.ie
[1]Biomaterials Cluster, Bernal Institute, University of Limerick, Limerick Ireland [2]Department of Vascular Surgery, University Hospital Limerick, Limerick, Ireland

INTRODUCTION: The wall shear stress (WSS) is the frictional force per unit area exerted at the interface of the flowing blood and the endothelium. WSS is a potent stimulus capable of evoking variable phenotypes in endothelial cells (EC)[1,2]. In adults, two pathways are alternatively involved in inducing EC quiescence conferring a protective effect on endothelium or EC activation and pro-inflammatory signalling inducing endothelium dysfunction and pro-inflammatory and pro-thrombotic effects. Physiological WSS patterns were decomposed correlating to imperative features such as TAWSS, peak WSS, temporal WSS gradient and OSI. The overarching aim is to systematically evaluate the features as mediators of venous EC activation and signalling.

METHODS: The study analyses the in vitro and ex-vivo WSS exposure using a Cone and Plate device with a custom designed ex-vivo perfusion system. In-vitro experiments: To decompose the driving factors correlated to WSS in-vivo, 10 idealised waveforms were created ranging for WSS peak magnitude of 0.5 to 2.5 Pa, t-WSSG from 2 to 20 Pa/s and from 0 to 0.5 of oscillatory shear index (OSI) value. Evaluation of differential protein and gene expression was completed using RT-PCR, Western Blot and Immunofluorescence staining. Preferential analysis was completed on NF-kB, PECAM-1, a-SMA and KLF-2 due to previously described relationship with vascular disease.
Ex-vivo experiments: Using a custom designed perfusion system, idealised physiological waveforms were used to exposed surgically created bovine AVF tissue samples for up to 2 weeks to correlate the tissue response to hemodynamics presented in in-vitro model. H&E and immunofluorescent staining techniques were used to compare the tissue's structural remodelling and differential protein response.

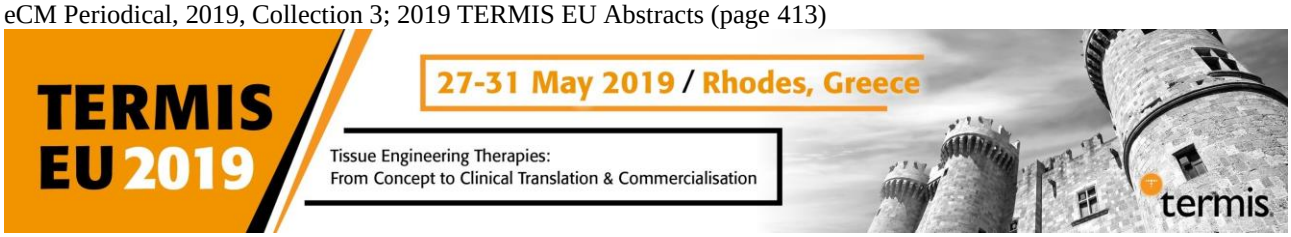
RESULTS & DISCUSSION: Our results indicate that WSS waveforms with a peak magnitude of 1.5 or 2.5 Pa can induce a protective phenotype in ECs in culture in terms of KLF-2, KLF-4, IL-8 and VCAM-1 expression. By presenting a reverse phase or decreasing TAWSS of the waveforms with a peak of 1.5 or 2.5 Pa, the flow-induced protective effect is maintained. A WSS peak of 1.5 Pa show to be sufficient to induce protective pathways. Pro-inflammatory gene expression showed no differences in the expression of two subunits of NFkB (NFkb-1, NFkB-3). Differential expression of protective and pro-inflammatory proteins colocalising with areas of disturbed hemodynamics in the ex-vivo model.

CONCLUSIONS: The effects of unsteady WSS profiles, atherogenic time-averaged WSS threshold should be reconsidered and reduced significantly, at least for venous EC. Similarly, preliminary results from ex-vivo tests suggest differential protein expression at the sites of disturbed WSS patterns within the tissue samples correlating with previous flow studies.

ACKNOWLEDGEMENTS: The authors would like to thank SFI (Grant no: 15/CDA/3323).

REFERENCES

[1] Nakajima, H. & Mochizuki, N. Cell Cycle **16,** 1893–1901 (2017).
[2] Chatterjee, S. Front. Physiol. **9,** 524 (2018).



Silica nanoparticle internalization by human mesenchymal stem cells enhances their adhesion properties in dynamic conditions

D. Fusco[1,2*], E. Vitale[1,2*], L. Gili Sole[2], G. Isu[2], G. Martra[3], C. Giachino[1*], A. Marsano[2*]
Presenting Author: Deborah Fusco, deborah.fusco@edu.unito.it
[1]Department of Clinical and Biological Sciences, University of Turin, Torino, Italy [2]Departments of Biomedicine and Surgery, University Hospital and University of Basel, Basel, Switzerland [3]Department of Chemistry, University of Turin, Torino, Italy *equally contributing authors

INTRODUCTION: Human mesenchymal stem cells (hMSC)-based therapies have shown some positive effects in regenerating injured myocardium. However, their therapeutic effect is limited by their engraftment capacity. Indeed, most of the MSCs die during the delivery and fail to adhere and therefore to survive once injected [1]. A method of fluorescent staining with silica nanoparticles (SiO_2NPs) was developed in order to track hMSCs in vivo. The uptake of SiO_2NPs was shown to be well-tolerated by hMSCs. It was shown that during in vitro static culture, SiO_2NP internalization increased the area and the maturation level of hMSC focal adhesion complexes, and enhanced the expression of Connexin-43 [2]. Based on these findings, we hypothesized that the internalization of SiO_2NPs could enhance MSC adhesion also in dynamic condition, resembling interstitial shear stresses physiologically present in the contractile myocardium [3].

METHODS: Human MSCs were isolated from three different bone marrow donors. hMSCs were incubated with 50 ug/ml SiO_2NPs overnight [2]. hMSCs without SiO_2NPs were used as control. Custom-made shear stress chambers were used to mimic the heart physiological shear stress of 0,43 dyne/cm^2. The adhesion capability of hMSCs (pre-loaded with Calcein) was evaluated first on a fibronectin-coated glass and later on a rat-origin cardiac cell monolayer (80% cardiomyocytes and 20% fibroblasts) following two hours. Connection between the hMSCs and the cardiac monolayer was evaluated by assessing the transfer of FITC-labeled Calcein.

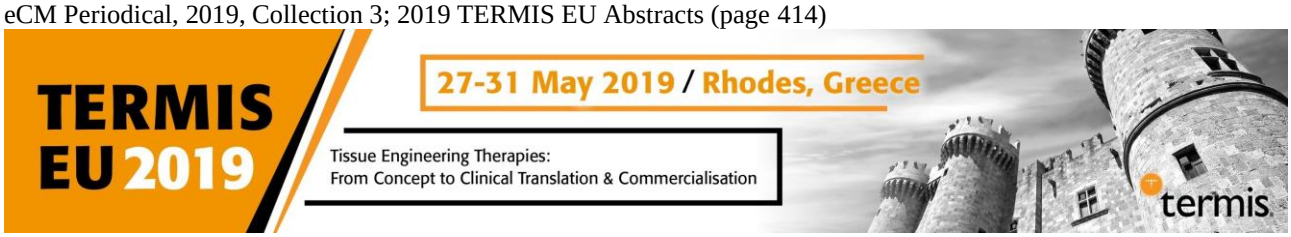
RESULTS & DISCUSSION: On fibronectin substrates the adhesion area of SiO_2NP-hMSC focal adhesion complexes was higher than in control cells (5122 ±701 and 1469 ± 236 cm^2, respectively). The adhesion area on a cardiac layer was also superior in SiO_2NP-treated MSCs. Nanoparticle-treated MSCs were also characterized by the highest number of polarized adhesion focal complexes (between 7-13 compared to 3-6 in control MSCs). Moreover, SiO_2NP-MSCs were capable to transfer Calcein in to the highest number of cardiac cells (20%±10 vs 10% ±12).

CONCLUSIONS: SiO_2NP incorporation clearly increases the hMSC adhesion capacity, showing a high potential to improve MSC engraftment and their therapeutic effect in vivo upon cell injection.

ACKNOWLEDGEMENTS: None from Basel. This study was supported by GIAC RILO 18 02

REFERENCES

[1] Satyam A et al. Adv Mater. 2014; 26(19):3024-34
[2] Popara J et al. Nanomedicine 2018; 13(10):1121-38
[3] Cigognini D et al. Sci Rep. 2016;6:30746



A novel mechanism for the protection of embryonic stem cell derived tenocytes from inflammatory cytokine interleukin 1 beta

A. McClellan[1], R. Evans[1], C. Sze[1], S. Kan[1], Y. Paterson[1,2], D.J. Guest[1]
Presenting Author: Debbie Guest, debbie.guest@aht.org.uk
[1]Centre for Preventive Medicine, Animal Health Trust, Newmarket, Suffolk, UK [2]Department of Veterinary Medicine, University of Cambridge, Cambridgeshire, UK

INTRODUCTION: Tendon injuries occur commonly in equine and human athletes and are associated with high re-injury rates due to poor regeneration and scar tissue formation. Equine ESCs survive[1] and differentiate into tenocytes[2] in the injured horse tendon and may provide a source of cells to aid tendon regeneration. Following a tendon injury there is an upregulation of inflammatory cytokines, including IL-1β[3], which may have a negative effect on the function of endogenous tendon cells and exogenous cells applied during therapies

METHODS: Equine tenocytes from adult, fetal and ESCs were exposed to 17 ng/ml IL-1β. MMP and tendon gene expression was measured using qPCR. Collagen gel contraction in 3D cultures was determined and the storage modulus of the resulting tendon-like constructs was quantified using Dynamic Shear Analysis. The expression of IL1 receptors was determined using qPCR and immunocytochemistry and nuclear translocation of the IL-1β signaling molecule, NFκB was quantified. The ability of IL1 receptor antagonist protein (IL1Ra) to protect tenocytes from the effect of IL-1β was also determined.

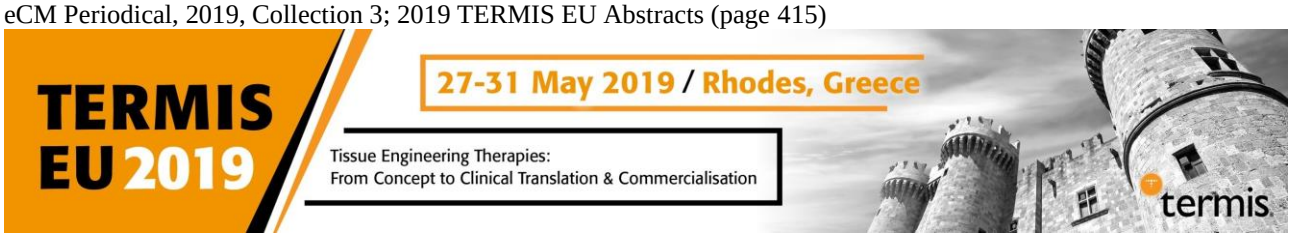
RESULTS & DISCUSSION: IL-1β upregulates MMP expression and changes the expression of tendon-associated genes in adult and fetal tenocytes. IL-1β inhibits collagen gel contraction by adult and fetal tenocytes, resulting in tendon-like constructs with a lower storage modulus. A high concentration IL1Ra can protect adult and fetal tenocytes from these effects. In contrast, equine ESC-tenocytes undergo no changes in gene expression or matrix remodeling when exposed to IL-1β. ESC-tenocytes express significantly less of the signaling receptor IL1R1 and more of the decoy receptor IL1R2 and the gene encoding IL1Ra, IL1RN. This corresponds to a lack of nuclear translocation NFκB in ESC-tenocytes following IL-1β exposure.

CONCLUSIONS: Defining the mechanisms by which ESC-tenocytes are protected from inflammation may enable the development of novel pharmaceuticals to protect endogenous adult tenocytes and produce better tendon regeneration to reduce re-injury rates.

ACKNOWLEDGEMENTS: This study was kindly funded by the Horserace Betting Levy Board (prj/782).

REFERENCES

[1] Guest D et al. Equine vet. J. 2010; 42: 636-642
[2] Barsby T et al. Tisse Eng Part A. 2013; 19: 2156-65
[3] Hosaka Y et al. J Vet Med Sci. 2002; 64: 945-947.



Designing programmable hydrogels for controlled vessel formation within engineered tissues

D. Rana[1], N. Salehi-Nik[1], J. Rouwkema[1]
Presenting Author: Deepti Rana, d.rana@utwente.nl
[1]Department of Biomechanical Engineering, Technical Medical Centre, University of Twente, 7522NB Enschede, The Netherlands

INTRODUCTION: Spatiotemporal control on the availability of growth factors is an important factor for controlling the vascularization within an engineered tissue [1]. Therefore, we have designed aptamer-functionalized hydrogel to evaluate their potential for growth factor sequestering, controlled release and study their effect on vessel formation by endothelial cells.

METHODS: The aptamer functionalized hydrogels were prepared via photo-polymerization of gelatin methacryloyl (GelMA) and acrydite functionalized aptamers containing a sequence that is optimized for the binding of vascular endothelial growth factor (VEGF). Irgacure 2959 was used as photoinitiator. The physicochemical properties of these aptamer functionalized hydrogels were evaluated and compared with control samples. To study the programmable release efficiency, the complementary sequences (CSs) were added on the hydrogel system to trigger the growth factor release from the aptamers which was evaluated using ELISA kits. For studying the effect of triggered growth factor release in co-culture system (human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs)), cells were encapsulated within aptamer functionalized hydrogels and the formation of vascular structures was investigated.

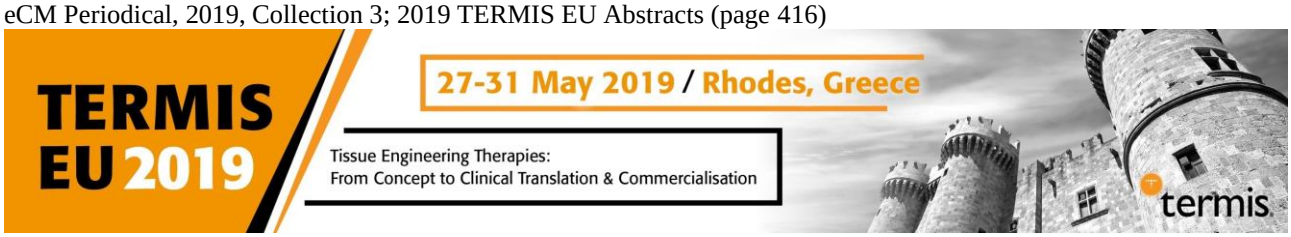
RESULTS & DISCUSSION: The results obtained from physicochemical analysis of the aptamer functionalized hydrogels confirmed the aptamer retaining capacity of acrydite functionalized aptamers, in comparison with the control aptamers for as long as 14 days at 37 °C. These results fit well with our hypothesis that the acrydite functionalized aptamers could covalently crosslink within the polymer network whereas control aptamers tend to get just physically entrapped within the hydrogels. The results obtained from the VEGF ELISA experiments showed the triggered release of VEGF from the aptamer functionalized hydrogels in response to CS addition. Without CS addition, these hydrogels could sustain a controlled release for until 10 days. Furthermore, in co-culture experiments, the developed aptamer functionalized programmable hydrogels supported cell viability and the formation of vascular structures by HUVECs and MSCs within the hydrogels for up to 8 days. These results further confirmed the bioactivity of the VEGF molecules after their loading within the aptamer functionalized hydrogels.

CONCLUSIONS: The present study clearly illustrate the beneficial effects of triggered VEGF release on the formation of vascular structures by HUVECs and MSCs in co-culture condition.

ACKNOWLEDGEMENTS: This work is supported by an ERC Consolidator Grant under grant agreement no 724469.

REFERENCES

[1] M.R. Battig et al. J. Am. Chem. Soc. 2012; 134: 12410-12413.



Injectable bone forming cells derived from human BM-MSC improve bone repair in a mouse model by promoting host and donor bone formation through two mechanisms of action

D. De Troy[1], L. Hertzog[1], S. Normand[1], A. Tury[2], R. Baron[3], S. Pietri[1]
Presenting Author: Delphine De Troy, delphine.detroy@bonetherapeutics.com
[1]Bone Therapeutics S.A., Gosselies, Belgium [2]Skeletal Cell Therapy Support, Gosselies, Belgium [3]Harvard Medical School, Boston, MA, USA

INTRODUCTION: Mesenchymal Stromal Cells (MSC)-based therapies are promising for bone regeneration. However, undifferentiated MSC may only exert an inductive paracrine effect without directly producing bone. Here, we developed an injectable product constituted of human bone-forming cells derived from bone marrow (BM)-MSC (ALLO-P2) and tested its bone formation and bone repair capacities in relevant animal models.

METHODS: Human BM cells from healthy volunteer donors were expanded in vitro into undifferentiated MSC (BM-MSC) or differentiated into bone-forming cells (ALLO-P2). Cells were characterized in vitro for the expression of chondro-osteogenic markers by RT-qPCR. Bone formation potential was assessed in vivo, 14 days after a single administration over the calvaria of 12-week old female NMRI-Nude mice, using X-ray and histological approaches. Bone healing capacity was tested in a segmental femoral sub-critical size defect (sub-CSD) model (2 mm, [1]) in female NMRI-Nude mice using X-Ray analyses over time (up to 6 weeks).

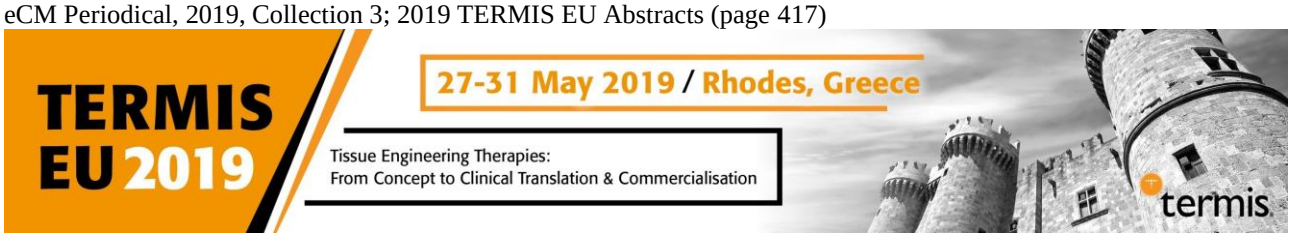
RESULTS & DISCUSSION: In vitro, ALLO-P2 cells expressed significantly higher levels of chondro-osteogenic markers such as RUNX2, BMP2, SOX9, MMP13 and ALP than undifferentiated BM-MSC derived from the same donors, indicating their engagement into the chondro-osteogenic lineage. In vivo, a single dose of ALLO-P2 significantly enhanced bone neoformation by about 2-fold, 14 days post-administration over the calvaria of NMRI-Nude mice compared to BM-MSC ($p<0.05$). Histological analyses and mouse/human type I collagen double-immunolabelling revealed the presence of an additional bone layer of mixed host and donor origins only in mice administered with ALLO-P2. Intramembranous ossification was induced along the calvarial surface by both types of cells, but only ALLO-P2 directly generated bone through endochondral ossification. In the segmental femoral sub-CSD model, ALLO-P2 improved fracture repair with a significant reduction of the bone defect size compared to the excipient.

CONCLUSIONS: ALLO-P2, enhanced bone formation and improved bone repair not only by stimulating host bone formation but also by promoting direct bone formation recapitulating endochondral ossification. ALLO-P2 is a potential promising clinical candidate for improving bone regeneration and fracture healing.

ACKNOWLEDGEMENTS: This work was supported by the Walloon Region (Belgium).

REFERENCES

[1] Manassero M et al. J. Vis. Exp. 2016; 116.



Correlation of cell number and exosome shedding of different cell lines

D.Y. Urey[1], N.G. Durmus[2], M.O. Ozen[1], R. Gupta[1], and U. Demirci[1, 2]
Presenting Author: Deniz Yagmur Urey, ureden.20@robcol.k12.tr
[1]Canary Center at Stanford for Cancer Early Detection, Department of Radiology, School of Medicine, Stanford, CA 94304, USA [2]Department of Biochemistry, School of Medicine, Stanford University, Stanford, CA 94304, USA

INTRODUCTION: Extracellular vesicles (EV) are a promising source of biomarkers for early detection, diagnosis and prognosis of different conditions. In this study, we discussed a method to isolate EVs and assess the dependence of exosome concentrations for 3 different cell lines.

METHODS: Exosome Total Isolation Chip (ExoTIC) is a new technology developed for the efficient isolation of extracellular vesicles (EVs) [1]. Using this unique technology, EVs from different cancer patient clinical samples such as plasma, urine and lavage can be run, and exosomes can be isolated based on their size. ExoTIC is made out of simple materials that cost under a dollar per device and achieves an extracellular vesicle yield ~ 4 - 1,000-fold higher than that with ultracentrifugation (UC), which is a widely-used method for isolating EVs. Here, we studied extracellular vesicles from 5 x 10^4 – 1 x 10^6 of AsPC-1 pancreatic cells, 3T3 fibroblast cells and MDA-MB-231 breast cancer cells. We show that EVs from these cell lines can be isolated based on their size using ExoTIC. We used RPMI media with 5% exosome depleted FBS for AsPC-1 and MDA-MB-231, and DMEM medium with 5% exosome depleted FBS for 3T3 cell line. We collected 1.5 mL of media from each cultured cell line samples. A total of 66 samples were prepared. We centrifuged them for 3 minutes at 1200 RPM, and then, filtered the collected media samples through 220nm filters, before running through ExoTIC. We used 30nm and 50nm membranes for EV isolation with ExoTIC. Isolated EV solutions were analyzed using NanoSight, utilizing Nanoparticle Tracking Analysis (NTA).

RESULTS & DISCUSSION: Isolation of EVs from biological samples is fundamental for clinical studies as well as experimental research. We developed and optimized the above protocol for the isolation of exosomes at >30nm and >50nm sizes. The relationship between exosome size based concentration and cell concentration is examined using NanoSight analysis.

CONCUSIONS: Our results can be used for a wide range of experiments on tissue or cell culture and clinical studies of diseases such as cancer.

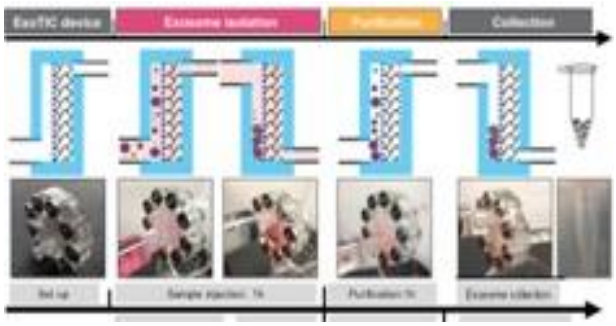
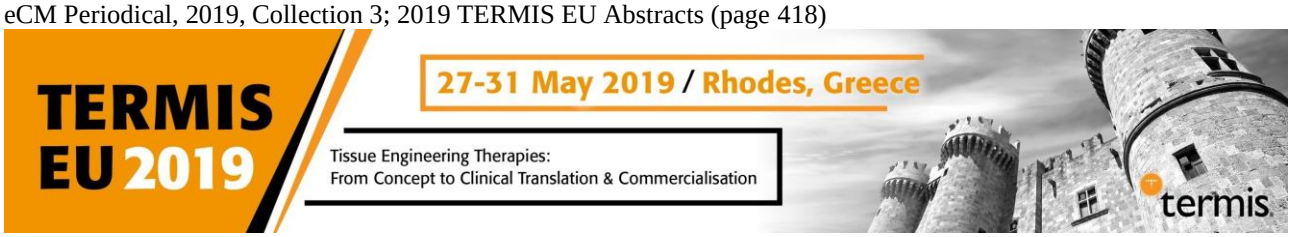


Figure 1: Design and fabrication of the ExoTIC device for exosome isolation.

REFERENCES

[1] Fei Liu et al. The Exosome Total Isolation Chip. ACS Nano, 2017; 11 (11): 10712–10723.



Macromolecular crowding and serum-free culture for stem cell phenotype maintenance

D. Gaspar[1,2], S. Korntner[1,2], D.I. Zeugolis[1,2]
Presenting Author: Diana Gaspar, diana.gaspar@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway [2]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

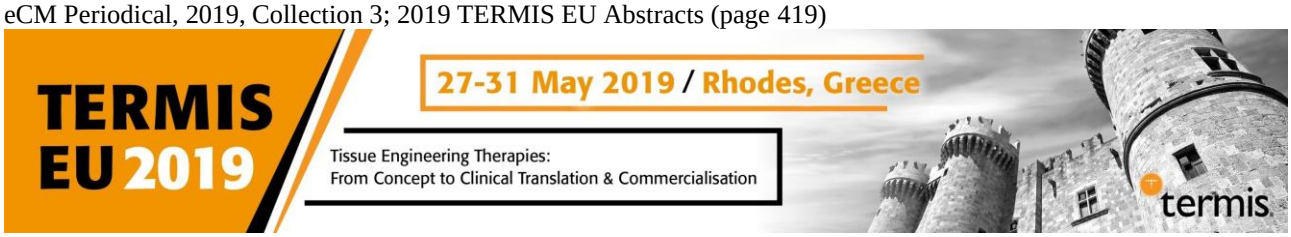
INTRODUCTION: Xenogeneic- and serum-free reagents are gaining increasing interest for stem cell culture. Serum contains several molecules that are crucial for initial cell attachment and subsequent survival in vitro, creating a need for added attachment substrates in serum-free culture. Further, the requirement for exogenous attachment proteins also stems from the slow extracellular deposition (ECM) typically observed in dilute culture media. ECM deposition can be accelerated through macromolecular crowding (MMC), a biophysical phenomenon based on the volume excluded by macromolecules in an environment, which accelerates the conversion of procollagen to insoluble collagen in vitro. Therefore, it is hypothesised that MMC in serum-free stem cell culture can accelerate ECM deposition which will support multipotent phenotype maintenance during expansion.

METHODS: Human bone marrow stem cells (BMSCs) were seeded at 25,000 cells/cm^2 in 24 well plates and were allowed to attach for 24 h. Subsequently, the medium was changed to medium with MMC (carrageenan or Ficoll) and 100 μM L-ascorbic acid phosphate (Sigma Aldrich). Medium without MMC was used as control. Media were changed every 3 days. SDS-PAGE, immunocytochemistry and FACS were conducted after 4, 7 and 10 days. Trilineage differentiation was performed at 21 days.

RESULTS & DISCUSSION: Isolation with different serum and animal component free media maintained the multipotent phenotype of BMSCs, evident by high expression of surface markers CD105, CD73, CD90 and CD44 and low expression of CD31, CD45 and CD146. Animal component free media maintained the spindle shaped morphology BMSCs up to passage 4. Cell viability and metabolic activity were not significantly altered by serum and animal component free conditions ($p<0.05$). Deposition of collagen type I was significantly enhanced in the presence of MMC in animal component free conditions ($p<0.05$).

CONCLUSIONS: This indicates the suitability of BMSC-secreted ECM for phenotype maintenance during expansion.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland, (15/CDA/3629 and 13/RC/2073); and H2020, Marie Skłodowska-Curie Actions (713690).



The effect of elastin-like recombinant on SMCs behavior and mechanical properties of tubular collagen gel-based scaffolds

D.B. Camasao[1], M. González-Pérez[2], J.C. Rodríguez-Cabello[2], D. Mantovani[1]
Presenting Author: Dimitria Bonizol Camasao, dimitria.bonizol-camasao.1@ulaval.ca
[1]Laboratory for Biomaterials and Bioengineering, CRC-I, Dept Min-Met-Materials Eng & Research Center of CHU de Québec, Laval University, Quebec City, Canada [2]G.I.R. Bioforge, University of Valladolid, Edificio LUCIA, Paseo de Belén, 19, 47011, Valladolid, Spain

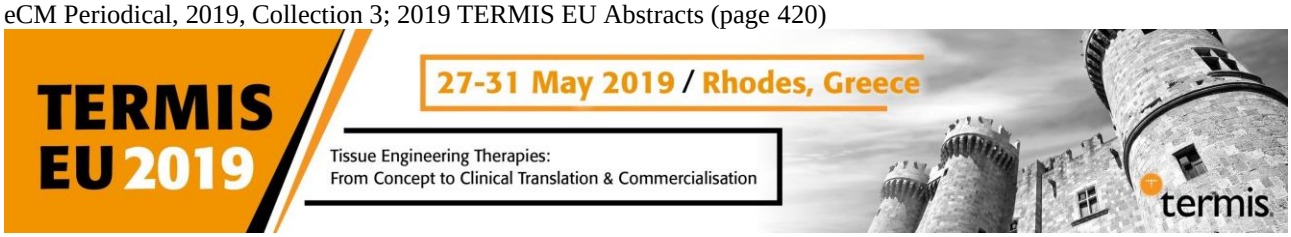
INTRODUCTION: Vascular tissue engineering has emerged from the need to develop functional conduits with similar properties as native vessels. Collagen and elastin are the dominant components of the extracellular matrix (ECM) of this tissue. While collagen is responsible for the stiffness, elastin confers the elasticity. These proteins also regulate vascular smooth muscle cells (SMCs) activity and phenotype. Although several models using collagen gel scaffold were proposed, the importance of elastin incorporation in these structures for cellular activity and final mechanical properties were not investigated. In fact, elastin has been reported to be a missing link in vascular tissue engineering approaches. Herein, the influence of elastin in collagen gels with SMCs was evaluated in terms of cell metabolic function, cell-mediated compaction, expression of key ECM proteins and mechanical properties.

METHODS: Collagen type I was extracted from rat tail tendon and solubilized in acetic acid at 4mg/mL. Genetically engineered elastin-like recombinamers (ELRs) were bioproduced in Escherichia Coli bacteria and dissolved in PBS at 4mg/mL. Human umbilical SMCs were used to prepare cellularized tubular collagen gels with 0, 0.3%, 3%, 30% and 60% in mass of ELR at a cell density of 0.5×10^6 cells/mL. Alamar Blue viability assay was used to evaluate cell metabolic function. Real-time PCR was performed to evaluate the expression of key proteins by SMCs (collagen, elastin, fibrilin-1, Ki67 and calponin). Immunostaining procedures were applied to observe collagen, elastin and SMCs organization. Finally, the elastic modulus of the tubular samples was evaluated through circumferential stress-relaxation tests. All the analyses were performed after 1, 3, 7 and 14 days of static maturation.

RESULTS & DISCUSSION: The presence of ELR in cellularized collagen gels did not compromise cell viability, showing even an increase in the metabolic activity in the gel containing 30% of elastin. Similarly, this same condition led to lower volumes of gel after maturation suggesting that the cells were more active to remodel the matrix. The elastic modulus increased with higher content of elastin after 14 days of maturation. Real-time PCR support this result as the expression of key ECM proteins, such as collagen were upregulated in presence of 30% and 60% of elastin.

CONCLUSIONS: Altogether, this study demonstrates that the presence of at least 30% of elastin in the collagenous matrix improve SMCs metabolic function, cell-mediated remodeling, expression of key ECM proteins and mechanical properties of the constructs. Interestingly, this condition is the one that closely mimic the native tissue composition.

ACKNOWLEDGEMENTS: This work was partially supported by NCPRM, NSERC, CFI, FQRS, FQRNT, the European Commission through the project ELASTISLET (NMP-2014-646075) and the Spanish Ministry of Education, Culture and Sports (FPU15-00448 to MGP).



Melt electrospinning writing of polycaprolactone-based scaffolds as electroconductive matrices for cardiac tissue engineering

D. Olvera[1], M. Sohrabi[1,2], G. Hendy[3], M. Monghan[1,2]
Presenting Author: Dinorath Olvera, olverad@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland [2]Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland [3]Spraybase, Avectas, MaynoothWorks, Maynooth University, Kildare, Ireland.

INTRODUCTION: Electroconductive matrices with defined architectures are warranted to control cell alignment and tissue reorganization of the heart wall after myocardial infraction (MI) [1]. Here, we produced electroconductive scaffolds with defined fibrous architecture by means of melt electrospinning writing (MEW) and the use of polycaprolactone (PCL) and a conductive polymer.

METHODS: We used a commercially available melt electrospinning writing (MEW) apparatus (MELT A-1204-0001-01D, Spraybase, Dublin, Ireland). PCL (80 kDa, Sigma) was blended with the conductive polymer under inert conditions. The electroconductive composite was extruded through a nozzle using gas pressure, a heated melt-head and a voltage field between the spinneret and collecting plate. Another set of PCL alone scaffolds were incubated in the conductive monomer and a dopant solution to deposit onto the PCL fibres. Polymerization of the conductive polymer occurred for 24 h at 4 °C with gentle rotation. FTIR, DSC, conductivity measurements, and SEM imaging were conducted on freshly produced samples and after 2, 7 and 14 days post processing.

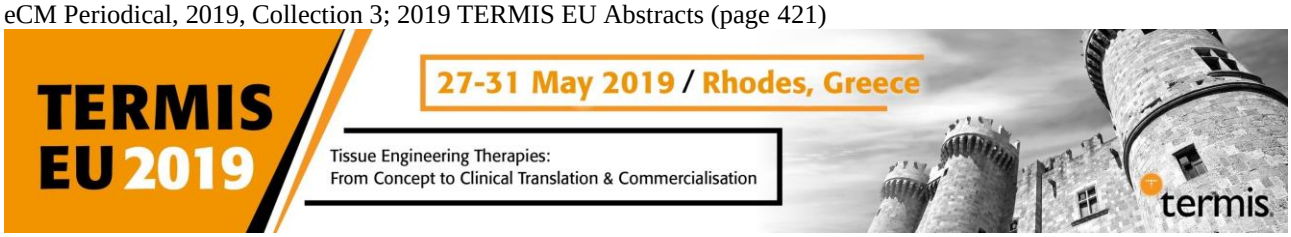
RESULTS & DISCUSSION: We characterized the effect of monomer content (1, 5, 10 and 40%), and polymerization time (0.25, 0.5, 0.75, 1, 2, 3 and 4 h) of the polymer composite on electro-conductivity. The highest conductivity was achieved with a polymerization time of 4 h. However, this polymer demonstrated low processability. Highly ordered PCL alone scaffolds were able to conduct the electrical stimulation when coated with the conductive polymer. We measured resistances of 1.8 ± 0.4 kΩ. These electroconductive scaffolds demonstrated conductivity stability after 14 days in PBS. Further, the electroconductive scaffolds demonstrated no cytotoxicity to human MSCs.

CONCLUSIONS: These data illustrate the successful generation of conductive PCL-based scaffolds. Future studies include providing the electrical, mechanical, and topographical cues of the electroconductive scaffolds presented here to induced human pluripotent stem cells (iPS).

ACKNOWLEDGEMENTS: To Enterprise Ireland (EI/IP/2017/0640), Ms. Song Wong, Dr. Nitika Grover and Matteo Solazzo for their help.

REFERENCES

[1] Wu, L. et al. ACS Nano. 2017; (11) 5646–59.



Tissue-engineered bone equivalent application for treatment of combat-related bone defects

R. Vasyliev[1,2], V. Oksymets[2], A. Rodnichenko[1,2], O. Gubar [2,3], A. Zlatska[1,2], I. Gordiienko[2,4], V. Khadzhinova[2], D. Zubov[1,2]
Presenting Author: Dmytro Zubov, d.zubov@ilaya.ua
[1]State Institute of Genetic & Regenerative Medicine NAMSU, Kiev, Ukraine [2]Medical Company ilaya®, Kiev, Ukraine [3]Institute of Molecular Biology & Genetics NASU, Kiev, Ukraine [4]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NASU, Kiev, Ukraine

INTRODUCTION: Management of critical sized bone defects is a genuine clinical challenge. High-energy combat-related bone defects are manifested in 10-12% of casualties, and 30% of these defects are complicated with osteomyelitis. Temporary and economic costs for treating patients with alterations of reparative regeneration processes, especially in the high-energy mechanism of trauma, the complexity of their social adaptation, justify the need to search for innovative organ-saving technologies of regenerative medicine for bone integrity restoration. Our aim was to develop and assess the clinical effectiveness of transplantation of three-dimensional pre-vascularized tissue-engineered bone equivalent/graft (3D-OPG) for restoration of combat-related bone defects of critical size (ClinicalTrials.gov Identifier: NCT03103295).

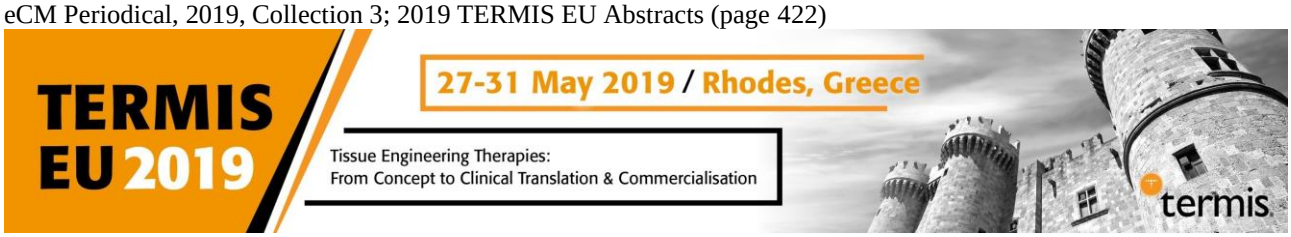
METHODS: Bone equivalent transplantation was performed in 47 combat-injured persons with 49 bone defects. Enrolled patients hadn't previous oncologic diseases reported and possessed circular and tangential critical sized defects of tubular bones and heel bone of various lengths and volume. New bone formation was assessed by X-ray examination and histological analysis. To manufacture 3D-OPG we used partially demineralized allo-/xenogeneic bone (blocks and chips) seeded with autologous cultured cells: BM-MSCs in mix with periosteum progenitor cells (PPCs) and endothelial progenitor cells (EPCs). The quality/identity of cell cultures was assured by flow cytometry (cell phenotype), cytogenetic analysis (GTG-banding), donor and cell cultures' infection screening (qPCR), functional analysis (cell kinetics, CFU analysis, multilineage differentiation assay), as well as vital FDA / PI combined staining and histological analysis of manufactured 3D-OPG random samples.

RESULTS & DISCUSSION: The casualties were included in a study 8-19 months after injury, due to illustrating the ineffectiveness of conventional surgical methods. All cell cultures had a normal phenotype and karyotype and differentiation potential to orthodoxal lineages. The vital FDA / PI combined staining and histological analysis of 3D-OPG samples showed their regular seeding with viable cells. Histological analysis of 3D-OPG samples taken within adoptive resection surgery 3 months after transplantation shows immature bone tissue formation and graft remodeling. Restoration of the bone defects was observed after 6 months (detected by X-ray examination and histological analysis) [1].

CONCLUSIONS: We discuss an effective method (90.4% effectiveness) for treating the consequences of combat-related limb injuries with use of 3D-OPG. The method allows restoring the integrity of the bone in combat-related bone defects of critical size and to maintain a functional state of a limb.

REFERENCES

[1] Vasyliev R et al. Exp Oncol. 2017; 39(3):191-196



Structural and biochemical analysis of extracellular matrix formed by jaw periosteal cells

D. Alexander[1], M. Danalache[2], S.M. Kliesch[3], M. Munz[1], S. Reinert[1]
Presenting Author: Dorothea Alexander, dorothea.alexander@med.uni-tuebingen.de
[1]Department of Oral and Maxillofacial Surgery, University Hospital of Tübingen, Germany [2]Department of Orthopaedic Surgery, University Hospital of Tübingen, Germany [3]Quality Analysis GmbH, Nürtingen, Germany

INTRODUCTION: For clinical applications of stem cell-based therapies, animal component-free culture conditions are required. In previous studies, we assessed the mineralization potential of jaw periosteal cells (JPCs) under supplementation of clinical approved human platelet lysate (hPL) compared to FCS cultivation [1]. In the present study, we performed qualitative analyses of the extracellular matrix (ECM) formed by JPCs under hPL and FCS culture conditions.

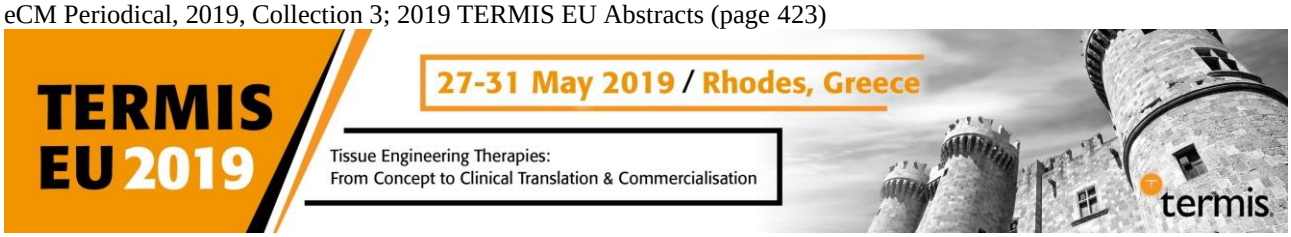
METHODS: JPCs were induced osteogenically under FCS and hPL culture conditions. At the end of differentiation, biochemical composition of the formed precipitates was analyzed by Raman micro-spectroscopy and mechanical properties were assessed by atomic force microscopic (AFM) measurements.

RESULTS & DISCUSSION: By Raman analyses, higher phosphate, lower carbonate content and higher crystallinity of hydroxyapatite minerals were detected under hPL culture conditions. Furthermore, regarding the quality of the collagen network, higher ratios of proline/hydroxyproline and higher collagen cross-linking were detected in hPL-cultured JPCs. Since hPL-supplementation leads to nearly equal production of the precursor protein proline and the mature protein hydroxyproline, these findings might indicate higher elastic properties of the collagen network in contrast to the FCS supplementation. Additionally, cross-linking seems to be higher under hPL culture conditions. When areas of cell monolayers without precipitates were monitored by AFM, higher Young's modulus of untreated and osteogenically induced JPCs under FCS compared to hPL culture conditions were detected. The opposite was the case for the formed precipitates: under hPL conditions, precipitates formed by JPCs showed significantly higher Young's modulus than those formed under FCS culturing.

CONCLUSIONS: In the present study, we detected significant differences in the biochemical composition and the mechanical properties of the mineralized extracellular matrix formed by hPL- and FCS-cultured JPCs. The combined technologies represent an optimal tool to assess the quality of in vitro formed bone-like tissue.

REFERENCES

[1] Wanner Y et al. Stem Cells Int. 2017;2017:8303959.



Dynamics of single cell migration on aligned, grid, wavy and looped fibre patterns

D. Zhang[1], N. Piano[1], E. Terentjev[2], Y.Y.S. Huang[1]
Presenting Author: Duo Zhang, dz301@cam.ac.uk
[1]Nanoscience Centre, Department of Engineering, University of Cambridge, CB3 0FF, UK.
[2]Cavendish Laboratory, Department of Physics, University of Cambridge, CB3 0HE, UK

INTRODUCTION: Understanding the interaction between cells and their physiological microenvironment has been a crucial topic in cell biology and tissue engineering [1-2]. In cellular microenvironment, natural extra-cellular matrices (ECMs) consist of fibril structures with topographical cues. As the physical architecture of native ECMs could be complex and normally comprised of multiple interaction of global patterns and local topographies, simplification of such effects may improve our understanding in the related governing principles on cell migration dynamics.

METHODS: Low-voltage Electrospinning Patterning (LEP) techniques to fabricate aligned, grid, wavy and loop polymeric fibres depositing on soft elastic substrates. Green fluorescent protein (GFP) transfected cancer cells (MB231) were seeded on such fiber structures, and their migration dynamics were monitored using time-lapse microscopy in a continuous 15-hour time period. Specific focuses were paid to derive the key morphological features for single cell migration.

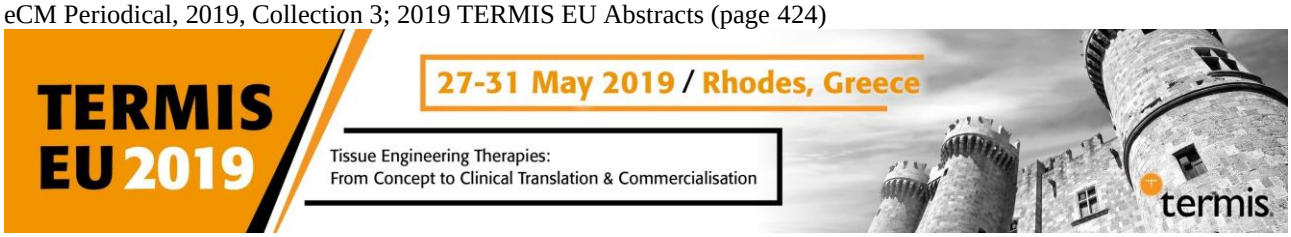
RESULTS & DISCUSSION: Cells' morphological responses to the fibril patterns as well as their correlation with the cellular kinetics were quantitatively studied. Cells were able to migrate along the fiber tracks, as well as adapting their morphologies to fit the global patterns and local topologies, especially when cells were trapped in fiber loops. The results are also tested and verified for a novel theoretical model developed for explaining the cell-fibre interactions.

CONCLUSIONS: Cellular behaviors are strongly influenced by ECM architecture and the local interfacing patterns. The results would promote vital application in identifying key morphological parameters and physiological mediators in cell-fibril interactions, as well as predicting cell migration behaviors in various ECM environments.

ACKNOWLEDGEMENTS: Engineering and Physical Sciences Research Council (EPSRC) UK; European Research Council; China Scholarship Council.

REFERENCES

[1] Haeger, Anna, et al. Trends in Cell Biology 25.9 (2015): 556-566.
[2] Dalby M J, et al. Nature Materials, 2014, 13(6): 558-569.



Dose-controlled release of placental growth factor from a collagen-based scaffold promotes angiogenesis and enhanced bone defect healing

E.J. Sheehy[1,2,3], G.J. Miller[1,2], A.G. Vazquez[1,2], R.M. Raftery[1,2], I. Amado[1,2], F.J. O'Brien[1,2,3]
Presenting Author: Eamon J. Sheehy, eamonsheehy@rcsi.ie
[1]Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland, Ireland [2]Trinity Centre for Bioengineering, Trinity College Dublin, Ireland [3]Advanced Materials and Bioengineering Research Centre (AMBER), Trinity College Dublin, Ireland

INTRODUCTION: Regeneration of critically-sized bone defects remains a significant challenge. We have recently identified placental growth factor (PlGF) as a mechanically augmented gene which promotes angiogenesis at high doses and osteogenesis at lower doses [1]. Herein, we sought to functionalize a collagen-hydroxyapatite (CHA) scaffold to deliver PlGF in a dose-controlled manner, with a high burst release to promote angiogenesis followed by a lower sustained release to promote osteogenesis. Furthermore, we aimed to investigate the capacity of this scaffold to promote regeneration of a critically-sized defect in vivo.

METHODS: Recombinant human PlGF-2 (1 µg/mL) was incorporated into alginate microparticles (MPs; 0.5% w/v) and dispersed within CHA slurries pre-loaded with 0, 5 or 10 µg/mL PlGF-2 and freeze dried to form scaffolds termed 'PlGF-single', 'PlGF-dual low' and 'PlGF-dual high', respectively. In vitro evaluations were performed using ELISAs, matrigel angiogenic assays and osteogenic differentiation assays (n=3-4). 7 mm calvarial defects in Wistar rats were treated with scaffolds (n=7-8) and assessed using µCT after 28 days. Statistical comparisons were performed using ANOVA. Significance; $p<0.05$.

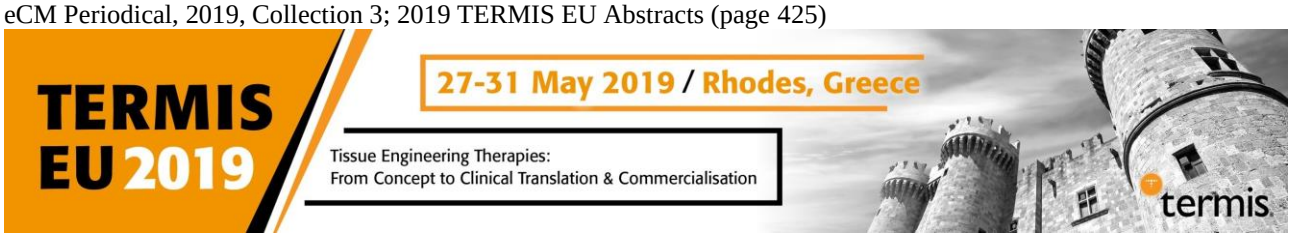
RESULTS & DISCUSSION: MPs were successfully incorporated within scaffolds. PlGF-single scaffolds showed a slow, consistent release whereas both PlGF-dual scaffolds demonstrated initial high burst release profiles followed by lower, sustained releases. When seeded with mesenchymal stem cells, PlGF-dual high scaffolds accumulated significantly more calcium compared to PlGF-free scaffolds ($p<0.001$). When added to human umbilical vein endothelial cells, elute from PlGF-dual high scaffolds promoted the formation of vessels with a significantly greater number of vascular junctions compared to PlGF-free groups ($p<0.001$). In vivo, PlGF-dual high scaffolds promoted significantly more bone formation compared to PlGF-free CHA scaffolds ($p<0.001$).

CONCLUSIONS: A dose-controlled release was achieved by combining the incorporation of PlGF directly within the scaffold, with the encapsulation of MPs, leading to a high burst release followed by a lower, sustained release. The harnessing of this dose-based effect allows for the delivery of pro-angiogenic and pro-osteogenic cues, key aspects of the regenerative process. This work provides a template for a mechanobiology-informed approach to regenerative medicine, by utilizing a therapeutic previously identified by leveraging the differential response of genes to mechanical loading [1].

ACKNOWLEDGEMENTS: Supported by the Health Research Board Ireland, Science oundation Ireland, AMBER, and the EU BlueHuman Interreg Atlantic Area project.

REFERENCES

[1] McCoy R et al., Stem Cells. 2014; 13:2420–31



Lens-free mechanical characterization of hydrogels

E. Fennell[1,2], T. Liu[3], Y. Zhang[3], A. Ozcan[3], J.M. Huyghe[1,2]
Presenting Author: Eanna Fennell, eanna.fennell@ul.ie
[1]Bernal Institute, University of Limerick, Limerick, Ireland [2]School of Engineering, University of Limerick, Limerick, Ireland. [3]Bioengineering Department, University of California, Los Angeles, USA

INTRODUCTION: Hydrogels are used extensively throughout tissue engineering to replicate the role of the Extracellular Matrix [1-2]. Understanding the mechanical properties of these materials is paramount to mimicking this process. However, the experimental quantification of hydrogels under loading from physiological fluid is very difficult. Therefore, this study focuses on quantifying the finite deformation of polyacrylate hydrogels under osmotic loading.

METHODS: Standard imaging techniques, such as confocal microscopy or particle tracking velocimetry, lack the requirements of a system needed to image such a highly dynamic three-dimensional process. Therefore, a novel Lens Free Imaging (LFI) system is developed. LFI combines a CMOS image sensor and partially coherent illumination to capture diffraction patterns of the specimen being imaged. The image is then focused through a variety of post processing algorithms allowing sub-micron resolution over an active imaging area of 20 mm^2. This technique can be extended to three-dimensional imaging by adding additional illumination sources. Here, transparent PMMA micro particles are embedded within the hydrogel and are tracked at 15 frames per second over an imaging volume of 80 mm [3]. As a result, the spatial and temporal coordinates of the micro-particles can be used to mechanically characterize the strain throughout the material.

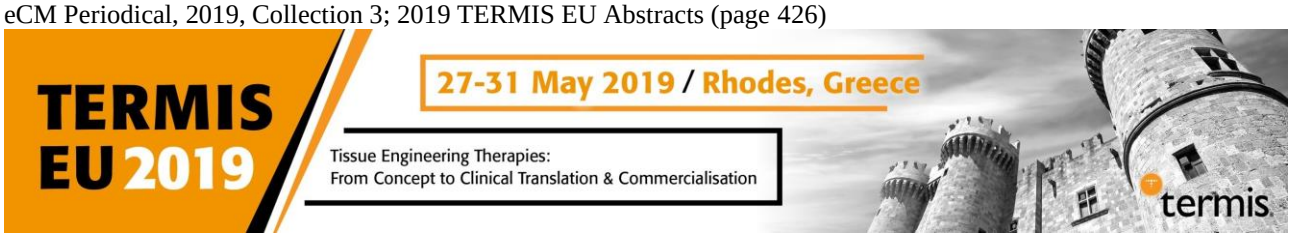
RESULTS & DISCUSSION: Preliminary results have indicated that the LFI system can track the swelling of these gels. The gels become fully transparent when fluid has permeated to the core. However, the boundary of the gel is still visible. The transparent micro particles can also be tracked after the implementation of a filtered back propagation algorithm.

CONCLUSIONS: The dynamic lens-free imaging of these gels has the potential to be extended to a variety of applications within the tissue engineering field. The advantages include low cost, small geometry, large field of view and dynamic imaging. Future work will concentrate on the dynamic imaging of cell adhesion to a gel matrix.

ACKNOWLEDGEMENTS: Financial support was received from Irish Research Council, (Project ID EPSPG/2017/230) and Procter & Gamble Service GmbH. The authors also wish to acknowledge Dr. Arsen Simonyan of Procter & Gamble Service GmbH for the creation of the protocol to polymerize the polyacrylate hydrogel beads.

REFERENCES

[1] Rowley JA et al. Biomaterials. 1999; 20 (1):45-53
[2] Geckil H et al. Nanomedicine. 2010; 5(3):469-84
[3] Isikman SO et al. IEEE. 2012; 18(3):1059-72



Combination of a gellan gum-based hydrogel with cell therapy for the treatment of cervical spinal cord injury

E.D. Gomes[1,2], B. Ghosh[3], R. Lima[1,2], M. Goulão[3], M. Urban[3], D. Silva[1,2], N. Sousa[1,2], A.C. Lepore[3], N.A. Silva[1,2], A.J. Salgado[1,2]
Presenting Author: E.D. Gomes, id5788@alunos.uminho.pt
[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]Vickie and Jack Farber Institute for Neuroscience, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, 19107, USA

INTRODUCTION: Cervical Spinal Cord Injuries (SCI) represent more than half of the SCI cases worldwide. Respiratory compromise, as well as severe motor deficits are among the main consequences of cervical lesions. Herein we propose to combine Gellan Gum-based hydrogel modified with GRGDS peptide, together with Adipose Stem Cells (ASCs) and Olfactory Ensheathing Cells (OECs) as a strategy for cervical SCI repair. This approach was previously validated in thoracic and lumbar SCI models [1, 2].

METHODS: A hemisection injury was performed in adult rats, at C2 level. 2×10^5 ASCs/OECs (1:1) encapsulated in 10 µl of GG-GRGDS were transplanted immediately after lesion at the injury site. Four weeks later, compound muscle action potentials (CMAPs) were performed, while five weeks post-injury diaphragm electromyograms (EMGs) were recorded. In another set of animals, motor and sensorial paradigms such as staircase, grooming and Von Frey tests were evaluated.

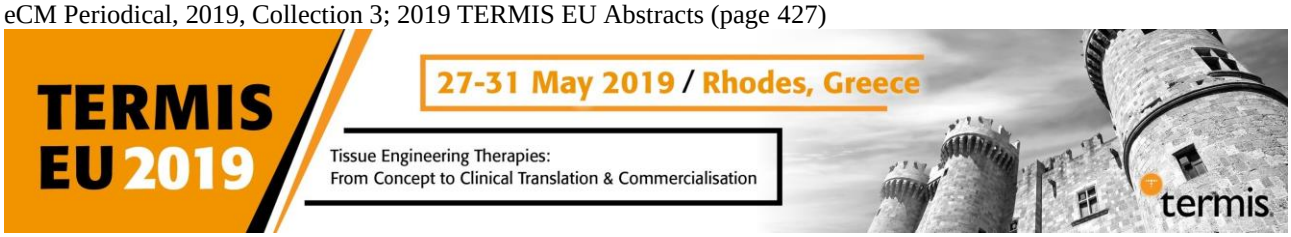
RESULTS & DISCUSSION: No differences were observed among groups regarding CMAPs recordings. Rats treated with hydrogel and cells presented increased EMG activity at the ventral and medial portions of the hemi-diaphragm. No differences were observed in forelimb-specific motor tests in any of the paradigms evaluated. Importantly, at the Von Frey test, rats treated with the combinatorial approach demonstrated a reduced hypersensitivity in comparison to non-treated animals.

CONCLUSIONS: These data indicate that the combination of GG-GRGDS hydrogel with ASCs/OECs has beneficial effects in cervical SCI-related respiratory and sensorial deficits.

ACKNOWLEDGEMENTS: Prize Melo e Castro for Spinal Cord Injury Research (MC-04/17); Funded by national funds through FCT under the scope of grant reference TUBITAK/0007/2014; Project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER); Projects POCI-01-0145-FEDER-007038 and POCI-01-0145-FEDER-029206 supported through FEDER funds, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT). ASCs were kindly provided by LaCell, USA.

REFERENCES

[1] Gomes ED et al. Stem Cells. 2018; 36(5):696-708;
[2] Gomes ED et al. Biomaterials. 2016; 105:38-51.



Fabrication of manganese dioxide (MnO_2)-loaded polymer capsules to prevent oxidative stress

E. Marin[1], A. Larrañaga[1], C. Tapeinos[2], G. Ciofani[3], J.R. Sarasua[1]
Presenting Author: Edurne Marin, edurne.marin@ehu.eus
[1]Department of Mining, Metallurgy and Materials Science, University of the Basque Country (UPV/EHU), Bilbao, Spain [2]Smart Bio-Interfaces, Italian Institute of Technology (IIT), Pontedera, Italy [3]Department of Mechanical and Aerospace Engineering, Polytechnic University of Torino, Torino, Italy

INTRODUCTION: Polymer capsules exhibit significant potential for therapeutic applications as micro- and nanoreactors, where the bio-chemical reactions of interest are efficiently performed in a spatial and time defined manner due to the encapsulation of an active entity and control over the transfer of reagents and products through the capsular membrane [1]. In this work, we fabricated layer-by-layer (LbL) polymer capsules loaded with MnO_2 nanoparticles and their potential to scavenge H_2O_2 and attenuate oxidative stress was tested in an in vitro model.

METHODS: Polymer micro- and nanocapsules loaded with MnO_2 were fabricated via the LbL approach using poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSS) as polyelectrolytes and MnO_2-decorated $CaCO_3$ particles as a sacrificial template. After thorough physicochemical, morphological and functional characterization, polymer capsules were incubated with HeLa cells and their potential to scavenge H_2O_2 and attenuate oxidative stress was evaluated.

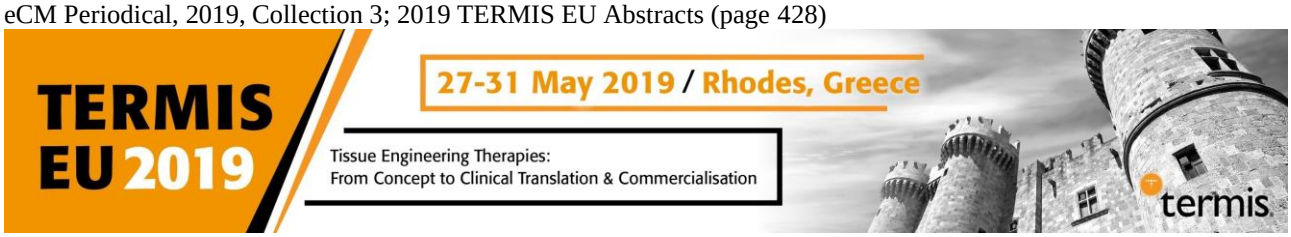
RESULTS & DISCUSSION: Polymer capsules of different sizes [submicron (~600 nm) vs. micron-sized (~4 μm)] were satisfactorily fabricated via a precise control of the synthesis conditions of the $CaCO_3$ template. The presence of MnO_2 nanoparticles within the capsules was confirmed by means of transmission electron microscopy (TEM) and thermogravimetric analysis (TGA). These hybrid polymer capsules efficiently scavenged H_2O_2 from solution at biologically relevant concentrations (10-50 μM of H_2O_2) and did not show any cytotoxic effect towards HeLa cells. Besides, MnO_2-loaded polymer capsules significantly improved the survival rate of HeLa cells exposed to oxidative stress.

CONCLUSIONS: The developed hybrid polymer capsules represent a promising approach for the treatment of oxidative stress, which is applicable for multiple inflammatory disease targets.

ACKNOWLEDGEMENTS: Government of the Basque Country for the financial support (GIC 15/52, IT-927).

REFERENCES

[1] Larrañaga A et al. Acta Biomaterialia 2018; 67:21-31



A bioresorbable carrier and passive stabilization device to improve heart function post-myocardial infarction

*E.B. Dolan[1,2,6,7], *B. Hofmann[3], H. de Vaal[3], G. Bellavia[4], S. Straino[4], L. Kovarova[5], D. Monahan[6], H. Kelly[1,2], S. Wildhirt[3], G.P. Duffy[2,6,7] *Joint co-first-authors
Presenting author: Eimear Dolan: Eimear.dolan@nuigalway.ie
[1]School of Pharmacy, Royal College of Surgeons in Ireland, Ireland [2]Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland, Ireland [3]AdjuCor GmbH, Lichtenbergstr, Germany [4]Explora Biotech Srl, Rome, Italy [5]R&D department, Contipro, Dolni Dobrouc, Czech Republic [6]Department of Anatomy, School of Medicine, National University of Ireland Galway, Ireland [7]Department of Biomedical Engineering, NUI Galway, Ireland

INTRODUCTION: Left ventricular (LV) remodeling following myocardial infarction (MI) involves progressive dilatation of the LV cavity, increased LV wall stress, cardiomyocyte hypertrophy and alterations in biochemical and molecular functions leading to heart failure [1]. Stabilization techniques in which the weakened LV wall is mechanically supported have shown promising clinical data [2]. However, the efficacy of these devices remains limited [2]. These results can potentially be explained by the inability of nonresorbable material used in these devices to conform to the LV as it remodels. We hypothesize that a biodegradable passive restraint carrier device will enable a targeted application of a biomaterial-based therapy to the infarcted LV and allow appropriate temporary stabilization, resulting in functional recovery. In this study, a minimally-invasive bioresorbable epicardial patch (SPREADS) was developed by AdjuCor, capable of encapsulating adipose derived stem cell (ADSC) loaded hyaluronic acid (HA) hydrogels to provide mechanical and regenerative support to the weakened LV wall post-MI. Efficacy of SPREADS was assessed in a chronic porcine study.

METHODS: Acute MI was induced in pigs (40 kg, Landrace, female) by occlusion of left anterior descending coronary artery for 90 minutes. 14 days post-MI, SPREADS device + HA hydrogel ± ADSCs (n=5) was positioned on the apex by a closed chest subxyphoideal approach and compared to clinical gold standard treatment (GS) (n=5). Heart function was assessed by monitoring the left ventricle ejection fraction (LVEF) via echocardiogram.

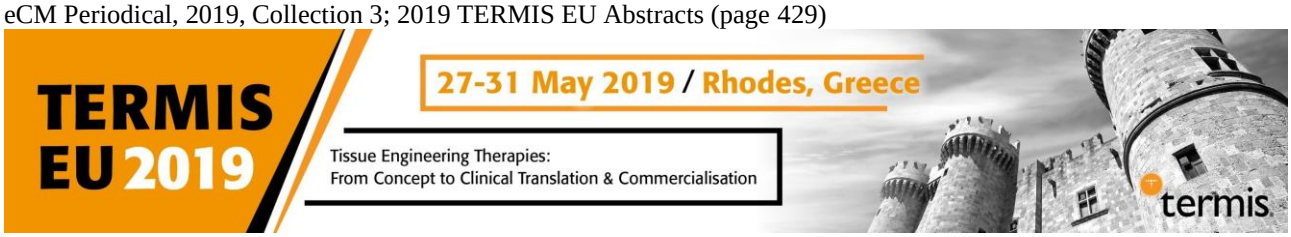
RESULTS & DISCUSSION: 28 days post-MI (14 days post-treatment) when GS was compared to GS + SPREADS + Gel ± ADSCs we saw a significant improvement in % LVEF ($p \leq 0.001$).

CONCLUSIONS: We have developed a novel, single-stage strategy to support the weakened myocardial region post-MI using a minimally-invasive, closed chest intervention. We show an improvement in % LVEF with the device ± cells compared to GS alone. The bioresorbable material enables a one-time procedure and the hydrogel encapsulated within the device can be modified to adjust both the mechanical characteristics and degradation rate to better suit stabilization needs of the patient.

ACKNOWLEDGEMENTS: AMCARE project funded by EU's 7th Framework Programme under Grant Agreement NMP3-SME-2013-604531.

REFERENCES

[1] Cohn, Circulation, 1995
[2] Kwon, J Thorac Cardiovasc Surg, 2012



Biomesh processing techniques: A synergy of cross-linking and $scCO_2$ extraction

E.A. Grebenik[1], L.P. Istranov[1], E.V. Istranova[1], S.N. Churbanov[1,2], B.S. Shavkuta[1,2], N.N. Veryasova[1], A.E. Lazhko[2], P.S. Timashev[1,2,3]
Presenting Author: Ekaterina A. Grebenik, grebeneka@gmail.com
[1]Institute for Regenerative Medicine, Sechenov First Moscow State Medical University, 8-2 Trubetskaya st., Moscow, 119991, Russia [2]Institute of Photonic Technologies, Research Center "Crystallography and Photonics", Russian Academy of Sciences, 2 Pionerskaya st., Troitsk, Moscow, 142190, Russia [3]Department of Polymers and Composites, N.N. Semenov Institute of Chemical Physics, 4 Kosygin st., Moscow, 119991, Russia

INTRODUCTION: Xenopericardial tube grafts are proposed as neoaortic segments to replace infected prosthetic or endovascular grafts [1]. To prevent graft rejection and biodegradation, xenogenic biomeshes are usually provided in the decellularized and chemically cross-linked form. However, the stiffness of these products is an order higher compared to the native arteries (Young's Modulus ~8-12 MPa) [2]. In our work, we describe an approach to tailor elasticity of chemically cross-linked decellularized bovine pericardium (DBP) towards the application in vascular reconstruction.

METHODS: First, bovine pericardium was decellularized using a modified alkaline treatment protocol. Next, DBP was treated with three different cross-linkers, namely ethylene glycol diglycidyl ether (EGDE), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and genipin; and then freeze-dried for further $scCO_2$ extraction. The acquired samples were thoroughly characterized by mechanical trials (nanoindentation, tensile testing), cytotoxicity (MTT-test), biodegradability (collagenase A digestion) and ultrastructural (scanning electron microscopy and two-photon laser scanning microscopy) studies. Finally, the effect of $scCO_2$ extraction during 3 h on the sample properties was estimated in static and dynamic modes. All tests were performed in a liquid at 37ºC.

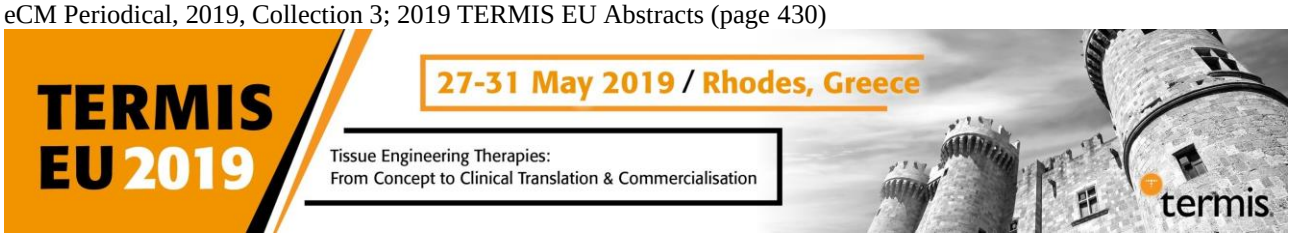
RESULTS & DISCUSSION: We demonstrated that the cross-linking chemistry strongly influences the biodegradation rate, but the values do not alter under $scCO_2$ extraction. The highest collagenase stability was achieved by EGDE treatment, and the lowest – by EDC. Scanning electron microscopy did not reveal structural derangements, as well as two-photon confocal laser scanning microscopy indicated the preservation of second harmonic generation produced by collagenous constituents. No effect of $scCO_2$ extraction on the cytotoxicity level was observed. However, Young's Modulus reduced after the $scCO_2$ extraction independent of the extraction mode as was demonstrated by both nanoindentation and tensile testing.

CONCLUSIONS: Collectively, these data clearly illustrate the beneficial effect of $scCO_2$ extraction on elastic properties of the biomeshes as candidates for vascular reconstruction with reduced hypertension.

ACKNOWLEDGEMENTS: Financial support was received from the Russian Science Foundation (Grant No. 18-15-00401).

REFERENCES

[1] Czerny M. et al. Ann. Thorac Surg. 2011; 92(5):1657-1662.
[2] Zhang X. et al. J Mater Sci Mater Med. 2010;21(2): 541-549.



Stable encapsulation of rifampicin in polymersome nanoparticles for delivery to intracellular bacteria

E. Porges[1], A. De Grazia[1], A. Taylor[2], D. Jenner[2], C.A. Rowland[2], T. A. Newman[1], N. D. Evans[1]
Presenting author: Eleanor Porges, E.Porges@soton.ac.uk
[1]Bioengineering and Clinical and Experimental Sciences, Faculty of Engineering and the Environment, University of Southampton, UK [2] Defence Science and Technology Laboratory, CBR Division, Porton Down, Salisbury, UK

INTRODUCTION: Intracellular bacterial infections are notoriously difficult to treat, in part due to poor membrane permeability and intracellular bioavailability of antibiotics. They can arise as a result of the implantation of biomaterials [1]. Current treatment options involve high doses of antibiotics for sustained periods of time, therefore contributing to the issues of antibiotic resistance [2]. Antibiotic encapsulation within nanoparticles, such as polymersomes (PMs), may provide an answer to this challenge by promoting intracellular uptake and targeted drug delivery. In this study we tested the hypothesis that polyethylene oxide-polycaprolactone (PEO-PCL) PMs can encapsulate the antibiotic rifampicin, with a view to treating intracellular infections in the future.

METHODS: PMs were prepared by dissolving 6 mg of the amphiphilic di-block copolymer PEO-PCL into 0.4 ml of dimethylformamide (DMF). Polymer solution was then added dropwise into phosphate buffered saline (PBS), under stirring, to facilitate the self-assembly of PMs by nanoprecipitation. To determine loading efficiency, rifampicin was passively incorporated at concentrations ranging from 0 – 20 mg/ml. Samples were dialysed, and the resulting PM-antibiotic concentrations determined using UV-vis spectrophotometry. To assess PM-antibiotic stability UV-vis readings were taken at various timepoints across a 14-day dialysis period.

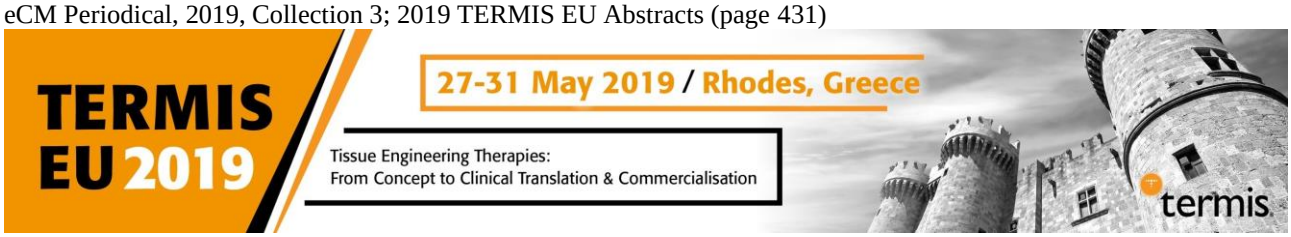
RESULTS & DISCUSSION: As the level of rifampicin loaded during PM preparation was increased, there was a concomitant proportional increase in the level of rifampicin successfully encapsulated within PMs. Encapsulation was linear with respect to loading concentration up to 10 mg/ml, where the highest level of drug encapsulation was achieved. PM-rifampicin preparations were stable for at least 14 days with no changes in hydrodynamic radius, as measured by dynamic light scattering, 88.5 nm ± 1.9. Antibiotic release and PM stability studies showed that the PM-rifampicin preparations had 8.5 μg/ml retained at Day 14 in bulk suspensions, equivalent to an estimated local nanoparticle concentration of 7 mg/ml.

CONCLUSIONS: Rifampicin can be successfully encapsulated into PEO-PCL PM nanoparticles, and retained for at least 14 days. We hypothesise this is due to the hydrophobic nature of rifampicin and its stabilisation within the hydrophobic membrane of the PMs. Future work will assess the efficacy of these nanoparticles on the killing of intracellular Burkholderia thailandensis.

ACKNOWLEDGEMENTS: We are grateful to DSTL, EPSRC, and the Institute for Life Sciences, Southampton for funding.

REFERENCES

[1] Rochford, E. T. J., Richards, R. G. and Moriarty, T. F. (2012); Clinical Microbiology and Infection, 18(12), pp. 1162–1167.
[2] Ladavière, C. and Gref, R. (2015); Nanomedicine, 10(19), pp. 3033–3055.



Cellular responses under static and dynamic conditions of polymeric micropatterned substrates fabricated via ultrafast laser direct writing

E. Babaliari[1,2], P. Kavatzikidou[1], A. Mitraki[1,2], A. Ranella[1], E. Stratakis[1,2]
Presenting Author: Eleftheria Babaliari, ebabaliari@iesl.forth.gr
[1]Foundation for Research and Technology - Hellas (F.O.R.T.H.), Institute of Electronic Structure and Laser (I.E.S.L.), Heraklion, Crete, Greece [2]Department of Materials Science and Technology, University of Crete, Heraklion, Crete, Greece

INTRODUCTION: Conventional cultures have been proven inadequate to provide sufficient levels of oxygen and nutrients to the interior of the scaffolds, and mechanical stimulation to the cells. On the contrary, dynamic cultures realized with the aid of microfluidics reflect more appropriately the in vivo environment of cells in tissues [1]. The purpose of the present work is to fabricate a novel microfluidic platform for the study of the combined effect of fluid shear forces and culture substrate morphology on cell proliferation and directionality [2].

METHODS: The microfluidic system is composed of a pressure controller, a flow sensor and a chamber containing the microstructured substrates with the cells. A Yb:KGW laser was used (170 fs pulse duration, 1 kHz repetition rate, 1026 nm wavelength) to fabricate the polymeric microstructured substrates. 25000 Schwann (SW10) cells were seeded in PET coverslips to perform the static and dynamic cultures.

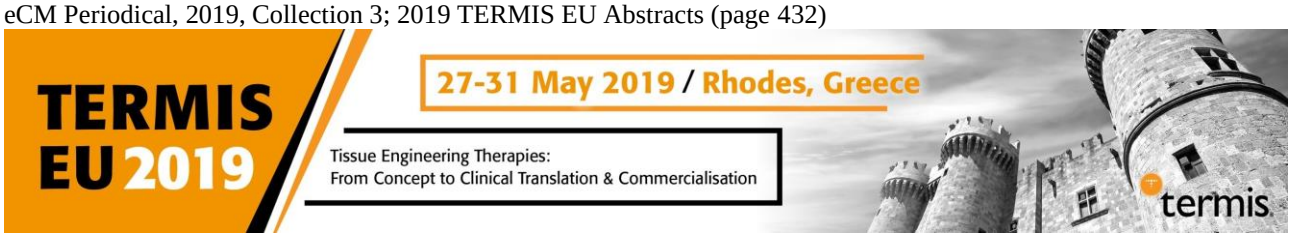
RESULTS & DISCUSSION: PET coverslips were ablated by the femtosecond laser at a constant fluence of 11.9 J/cm^2, scan velocity of 7 mm/s and a x_{step} of 50 μm to fabricate the microgrooves [3].The cytoskeleton of the SW10 cells was elongated along the direction of the microgrooves whereas a random orientation observed on the flat PET. By applying a flow rate of 50 μL/min, cells oriented along the direction of the microgrooves and parallel to flow. Interestingly, cells oriented parallel to flow on the flat PET. An increased cell length was noticed under flow conditions (50 μL/min) compared to static. Finally, a further enhancement of cell length was observed under flow conditions
combined with the microgrooves (Figure 2).

CONCLUSIONS: Our results indicate the ability to engineer cell alignment in vitro that could be potentially useful in the field of neural tissue engineering.

ACKNOWLEDGEMENTS: Financial support was received from State Scholarships Foundation (IKY) and Onassis Foundation.

REFERENCES

[1] Leclerc E et al. Biomaterials. 2006; 27(4):586-95
[2] Stratakis E et al. Biomicrofluidics. 2011; 5(1):013411
[3] Babaliari E et al. Int. J. Mol. Sci. 2018; 19(7):2053



Formative biofabrication: From scaffold to scaffield

E.A. Bulanova[1], V.A. Parfenov[1], E.V. Koudan[1], P.A. Karalkin[1], F.DAS. Pereira[1], A.A. Gryadunova[1], O.F. Petrov[2], M.M. Vasiliev[2], M.I. Myasnikov[2], K. Brakke[3], Y.D. Khesuani[1], U. Demirci[4], V.A. Mironov[1]

Presenting Author: Elena Bulanova, elabulanova@gmail.com

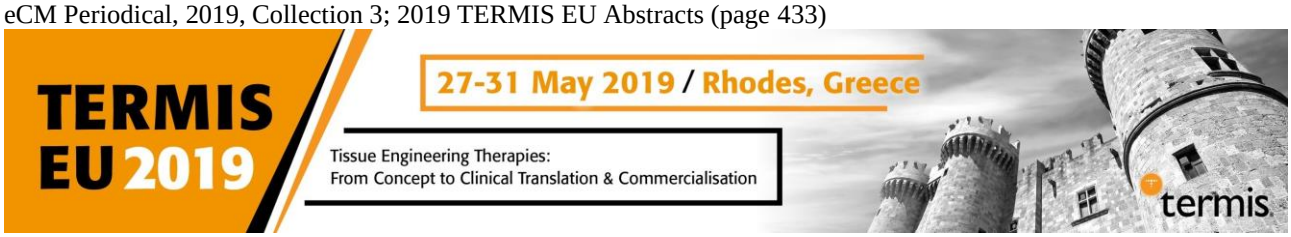
[1]Laboratory for Biotechnological Research 3D Bioprinting Solutions, Moscow, Russia [2]Joint Institute for High Temperatures, Russian Academy of Sciences, Moscow, Russia [3]Susquehanna University, Selinsgrove, Pennsylvania 17870, USA [4]Stanford University, Palo Alto, USA

INTRODUCTION: Formative biofabrication is an alternative strategy for tissue constructs engineering applying large preformed multicellular fabrication units, tissue spheroids (TS). By this approach engineered tissue is assembled quickly, within 30-45 seconds, and scaffold-free. We are developing a new scaffold-free approach as an alternative or addition to scaffold-based one. In this approach TS were patterned and hold together by physical forces or physical fields. Magnetic field as well as acoustic field provide temporal and removable support and it could be considered as a new form of scaffold. To escape a possible confusion, we propose to call this new type of temporal support based on employment of physical fields as a scaffield.

METHODS: TS were prepared from sheep and human primary chondrocytes, chondrocytes immortalized line SW1353, rat thyrocytes using non-adhesive technology. Biofabricated TS were used for magnetic, acoustic and acoustic-magnetic levitation. Magnetic levitation was performed applying an experimental custom-made magnetic printer in presence of different Gd^{3+} gadolinium concentrations, depending on gravity conditions and magnetic field strength. Acoustic and acoustic-magnetic levitation was performed on custom-made experimental devices too.

RESULTS & DISCUSSION: TS of standard geometry were applied for magnetic levitation. Our experiments confirmed that relatively weak magnetic fields allow quick levitational assembly of tissue spheroids only in the presence of high 50 mM Gd^{3+} concentrations which represents borderline toxicity state. We hypothesized that employing higher gradient magnetic field we could decrease Gd^{3+} gadolinium concentration and perform TS assembly at non-toxic Gd^{3+}concentration in paramagnetic medium. TS magnetic levitational assembly was performed at The European High Magnet Field Laboratory and showed that employing 22 Tesla we were able to decrease Gd^{3+} gadolinium concentration down to 0.8M. Another approach involves the use of space microgravity conditions. We developed a magnetic bioprinter to produce cartilage construct and rat thyroid gland construct in Space applying 0.8mM, 10mM and 50mM Gd^{3+} concentrations.

CONCLUSIONS: 3D tissue constructs have been biofabricated from TS in paramagnetic medium at different gravity conditions and various Gd^{3+} concentration. We report for the first time a successful rapid assembly of 3D tissue engineered construct using scaffold-free, label-free and nozzle-free magnetic levitation of TS in gravity and in zero gravity conditions in Space. In microgravity conditions the tissue construct was assembled at 10mM, 50Mm Gd^{3+} concentrations but not at 0.8mM one. These data provide a proof of concept for new strategies in tissue engineering. Formative biofabrication enables rapid assembly of various tissues and organs. Additionally, the systematical investigation of the scaffold-free and label-free acoustic and acoustic-magnetic levitation has been started as additional potential technologies.



TRPV4 inhibition reduces stretch-induced inflammation in human intervertebral discs

E. Cambria[1], F. Passini[1], O. Krupkova[1], W. Hitzl[2], J. Snedeker[1], O.N. Hausmann[3], S.J. Ferguson[1], K. Wuertz-Kozak[1]
Presenting Author: Elena Cambria, ecambria@ethz.ch
[1]Institute for Biomechanics, ETH Zurich, Zurich, Switzerland [2]Research Office (Biostatistics), PMU Salzburg, Salzburg, Austria [3]Klinik Sankt-Anna Hirslanden, Lucerne, Switzerland

INTRODUCTION: Low back pain (LBP) and degenerative disc disease (DDD) profoundly affect the health and economics of our society. Mechanical loading and inflammation are interacting causes of DDD. Cyclic stretching of intervertebral disc (IVD) cells was shown to induce secretion of inflammatory mediators [1]. However, little is known about the underlying mechanisms. Recently, the transient receptor potential vanilloid 4 (TRPV4) channel was associated with reduced osmolarity and pro-inflammatory cytokines in the IVD [2]. Nevertheless, its role in IVD mechanotransduction remains to be fully elucidated. The aim of this study is to test whether stretch-induced inflammation of IVD cells is regulated by TRPV4.

METHODS: Primary human annulus fibrosus cells (n=3-4 donors) were seeded on fibronectin-coated PDMS chambers and cyclically stretched for different durations (1 to 24 hours) at 20% strain and 1 Hz frequency on a commercial bioreactor (STB-140-10, Strex). In TRPV4 inhibition experiments (n=4), cells were stretched for 1 hour with/without the specific TRPV4 antagonist GSK2193874 at different concentrations (20 to 500 nM). Gene/protein expression were quantified by RT-qPCR/ELISA. Calcium flux was analyzed by live imaging with the Fluo-4AM dye and activation of MAP kinases (p-38, JNK, ERK) was tested after 15 min stretching (n=3) by Western blot.

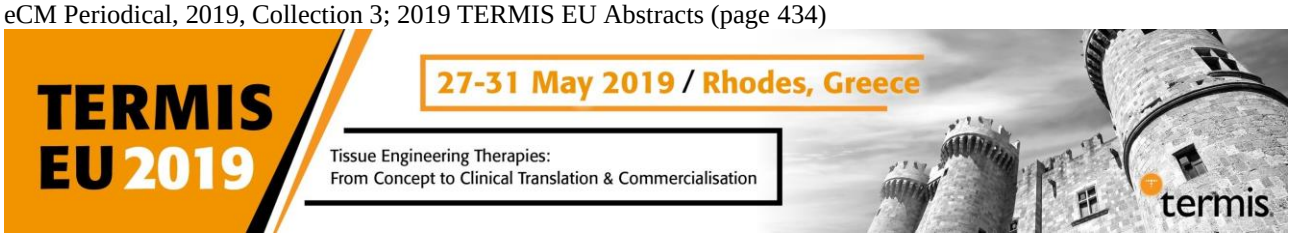
RESULTS & DISCUSSION: Gene expression of IL6, IL8 and COX2 was significantly upregulated by 1 hour stretching compared to controls, and decreased with longer durations. Stretch-induced upregulation of IL6 and COX2 mRNA expression was reduced by GSK2193874 (Fig.1). On the protein level, PGE2 was only slightly increased by stretching and reduced by TRPV4 blocking. Stretching caused calcium influx and phosphorylation of p-38, JNK and ERK compared to controls, and p38 activation was reduced by GSK2193874.

CONCLUSIONS: Stretch-induced inflammation may be mediated at least in part by TRPV4. TRPV4 may thus constitute a potential therapeutic target to tackle DDD and LBP. Current work includes experiments with CRISPR/Cas9-based TRPV4 knockout cells.

ACKNOWLEDGEMENTS: Financial support was received from Swiss National Science Foundation (SNF PP00P2_163678/1) and Eurospine (2016_4).

REFERENCES

[1] Gawri R et al. Arthritis Res Ther. 2014; 16(1):R21
[2] Walter B et al. Eur Cell Mater. 2016; 32:123-36



Dexamethasone drives early osteogenic differentiation by modulation of SOX9 and PPARG expression

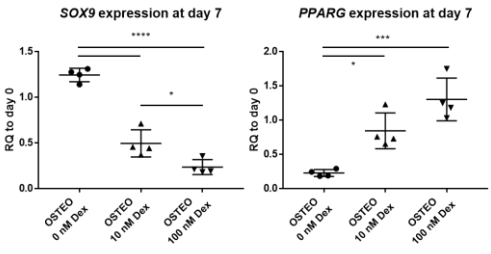
E. Della Bella, V. Basoli, M. Alini, M.J. Stoddart
Presenting Author: Elena Della Bella, elena.dellabella@aofoundation.org
AO Research Institute Davos, Davos Platz, Switzerland

INTRODUCTION: Dexamethasone is widely used in protocols for osteogenic differentiation and it has been associated with an increase in RUNX2 expression. However, it also inhibits osteocalcin expression and terminal differentiation of osteoblasts. The present study aims to clarify the role of dexamethasone during early stages of osteogenic differentiation and to improve currently used protocols with a potential for a higher clinical translation of in vitro results in the field of bone research.

METHODS: Human bone marrow-derived mesenchymal stromal cells were obtained from 4 donors with full ethical approval and induced towards osteogenic differentiation. Differentiation medium was DMEM 1 g/L glucose with 10% FBS containing 50 µg/mL ascorbic acid 2-phosphate and 5 mM beta-glycerol phosphate. Dexamethasone (dex) was used at 0, 10, or 100 nM for the first week of induction, then different combinations of 0 or 10 nM were used thereafter. Osteogenic gene expression was assessed after 7 and 21 days, while mineral deposition was analyzed at day 21.

RESULTS & DISCUSSION: Day 7 gene expression analysis showed no effect of dex on RUNX2, RUNX3, or SP7. However, dex inhibited SOX9 expression and upregulated PPARG in a dose-dependent manner. The RUNX2/SOX9 ratio at day 7 positively correlated with Alizarin red staining at day 21, even when dex was reduced to 0 or 10 nM after the first week.

CONCLUSIONS: Preliminary results suggest that the outcome of differentiation is mainly decided within the first week of induction. Comprehensive analysis of RUNX2, SOX9, and PPARG expression suggested that, while RUNX2 levels are constant despite increasing concentrations of dex, regulation of the other transcription factors is more influential on differentiation. In particular, the results showed an inverse correlation between early SOX9 expression and mineralization at day 21, supporting the hypothesis that a drop in SOX9 levels is the main event driving osteogenesis, potentially by unleashing inhibition of RUNX2.



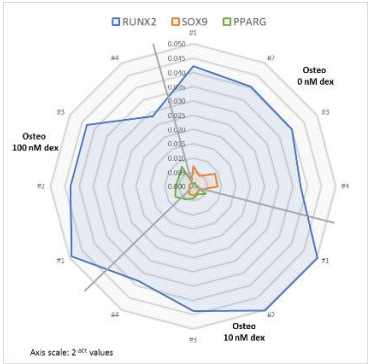
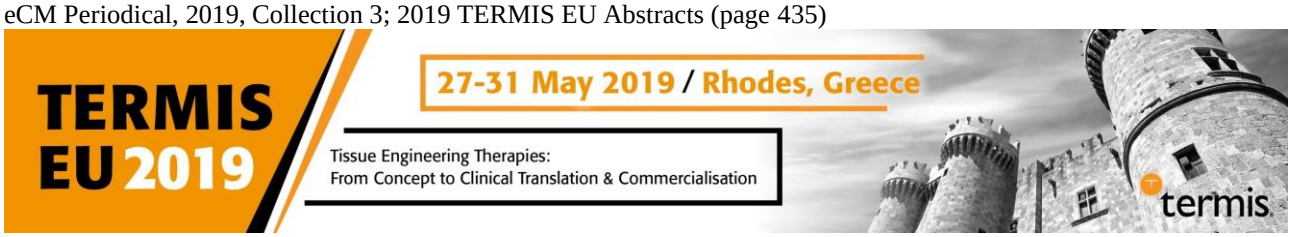


Figure 1: (Left) Dexamethasone inhibits SOX9 expression and upregulates PPARG in a dose-dependent manner. **Figure 2:** (Right) Overview of the expression of key transcription factors during early osteogenic differentiation.

ACKNOWLEDGEMENTS: This work was funded by the AO Foundation.



Engineering the migration and attachment behavior of primary dermal fibroblasts

E. García-Gareta[1], A. Levin[1], L. Hook[2]
Presenting Author: Elena García-Gareta, garciae@raft.ac.uk
[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood, United Kingdom [2]Smart Matrix Limited, Leopold Muller Building, Mount Vernon Hospital, Northwood, United Kingdom.

INTRODUCTION: The availability of primary cells present in pathological conditions is often very limited due to stringent ethical regulation and patient consent. One such condition is chronic wounds, where dermal fibroblasts show a deficient migration. In vitro models with cellular tools that mimic the in vivo scenario would be advantageous to test new tissue-engineered therapies for these challenging wounds. Since the availability of primary dermal fibroblasts present in chronic wounds is restricted and their "shelf-life" limited due to increased senescence, our aim was to engineer human dermal fibroblasts with impaired migration using synthetic RGD peptides.

METHODS: First, the study was carried out on 3 different 2D surfaces representative of the materials used to develop tissue-engineered dermal scaffolds: hydrophilic synthetic polymeric surface, and fibrinogen and gelatin surfaces. Different variables and RGD peptide concentrations were tested. Cell attachment, migration, and integrin expression were studied. Next, RGD treated cells were seeded in commercially available, clinically used, collagen-based 3D dermal scaffolds (Integra® and Matriderm®) and their attachment and migration studied. The mechanism of action of both scaffolds is through integrin ligands.

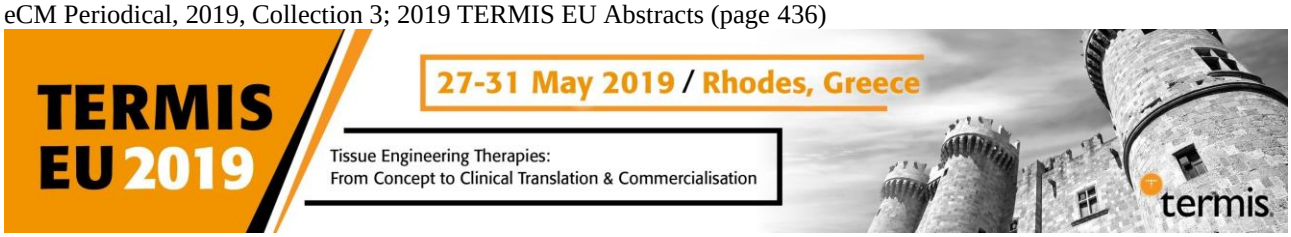
RESULTS & DISCUSSION: Results on 2D surfaces showed cell attachment is reduced in a concentration dependent manner. Cells cultured on gelatin surfaces looked the most spread and obtained overall the least reduction in cell migration. Expression of integrins was not affected and the components of the migration pathway were not altered by the RGD peptides. Results in 3D scaffolds showed that the concentration of synthetic RGD peptides necessary to impair migration of dermal fibroblasts should be tailored to the number of RGD sites present in the 3D matrix.

CONCLUSIONS: The concentration of synthetic RGD peptides necessary to impair migration of dermal fibroblasts should be tailored to the particular surface/material and cell population used. The described technology could be translated to other cell types including established cell lines. A wide range of synthetic peptides exist, which differ in the amino acid sequence, thus increasing the possibilities of this technology [1].

ACKNOWLEDGEMENTS: Work funded by the Restoration of Appearance and Function Trust (RAFT, UK, registered charity number 299811) and Smart Matrix Ltd.

REFERENCES

[1] García-Gareta et al. Biotechnol Bioeng. 2019; In revision.



3D bioplotted osteochondral tissue-like scaffolds: Effect of architecture and material composition

D. Pierantozzi[1], A. Scalzone[2], P. Gentile[2], E. Mancuso[1]
Presenting Author: Elena Mancuso, e.mancuso@ulster.ac.uk
[1]Nanotechnology and Integrated BioEngineering Centre, Ulster University, Jordanstown campus, UK
[2]School of Engineering, Newcastle University, Newcastle Upon Tyne, UK

INTRODUCTION: In recent years, bi- and triphasic osteochondral (OC) scaffolds have been investigated as an alternative to traditional procedures. However, the issues of delamination, less accurate graded structure, as well as poor mechanical properties are limiting their application. These actual challenges form the focus of this work, which aims to design and develop novel 3D scaffolds in order to best mimic the multilayered nature of OC tissue in its integrity and function.

METHODS: A 3D-Bioplotter (EnvisionTEC Germany) was used to produce cylindrical scaffolds with strand thickness of 400 µm and different inner patterns. Three powder-derived formulations (combined with a solvent-free approach) were investigated: poly-ε-caprolactone (PCL), PCL/hydroxyapatite (HA) and PCL/strontium-doped (HA) at different ratios (see Table 1). The effect of compositional and architectural variations of the resulting bioplotted scaffolds was analysed in terms of physico-chemical (FTIR, TGA, SEM, contact angle and micro-CT) and mechanical properties, as well as biological behavior (cytotoxicity, attachment and proliferation).

RESULTS & DISCUSSION: The ceramic phase slightly affected the printing temperature of the formulations (140°C composites vs 130°C PCL alone). TGA and FTIR analysis confirmed the presence of HA in the raw powders as well as extruded materials, demonstrating a homogenous mixing of the two phases. 3D bioplotted scaffolds exhibited a range of customizable hierarchical structure (see Fig.1) and mechanical properties (Young's modulus in the range 23.27±2.2÷117.73±7.1 MPa) appropriate to match those of OC tissue layers. SEM analysis showed how the presence of the ceramic phase affected the scaffold's surface roughness, which in turn enhanced the hydrophilicity as well as the in vitro behavior in contact with Y201 cells.

CONCLUSIONS: The use of a 3D-Bioplotter revealed promising to manufacture custom design, OC-like substitutes with a unique multilayered structure. Also, the inorganic phase favored the development of 3D scaffolds with greater biomechanical stability and biocompatibility.

CODE	COMPOSITION [wt%]
PCL	100% PCL
PCL/10HA	90% PCL/10% HA
PCL/10HASr	90% PCL/10% HASr
PCL/20HA	80% PCL/20% HA
PCL/20HASr	80% PCL/20% HASr

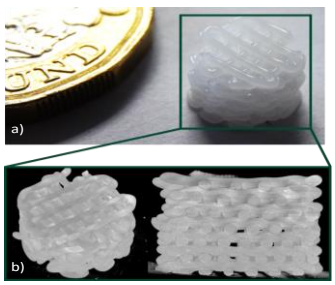
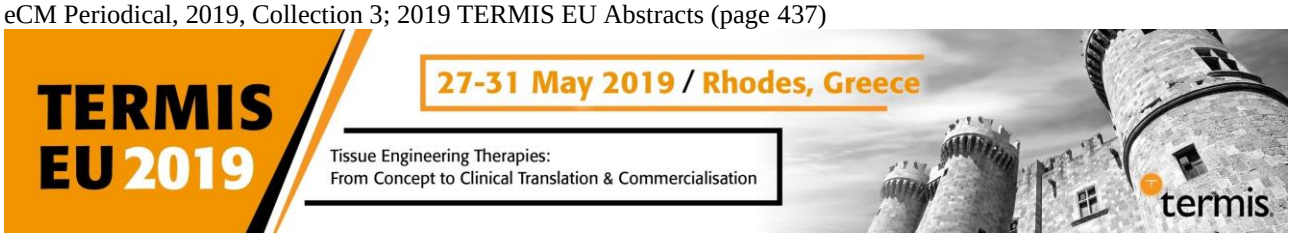


Table1. Scaffold compositions. **Figure 1:** a) PCL/10HA scaffold and (b) micro CT analysis showing the hierarchical structure.

ACKNOWLEDGEMENTS: This work was financially supported by Invest Northern Ireland's, the EU ERDF and NWCAM project (EU's INTERREG VA programme).



Extracellular matrix deposition and matrix-associated gene profile of multipotent mesenchymal stromal cells at "physiological" hypoxia

E.R. Andreeva[1], D.K. Matveeva[1,2], S.V. Buravkov[2], I.V. Zhivodernikov, L.B. Buravkova[1,2]
Presenting Author: Elena R Andreeva, andreeva1564@gmail.com
[1]Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia [2]Moscow State University, Moscow, Russia

INTRODUCTION: Tissue related O_2 - "physiological" hypoxia, is considered as an important physical governor of multipotent mesenchymal stromal cells (MSC) fate in local tissue milieu. Here we examined the extracellular matrix (ECM) deposition and hypoxia-regulated gene expression in MSCs under "physiological" hypoxia.

METHODS: Human adipose tissue MSCs were permanently expanded at ambient (20%) and "physiological" hypoxia (5%) O_2. Collagen and noncollagen ECM proteins were stained with Sirius Red F3BA and Fast Green FC, respectively and then quantified by colorimetric analyses. Gene transcription profile was examined with Illimina global gene expression analysis and verified with RT-PCR.

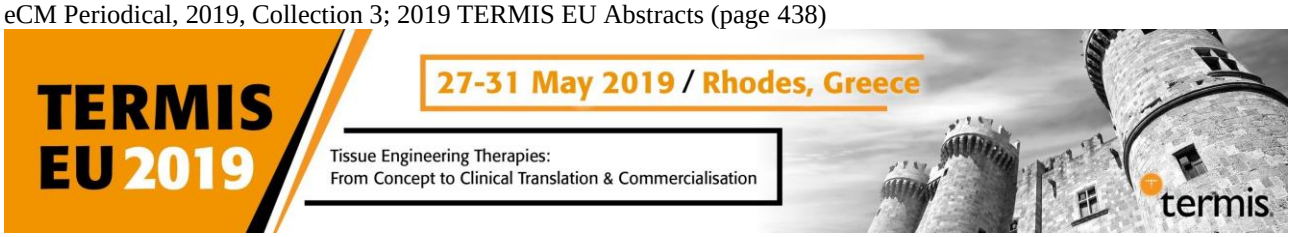
RESULTS & DISCUSSION: Histochemical staining with subsequent colorimetry, did not reveal difference in deposition and amount of collagen and noncollagen proteins in ECM of MSCs at 5 vs 20% O_2. According whole genome profiling, among 86 ECM-related genes only 11 were upregulated. "Core" matrisome (collagens, glycoproteins, proteoglycans) comprised 70% of ECM genes, while the other included proteases and ECM-associated molecules. Using a database of hypoxia-regulated proteins [3] the 60 corresponding genes have been identified. The vast majority of those genes were downregulated: a half among of "core" and most of all matrisome-affiliated (Tabl. 1). RT-PCR analysis of several genes encoding ECM molecules of different groups revealed changes of FN, COMP, MMP1, MMP2, PLAU, TIMP3, TGFB, SPP1 transcription coincided with the results of full-genome analysis.

CONCLUSIONS: MSCs demonstrate the substantial ECM accumulation both at ambient O_2 and "physiological" hypoxia. The shift in the ECM-associated genes transcriptomic profile suggests that in case of tissue damage these cells will be more "predisposed" for the realization of reparative properties, such as migration and proliferation.

Matrisome groups	Altered expression, total	Hypoxia-regulated
"Core"matrisome		
Collagens	14	7
Glycoproteins	23	12
Proteoglycans	4	2
Matrisome-associated		
Proteases	7	6
ECM-affiliated	11	8

Table 1: Hypoxic regulation of MSC matrisome genes

ACKNOWLEDGEMENTS: This work was supported in part by Programm #P43 of Presidium of Russian Academy of Sciences



Adipose tissue-derived therapeutic cells in their natural environment as autologous cell therapy strategy: The microtissue-stromal vascular fraction

S. Nürnberger[1,2,3], C. Lindner[1,2], J. Maier[1,2], K. Strohmeier[1,2,4], C. Wurzer[1,2,5], P. Slezak[1,2], S. Suessner[2,6], W. Holnthoner[1,2], H. Redl[1,2], S. Wolbank[1,2], E. Priglinger[1,2]
Presenting Author: Eleni Priglinger, Eleni.Priglinger@trauma.lbg.ac.at
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Linz/Vienna, Austria [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria [3]Medical University of Vienna, Department of Orthopedics and Trauma-Surgery, Vienna, Austria [4]School of Medical Engineering and Applied Social Sciences, University of Applied Sciences Upper Austria, Linz, Austria [5]Liporegena GmbH, Breitenfurt, Austria [6]Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria

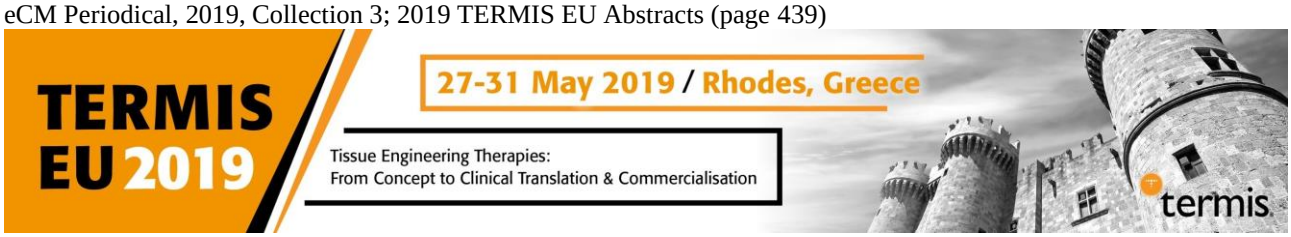
INTRODUCTION: Prerequisite for successful clinical use of autologous adipose tissue-derived cells is the highest possible regenerative potential of the applied cell population, the stromal vascular fraction (SVF). Current isolation methods depend on high enzyme concentration, lysis buffer, long incubation steps and mechanical stress resulting in single cell dissociation from their natural microenvironment. The aim of this study was to limit cell manipulation and obtain a derivative comprising therapeutic cells (Microtissue-SVF) without dissociation from their natural extracellular matrix by employing a gentle GMP-grade isolation with low enzyme concentration.

METHODS: Within this study, our optimized Standard-SVF isolation protocol was adapted resulting in SVF cells protected within their extracellular matrix and analyzed regarding structure, yield and viability, presence of regenerative cells and subpopulations, trophic factor release, vascularization, trilineage differentiation potential and integration after intramuscular injection into nude mice.

RESULTS & DISCUSSION: Microtissue-SVF yielded higher numbers of viable cells compared to standard isolation and a minimal content of dead cells. It comprised stromal tissue compounds (collagen, glycosaminoglycans, fibroblasts), capillaries and vessel structures (CD31+, smooth muscle actin+). A broad range of cell types were identified by surface marker characterization including mesenchymal, hematopoietic, pericytic, blood and lymphatic vascular and epithelial cells. Subpopulations such as supra-adventitial adipose-derived stromal/stem cells and endothelial progenitor cells were significantly higher in Microtissue-SVF (Figure 1) corroborated by significantly higher potency for angiogenic tube-like structure formation in vitro. Microtissue-SVF showed the characteristic phenotype and tri-lineage mesenchymal differentiation potential in vitro according to the ISCT/IFATS, as well as an immunomodulatory and pro-angiogenic secretome. In vivo implantation of Microtissue-SVF combined with fat demonstrated successful graft integration in nude mice.

CONCLUSIONS: In this study, we present a fast and gentle isolation by minor manipulation of liposuction material, achieving a therapeutically relevant cell population with high vascularization potential and immunomodulatory properties still embedded in a fraction of its original matrix.

ACKNOWLEDGEMENTS: We would like to acknowledge Dr. Hajnal Kiprov and Dr. Matthias Sandhofer for providing liposuction material and valuable discussion. This work was funded by a grant from the Austrian Research Promotion Agency (FFG) (Bridge1 programme, grant No.846062).



Engineering blood vessel like structures: A preliminary study

E. Bosch-Rue[1], L.M. Delgado[1], R.A. Pérez[1]
Presenting Author: Èlia Bosch, ebosch@uic.es
[1]Bioengineering Institute of Technology (BIT), Universitat Internacional de Catalunya (UIC), Barcelona, Spain

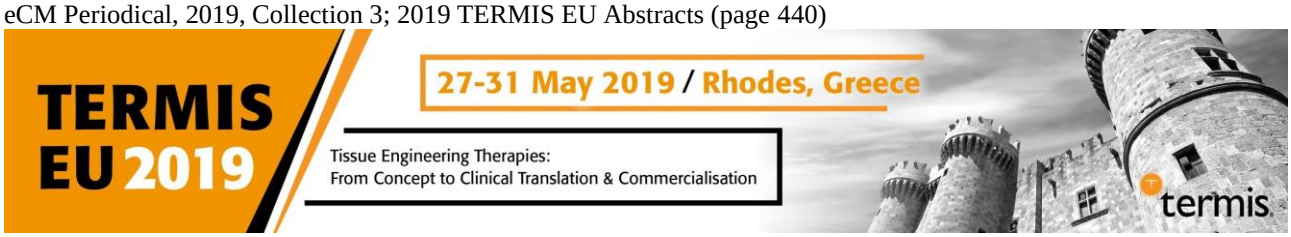
INTRODUCTION: There is a demand for replacing vascular conduits in some clinical situations, such as vascular occlusions or aneurysms. Tissue engineering has focused on developing tubular structures similar to the native structure of blood vessels: endothelial cells (EC) parallelly aligned to the vessel in the inner layer and smooth muscle cells (SMCs) perpendicularly aligned to the vessel in the outer layer. The aim of this work is to mimic the architecture of a blood vessel through a multilayered hollow hydrogel-based fiber.

METHODS: The hollow hydrogel-based fiber was obtained through an extrusion technique, using a triple core-shell nozzle. The composition was: i) alginate with gelatin or collagen in the shell; ii) collagen in the middle-core; iii) pluronic in the inner-core. Once the multilayered fiber was formed, pluronic was removed to obtain a hollowed tubular structure. Hydrogel compositions were optimized to maintain geometrical stability and to allow the encapsulation of human umbilical vein endothelial cells (HUVEC) in the core and human SMCs in the shell. Different cell concentrations and ratios were studied to validate the elongation, proliferation and alignment of the cells and was compared to the native conformation.

RESULTS & DISCUSSION: Preliminary results with non-hollowed core-shell fibers showed that HUVEC were able to elongate, proliferate and align parallel to the direction of the fiber. The optimal HUVEC concentration for cell elongation was around $2x10^6$ cells/mL. With the hollowed core-shell fibers, 5 mg/mL collagen maintained the tubular structure once pluronic was removed and HUVEC cells were also able to maintain its parallel alignment in the fiber direction. However, the highest concentration tested ($5x10^6$ cells/mL) was not enough to cover all the fiber. The shell composition was then optimized mixing 1,5-3% alginate with collagen at low concentrations, allowing the encapsulation of $5x10^6$ cells/mL of hSMC, showing that the composition induced the elongation of most hSMC. However, their orientation was completely random.

CONCLUSIONS: These preliminary results showed similarities with the native structure of blood vessels and the survival of HUVEC with the right orientation. Although hSMC could also survive, further experiments are needed to optimize the hSMC alignment and proper cell concentrations to completely cover the hollow fiber. Furthermore, co-cultures need to be further explored to analyze the possible cross-talks.

ACKNOWLEDGEMENTS: Financial support was received from UIC PhD student grant; SGR (2017SGR708); Juan de la Cierva (IJCI-2015-27006).



Cyclic stretch and shear stress differentially modulate early vascular tissue regeneration

E.E. van Haaften[1,2], T.B. Wissing[1,2], A.I.P.M. Smits[1,2], N.A. Kurniawan[1,2], C.V.C. Bouten[1,2]
Presenting Author: Eline van Haaften, e.e.v.haaften@tue.nl
[1]Soft Tissue Engineering and Mechanobiology, Eindhoven University of Technology, Eindhoven, The Netherlands [2]Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands

INTRODUCTION: In situ vascular tissue engineering (TE) is rapidly moving towards clinical application, however, early stenosis and aneurysm formation remain prevalent occurrences. These complications are often attributed to a mismatch between scaffold properties and hemodynamic loading, i.e., cyclic stretch and shear stress. Here we aimed to understand the relative roles of hemodynamic loads on the regeneration of vascular tissues using a custom-made bioreactor that allows shear stress and cyclic stretch to be effectively decoupled.

METHODS: Human vena saphena cells (HVSCs) and primary monocytes (MQs) were seeded in electrospun polycaprolactone-based scaffolds using fibrin. The constructs were subjected to shear stress (τ_w; 1 Pa), cyclic stretch (λ; 1.05), or combined loads (1 Pa τ_w+1.05 λ). After 3 and 20 days, samples were analyzed for cell phenotype, inflammatory state, and matrix G&R by a wide range of analysis techniques (qPCR, tissue assays, immunohistochemistry, mechanical tests, zymography, ELISA).

RESULTS & DISCUSSION: Both cell types remained viable throughout the duration of the experiment. Cyclic stretch stimulated HVSC proliferation and neotissue formation and reduced the secretion of pro-inflammatory cytokines. Shear stress, on the other hand, attenuated cyclic-stretch-induced tissue growth, but enhanced MMP1- and TIMP1-mediated collagen remodeling, which are reflected in the mechanical properties of the constructs. The combination of both loads resulted in a synergistic increase in IL-10 secretion and suppression of HVSC contractile phenotype.

CONCLUSIONS: Together, the results suggest that shear stress and cyclic stretch differentially act as regulating factors during early in situ regeneration. These insights are critical for understanding and improving the long-term clinical performance of in situ tissue engineered blood vessels.

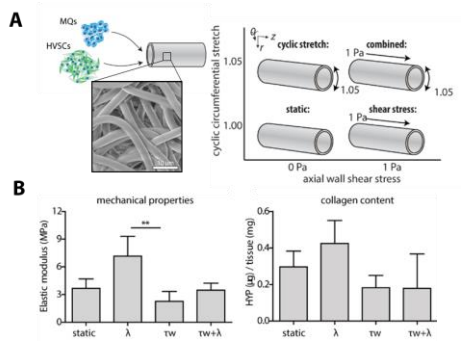
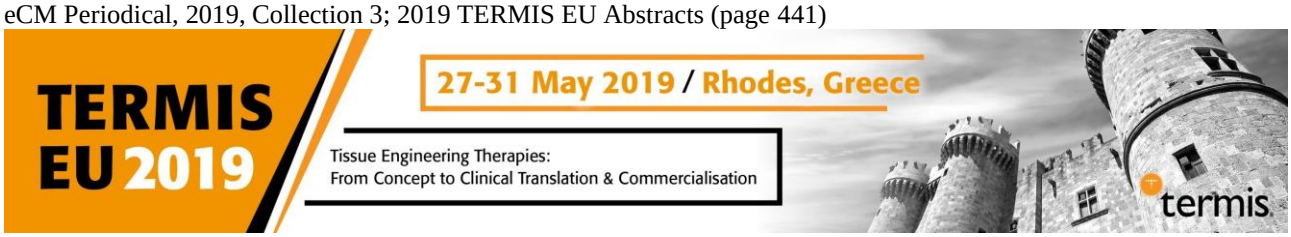


Figure 1: A): scaffolds containing MQs and HVSCs are exposed to either shear stress, cyclic stretch, or a combination thereof. (B): Elastic modulus in circumferential direction and total hydroxyproline (HYP) content.

ACKNOWLEDGEMENTS: This study (436001003) is financially supported by ZonMw and Dutch Kidney Foundation



Suspended microfibre patterning guides spheroid assembly

E.L. Gill[1,2], M. Gerigk[1,2], D. Zhang[1,2], Y.Y.S. Huang[1,2]
Presenting Author: Elisabeth Gill, elg54@cam.ac.uk
[1]The Nanoscience Centre, University of Cambridge, 11 JJ Thomson Ave, Cambridge CB3 0FF, UK
[2]Dept. of Engineering, University of Cambridge, Trumpington St, Cambridge CB2 1PZ, UK

INTRODUCTION: Fibril topography is a significant environmental cue that the extracellular matrix (ECM) provides in tissues [1]. Existing work has explored the effect of surface topography at single cell level [2]. We developed a 3D printing platform capable of patterning suspended fibres in 3D, which mimic the resolution of the large fibril components of the ECM. Here, we show the effect of topographic features in 3D space, interfacing cell aggregates. Our results demonstrate fibres creating a local topographic effect whilst steering global spheroid morphology.

METHODS: Human glioblastoma cell aggregates (U87) were seeded on suspended fibre devices. Devices consist of suspended electrospun gelatin fibres patterned with low voltages (100-130V), around 1-10 μm in diameter, suspended over 3D printed PLA support structures. Cell aggregates were cultured on four different suspended fibre patterns, with media refreshed every 48 hours. The different spheroid morphologies were stained for viability and cell response was measured over a spatial distribution after 1, 3 and 10 days of culture.

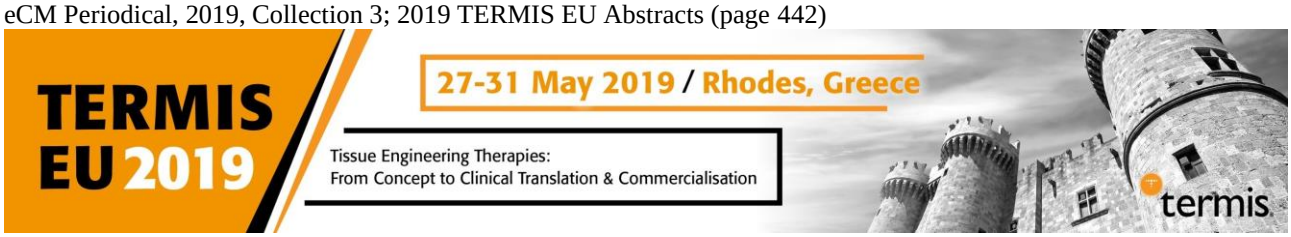
RESULTS & DISCUSSION: The topographic guidance and biochemical similarity to collagen provided by suspended fibre scaffolds encourages cell strings to form rapidly, developing into dense suspended cell structures. The low fibre stiffness enables cells to perform non-axial deformation on the scaffold and cell-cell interaction encourages clusters of cells to fuse. Cell strings are highly viable and larger spheroids are viable at the surface but beyond the typical thickness, internal cells become necrotic. Despite the ability of the cells to remodel the scaffold, the overall morphology of the suspended cell structure reflects the in-plane fibre patterns.

CONCLUSIONS: This study demonstrates suspended fibre architecture for guiding self-assembly of cells cultured in 3D, such as [3]. Suspended fibre architecture influences spheroid morphology and growth characteristics. Topography guided 3D cell culture is still largely unexplored, with significance in cancer research.

ACKNOWLEDGEMENTS: Engineering and Physical Sciences Research Council (EPSRC) UK (EP/M018989/1); European Research Council (ERC-StG, 758865); WD Armstrong Trust; Cambridge CRUK multidisciplinary studentship; China Scholarship Council

REFERENCES

[1] Dvir, T et al. Nat. Nanotech. 2011: 6(1): 13-22
[2] Doyle, A et al. J of Cell Bio. 2009: 184(4): 481-490
[3] Lancaster, M et al. Nat. Biotech. 2017: 35 (659-666)



Development of an in vitro generated 3D human melanoma-in-skin model as a research platform for therapeutic testing

E. Michielon[1], M.L. Gonzalez[2], J.L.A. Burm[1], T. Waaijman[1], T.D. de Gruijl[2], S. Gibbs[1,3]
Presenting Author: Elisabetta Michielon, e.michielon@vumc.nl
[1]Department of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit, Amsterdam, The Netherlands [2]Department of Medical Oncology, Amsterdam UMC, Vrije Universiteit, Cancer Center Amsterdam, Amsterdam Infection & Immunity Institute, Amsterdam, The Netherlands [3]Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit, Amsterdam, The Netherlands

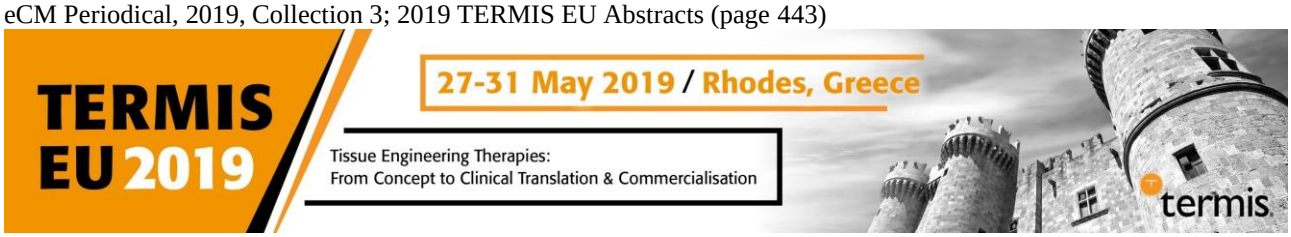
INTRODUCTION: Validation of novel therapies against cancers, such as melanoma, requires models that reflect the human physiology and immune response. The current use of humanized animal models is associated with high costs and low prediction of success in clinical trials, while two-dimensional (2D) cultures fail to reflect the complex tumor microenvironment. There is thus a need for improved three-dimensional (3D) organotypic in vitro melanoma models to facilitate development of therapies.

METHODS: Melanoma reconstructed human skin (Mel-RhS) models were constructed by co-seeding Sk-Mel-28 melanoma cells and keratinocytes onto a fibroblast-populated dermal equivalent, followed by culture in air-lifted conditions for up to six weeks. (Immuno)histochemical staining was used to identify tumor cells and compare the developed Mel-RhS model to melanoma patient material. Culture supernatant was used to detect cytokine secretion by ELISA and to assess changes in the expression of surface markers during monocyte to monocyte-derived dendritic cell (mo-DC) differentiation by flow cytometry.

RESULTS & DISCUSSION: Tumor nests were observed to develop over time at the epidermal-dermal junction and to spread towards the dermis, disrupting the basement membrane. We also observed increased secretion of cytokine IL-10 in Mel-RhS compared to healthy controls. Of note, Sk-Mel-28 cultured in 2D monolayers did not produce detectable IL-10 levels. Supernatant collected from Mel-RhS stimulated differentiation of mo-DC into M2-like machrophages.

CONCLUSIONS: Features of the Mel-RhS resemble the initial stages of invasive melanoma in humans. In addition, the ability of the Mel-RhS culture supernatant to promote a tolerogenic M2-like phenotype in mo-DC cultures is consistent with a switch towards a more immunosuppressive tissue microenvironment. Collectively, this data demonstrates that the developed melanoma model has the potential to be used for research on melanoma development and invasion, in the setting of an immune competent skin microenvironment. In the future, the model may also provide an in vitro tool for preclinical testing of novel therapeutics, bringing us one step closer to personalized medicine.

ACKNOWLEDGEMENTS: Financial support was received from EuroStar (IMAGINe, Project ID 11 275).



P_2O_5-free bioactive glasses with osteogenic and angiogenic potential

E.R. Balmayor[1], S. Font Tellado[1], J.A. Delgado[2], P.S.P. Poh[1], W. Zhang[1], M. García-Vallés[3], S. Martínez[3], A. Gorustovich[4], L. Morejón[2], M. van Griensven[1]
Presenting Author: Elizabeth R. Balmayor, elizabeth.rosado-balmayor@tum.de
[1]Experimental Trauma Surgery, Klinikum rechts der Isar, Technical University of Munich, Germany
[2]Dept. of Ceramic and Composites, Center of Biomaterials - BIOMAT, Univ. of Havana, Cuba
[3]Department of Crystallography, Mineralogy and Mineral Deposits, Univ. of Barcelona, Spain
[4]Interdisciplinary Materials Group, Univ. Salta, Argentina

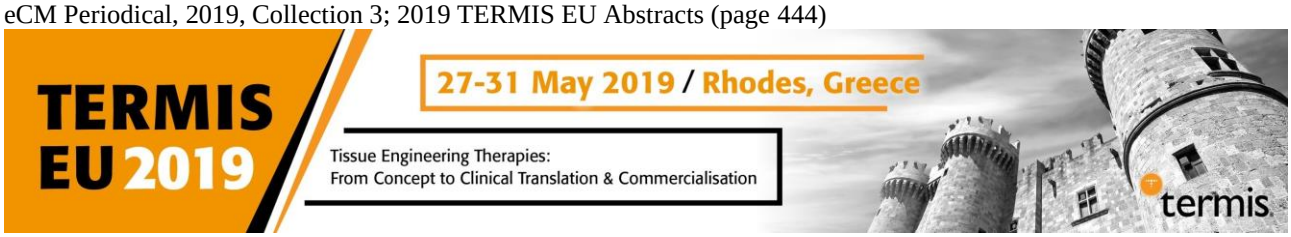
INTRODUCTION: Bioactive glasses (BG) are attractive materials for bone tissue engineering due to their bioactivity and osteoinductivity. We report the synthesis and characterization of novel silicate–based bioactive glasses (S-BG) with composition of 49.9-52% SiO_2, 23-23.7% Na_2O and 22.8-23.3% CaO (%wt). The biocompatibility, osteogenic and angiogenic properties of S-BG particles were analyzed in vitro with human adipose-derived mesenchymal stem cells (AdMSCs) and human osteoblasts (OBs). Angiogenesis was further tested in vivo in a zebrafish embryo model.

METHODS: Glass synthesis was performed by melt quenching. Elemental composition and identification of the S-BGs crystalline phases was performed by X-ray. Thermal analysis was performed by TG-DTA. The bioactivity of produced S-BGs was performed by immersion in Simulated Body Fluid, and subsequent mineral deposition was analyzed by SEM. S-BGs biocompatibility was evaluated by means of MTT and LDH using AdMSCs and OBs. Proliferation was assessed in both cell types by PicoGreen. Furthermore, gene expression was evaluated by RT-PCR. VEGF production was investigated by ELISA, and using a zebrafish embryo model. The best performing S-BG was selected and glass particles were incorporated into a polycaprolactone solution 10% (w/v). PCL/S-BG scaffolds were fabricated by additive manufacturing, and evaluated in vitro for biocompatibility, osteogenic and angiogenic properties using AdMSCs.

RESULTS & DISCUSSION: S-BGs were biocompatible and induced the deposition of a mineralized matrix and the expression of osteogenic and angiogenic markers (RunX2, ALP, Osteocalcin, Osteopontin, Collagen I and VEGF). VEGF was secreted in vitro. When tested in a zebrafish embryo model, the solutions containing the S-BG ions showed abundant vessel formation that was similar to the positive controls (i.e. treated with bFGF). PCL scaffolds doped with 10%wt glass showed high porosity (76%) and pore interconnectivity. The incorporation of S-BG particles increased AdMSCs proliferation compared to plain PCL scaffolds, indicating improved biocompatibility. Finally, S-BG incorporation improved the scaffolds' osteogenic and angiogenic properties by increasing mineral deposition and inducing relevant gene expression as well as VEGF protein secretion.

CONCLUSIONS: Collectively, these data clearly illustrate P_2O_5-free BG particles as attractive for bone engineering applications with relevant angiogenic properties.

ACKNOWLEDGEMENTS: The authors thanks to BayLat for the project 914-20.1.1



Microstructural and molecular signals enhance osteogenic differentiation of human mesenchymal stem cells on polymeric scaffolds

I.M. Wojak-Ćwik[1], D. Scharnweber[2], E. Pamuła[1]
Presenting Author: Elżbieta Pamuła, epamula@agh.edu.pl
[1]AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Biomaterials, Al. Mickiewicza 30, 30-059 Krakow, Poland [2]Technische Universität Dresden, Institute of Materials Science, Max Bergmann Center of Biomaterials, Budapester Str. 27, 01069 Dresden, Germany

INTRODUCTION: Numerous reports have indicated a strong dependence of cell adhesion and proliferation on the scaffold microstructure. Bioengineered artificial extracellular matrices (aECMs) containing glycosaminoglycans (GAGs) with a specific sulphation pattern have been found to regulate cellular responses during healing process [1]. Here we evaluate the synergistic effect of microstructure (monomodal vs. bimodal porosity) and aECM surface modification of poly(L-lactide-co-glycolide) (PLGA) scaffolds on the osteogenic differentiation of human mesenchymal stem cells (hMSCs).

METHODS: PLGA bimodal scaffolds were produced by solvent casting/porogen leaching with the use of salt and PEG as porogens [2]. The scaffolds were coated with aECMs containing collagen type I and highly sulphated hyaluronic acid (coll/sHya) and were characterised by µCT, SEM-EDX and compression test; content of coll and sHya was measured by Sirius red and toluidine blue, respectively. HMSCs were seeded at 45,000 cells/scaffold and cultured up 28 days. Cell adhesion, proliferation (LDH), differentiation (ALP activity, mineralization and gene expression by real-time PCR) were evaluated.

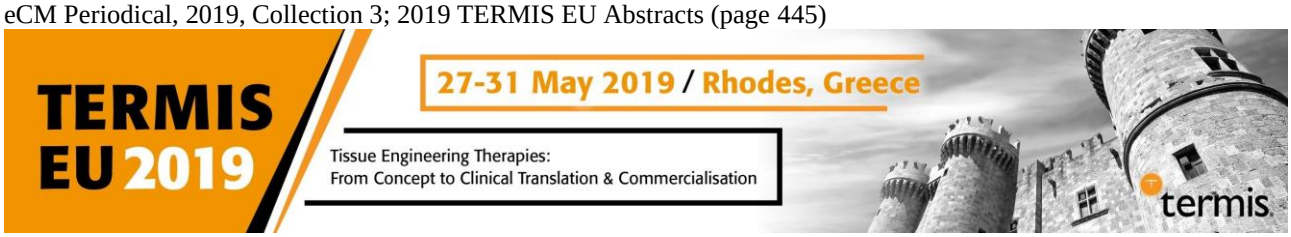
RESULTS & DISCUSSION: The application of two porogens resulted in bimodal pore distribution within the PLGA scaffolds as shown by SEM and micro-CT. Two types of pores with diameters 400 – 600 µm and 2 – 20 µm were obtained. The scaffolds were successfully coated with a homogenous layer of aECM. In vitro study showed that presence of bimodal pore distribution in combination with collagen/sHya did not significantly influence hMSC proliferation and early osteogenic differentiation compared to scaffolds with monomodal pore distribution. However, it enhanced mineralization as well as the expression of Runt-related transcription factor 2 (RUNX-2), osteopontin and bone sialoprotein II.

CONCLUSIONS: As a result PLGA scaffolds with bimodal pore distribution modified with collagen/sHya can be considered as prospective material promoting bone regeneration.

ACKNOWLEDGEMENTS: Financial support was received from National Science Center, Poland (UMO-2016/21/D/ST8/0185), the BMBF, Germany (GoBone, No.01DS16010A) and Polish National Agency for Academic Exchange (PPI/PRO/2018/1/00026/U/001).

REFERENCES

[1] I. Wojak-Cwik et al. J Biomed Mater Res A. 2013; 101:3109-22
[2] I. Wojak-Cwik et al. et al. Mat Sci Eng C 2019;97:12-22



Effect of the nichoid culture substrate on MSC movement

E. Jacchetti[1], F. Donnaloja[1], L. Boeri[1], R. Osellame[2], G. Cerullo[2], M.T. Raimondi[1].
Presenting Author: emanuela.jacchetti@polimi.it
[1]Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Italy [2]Istituto di Fotonica e Nanotecnologie (IFN)-CNR and Department of Physics, Politecnico di Milano, Italy

INTRODUCTION: The environment transmits forces through the cell cytoskeleton, inducing nuclear mechanotransduction. We developed an innovative microstructured 3D culture scaffold named "nichoid" that provides a geometric constraint to adhering cells, favoring maintainance of the stemness of mesenchymal stem cells (MSC) [1]. Here we want to prove that the nichoid affects not only cell adhesion, but also some aspects of cell migration.

METHODS: Rats bone marrow MSC were seeded in 3D nichoids and on flat glass coverslips (controls). Cells were processed for immunofluorescence analysis by using the fluorescent antibody anti-vinculin and the fluorescent dye phalloidin-FITC and Hoecst33342 to stain actin cytoskeleton and DNA. In order to study cell migration, MSC nuclei were stained by Hoecst-33342 fluorescent probe. Z-stack (1 □m depth) and time lapse (one acquisition every 20 min for 6h) were acquired by a confocal microscope. The software ImageJ and Origin Pro were used to analyze images and to quantify cell displacements and path.

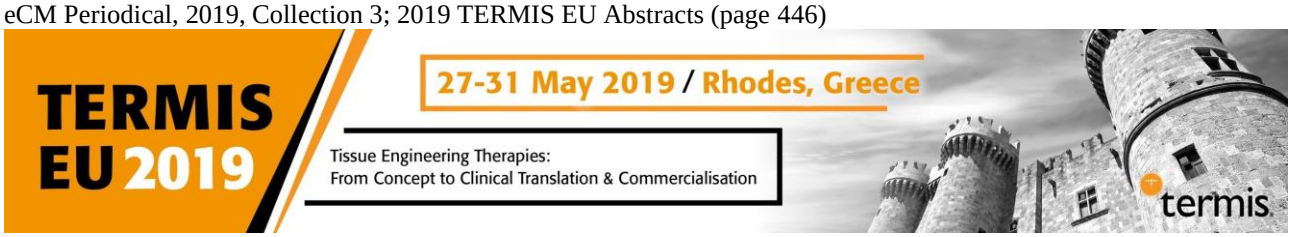
RESULTS & DISCUSSION: Our results show that the structure of the 3D-nichoid affects cell migration. MSC grown in the nichoid have a migratory capability reduced by 33% compared to controls in terms of displacement and total distance covered (so-called path). Nevertheless, MSC adhering to the nichoid lateral walls showed a mobility comparable to flat controls. These results were related the cell morphology: immunofluorescence measures revealed that MSC in the nichoid had focal adhesions reduced by 19% in number compared to controls. In addition, the cell cytoskeleton was modulated by interaction with the nichoid scaffold: in MSC adhering in the nichoid, the cytoskeleton was mainly composed by cortical actin networks, while in cells on flat controls we observed thick stress fibers also inside the cell cytosol, that likely transmitted higher forces from the cell periphery to the nucleus.

CONCLUSIONS: Our results show how the properties of the nichoid scaffold affect drastically the migratory cell behavior. In the nichoid, the cell capability to migrate is reduced by the absence of a flat substrate for cell adhesion. We hypothesize that such motion constraint is a key feature causing stemness maintenance in the 3D nichoid scaffold and we plan to further investigate this effect on the expression of motility-related genes.

ACKNOWLEDGEMENTS:
ERC grant NICHOID n. 646990. We would like to thank also Lara Giove and Tommaso Zandrini for their support in the nichoid production.

REFERENCES

[1] Nava M. et al. J Tissue Eng Regen Med 2017; 11: 2836–2845



Novel production process of xenograft demonstrates safety, biological and preclinical performance

E. Rompen[1], E. Dory[2], F. Lambert[3,4,5]
Presenting Author: Emilie Dory, emilie.dory@wishbone-biotech.com
[1]Department of Dental Science, Faculty of Medicine, University of Liege, eric.rompen@chu.ulg.ac.be, 4020 Liege, Belgium. [2]Wishbone SA, Belgium, Dental Biomaterials Research Unit (d-BRU), Faculty of Medicine, University of Liege, Liège, Belgium [3]Department of Periodontology and Oral Surgery, Faculty of Medicine [4]University of Liege, Liège, Belgium [5]Service de Médecine Dentaire, Domaine du Sart Tilman Bat B-35, 4000, Liège, Belgium

INTRODUCTION: Xenografts are commonly used in dental patients requiring bone volume restoration and regeneration[1]. However, common origin of procurement & processing does not guarantee a predictable and uniform clinical response. The processing of xenografts subsequently influences physicochemical features, biocompatibility and preclinical performance. Herein, we report a unique proprietary processing method with using a subcritical semi-continuous reactor and subsequent investigation of the resultant HA material.

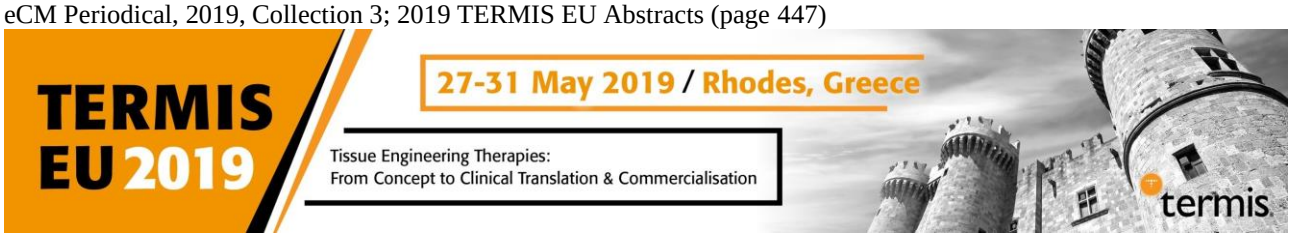
METHODS: Carefully selected femurs from young bovine were washed with water and extracted in a semi-continuous reactor inside a clean room using controlled high values of pressure and temperature with alkali solution for time periods between 5-8 hours. The HA materials were subsequently washed with a 15% wt.% H_2O_2 solution for 10min and heat treated. The chemical composition was investigated with XRD, FTIR, ICP-MS and physical/surface properties investigated with SEM and BET. Inflammatory & foreign body response i.e biocompatibility was investigated in vivo by sensitization, irritation, cytotoxicity and genotoxicity assays.

RESULTS & DISCUSSION: Chemical investigation confirms no organic impurities including residual proteins, main component being natural carbonate rich hydroxyapatite followed by magnesium (MgO_2) in the periclase or brucite phase. Other elements present in the materials were calcium phosphorus and sodium. Additionally, trace amounts of barium, manganese and iron ions present in quantities similar to natural bone were found. Investigation of physical properties before & after processing reveals minor or no change in physical architecture and preservation of bone-like topography. In vivo biocompatibility reveals no sensitizing, irritating, cytotoxic, genotoxic or pyrogenic reactions as response to materials exposure. Also, no systemic toxicity was detected.

CONCLUSIONS: The rigorous approach to start with clean raw material followed by a unique extraction technology based on high temperature & pressure results in fast extraction of proteins without damage to natural bone surface topography and porosity. Additionally, biocompatibility results of HA are overwhelmingly positive.

REFERENCES

[1] Baldini N, et al, Dental materials:official publication of the Academy of Dental Materials. 2011;27(1):61–70
[2] Block MS, J Oral Maxillofac Surg, 2018, Oct 12.



Characterization of nanowire substrates for neural integration

E.G. Kalaf[1,2], A. Selby[2], J. Fairfield[1,2]
Presenting Author: Emily Growney Kalaf, emily.growneykalaf@nuigalway.ie
[1]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]Department of Physics, National University of Ireland Galway (NUI Galway), Ireland.

INTRODUCTION: Nanowires for use in neural electrodes is a burgeoning field of research that has not been thoroughly characterized in terms of artificial synaptic properties. Nanowires alone are subject to traction forces due to loose connectivity to the electrode. This abstract characterizes various biocompatible substrates for use as an integrating layer for nanowires by way of physical, electrical, and biological measurements.

METHODS: Rat Dorsal Root Ganglion (DRG) explants were extracted and cultured on titanium dioxide (TiO_2) or silver (Ag) nanowire networks that had been mixed or layered with various biocompatible coatings (Table 1). Confocal images measured neurite outgrowth. Oxygen-plasma treated coated coverslips were analyzed for impedance and conductance via potentiostat and Electrostatic Force Microscopy. Substrate topology was characterized by SEM. Memristive properties of the substrates was analyzed by an in-lab probe station.

RESULTS & DISCUSSION: DRG explants cultured on nanowire substrates qualitatively exhibited preferential neurite outgrowth in substrates with TiO_2 nanowires over Ag nanowires (Figure 1). The sterilization procedure used was found to cause morphological changes to nanowire junctions which could affect electrical behaviour, which is a factor that must be considered when preparing nanomaterial substrates for biological use. Neurite outgrowth results are forthcoming.

CONCLUSIONS: Collectively, these data illustrate the beneficial effect of a coating layer in the promotion of neural culture on nanowire substrates. This project aims to optimize for both biological and electrical applications; further memristive testing will be performed on the optimized substrates.

Coating	Concentration	Layering Method
Poly-L-Lysine	0.01%	All substrates were either mixed with nanowires, layered beneath nanowires, or deposited above nanowires.
Poly-D-Lysine	0.01%	
Laminin-111	0.002%	
Collagen I	0.003%	
Silk Fibroin	2.5%	
Chitin	0.0025%	

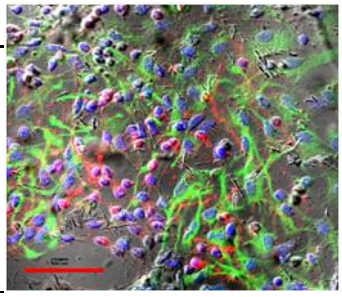
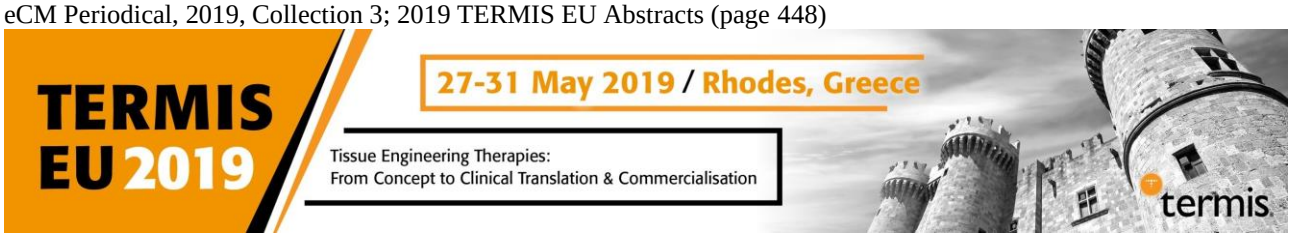


Table 1: Substrates and layering method used for viability and neurite outgrowth assays. **Figure 1:** Embryonic (E17) DRGs cultured on a mixed substrate of PLL and TiO2 nanowires, shown as a combined confocal/differential interference contrast image. Red staining indicates beta (III) tubulin, green staining indicates glial fibrillary acidic protein, and blue indicates cell nuclei. Scale bar indicates 50 microns.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland, (15/CDA/3629 and 13/RC/2073); and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713690.



Olfactory bulb and DOPAL: A novel organotypic model of pre-clinical Parkinson's disease

E. Bagnoli[1,2], J. MacMahon[1,2], U. FitzGerald[1,2]
Presenting Author: Enrico Bagnoli, enrico.bagnoli@nuigalway.ie
[1]Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]Galway Neuroscience Centre, National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting more than 10 million people worldwide. The olfactory bulb (OB) is one of the first places affected in PD and it is thought to be involved in the disease pathogenesis [1]-[2]. Herein, we combine OB organotypic slices and DOPAL, a metabolite of dopamine involved in PD progression [3], to create a new platform for testing new therapeutic strategies.

METHODS: OB organotypic slices (300 µm thick) have been obtained from postnatal SD rats (p10) with a McIlwain tissue chopper. The slices have been cultured for 7 days in vitro (DIV) followed by 3 DIV of DOPAL treatment and other 4 DIV of recovery. Slice viability has been assessed using the alamarBlue® assay and the media collected for analysis of nitrite and reactive oxygen species (ROS) at DIV 7, 10, 14. The effects of DOPAL on the bioenergetic performances of OB cells have been studied using the MitoStress Test assay and the Seahorse XFp analyser. Furthermore the OB cell composition have been characterized using immunohistochemistry.

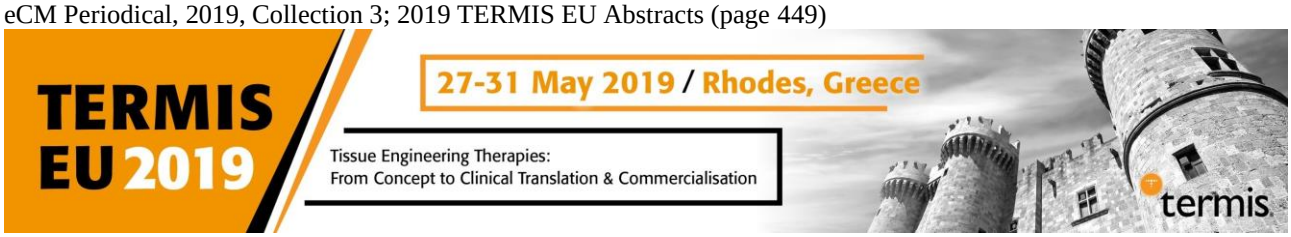
RESULTS & DISCUSSION: OB slices preserve the 3D anatomical structures during culture. DOPAL treatment significantly increases the content of ROS and nitrites ($p<0.05$), resulting in a reduced viability of treated slices. A possible mechanism of action is through the damage of mitochondria and the impairment of the physiological energy homeostasis in the cells. Signs of mitochondrial damage, like an increased proton leak or a decreased coupling efficacy and spare respiratory capacity, appear minutes after DOPAL is added to the culture media. The bioenergetic health index (BHI) is significantly ($p<0.001$) reduced upon DOPAL exposure.

CONCLUSIONS: Exposure of cultured olfactory bulb slices to DOPAL causes toxicity that mimics aspects of PD pathology, in particular increased oxidative stress, mitochondrial dysfunction and, lastly, neurodegeneration. This novel experimental platform shows great promise as an innovative, easy and accessible technology for the development of new restorative and regenerative treatments for PD, furthermore raising the enticing possibility of early-stage intervention.

ACKNOWLEDGEMENTS: This project has been funded by the European Union Horizon 2020 Programme (H2020-MSCA-ITN-2015) under the Marie Skłodowska-Curie Innovative Training Networks and Grant Agreement No. 676408.

REFERENCES

[1] Hawkes C et al. Q J Med. 1999. 92(8), pp. 473–480.
[2] Doty, R. L. Nat. Rev. Neurol. 2012. 8, 329–339
[3] Goldstein D et al. Eur. J. Neurol. 2011, 18(5) 703-710



Cartilage oligomeric matrix protein inhibits angiogenesis in vitro, depending upon its oligomerisation state

E.A. Sastre[1], F. Zaucke[2], J.W. Bouma[1], K. Maly[2], G.J.V.M van Osch[3], E. Farrell[1]
Presenting Author: E. Andrés Sastre, e.andressastre@erasmusmc.nl
[1]Department of Oral and Maxillofacial Surgery, Erasmus MC, University Medical Centre Rotterdam, the Netherlands [2]Dr. Rolf Schwiete Research Unit for Osteoarthritis, Orthopaedic University Hospital Friedrichsheim, Frankfurt, Germany [3]Departments of Orthopaedics and Otorhinolaryngology, Erasmus MC University Medical Centre, Rotterdam, the Netherlands.

INTRODUCTION: The thrombospondin family is composed of 5 members. TSP-1 and TSP-2 exert potent antiangiogenic functions via their Type 1 repeats, which are absent on the other three thrombospondins [1]. TSP-5 (also known as Cartilage Oligomeric Matrix Protein, COMP) is a homopentameric protein and found predominantly in cartilage and tendon, and during matrix remodeling its oligomeric state is lost [2]. Here we asked if COMP may have an anti-angiogenic role like its other family members TSP-1 and 2, and if that depends on its oligomeric state.

METHODS: The angiogenic effect of COMP on endothelial cells (ECs) was assessed via standard in vitro assays using human umbilical vein endothelial cells (HUVECs). On each assay, media was supplemented with either 10 µg/mL of the recombinant protein (COMP) or with a monomeric COMP derived construct lacking the oligomerisation N-terminal domain (mCOMP). Tube formation assay. ECs were seeded on 96 well plates coated with reduced growth factor basement membrane [Geltrex]. After 24h, tubes were imaged and quantified. Migration assay. ECs were seeded on modified Boyden chambers of 8 µm pore size [Falcon] and allowed to migrate either towards COMP and mCOMP, or from a media containing them towards a chemoattractant media (EGM-2). After 10h, migrated cells were counted.

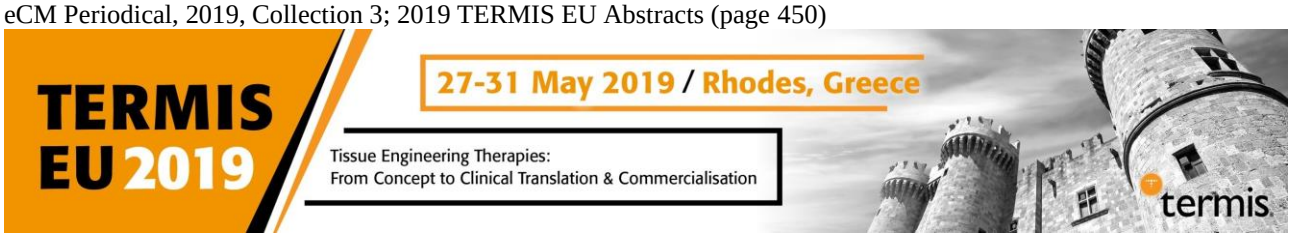
RESULTS & DISCUSSION: COMP inhibited tube formation of ECs ($p<0.01$), while at the same concentration mCOMP showed no significant differences ($p>0.05$). Neither of the forms of the protein induced EC migration ($p>0.05$). However, when COMP was placed in direct contact with the cells that were allowed to migrate towards a chemoattract medium, a significant reduction of migration was observed ($p<0.01$), which did not occur with addition of mCOMP ($p>0.05$).

CONCLUSIONS: COMP plays an antiangiogenic role that depends on its oligomerisation state, which probably involves a different mechanism than that from TSP-1 and -2. This finding supports the idea that an avascular tissue such as cartilage is protected from angiogenesis until its extracellular matrix is degraded.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 721432.

REFERENCES

[1] Bornstein, P. J. Cell Commun. Signal. 2009;3:189.
[2] Ahrman, E. et al J Biol Chem. 2014; 289(30):20908-16



Novel microfluidic protein patterning method for human-organs-on-chip: Liver-tumor application

E. Ferrari[1], G.S. Ugolini[1], M. Rasponi[1]
Presenting Author: Erika Ferrari, erika1.ferrari@polimi.it
[1]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy

INTRODUCTION: Micropatterned co-cultures (MPCCs) of hepatocytes and 3T3 fibroblasts can maintain higher hepatic functions for several days in vitro, compared to standard culture techniques [1]. Despite the micrometer resolution of protein patterns, so far MPCCs have been used in standard multi-well plates or glass substrates only. Indeed, no methods were described to combine this technology with microfluidic systems. Possible advantages in coupling MPCCs with microfluidic designs include: higher throughput, lower reagent consumption, compatibility with organotypic or multi-organ designs [2]. Here we present a novel technique that allows culture and analysis of MPCCs within a microdevice specifically designed for liver-tumor compartmentalized cultures. The platform is of use in studying drug effects on tumor tissue in the presence of liver metabolism.

METHODS: Microfluidic devices were fabricated following standard photo (SU-8) and soft (PDMS) -lithographies. The liver and tumor chambers were separated by an array of microchannels (5µm high, 3µm wide, 500µm long) to impose a diffusion-based exchange between the compartments. Collagen islands (500µm diameter, 1.2mm center-to-center) were obtained inside microfluidic devices by means of consecutive operations: protein coating, stamp positioning, plasma ablation and bonding of the device. HepG2 cells were seeded at $20 \cdot 10^6$ cells/mL and allowed to adhere for 20 minutes before a washing step. The following day, 3T3 fibroblasts were seeded at $5 \cdot 10^6$ cells/mL forming MPCCs. HCT-116 colon cancer cells were seeded at $10 \cdot 10^6$ cells/mL in the tumor compartment and medium was changed every 24h. Medium supplemented with 100µM Tegafur was added in the liver compartment and the viability of the tumor cells was then evaluated.

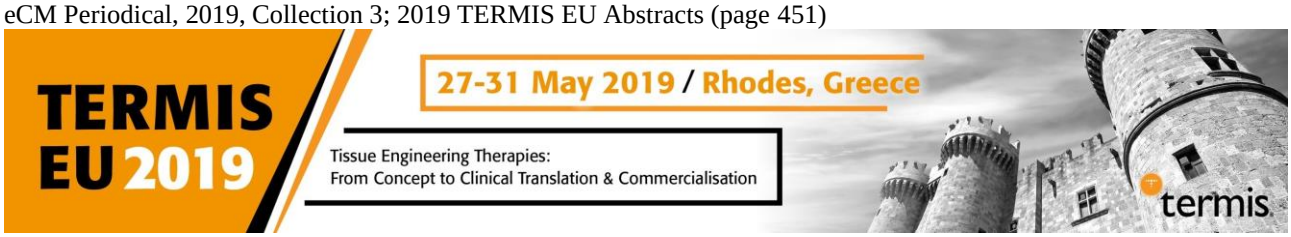
RESULTS & DISCUSSION: Microfluidic devices were fabricated and evaluated by dye diffusion to assess the integrity of the microchannels. The patterning and bonding technique resulted efficient with collagen islands of defined circularity successfully obtained inside the liver compartment of the microdevice. MPCCs resulted viable and stable in size. MPCCs were assessed with LIVE/DEAD assay and showed high viability (82%) even after 8 days of culture. Morphologically, HepG2 islands were well maintained during the culture period even though a small loss of circularity was observed after the third day of culture. Ongoing experiments are aimed at validating the platform for pharmacokinetic-based drug screenings. Indeed, Tegafur is a known model drug that increases tumor cytotoxicity after being metabolized by the liver.

CONCLUSIONS: Owing to the fine control of microfluidics, the device described is particularly suitable to study the effect of liver metabolism on anti-tumor drugs. However, our new technology can be applied to potentially all combinations of protein patterns and microfluidic layouts, enabling for new human in vitro studies.

ACKNOWLEDGEMENTS: Italian Ministry of Health (Ricerca Finalizzata GR-2016-02364990).

REFERENCES

[1] Nakazawa K et al. J Bioprocess Biotech. 2011; S3:002
[2] Ugolini GS et al. Micromachines. 2016; 7:233



Modulating the architecture of custom-made macroporous bioceramics to improve their load-bearing properties in bone

B. Charbonnier[1], M. Manassero[2], K. Oudina[2], H. El-Hafci[2], A. Decambron[2], S. Corsia[2], M. Bourguignon[2], G. Mitton[2], D. Leon[1], M. Bensidhoum[2], H. Petite[2], D. Marchat[1], E. Potier[2]
Presenting Author: Esther Potier, esther.potier@cnrs.fr
[1]Ecole Nationale Supérieure des Mines de Saint-Etienne, CIS-EMSE, INSERM U1059, SAINBIOSE, F-42023 Saint-Etienne, France [2]UMR 7052 Laboratoire B3OA, CNRS, INSERM, Univ Paris Diderot Sorbonne Paris Cité, École nationale vétérinaire d'Alfort, F-75010, Paris, France

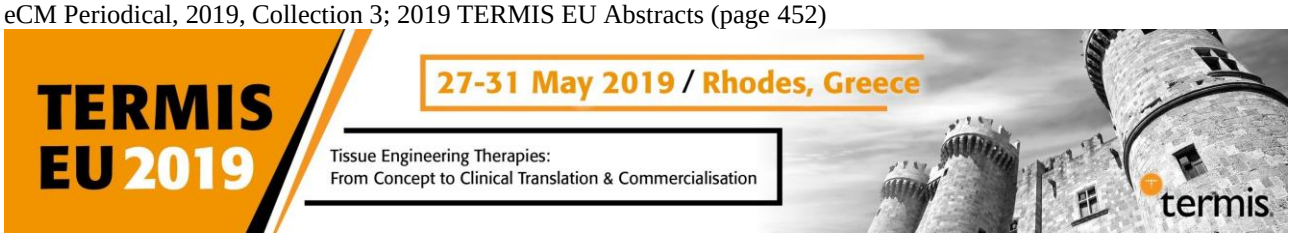
INTRODUCTION: Scaffold architecture, by regulating cell invasion and nutrient transports, is a key parameter for the success of bone tissue engineering. Additive manufacturing techniques allow now to produce scaffolds with controlled architectural features and improved permeability. Such porous scaffolds must, however, be able to sustain high mechanical strain when implanted in load bearing site, even when osteosynthesis material is used. The objective of the study was, therefore, to assess the ability of a cortical reinforcement to improve the mechanical resistance of hydroxyapatite (HA) scaffolds with open and continuous tri-periodic gyroid porosity without impeding bone invasion when implanted in a femoral defect.

METHODS: HA-scaffolds were designed and produced using an innovative process to the anatomic shape of a 3 mm femoral defect in rats, with (i) a macroporous gyroid porosity of 430 μm in diameter (GP) or (ii) with the same gyroid porosity reinforced by a semi-cortical 200 μm thick (GPRC) (total porosity of 60% and 43%, respectively). After manufacturing, morphological characteristics were assessed by micro-computed tomography (CT). Macro- to nano-pores were examined with scanning electron microscopy (SEM). Scaffold mechanical properties were measured with an Instron Universal Testing at constant speed (20 μm/min, n=5/group). Scaffold osteoconductive properties were measured by micro-CT 2, 4, 6 and 8 weeks after implantation in non-critical (3 mm) femoral defects, stabilized with PEEK plates, in female Lewis rats (12-15 weeks of age, n=8/group). 8 weeks after implantation, femurs were explanted and analyzed by high definition micro-CT and histomorphometry for bone formation. Mineralization levels (Ca/P ratio) of newly formed bone were determined by back-scattered electron-SEM (n=5/group).

RESULTS & DISCUSSION: GP and GPRC scaffolds were manufactured with high accuracy in reference to the computerized models. SEM images confirmed that macro- to nano-topography and porosity were similar between both scaffolds. Cortical reinforcement significantly improved scaffold stiffness (GP = 463 ± 68 MPa; GPRC = 664 ± 82 MPa) and resistance to fracture in load bearing site (8/8 GP and 2/8 GPRC scaffolds fractured). Quantity of newly formed bone was similar between both scaffolds, as determined by micro-CT and histomorphometry. Bone, however, was preferentially formed on scaffold borders for the GP and in scaffold center for GPRC scaffolds. Mineralization levels of newly formed bone were also lower for the GPRC than for the GP scaffolds (GP = 1.56 ± 0.07; GPRC = 1.46 ± 0.09).

CONCLUSIONS: For the first time, this study showed that, with a cortical reinforcement, macroporous HA scaffolds can be successfully implanted in load bearing site and that this cortical reinforcement did not impede bone formation within the scaffold.

ACKNOWLEDGEMENTS: Financial support was received from ANR 2011 Orthoflase and CNRS PEPS 2017 Archibone



Exploring the use of dental pulp stem cells in the treatment of head and neck cancer

E. Wolfs[1], P. Gervois[1], C. Erens[1], Y. Dillen[1], K. Libberecht[1], L. Rasking[1], U. Himmelreich[2,3], I. Lambrichts[1]
Presenting Author: Esther Wolfs, esther.wolfs@uhasselt.be
[1]Morphology research group, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium [2]Molecular Small Animal Imaging Center (MoSAIC), University of Leuven, Leuven, Belgium [3]Biomedical MRI Unit, Department of Imaging and Pathology, University of Leuven, Leuven, Belgium

INTRODUCTION: Dental pulp stem cells (DPSC) are a subset of the mesenchymal stem cell family (MSC) and appreciated for their differentiation capacity, paracrine actions as well as their easy isolation method. As MSC are known to migrate towards tumor lesions (1, 2) and we have shown in vitro migration of DPSC towards a number of different tumor cell lines, we believe that DPSC are suitable carriers to specifically deliver therapy to tumor lesions. Because of the severe side-effects and poor quality of life after current treatments in head and neck cancer patients, there is an urgent need for new personalized and targeted therapies. We hypothesize that DPSC are suitable carriers for gap junction-mediated tumor therapy using the so-called "suicide gene" Herpes Simplex Virus type 1 thymidine kinase (HSV1-tk) in head and neck cancer.

METHODS: Co-cultures of human DPSC and an oral squamous cell carcinoma (OSCC) cell line were set up in different ratios (1:1 to 1:3) to study the formation of gap junctions, through immunocytochemistry against connexin-43 and transmission electron microscopy (TEM).A transwell assay was performed to assess migration of DPSC towards OSCC cells. Interdonor differences in DPSC were assessed regarding gap junction formation and migration. DPSC stably expressing firefly luciferase (Fluc) and HSV1-tk were generated using a lentiviral vector. Transgene functionality was assessed through luminescence assays and cell killing experiments via ganciclovir. A head and neck cancer rat model was optimized using the carcinogen (4-Nitroquinoline 1-oxide) 4NQO dissolved in the drinking water or administered locally. Tumor development was assessed with ex vivo MRI and histology.

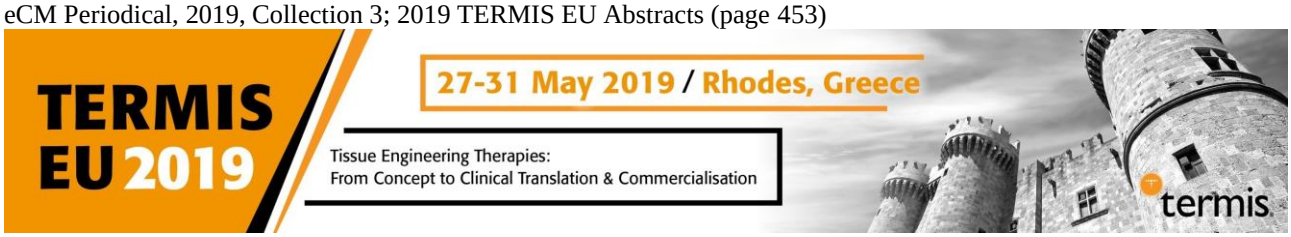
RESULTS & DISCUSSION: All DPSC donor lines formed gap junctions with the OSCC cell line in all ratios, confirmed by connexin-43 immunostaining and TEM. We did observe a difference in migration capacity between DPSC donors. Therapeutic DPSC were generated successfully, functionality of Fluc and HSV1-tk was confirmed. Furthermore, a clinically representative rat model for head and neck cancer was optimized using the carcinogen 4NQO, with clear lesions in both models (drinking water vs local administration) as seen with ex vivo MRI and histological stainings.

CONCLUSIONS: These data indicate that DPSC are attractive candidates as vehicles for gap junction-mediated suicide gene therapy. Further in vivo studies will be performed in order to development a novel, targeted and specific therapy for head and neck cancer.

ACKNOWLEDGEMENTS: Research Foundation-Flanders ("Fonds Wetenschappelijk Onderzoek Vlaanderen" FWO, grant G0A7514N). Y. D. and P. G are FWO pre- and postdoctoral fellows.

REFERENCES

[1] Kidd S et al. Stem Cells. 2009;27(10):2614-23.
[2] Wu X et al. J Neurosurg. 2008;108(2):320-9.



A novel multi-layer functionalized collagen scaffold to regenerate the tendon-to-bone interface

E. Pugliese[1,2], D.I. Zeugolis[1,2]
Presenting Author: Eugenia Pugliese: eugenia.pugliese@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland[2] Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: The enthesis is a transitional tissue interface between tendon and bone essential for adequate force transmission [1]. Traditionally used monotherapies and tissue substitutes lack to reproduce the complexity of the native enthesis, posing the need for the next generation of multi-domain delivery vehicles [2]. We hypothesized that a multi-layer collagen scaffold functionalized with specific growth factors will enhance the functional regeneration of the enthesis.

METHODS: A 3-layer sponge composed by a tendon-like layer of collagen type I, a cartilage-like layer of collagen type II and a bone-like layer of collagen type I and hydroxyapatite was fabricated and cross-linked with poly-ethylene glycol. The cartilage-like layer was functionalized with insulin growth factor 1 (IGF-1) and the tendon-like layer with platelet-derived growth factor (PDGF) and Elisa assay was performed to assess the daily dose released in the media. Histological stainings were performed to assess chondrogenic and osteogenic differentiation of bone-marrow stem cells (BMSCs) seeded on functionalized and non-functionalized scaffolds (Alcian blue and Fast red for proteoglycan production and Alizarin red for calcium deposition, respectively). Tenogenic differentiation of BMSCs was evaluated through expression of specific markers (scleraxis and tenomodulin) by PCR.

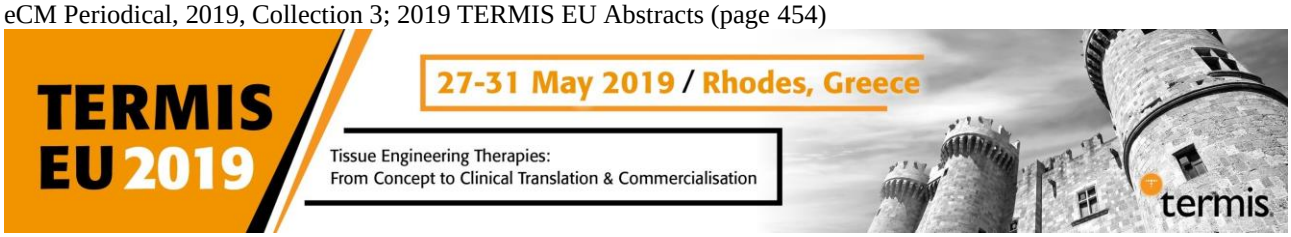
RESULTS & DISCUSSION: Elisa assay showed a release of approximately 5 ng/mL in the media up to 21 days. Osteogenic and chondrogenic differentiation of BMSCs cultured in basal media was observed after 21 days specifically localized in the bone-like layer and in the cartilage-like layer, respectively of the non-functionalized scaffold. The IGF-1 functionalized scaffold increased and accelerated proteoglycan production up to day 14 in the cartilage-like layer. Increased expression of tenogenic markers was observed in the PDGF-functionalized scaffold in the tendon-like layer.

CONCLUSIONS: The different collagen composition of the 3-layer sponge together with the growth factor functionalization was able to promote localized differentiation of BMSC into tenogenic, chondrogenic and osteogenic lineage, establishing a promising multi-domain delivery vehicle for the functional regeneration of the enthesis. On-going work is establishing the synergistic effect between different growth factors.

ACKNOWLEDGEMENTS: Financial support was received from the European Union, Horizon 2020 Programme, Grant Agreement 676338.

REFERENCES

[1] S. Tellado et al., Advanced Drug Delivery Reviews, 94, 126–140, (2015)
[2] E. Pugliese et al., Advanced Materials, 30 (2018)



Characterization of endometriosis and comparison to endometrial cancer using Raman microspectroscopy

M. Weiss[1,2], T. Beyer[1], A. Hornberger[1], S.Y. Brucker[1], K.K. Rall[1], K. Schenke-Layland[1,3,4], E. Brauchle[1,3]
Presenting Author: Eva Brauchle, eva-maria.brauchle@uni-tuebingen.de
[1]Department of Women's Health, Research Institute for Women's Health, Eberhard-Karls-University Tübingen, Tübingen, Germany [2]Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany [3]Department of Medicine / Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA [4]Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB), Stuttgart, Germany

INTRODUCTION: Endometriosis is one of the most common gynecological diseases affecting millions of women worldwide and causing chronic pelvic pain and infertility. It is defined by the presence of endometrial epithelium and stroma outside the uterine cavity. The current gold standard for treatment is purely symptomatic and includes surgical removal of the affected tissue. Endometriosis is considered as a benign disease; however it shares features with cancer, such as neovascularization and metastatic cell behavior [1, 2]. In this study, we aimed to gain insight into the molecular composition of healthy endometrium, endometriosis and endometrial cancer by employing Raman microspectroscopy.

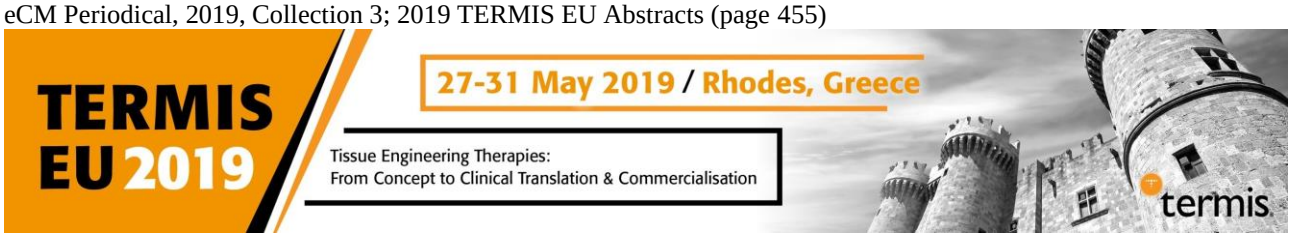
METHODS: Sections of normal endometrium (proliferative phase, n=3), endometriosis (deep infiltrating endometriosis, n=3) and endometrial cancer tissues (n=3) were investigated using Raman microspectroscopy. Raman spectra were collected from epithelial gland cells, their surrounding stromal cells and the associated extracellular matrix (ECM). Molecular profiles of epithelial cells and stroma were compared using multivariate analysis.

RESULTS& DISCUSSION: Raman spectra from epithelial cells in healthy endometrium and endometriosis tissues showed clearly distinct molecular profiles. Notably, glands in endometriosis tissues of different patients were highly heterogeneous in morphology and the obtained Raman spectra. Healthy and cancer tissues showed less patient-variability, indicating potential pathological subtypes in endometriosis. Raman microspectroscopy identified molecular similarities of epithelial gland cells in deep-infiltrating endometriosis with those in endometrial cancer. Moreover, multivariate analysis resolved disease-specific Raman spectral patterns from the ECM surrounding the endometrial glands. These findings indicate a potential role of the microenvironment in the pathogenesis of endometriosis.

CONCLUSIONS: Raman microspectroscopy is a suitable method to differentiate between healthy endometrium and endometriosis. Moreover, it identified similarities of endometriosis and endometrial cancer. This study demonstrates the potential of Raman microspectroscopy as a new tool for the marker-independent identification and characterization of endometriosis.

REFERENCES

[1] Ahn SH, Singh V, Tayade C. Biomarkers in endometriosis: challenges and opportunities. Fertility and Sterility. 2017;107(3):523-32
[2] Swiersz LM. Role of endometriosis in cancer and tumor development. Annals of the New York Academy of Sciences. 2002;955:281-92; discussion 93-5, 396-406.



Cytotoxicity studies of foamed calcium phosphate bone cements – Safe concentration of selected surfactants

E. Cichoń[1], B. Mielan[1], A. Zima[1], E. Pamuła[1], A. Ślósarczyk[1]
Presenting Author: Ewelina Cichoń, ecichon@agh.edu.pl
[1]Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Krakow, Poland

INTRODUCTION: Foamed calcium phosphate bone cements (fCPCs) constitute a new type of biomaterials. For their preparation as foaming agents usually surfactants are used. To our best knowledge the safe concentration of surfactants in the cement for cells has not been established yet. In this study we have determined non-cytotoxic concentration of three selected surfactants in contact with bone cells.

METHODS: FCPCs used in this study consisted of highly reactive alpha tricalcium phosphate (α-TCP) as the solid phase. α-TCP was obtained by the wet chemical method. The liquid phase for cement pastes preparation was 2 wt% Na_2HPO_4 with addition of 10 wt% of selected non-ionic surfactants: Tween 20 (TW20), Tween 80 (TW80) and Tetronic 90R4 (90R4). The cement without any surfactant served as a control (TCP). Liquid to powder weight ratio was 0.7. The obtained cements were sterilized with ethylene oxide and subjected to MG-63 cell studies. Extracts from the cements were prepared according to ISO 10993 with 1:10 sample to medium ratio. The series of extract dilution were prepared: 1 (undiluted), 2, 4, 8, 16, 32 times. Cells were seeded in 48-well plates at concentration of $1x10^4$ cells/well and after 24 h prepared extracts dilutions were added. Live/dead staining and Alamar Blue assays were done on days 1, 3 and 7.

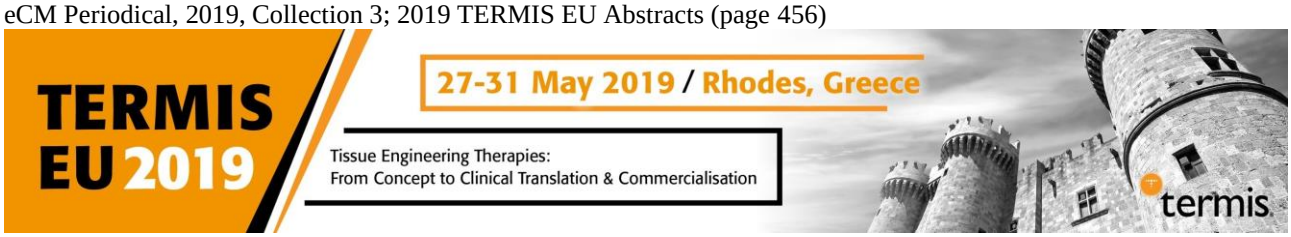
RESULTS & DISCUSSION: Viability of MG-63 cells by Alamar Blue reduction (%) on day 7 is shown in Fig. 1. Numbers (1, 2, 4, 8, 16, 32) stand for the extract dilution level.

CONCLUSIONS: Our results show that even the extract from TCP material without surfactants diluted by factor 16 resulted in decreased cell viability as compared to cells cultured in pure medium (Ctrl). The mechanism of „false cytotoxicity" of bioactive calcium phosphate ceramics of high surface area has been already explained [1]. TW20 was the most cytotoxic. Eight times diluted extracts from cements with the individual surfactant addition resulted in the same cell viability as compared to Ctrl. Taking this into account we can establish that 1.25 wt% addition of studied surfactants in liquid phase of a cement is a safe concentration for cells. Surfactants in such concentration in liquid phase provide sufficient foaming ability for preparation of fCPCs.

ACKNOWLEDGEMENTS: This study was supported by the National Science Centre, Poland (project no. 2017/27/N/ST8/00913) and by Polish National Agency for Academic Exchange – project no. PPI/PRO/2018/1/00026/U/001.

REFERENCES

[1] Klimek K (et al.) Mater Sci Eng C 2016; 65:70-79



Fluid flow supported angiogenic sprouting into granular hydrogels

F. Stein[1], V.D. Trikalitis[1], J. Rouwkema[1]
Presenting Author: Fabian Stein,mailto:diana.gaspar@nuigalway.ie f.stein@utwente.nl
[1]Department of Biomechanical Engineering, Technical Medical Centre, University of Twente, 7522NB Enschede, The Netherlands

INTRODUCTION: The unique physical properties of granular hydrogels making them an increasingly promising tool for the field of tissue engineering [1]. Depending on the hydrogel material used, it has been shown that granular hydrogels support cell survival, cell growth, and network formation of single cells and multicellular spheroids [2,3]. Herein, we analyzed the first time the effect of fluid flow in combination of the angiogenic growth factor $VEGF_{165}$ in granular hydrogels on vascular sprouting in a microfluidic system.

METHODS: A five-channel microfluidic plat-form was designed and fabricated, with a channel height of 100 µm. Human umbilical vein endothelial cells (HUVECs) were seeded in one of the two outer fluid flow channels at $6*10^6$ cells/mL to obtain a confluent monolayer. Collagen type I from rat tail (Sigma Aldrich) at a concentration of 6 mg/mL was used to create a barrier to entrap the granular hydrogel suspension made of agarose-collagen in the middle channel. The remaining second outer channel was used as a source of $VEGF_{165}$ in a concentration of 50 ng/mL to stimulate angiogenesis and proliferation. Cells were pre-labelled with the cell tracker CMFDA (Thermo Fisher Scientific) to allow for imaging of the formation of vascular sprouts into the granular hydrogels over a period of 7 days using confocal microscopy. Images were subsequently analysed using ImageJ. To characterize the diffusion of growth factors added into the microfluidic device through the solidified hydrogel and hydrogel suspension, diffusion experiment were performed using FITC-conjugated dextran.

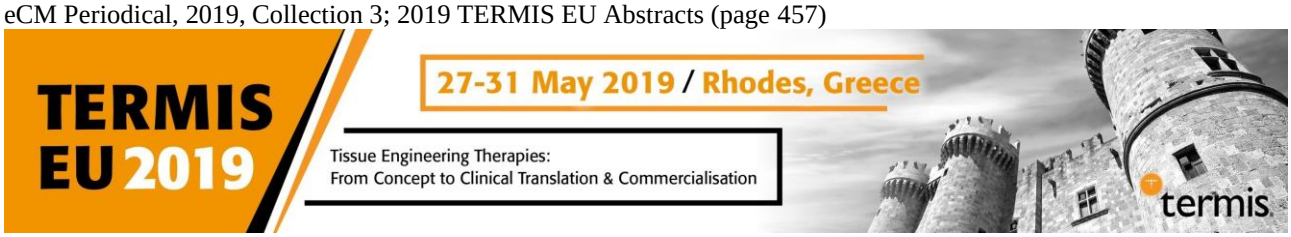
RESULTS & DISCUSSION: The results obtained showed that the granular hydrogels could indeed be isolated and stabilized in the central microfluidic channel by the incorporation of a collagen hydrogel barrier. Furthermore, by instituting a pressure difference over the two side channels, a fluid flow over the hydrogel compartment could be instituted. By addition of soluble exogenous angiogenic growth factor $VEGF_{165}$ an increased vessel growth into the granular hydrogels could be observed. The extent of vessel growth could further be controlled by varying the composition of the hydrogel suspension.

CONCLUSIONS: One of the main limitations in tissue engineering is the lack of a sufficient blood vessel system. By showing that granular hydrogels can facilitate the formation of a capillary bed in combination with fluid flow and gradients of growth factors, we have developed a promising platform to further investigate the creation of larger vascularized tissue constructs based on granular hydrogels.

ACKNOWLEDGEMENTS: This work is supported by an ERC Consolidator Grant under grant agreement no 724469.

REFERENCES

[1] Loebel C. et al. Nat Protoc 2017; 12: 1521– 1541
[2] Trikalitis V. D et al. 5th TERMIS World Congress 2018, Kyoto, Japan
[3] Sideris E. et al. 2016. doi:10.1021/acsbiomaterials.6b00444.



Yap silencing in synovial MSCs using gapmer/$CaCO_3$ microparticles

F. Colella[1*], M. Sorbona[2*], K. Howard[3], Y. Cai[3], A. Roelofs[1], M.M. Stevens[2], C. De Bari[1]
Presenting Author: Fabio Colella, fabio.colella@abdn.ac.uk
[1]Arthitis and Regenerative Medicine Laboratory, Aberdeen Centre for Arthritis and Musculoskeletal Health, Institute of Medical Sciences, University of Aberdeen, United Kingdom [2]Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London, United Kingdom [3]Interdisciplinary NanoScience Centrer (iNANO), Department of Molecular Biology and Genetics, Aarhus University, Denmark. [*] The authors contributed equally

INTRODUCTION: Over the last years, the transcriptional co-factor Yes-associated protein (Yap) has gained increasing interest as a potential therapeutic target for the treatment of osteoarthritis, a very common and debilitating joint disease that affects 9 million people in the UK only [1][2]. Here, anti Yap gapmers (5 kDa), single stranded RNAi oligos able to be self-internalized by cells, were designed and encapsulated for controlled release. The delivery of small compounds (<1 MDa) within the joint cavity is strongly impaired by their rapid clearance (1-24 hours), in contrast to bigger ones (>200 nM) with longer joint-residence times (2-35 days) [3]. Therefore, to increase retention and obtain sustained delivery of gapmers, we developed $CaCO_3$ microparticles for gapmer encapsulation and release.

METHODS: A co-precipitation method was used to encapsulate 60 µg of gapmers into 0.65 mg of $CaCO_3$ microparticles. Microparticles size was determined by transmission electron microscopy. Encapsulation and release were quantified by spectrophotometry. To test silencing efficiency, the media of primary synovial MSCs was replaced daily and incubated with the released gapmers from $CaCO_3$ microparticles.

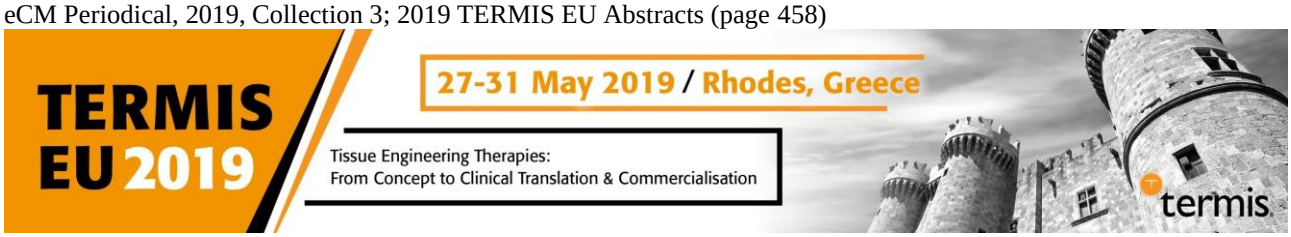
RESULTS & DISCUSSION: After gapmer/$CaCO_3$ microparticles formation, a size of 3.03±0.38 µm (mean + SD, n=3) and an encapsulation efficiency of 96.4%±1 (mean + SD, n=3) were obtained. Release studies highlighted an initial burst release of 41.3% of the cargo within the first 24 hours and a continuous release of an additional 18.8% over 7 days. Silencing studies led to reduced Yap mRNA expression of 61%±9 and 36%±22 (mean + SD, n=3) after 2 and 4 days, respectively.

CONCLUSIONS: Collectively, this work introduces a novel gapmer deliver strategy with favorable size properties for joint retention and sustained release.

ACKNOWLEDGEMENTS: The work was supported by the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska Curie grant agreement No 642414.

REFERENCES

[1] Roelofs A et al. Nat. Commun. 2017;8:15040
[2] Gong Y et al. J. Mol. Med. 2018;
[3] Di Domenico C et al. Nat. Rev. Rheumatol. 2018; 14:393-403.



Hydrogel stiffness regulates the response of mesenchymal stem cells to dynamic loading

F. Chariyev-Prinz[1,2], D.J. Kelly [1,2]
Presenting Author: Farhad Chariyev-Prinz, chariyef@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland [2]Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Ireland

INTRODUCTION There is increasing clear that mechanical loading plays a key role in regulating the differentiation of mesenchymal stem cells (MSCs) into matrix producing chondrocytes [1, 2]. The aim of this study was to optimize the properties of fibrin hydrogels for tissue engineering (TE) and mechanobiology studies. Subsequently, the response of MSCs embedded in soft and stiff fibrin hydrogels to dynamic compression (DC) was investigated in the absence of any specific chondrogenic growth factor.

METHODS: MSCs were encapsulated in soft and stiff fibrin hydrogels (fibrinogen: 50 mg/ml in aprotinin-$NaCl_2$ solution; thrombin: 2.5 U/ml in DMEM with 1 mM $CaCl_2$ (soft) or 20 mM $CaCl_2$ (stiff) using silicon moulds and polymerized at 37 °C for 40 min. The elastic modulus of the fibrin hydrogels was determined using an unconfined compression test. The obtained cylindrical hydrogel constructs were subjected to DC for 2 weeks (2h/day; 5days/week; 1 Hz; 10% strain; 37 °C; 5% O_2; 5% CO_2) and the expression of chondrogenic marker genes SOX9 and aggrecan (ACAN) was examined.

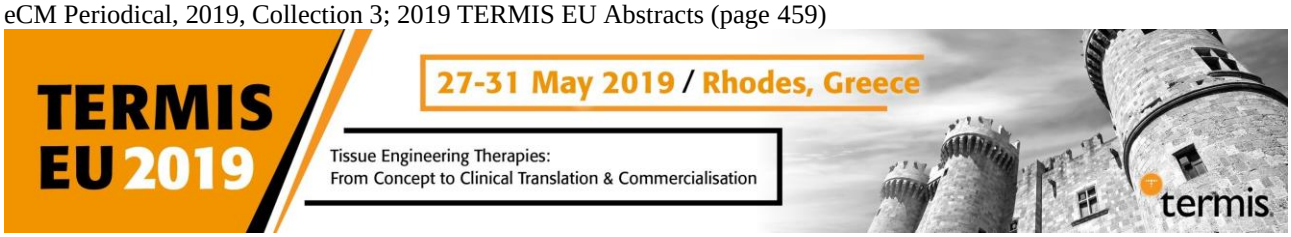
RESULTS & DISCUSSION: hMSCs were encapsulated in **s**oft (≈21.9 kPa) and stiff (≈30.6 kPa) hydrogels and loaded for two weeks in the absence of specific chondrogenic growth factors. RT-qPCR indicated an increase in SOX9 after one ($p=0.015$) and two weeks ($p=0.0052$) in DC samples in stiffer gels. DC had no significant effect on the expression of chondrogenic genes within the softer hydrogels. In fact, DC was found to reduce the expression of ACAN in soft fibrin hydrogels.

CONCLUSIONS: The significant increase of the chondrogenic transcription factor SOX9 in stiffer gels upon the application of DC indicates the importance of hydrogel properties in determining the cellular response to such extrinsic mechanical cues. Hence it is crucial to consider stem cell mechanobiology when designing hydrogels for cartilage TE.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 721432.

REFERENCES

[1] Thorpe et al. J. Biomech. 2012; 45: 2483-2492
[2] Steward et al. Eur. Cell. Mater. 2013, 25: 167-178



Ready-to-use regenerative medicinal product: Universal human platelet rich plasma

F.E. Unuvar[1], R. Vural[1], I.P. Eyuboglu[2], N.B. Pelit[1], E. Ovali[1]
Presenting Author: Fatma Eyuboglu Unuvar, fteyuboglu@gmail.com
[1]Acibadem Labcell Cell Laboratory [2]Dep. of Medical Bio., Sch. of Med., Marmara University, Turkey

INTRODUCTION: Platelet-Rich Plasma (PRP) is a concentrated form of platelets in plasma. Growth factors (GF) with rich content of chemokine and cytokines can stimulate cell chemotaxis, proliferation, differentiation, angiogenesis and production of extracellular matrix. Thanks to these properties, it is used to support tissue regeneration in many different clinical areas [1-3]. Nowadays, PRP used for this purpose is prepared just as autolog and produced before application. Therefore, quality, content and effectiveness of product vary by the manufacturer, the current blood values of the donor or the method used. The aim of this study is to investigate the most suitable method for universal PRP product which is ready to use, standard, sterile, safe, and concentrated.

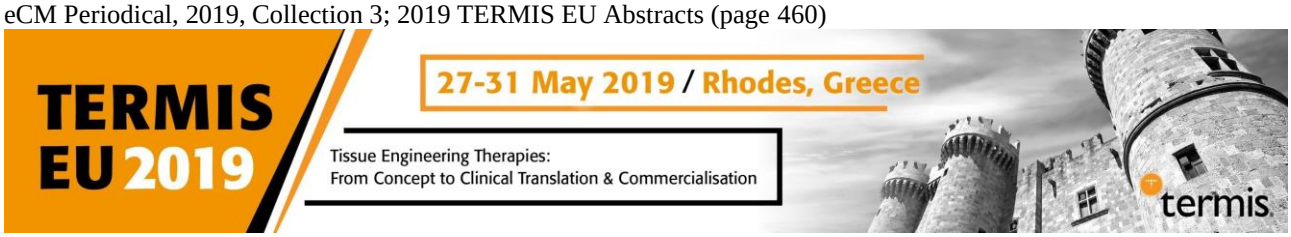
METHODS: Three different male volunteers at the ages of 21, 27 and 28 were treated with thrombocyte apheresis. After the obtained platelet suspension (PRP) was irradiated, some parts were directly frozen and some were stored at -20° C after lyophilization. With cell samples, pH, osmolarity, Eliza, GF content measurements (EGF, VEGF, PDGF) and efficacy analysis (MSC Proliferation Assay) were performed from fresh samples and samples thawed at 1st, 3rd and 12th months after storage. The assessments were done with the average of the independent 3 donors.

RESULTS & DISCUSSION: Compared to fresh PRP, no negative effect of irradiation has been detected on cell number, osmolarity, pH and GF release. The number of cells in the lyophilized group decreased significantly ($p<0,05$). The groups stored in lyophilized form were found to retain the GF concentration better than the freeze-conserved groups. In the 12th month, no change in the pH measurements was observed ($p>0,05$), but the osmolarity was lower ($p<0,05$). In the MSC proliferation test compared to fresh PRP, it was observed that in the lyophilized group there was found no significant difference ($p>0,05$).

CONCLUSIONS: The data obtained shows that PRP collected by platelet apheresis preserve its efficiency for 3 months after irradiation and lyophilization and with this way it can be used as a ready for use, safe, effective and universal product. The low osmolarity values measured due to cell loss in the lyophilized PRP groups will reach fresh PRP value by optimizing cell concentration with taking into account the amount of loss. The study will be developed at an advanced level by increasing the number of samples, adding different GF/cytokine assays, and supported by diagnostic-based in vivo studies.

REFERENCES

[1] Marmotti A. et al. BioMed Res. Int. 2015, 542502,
[2] M. D. Lynch & S. Bashir J Derm. Treat, 2016; 27(3): 285–289
[3] Yuan T. et al. Current Phar. Biotec., 2012, 13, 1173-1184



The effect of BMP signaling on neural and keratinocyte differentiation of placenta derived cells

F. Biniazan[*1], A. Manzari-Tavakoli *[2], F. Safaeinejad*[3], A. Moghimi[2], F. Rajaei[1], H. Niknejad[3]
Presenting Author: Felor Biniazan, felorbiniazan@yahoo.com
[1]Cellular and Molecular Research Center, Qazvin University of Medical Science, Qazvin, Iran [2]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Iran [3]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran.
*Co-first author

INTRODUCTION: Stem cell therapy has a great promise for the treatment of various diseases. Placenta derived stem cells have desirable and unique characteristics that make them highlighted among the other stem cells and attractive for cell therapy [1]. The aim of this research was to evaluate the effect of BMP and its antagonist, noggin, on neural and keratinocyte differentiation.

METHODS: The amnion layer was mechanically peeled off from the chorion and human amniotic epithelial cells were isolated with trypsin-EDTA in 37°C. Then, cells were exposured by differentiation medium include BMP-4 (25 and 50 ng/ml), noggin (50 and 100 ng/ml), heparin (1ul/ml). After 21 days, cells were fixed and characteristic analysis of AEC-derived ectodermal (neurons and keratinocytes) was performed by flow cytometry. The following primary antibodies were applied for flow cytometer: Map2 (neuron marker), Olig2 (oligodendrocyte marker), K14 (keratinocyte marker).

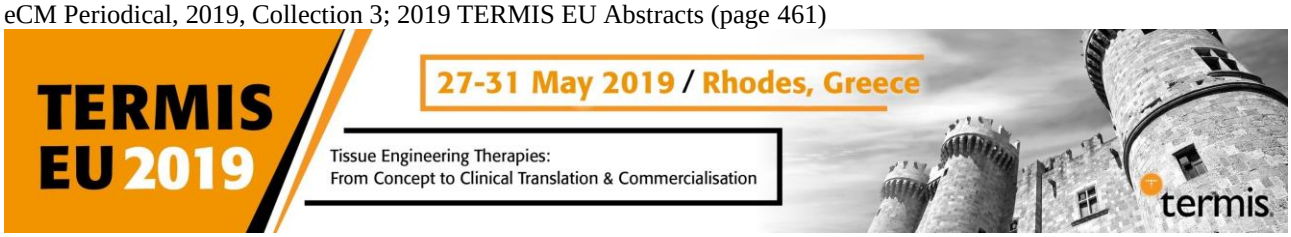
RESULTS & DISCUSSION: These results showed that percent of MAP2 expression was 74.07± 1.8 for 50 ng noggin, Olig2 expression 60.8 ± % 0.7 for 25ng/ml BMP-4 and k14 expression 64.45% ± 0.5 for heparin group compared with control group.

CONCLUSIONS: The results of this study illustrated the AECs have capability to differentiate into neuronal, glial and keratinocyte cells by noggin, BMP4 and Heparin, respectivel

ACKNOWLEDGEMENTS: The authors would like to thank the operation room personal of Erfan hospital for their assistance in this research. Research reported in this publication was supported by Elite Research Grant Committee from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Niknejad et al. Neuroscience Letters. 2012; 506 (1); 22–27.



A fully automated pipeline for the large-scale production of mesenchymal stem cells (MSC)

F. Biermann[1], G. Shaw[2], J. Ochs[1], S. Jung[1], N. König[1], N. Duffy[2], M. Murphy[2], R. Schmitt[3]
Presenting Author: Ferdinand Biermann, ferdinand.biermann@ipt.fraunhofer.de
[1]Fraunhofer Institute for Production Technology IPT, Aachen, Germany [2]National Centre for Biomedical Engineering Science (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland [3]Laboratory for Machine Tools and Production Engineering (WZL), RWTH Aachen, Germany

INTRODUCTION: The AUTOSTEM platform is a pipeline to fully automate all steps necessary for producing stem cells on a multi litre scale, including cell expansion, formulation, and cryopreservation.

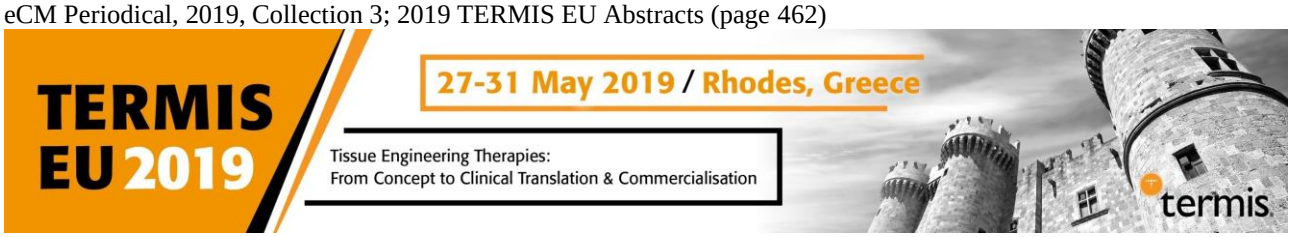
MOTIVATION: Mesenchymal stem cells (MSC) are a promising therapeutic agent in the field of regenerative medicine. They can be isolated from a variety of tissues and are in advanced clinical testing for a number of clinical indications. The manual production of MSCs currently depends largely on manual labour and is furthermore highly sensitive to production variations and errors. In order to increase the capacity for production and the reproducibility, the robotic platform AUTOSTEM has been developed.

METHODS: The biological process of cultivating MSCs covers extracting the cells from bone marrow, growing them in a bioreactor, performing frequent cell counts, and finally transferring the cells into cryo-tubes for freezing. In the AUTOSTEM platform, all processes after cell extraction are executed automatically while being GMP-ready. Therefore, medical and biological devices, like bioreactors, a cell counter, and a centrifuge, have been linked to a central control system responsible for an adaptive process control. Furthermore, devices like a pumping station, a sampling station, decappers, and an automated pipette have been developed especially for AUTOSTEM. Two robots transport cells and material from one station to another. In order to provide a safe environment for the different production steps, the platform consists of different biosafety levels. Cells can be transferred from grade A to D through a hatch and from grade D to A trough an innovative fluid transferring device.

RESULTS & DISCUSSION: The automated handling functions for cells and materials as well as the sterility of the bioreactor have been successfully tested. Furthermore, it has been shown that the bioreactors can produce cells. The characterisation of the cell products was undertaken and assay results showed the quality of the MSC product to be comparable to cells grown on microcarriers in spinner units or those grown in traditional T flasks in terms of surface marker expression and differentiation potential.

CONCLUSIONS: Further optimizations are planned for AUTOSTEM. Next steps to clinical application are the demonstration of full GMP compliance as well as further improvements of several modules. There is potential to adapt the AUTOSTEM platform for the automated production of other cell types.

ACKNOWLEDGEMENTS: This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 667932.



Immunomodulatory hyaluronic acid gels for cell encapsulation strategies

F. Zamboni[1,2], E. Ryan [2], K. McGourty[1,2], M.N. Collins[1,2]
Presenting Author: Fernanda Zamboni, fernanda.zamboni@ul.ie
[1]Bernal Institute, University of Limerick, Limerick, Ireland [2]Health Research Institute, University of Limerick, Limerick, Ireland

INTRODUCTION: Hyaluronic acid (HA) is a polysaccharide found in the extra cellular matrix (ECM) of various tissues in the body [1]. One of the most important biological roles of HA is linked to its immunomodulatory properties, which depend on HA molecular size [2]. For this reason, HA is a material of increasing significance to biomaterials science and it is finding applications in a wide range of areas, including drug delivery and regenerative medicine. However, since HA is readily degraded in physiological conditions due to hydrolysis and enzymatic degradation, chemical modification is necessary in order to increase HA structural stability. In this project, HA was successfully crosslinked using a new biocompatible di-isocyanate crosslinker. In order to verify the biocompatibility and the immune response triggered by these novel gels, L929 fibroblast cell line originated from mice are used as a first line of testing.

METHODS: A range of samples was produced utilizing HA with 0.1 MDa (low molecular weight - LMW) and with 1.2 MDa (high molecular weight - HMW). Heterogeneously crosslinked HA films (7% wt/v) using 200 mg/mL of crosslinker were selected for proliferation and immune response tests (Figure 1). Proliferation was assessed using Alamar Blue metabolic activity assay. L929 fibroblasts secrete macrophage colony-stimulating factor (GM-CSF), a cytokine that induce macrophage differentiation as part of the immune response chain. Upon culturing L929 cells on our novel HA gels, GM-CSF is measured at different time intervals by taking aliquots of media using an ELISA kit.

CONCLUSIONS: LMW HA gels show decreased proliferation rates than HMW gels, which correlates with immune activation and GM-CSF cytokine production. Thus, it is envisaged that LMW HA gels (0.1 MDa) due to its pro-inflammatory characteristics can be exploited for the delivery of vaccines, in order to further instigate the immunisation and recruitment of immune cells. Moreover, the HMW HA gels (1.2 MDa) can be used for cell encapsulation in order to decrease the immune rejection of transplanted cells.

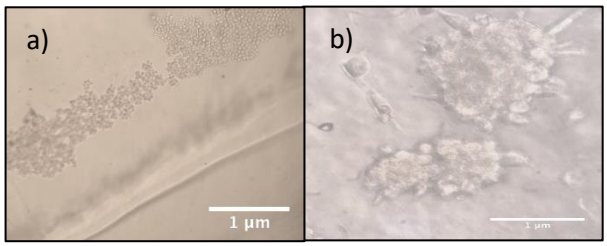


Figure 1: Bright field images of 1.2 MDa gels at 4 hours (a) and 48 hours (b) after L929 cell seeding.

ACKNOWLEDGEMENTS: Irish Research Council (GOIPG/2015/3577)

REFERENCES

[1] Zamboni F et al., International Journal of Pharmaceutics. 2017; (521):346.
[2] Zamboni F et al. Progress in Materials Science. 2018; (97):25

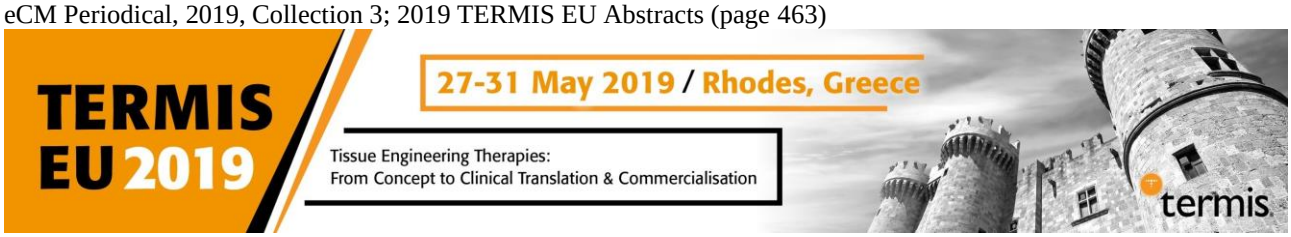


Photo-crosslinkable mono-component elastin-like recombinamer bioink for 3D printing

F. González-Pérez[1], M. Alonso[1], J.C. Rodríguez-Cabello[1]
Presenting Author: Fernando González-Pérez, fgonzalez@bioforge.uva.es
[1]G.I.R. BIOFORGE, Edificio Lucia, Universidad de Valladolid CIBER-BBN, Paseo Belén 19, 47011 Valladolid, Spain

INTRODUCTION: 3D bioprinting is a technology that allows the creation of stable-high-defined structures containing cells mimicking the biological environments [1]. Its applications range from ex vivo models for drug design and cancer studies to the development of organoids and clinical translation. Proper bioinks must be capable of maintaining the depicted structure without losing fidelity offering simultaneously an adhesive and biomimetic extracellular matrix (ECM). Herein a novel photo-crosslinkable mono-component Elastin-Like Recombinamer (ELR) bioink was developed. Concentration and temperature printing conditions were optimized as well. ELRs were characterized in terms of viscosity, dynamics modulus and intern cross-sections. Citocompatibility was evaluated by proliferation and viability studies in presence of fibroblasts.

METHODS: Genetically engineering Elastin-Like Recombinamer (ELR) bearing a silk domain ([(VPGKG)48-(IPGVG)60-(GAGAGS)5]2-RGD6) was biosynthesize in Escherichia Coli bacteria. The purified recombinamer was modified with methacrylamide (MA) by an amidation reaction using the ε-amino groups present in the lysine aas. ELR-MA was dissolved in PBS at different concentrations conducting printing tests at temperatures over and below transition temperature, exposing UV during printing with an extrudable printer. Deposition of several layers was studied. Bioink viscosity and dynamic modulus of photo-crosslinked structures at selected concentration was measure. Internal porous cross-sections were characterized by SEM. Proliferation and viability of printed patterns loaded with fibroblasts were studied by Life/Dead and Alamar blue assays.

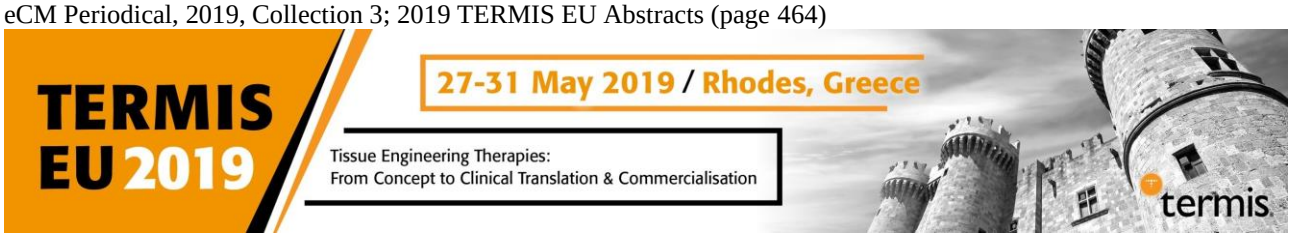
RESULTS & DISCUSSION: ELR-MA at best temperature (14ºC) and concentration (190mg/mL) conditions exhibited great printability (~1) in small mesh constructs (1.5mm pore size). Bioink viscosity at selected concentration was high (10Pa·s), showing a non-Newtonian liquid behavior decreasing viscosity with %shear rate allowing better extrudability. Dynamic modulus of photo-crosslinked structures displayed hydrogel characteristic G´>>G``. SEM analysis showed an intern porosity of (~8um). RGD bioactive sequences included in hydrogel ensured fibroblasts adhesion, viability and proliferation studied by Life/Dead and Alamar blue assays.

CONCLUSIONS: These results show the potential of an ELR-MA photo-crosslinkable hydrogel for the 3D bioprinting of structures in the field of tissue-engineering applications.

ACKNOWLEDGEMENTS: Financial support was received from MINECO (PCIN-2015-010, MAT2016-78903-R, FPU 16/04015), Junta de Castilla y León (VA317P18) and Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León.

REFERENCES

[1] Sean V Murphy & Anthony Atala. 2014; 32(8):773–785



Dual enzyme-responsive smart-ELRs for switchable catalytic activity

F. Cipriani[1], M.G. Pérez[2], I.G. de la Torre[1], M.A. Rodrigo[1,2], J.C. Rodríguez Cabello[1,2]
Presenting Author: Filippo Cipriani, fcipriani@tpnbt.com
[1]Technical Proteins Nanobiotechnology, S.L. Parque Cientifico Universidad de Valladolid, Spain
[2]BIOFORGE Lab, CIBER-BBN, Universidad de Valladolid, Valladolid, Spain

INTRODUCTION: Smart materials have gained widespread interest in the material science, they have been used in a large variety of applications [1]. Elastin-like Recombinamers (ELRs) show extraordinary properties in numerous biomedical fields [2]. Moreover it has been demonstrated that the Inverse Temperature transition (T_t) which, is a typical feature of ELRs, can be modulated by the addiction and/or the removal of chemical groups such as phosphate groups [3].

METHODS: Recombinant DNA techniques were used for the synthesis of smart-ELRs containing kinase consensus sequences and RNAse A sequence. A set of smart-ELRs were designed, expressed and bioproduced in E. coli. The ELRs were composed by the inclusion of 10 consensus sequence phosphorylation sites regularly distributed along the (VPGIG)n chain. The phosphorylation was performed by cAMP - dependent protein kinase (PKA) catalytic subunit, whereas de-phosphorylation was performed by alkaline phosphatase Calf Intestinal (CIP). In order to verify the effectiveness of the phosphorylation and to quantify the number of phosphate groups transferred and removed from and to the smart-ELRs, the samples were analyzed by HR-Massa and the T_t was characterized by turbidity analysis. Finally, the RNAse catalytic activity was monitored following a RNAse quantification method [4].

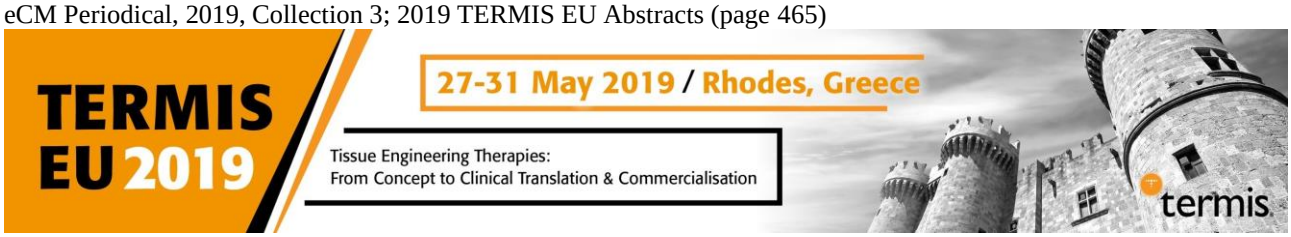
RESULTS & DISCUSSION: All the smart-ELRs were subjected to (de-)phosphorylation reactions. In all cases, the Mass spectrometry showed a difference in molecular weight of 796 Da that corresponds exactly to 10 phosphate groups. The turbidity analysis revealed an evident shift in T_t (around 12 °C) between the phosphorylated and the de-phosphorylated ELRs. The Mass spectrometry and the turbidity analysis revealed a completely reversibility of the reaction. At mild conditions the de-phosphorylated ELRs formed aggregates, contrary to the phosphorylated ones. The RNAse activity assay revealed a modular RNAse catalytic activity depending from the smart-ELRs state.

CONCLUSIONS:
A new smart-ELR having a dual enzyme-responsiveness was developed. The selective (de-)phosphorylation response of the serine residues modulate the smart-ELR state, with a consequent change for the RNAse catalytic activity.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 642687.

REFERENCES

[1] Furth, M.E., et al., Biomaterials, 2007. 28(34): p. 5068-5073.
[2] Rodriguez-Cabello, J. C. et al., Nanomedicine 6, 111−122 (2011).
[3] Pattanaik A. et al., Biochemical and Biophysical Research Communications 178, 539-545 (1991).
[4] Greiner-Stoeffele, T., et al., Anal Biochem, 1996. 240(1): p. 24-8.



Bioprinting silk hydrogels using two-photon stereolithography regulates the biomaterial properties and the cell behaviour

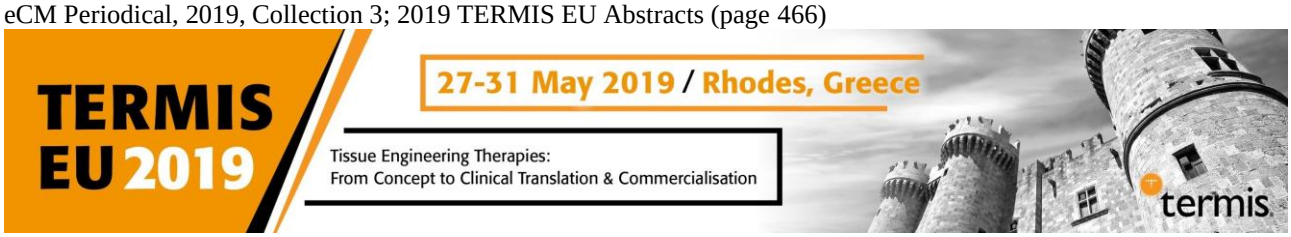
F. Valente[1], J. Chen[2], M. Hepburn[3], B. Allardyce[2], B. Kennedy[3], S. Redmond[1] and R. Dilley[1, 4]
Presenting author: Filippo Valente, filippo.valente@earscience.org.au
[1]Ear Science Institute Australia; Ear Sciences Centre, School of Medicine, University of Western Australia, Nedlands, Australia [2]Deakin University, Institute for Frontier Materials, Geelong, Australia [3]BRITElab, Harry Perkins Institute of Medical Research, Nedlands, Australia [4]Centre for Cell Therapy and Regenerative Medicine, School of Biomedical Sciences, University of Western Australia, Nedlands Australia

INTRODUCTION: Bioprinting using micro-stereolithography offers advantages in printing resolution and viability rate of the encapsulated cells. In this work we demonstrate that by regulating the printing conditions, it is possible to generate different hydrogels starting from a single bioink solution, and the resulting bioprinted environment influences the cell proliferation.

METHODS: Silk fibroin solution including high-density human dermal fibroblasts was bioprinted into a solid hydrogel using a ruthenium-based cross-linked method and two-photon infrared laser polymerization. The resulting hydrogels were analyzed by SEM, FTIR and optical coherence elastography. The cells included in the hydrogels were cultured in vitro and their viability and proliferation rate were assessed up to four weeks using a live-dead assay.

RESULTS & DISCUSSION: tuning the intensity of the two-photon infrared laser it was possible to regulate the mechanical properties, cross-link percentage and microstructure of the resulting hydrogels. Cells were observed to engraft within the hydrogel in a 3-dimensional way during a 4 weeks' timeframe and proliferated from it, with a different rate depending on the hydrogel stiffness and cross-linking percentage.

CONCLUSIONS: This approach could be used to produce patterned silk biomaterials, capable of influence the cell behavior and proliferation, for soft tissue engineering and cell delivery.



Biofabrication of multiscale bone ECM scaffolds to support vascularization and osteogenesis

F.E. Freeman[1,2], D.C. Browe[1,2], J. Nulty[1,2], S. Von Euw[1,2], W.L. Grayson[3], D.J. Kelly[1,2]
Presenting Author: Fiona Freeman, freemanf@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, TCD, Ireland [2]Department of Mechanical and Manufacturing Engineering, School of Engineering, TCD, Ireland [3]Department of Biomedical Engineering, Translational Tissue Engineering Centre, Johns Hopkins University School of Medicine, Baltimore, MD, USA

INTRODUCTION: Interconnected porosity is critical to the design of regenerative scaffolds, as it permits cell migration, vascularization and the diffusion of nutrients and regulatory molecules to the inner parts of the scaffold. 3D printing is a promising strategy to achieve this as it allows for control over scaffold pore size, porosity and interconnectivity. The aim of this study was to integrate distinct biofabrication strategies to develop multiscale bone ECM scaffolds that were not only mechanically functional at the time of implantation, but which also supported rapid vascularization and osteogenesis of recruited stem cells. To this end, 3D printing was used to control the macro-porosity of bone ECM functionalized scaffolds, while controlled micro-porosity was introduced by freeze-drying solubilized bone ECM within these 3D printed scaffolds.

METHODS: Decellularised bone (DCB) extracellular matrix (ECM) was generated as previously described [1]. 30% (w/w) DCB was added to PCL to generate a composite thermopolymer. Scaffolds of Ø 4mm x 4mm high, a fiber spacing of either 0.8 mm (70% porosity) or 1.2mm (80% porosity) were printed with PCL+DCB (macroscale porosity). Using the DCB a solubilised bone ECM (sbECM) was generated using pepsin and acetic acid. The sbECM was lyophilized in between the printed filaments of the PCL+DCB scaffold (multiscale porosity). Porcine MSCs ($1x10^6$) were seeded on scaffolds 24 hours prior to subcutaneous implantation into nude mice. Microct (µCT) and quantitative histological analysis were assessed at 2 and 8 weeks.

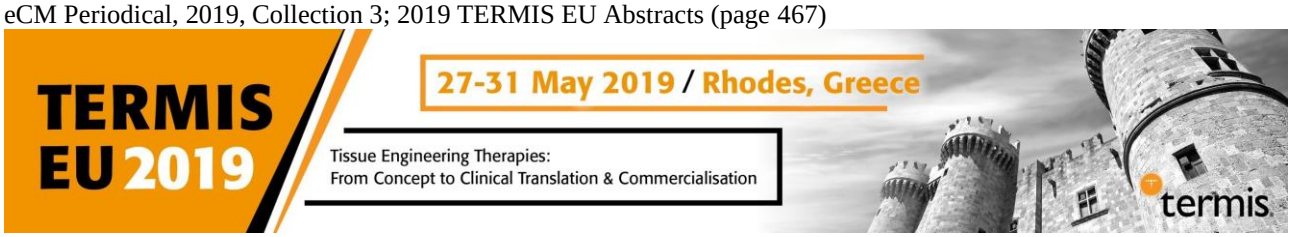
RESULTS & DISCUSSION: For the macroscale porous scaffolds, a larger fiber spacing/porosity (80%) was found to accelerate vascularization compared to a smaller fiber spacing/porosity (70%). Furthermore, there was significantly more vessels present in the centre of the multiscale porous scaffold compared to the macroscale porous scaffold after 2 weeks of implantation

CONCLUSIONS: We have developed a bone ECM derived scaffold that is mechanically competent and appropriately porous at multiple scales, thereby enhancing vessel infiltration and bone formation in vivo.

ACKNOWLEDGEMENTS: Funding from ERC grant 647004 & the IRC grant GOIPD/2016/324.

REFERENCES

[1] Nyberg, E., et al. Tissue Engineering Part A. 2017.



Use of tannic acid as a versatile tool for surface modification in tissue engineering

F. Reitzer[1,2], L. Ploux[1], V. Ball[1], F. Meyer[1,2]
Presenting Author: Florent Meyer, fmeyer@unistra.fr
[1]Université de Strasbourg, INSERM UMR_S 1121 Biomaterials and Bioengineering, Strasbourg, France [2]Hôpitaux universitaires de Strasbourg, Strasbourg, France

INTRODUCTION: Tannic acid (TA), a polyphenol, gathers several biological activities that are of particular interest in tissue engineering. Amongst them antimicrobial and antioxidative properties are the most cited [1]. It could be an interesting choice as well for biomaterial shaping or cell adhesion promotion due to its interaction with proteins [2-3]. Herein, we ventured to assess the influence of TA as surface coating agent on the adhesion, migration and survival of both fibroblasts and bacteria.

METHODS: Glass coverslips were coated with poly(2-hydroxyethyl methacrylate) (poly-HEMA)) doped with various concentration of TA (0, 0,0078, 0,0625, 0,125, 0,25, 0,5, 1 mg/mL). NIH 3T3, mouse fibroblasts, were seeded at 25,000 cells/cm^2 on such glass coverslips in 24 well plates and were allowed to attach for 4 h. Subsequently, the glass coverslips were transferred in a new 24 well plate to insure to discard none-attached cells. Cell adhesion, proliferation and migration were subsequently studied. Aside the same surfaces were challenged for antimicrobial activity as well as biofilm formation on Escherichia coli and Enterococcus faecalis.

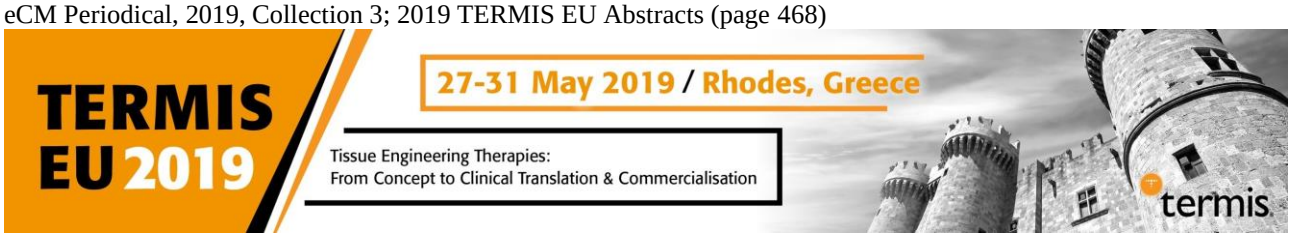
RESULTS & DISCUSSION: NIH 3T3 adhesion is directly correlated to the concentration of TA incorporated in the poly-HEMA up to 0,25 mg/mL. Above, no cell adhesion is recorded. Cell adhesion is identical to a control surface with a number of focal point comparable. Adhesion is driven by direct interaction between integrin and tannic acid as demonstrated by adhesion test with culture medium without protein or surface modification by sodium periodate treatment. However, no substantial modification of the hydrophilicity was recorded by contact angle measurement whatever the concentration of tannic. Surface antimicrobial activity of TA is conserved once mixed to poly-HEMA, but under a threshold concentration (MIC = 100 ug/mL) bacterial adhesion is favored.

CONCLUSIONS: TA represents a versatile tool for surface coating that can favor eukaryotic cell adhesion and migration and, in the meantime, displays antimicrobial activity. However, the concentration must be tuned as over a threshold concentration, tannic acid is toxic for eukaryotic cell and above can favor bacterial adhesion and growth.

ACKNOWLEDGEMENTS: Financial support was received from “Institut Carnot” MICA, France (ElectrATPulpe project).

REFERENCES

[1] Reitzer F et al. Adv Colloid Interface Sci. 2018; 257:31-41
[2] Allais M et al. J Colloid Interface Sci. 2018; 512:722-729
[3] Guo J et al. Acta Biomater. 2018; 72:35-44



Evaluation of extracellular matrix produced during osteogenic differentiation of human bone marrow-derived mesenchymal stem cells in chitosan/gelatin scaffolds

F. Papadogiannis[1,2], A. Batsali[2], A. Georgopoulou[1], C. Pontikoglou[2], M. Chatzinikolaidou[1,3]
Presenting Author: Fotios Papadogiannis, fotis4393@gmail.com
[1]Department of Materials Science and Technology, University of Crete, Heraklion, Greece [2]Haemopoiesis Research Laboratory, Department of Haematology, School of Medicine, University of Crete, Heraklion, Greece [3]Institute of Electronic Structure and Laser, Foundation for Research & Technology Hellas, Heraklion, Greece

INTRODUCTION: We have previously shown that 3D porous scaffolds comprising of 40:60% chitosan/gelatin (CS/Gel) crosslinked with 0.1% v/v glutaraldehyde, promote the survival, proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells (hBM-MSCs) [1]. Herein we evaluate the production and composition of extracellular matrix (ECM) derived from hBM-MSCs following osteoblastic differentiation in CS/Gel scaffolds. Results were compared to respective hBM-MSC cultures in tissue culture polystyrene (TCPS) controls.

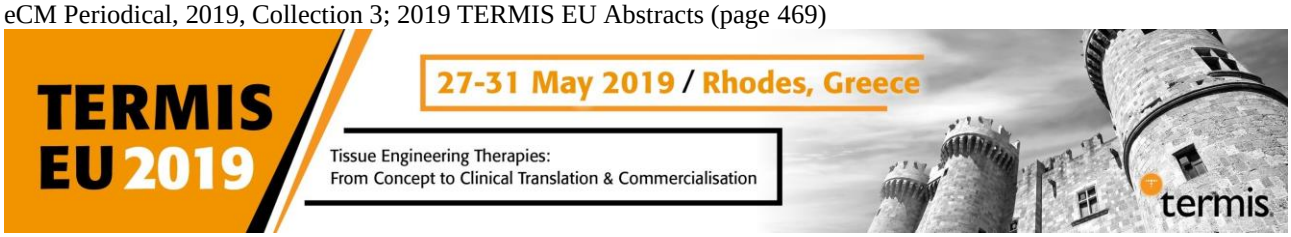
METHODS: hBM-MSCs were isolated from BM aspirates of healthy donors (n=6) after informed consent. The cells were ex vivo expanded until passage (P)-2, phenotypically characterized by flow cytometry and differentiated towards adipocytes and osteoblasts. Differentiation was assessed by specific mRNA gene expression and immunostaining. P2 hBM-MSCs were seeded on CS/Gel scaffolds and induced towards osteoblasts. We then assessed (a) hBM-MSCs adhesion potential by electron microscopy, (b) their osteogenic potential by mRNA expression of RUNX-2, DLX-5, OSC and ALP using real time-RT PCR, (c) the expression of ECM related genes using a PCR array and (d) the endogenous expression of osteopontin, osteocalcin and collagen type I A1 proteins using confocal laser fluorescence microscopy (CLFM).

RESULTS & DISCUSSION: hBM-MSCs were expanded successfully and fulfilled the minimal criteria for MSC definition (spindle-shape morphology, expression of CD90, CD105, CD29, CD73, lack of CD45, CD14, CD34 and ability to differentiate into adipocytes and osteocytes). Cultured hBM-MSCs on CS/Gel scaffolds showed strong adhesion potential and displayed increased osteogenic capacity as shown by the increased expression of ALP and OSC at days 7 and 14 compared to the TCPS cultures and the increased expression of RUNX2 and DLX5 time course during the differentiation. PCR array analysis showed differential expression of ECM-related genes between hBM-MSC cultures differentiated towards osteoblasts in CS/Gel scaffolds and TCPS controls. In specific, we found increased MMP1, MMP3, MMP9-13, MMP16, and VTN and decreased COL1A1 COL5A1, SPP1, THBS1-2, TIMP1-3 mRNA expression in CS/Gel scaffolds compared to TCPS. CLFM images showed collagen type I A1 and osteopontin production in both CS/Gel scaffolds and TCPS; osteocalcin, as a late marker of osteogenic differentiation, was identified at low levels in both CS/Gel scaffold and TCPS.

CONCLUSIONS: We have evaluated for the first time the ECM production by hBM-MSCs cultured on 40:60% CS/Gel scaffolds providing additional evidence that this biomaterial supports osteogenesis. Our results present a promising strategy using hBM-MSCs within CS/Gel scaffolds for bone regeneration.

REFERENCES

[1] Georgopoulou A et al. J of Mater Sci:Mater in Medicine. 2018;29:59



Cycling fibroblasts through iPS-reprogramming and redifferentiating them into fibroblasts enhances their matrix production in 2D and 3D

F. Santarella[1], B. Cavanagh[1], R.J.F.C.D. Amaral[1], M. Marinkovic[1], R. Sridharan[1], A. Smith[2], F.J. O'Brien[1], J. Garlick[2], C.J. Kearney[1]
Presenting Author: Francesco Santarella, FrancescoSantarella@rcsi.ie
[1]Kearney Lab/Tissue Engineering Research Group, Royal College of Surgeons in Ireland, Ireland
[2]Dept. Diagnostic Sciences, School of Dental Medicine, Tufts University, Boston, USA

INTRODUCTION: Diabetic foot ulcers (DFU) are chronic, non-healing wounds that often lead to lower leg amputation [1]. Fibroblasts differentiated from iPS-reprogrammed fibroblasts (iPSF - Generated in Garlick lab) produce a more pro-repair, matrix [2]. Our lab aims to use this matrix as a source material for scaffold fabrication for DFU treatment. This work explores techniques to enhance post-iPSF matrix production.

METHODS: 2D cultures were used to test matrix enhancement. Human pre- and post-iPSF were seeded at 16k and 64k/cm^2, and 0 – 100 µg/mL Ascorbic Acid (AA) was added to media. In 3D: collagen-GAG scaffolds (CG) [3] were used as a template to grow pre- & post-iPSF (500k/CG) and cultured with 2D-optimized media. Confocal images (DAPI/Phalloidin) and SafraninO were used to assess cell penetration and matrix production. Coll1, 3, 4, FN1, LAMA1 and 5 RNA (key structural protein genes) were analyzed via RT-PCR in 2D and 3D. Analyses were conducted at 0, 1 and 3wk (n=3). 2-way ANOVA (Bonferroni post-hoc) was used for statistical analysis.

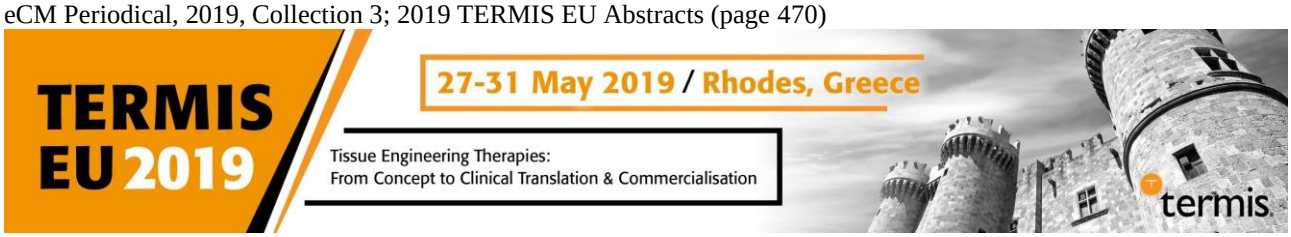
RESULTS & DISCUSSION: 100 µg/mL AA boosts matrix production in both pre- and post-iPSF groups increasing Col3, Col4, LAMA5 RNA in 2D 3wk post-iPSF ($p<0.05$). Pre- or post-iPSF 3wk seeded CGs (3D cultures) had no observable differences macroscopically; but handling post-iPSF scaffolds revealed a lower surface friction. Post-iPSF were more elongated on the CG surface and fully penetrated the CG at 3wks. Histology confirmed post-iPSF fill the CGs by 1wk, producing matrix. Col1,3,4; FN1; LAMA1,5 RNA are higher in post-iPSF at 24hrs ($p<0.05$); but not at 1 wk. Notably, post-iPSF Col3 RNA is higher at 3wk ($p<0.05$).

CONCLUSIONS: This work demonstrates the ability of post-iPSF cells to produce matrix in higher volumes than pre-iPSFs. Furthermore, we demonstrate the possibility of enhancing Col3 matrix production (pro-repair), in 2D and 3D. Ongoing work will confirm protein levels in the matrix. This matrix will ultimately be used for scaffold fabrication for DFU treatment.

ACKNOWLEDGEMENTS: ERC Proj. #758064

REFERENCES

[1] Kearney et al.Tiss Eng A. 2016, 22(5-6):401-2
[2] Shamis et al. In Vitro Cell Dev Biol Anim. 2012, 48(2):112-122
[3] O'Brien F. Materials Today. 2011;14(3):88-95



In vitro replication of tumor microenvironment activation

F. Urciuolo[1], C. Mazio[2], C. Casale[2], G. Imparato[3], P.A. Netti[1,2,3]
Presenting Author: Francesco Urciuolo, urciuolo@unina.it
[1]Department of Chemical, Materials and Industrial Production Engineering (DICMAPI), University of Napoli Federico II, P.le Tecchio 80, 80125 Napoli, Italy [2]Center for Advanced Biomaterials for Health Care@CRIB Istituto Italiano di Tecnologia, Largo Barsanti e Matteucci n. 53, 80125 Napoli, Italy [3]Interdisciplinary Research Centre on Biomaterials (CRIB), University of Napoli Federico II, P.le Tecchio 80, 80125 Napoli, Italy

INTRODUCTION: Tumor microenvironment (TME) heterogeneity and dynamics are scarcely replicated in vitro [1] making hurdle to obtain a reliable experimental model to study breast cancer. By inducing the assembling of a fibroblast-synthesized extracellular matrix (ECM), here we propose a 3D tumor micro tissue mimicking relevant cellular and extracellular events occurring in native cancers.

METHODS: Breast cancer micro tissues (BC-T) were obtained by seeding human breast fibroblasts within engineered porous gelatin microspheres in a suspension bioreactor. Fluid dynamic conditions were optimized to induce the assembly of a fibroblast-synthesized ECM. Porous gelatin microspheres were designed to degrade in concert with the assembly of new ECM molecules [2]. At 14 and 21 days HUVECs and malignant MCF7 cells were added respectively. Normal micro-tissues (N-T) were obtained in a similar way by using non-malignant (MCF10) breast cancer cells instead of MCF7. By means of both on-line multiphoton microscopy and immunofluorescence we studied the micro-tissues modification in terms of textural parameters and composition of ECM, and variation of vascular network architecture.

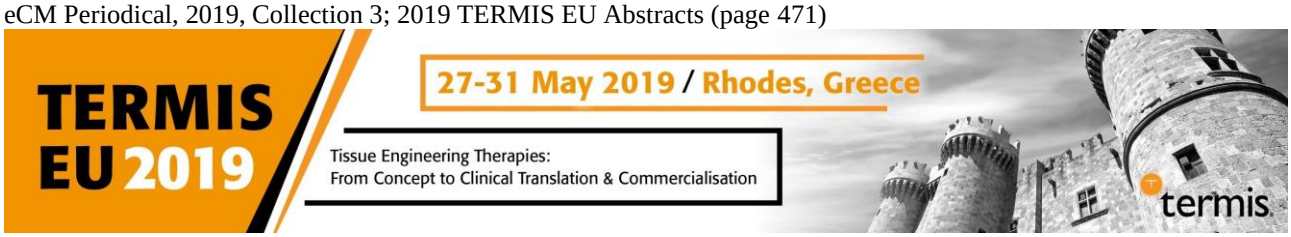
RESULTS & DISCUSSION: At 3 days after bioreactor cultivation, both BC-T and N-T (200 m in diameter) presented an abundant production of neo-formed collagen network. In the extracellular space fibronectin (FN), hyaluronic acid (HA) and capillary-like network (CLN) were also present. Despite to N-T, after 10 days BC-T displayed strong variation of the extracellular space. We found: (i) alterations of both textural [3] and architectural parameters of collagen network (i.e. collagen correlation length, polarization of collagen fibers); (ii) increasing of fibronectin, HA, MMPs; (iii) increasing of volumetric fraction and ramification points of CLN. Interestingly, malignant cells formed heterogeneous structures inside each BC-T (i.e. single file, solid strand).

CONCLUSIONS: BC-TP possess a reactive TME showing an in vivo-like activation. This, coupled with its sub-millimeter size, makes BC-TP a powerful platform for therapeutic candidates screening, in tissue on chip applications also [**3**].

ACKNOWLEDGEMENTS: we acknowledge FIRB project Newton (RBAP11BYNP_004) Italian Ministry of Education, University and Research (MIUR).

REFERENCES

[1] Peela N et al. Biom. (2017) 133:176-207.
[2] Brancato V, et al. Acta Biom. (2017) 47:1-13.
[3] Urciuolo F, Gioiella F et al. Adv. Heatl. Mat. (2016)5.23: 3074-84.



Recombinant human tropoelastin induces elastogenesis in human vascular smooth muscle cells seeded in fibrin-collagen-glycosaminoglycan scaffolds

F. Almeida-González [1,2], A. González-Vázquez[2], A.S. Weiss[3], F.J. O'Brien[2], C.M. Brougham[1,2]
Presenting Author: Francisco Almeida-González, francisco.almeida@mydit.ie
[1]School of Mechanical and Design Engineering, Dublin Institute of Technology (DIT), Dublin, Ireland [2]Tissue Engineering Research Group (TERG), Department of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [3]Charles Perkins Centre, University of Sydney, Sydney, Australia.

INTRODUCTION: In tissue engineering, the aim is to fabricate materials capable of mimicking native tissue. For tissue engineered heart valves (TEHVs), this requirement means they must withstand constant opening/closing cycles, and also remodel and grow as required. Our approach combines autologous cells in a scaffold made of fibrin collagen and glycosaminoglycan (FCG), which we have shown to be a potential platform for developing a fully functional TEHV [1,2]. TEHVs generally lack the ability to form new elastic fibers, resulting in a reduced fatigue life.
We propose that elastic fiber deposition, or elastogenesis, can be induced by adding recombinant human tropoelastin lacking domain 26A to human vascular smooth muscle cells (hVSMCs) seeded in FCG scaffolds [3].

METHODS: hVSMCs were seeded in FCG scaffolds [1] and 10 mg/ml tropoelastin was added onto the scaffolds on day 1, with a second addition on day 7. Samples were collected on days, 3, 7 and 14 for dsDNA quantification, compression testing, dimensional analysis and RT-qPCR.

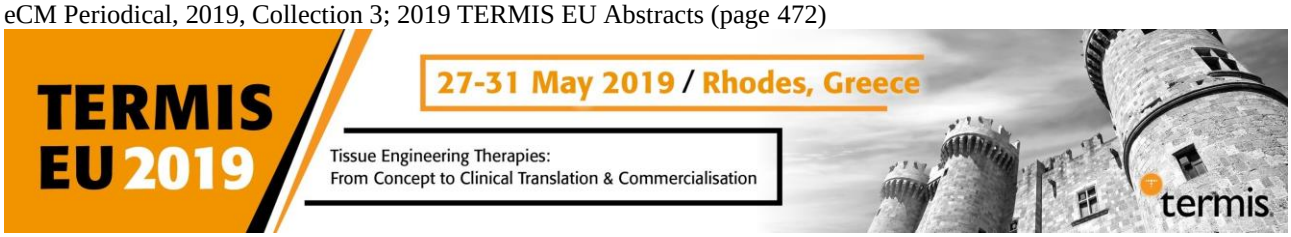
RESULTS & DISCUSSION: Elastic fibers were present in all samples treated with tropoelastin. A second addition of tropoelastin increased the elastic fiber coverage in the scaffolds. Cell proliferation and compressive modulus did not change by the addition of tropoelastin. No dimensional changes were observed in the scaffolds. A two-fold increase in elastin expression occurred by day 3 which has not been seen in previous publications.

CONCLUSIONS: The results from this study have shown that elastogenesis can be induced in hVSMCs by adding recombinant human tropoelastin lacking domain 26A in order to promote elastic fiber formation in FCG scaffolds. The amount of elastic fibers deposited was dependent on the content of ECM elastin-associated proteins. These promising results demonstrate the potential in this elastic fiber formation method, which may advance the fabrication of fully functional TEHVs with enhanced elastic recovery and fatigue life.

ACKNOWLEDGEMENTS: Financial support was received through the DIT Dean of Graduate Research School Award and the DIT School of Mechanical and Design Engineering.

REFERENCES

[1] Brougham CM et al. Acta Biomater. 2015; 26:205-14
[2] Brougham CM et al. Adv Healthc Mater. 2017; 6:1-7
[3] Martin S et al. Gene. 1995; 154(2):159-66



Biomimetic, multi-responsive and self-healing matrices based on a lactose-modified chitosan for regenerative medicine

F. Furlani[1], P. Sacco[1], M. Cok[1], E. Marsich[2], S. Paoletti[1], I. Donati[1]
Presenting Author: Franco Furlani, FRANCO.FURLANI@phd.units.it
[1]Department of Life Sciences, University of Trieste, Trieste, Italy [2]Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy

INTRODUCTION: Mechanical factors are able to influence biological processes according to a mechanism termed mechanotrasduction. [1] There is a growing effort to recreate rheological properties of natural networks by exploiting synthetic polymers. [2] Chitosan is one of the most widely used polysaccharides for biomedical applications. [3] The present contribution describes an active network based on a semi-synthetic polymer, a lactitol-bearing chitosan derivative (CTL, also termed Chitlac), and a transient inorganic cross-linker, boric acid. CTL is bioactive vs. different cell types such as chondrocytes, osteoblasts and neural cells. [4-6]

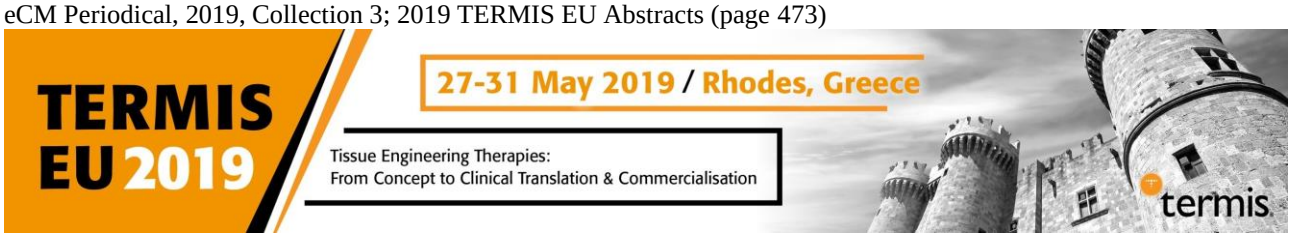
METHODS: Rheological behavior of CTL upon treatment with boric acid was explored. Self-healing ability and responsiveness to different stimuli (i.e. temperature and the presence of small molecules) were investigated by rheological tests. Mouse fibroblasts (3T3) and pig primary chondrocytes were seeded in multi-well plates and were allowed to attach for 24 h. Subsequently, the medium was changed with CTL-based networks. In a parallel experiment cells were encapsulated into networks. Cell viability was evaluated by Alamar blue assay after 1 and 3 days.

RESULTS & DISCUSSION: An uncommonly high dependence in the scaling law between the zero-shear viscosity and the concentration of CTL emerged, pointing to interesting potential implications in the field of viscosupplementation. [7] Strain hardening effects and non-linear response to stress were noticed. [8] Such trends are considered as hallmarks of cytoskeletal and biopolymer networks. Obtained networks were able to self-heal after breakage. Notably, herein we report that CTL-boric acid gels are multi-responsive systems, whose mechanics can be tailored by different stimuli such as the presence of small molecules (e.g. glucose). CTL-boric acid networks resulted to be biocompatible toward two different cell models, namely pig primary chondrocytes and mouse fibroblasts.

CONCLUSIONS: CTL based networks resulted to be biocompatible, multi-responsive and able to self-heal after breakage. Furthermore, these networks displayed mechanical properties similar to natural tissues. Giving the peculiar properties of the obtained system, bioactivity of CTL could be linked to a mechanotrasduction mechanism. These networks are promising candidates as in vitro mimics of natural active matrices and for regenerative medicine applications.

REFERENCES

[1] Hoffman B et al. Nature 2011; 475:316–323
[2] Varnosfaderani M et al. Nature 2017; 549:497–501
[3] Sacco P, Furlani F et al., Gels 2018; 4:1-29
[4] Donati I et al. Biomaterials 2005; 26:987–998
[5] Travan A et al. Biomacromolecules 2010; 11:583–592
[6] Medelin M et al. Acta Biomaterialia 2018; 73: 285-301
[7] Sacco P, Furlani F et al., Biomacromolecules 2017; 18:4206−4213
[8] Furlani F et al., Carbohydrate polymers 2019; 208:451–456



Establishment of an inflamed adipose tissue model under defined conditions

F. F. Schmid [1], A.-C. Volz [1], F.B. Atzinger[1], A. Komitakis[2], T. Maucher [2], L. Grupp[2], B. Weiß[2]. P.J. Kluger[1]
Presenting Author: Franziska Atzinger, Franziska.Atzinger@Reutlingen-University.DE
[1]Smart Biomaterials, Reutlingen University, Reutlingen, Germany [2]Esslingen University of Applied Sciences, Germany

INTRODUCTION: In recent decades, the link between many diseases such as diabetes or pancreatitis and existing obesity has been uncovered [1, 2]. In vitro models of inflamed adipose tissue could help to gain insights into the development of the pathological condition. Moreover, such a model could be used to screen for new potential drugs to contain a further epidemic increase of related secondary diseases as far as possible. There are various current attempts on the setup of an adipose tissue model, however most of them are based on the use of animal derived sera which come along with the potential risk of contaminations and high batch-to-batch variations [3]. We aimed to set up an adipose tissue model under defined conditions by the development of a defined adipocyte medium. Furthermore, we sought to lift the model to an inflammatory state by the incorporation of an immunological component in form of macrophages.

METHODS: Mature adipocytes (MAs) and adipose-derived stem cells (hASCs) were isolated from human subcutaneous adipose tissue and encapsulated in 3D hydrogels based on methacrylated gelatin (GM). 3D constructs were built by the manual setup or by an extrusion based bioprinting process and crosslinked via UV radiation at 365nm. While isolated MAs were cultured in adipocyte medium, hASCs were differentiated to MAs for 14 days.
For the integration of an immunological component, a defined co-culture medium for adipocytes and macrophages was developed. Monocytes' ability to differentiate to macrophages was evaluated in mono-culture. Consecutive a functional co-culture of mature adipocytes and monocytes was set up under defined conditions.

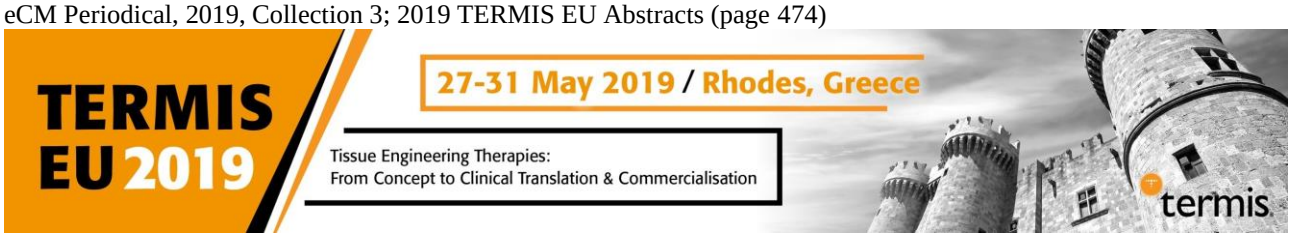
RESULTS & DISCUSSION: A 3D printed adipose tissue simulating an inflammatory state was successfully established. The defined medium led to a co-culture with cell-specific characteristics of mature adipocytes and macrophages. The inflammatory state was proven by the expression of cytokines like Interleukin 6. The adipose tissue model morphologically recreates the in vivo situation which was confirmed by histological and immunofluorescence staining.

CONCLUSIONS: An inflamed adipose tissue model was implemented for the first time. This represents a fundamental achievement for future applications of in vitro adipose tissue models in the field of drug development. Hence, the model provides great potential for meeting the challenges to alternative methods for animal testing and the transfer of the results will be of great value for other tissue engineering approaches.

ACKNOWLEDGEMENTS: Financial support was received from the Ministry of Rural Affairs, Food and Consumer Protection Baden-Wuerttemberg (#1099895).

REFERENCES

[1] Xu H et al. The Journal of clinical investigation. 2003; 112(12):1821-1830.
[2] Franco-Pons N. et al.J Pathol. 2010; 221(2):175-82.
[3] Jochems CE et al. ATLA-NOTTINGHAM. 2002;30(2): 219-228.



Cell-laden biomimetically mineralized shark skin collagen-based 3D printed scaffolds for the engineering of hard tissues

G.S. Diogo[1,2], C.F. Marques[1,2], C.G. Sotelo[3], R.I. Pérez-Martín[3], R.P. Pirraco[1,2], R.L. Reis[1,2,4], T.H. Silva[1,2]

Presenting Author: Gabriela Soares Diogo, gabriela.carlos@i3bs.uminho.pt

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain [4]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: 3D approaches made of collagen-apatite materials have been targeted envisioning mineralized tissues applications. Direct mixing of calcium phosphates powder with collagen solutions or scaffolds immersion in simulated body fluid (SBF) are the most common methods. However, loss of efficiency has been reported, pointing to uncontrollable methods and long processing time, which might result in a limited bioactivity. Alternatively, we propose for the first time the in situ mineralization of Prionace glauca (blue shark) skin collagen (PGColl) and its use on inks for the 3D bioprinting of encapsulated cells envisioning the engineering of mineralized tissues.

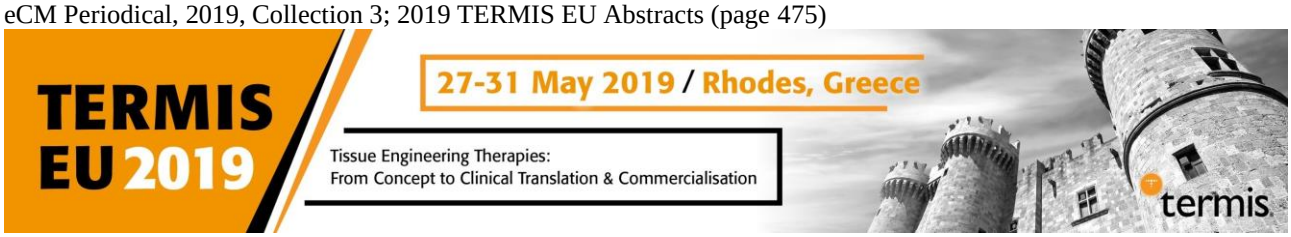
METHODS: Collagen extracted from blue shark skin through an acidic method was solubilized in 10 mM HCL. To induce hydroxyapatite formation, calcium chloride and ammonium hydrogenphosphate were added as calcium and phosphate sources (Calcium-to-phosphate ratio =1.67), respectively. To prepare the cell-laden bioinks, 2% (w/v) PGColl was combined with 12% (w/v) alginate at the following volume ratios: 1:1, 1:2, 1:3 and only alginate (AG). The printing process was carried out using sterile material in a REGEMAT 3D dispensing system, at room temperature (25 ºC).

RESULTS & DISCUSSION: FT-IR and XRD analyses confirmed hydroxyapatite formation of the in situ mineralized collagen. The printed constructs using the different bioinks exhibited a homogeneously distribution of cells, which indicates a successful cell encapsulation. The intensity of the red signal (dead cells) decreased with alginate ratio, revealing the positive effect of mineralized PGColl with enhanced cell metabolic activity and proliferation (DNA quantification).

CONCLUSIONS: Successfully designed and printed cell-laden constructs made of in situ mineralized PGColl through a biomimetic approach were achieved. The survival and spread of cells were favored by the presence of mineralized PGColl.

REFERENCES

[1] Xia Z. el tal, J. Mater. Chem. B, 2014. 2(14): p. 1998-2007.



Osteoblast-osteocyte differentiation in microfluidic devices

G. Nasello[1], P.A. Diéz[1], J. Schiavi[2], M.A. Pérez[1], L. McNamara[2], J.M. García-Aznar[1]
Presenting Author: Gabriele Nasello, gnasello@unizar.es
[1]Multiscale in Mechanical and Biological Engineering, University of Zaragoza, Spain
[2]Mechanobiology and Medical Device Research Group, NUI Galway, Ireland

INTRODUCTION: In-vitro bone tissue models are a prerequisite tool for a deeper comprehension of the biological mechanisms in bones [1]. Mineralized bone matrix formation as well as bone cell differentiation are essential requirements for reliable bone tissue models. In this work, a microengineered 3D cell culture system recapitulates the structural and biochemical microenvironment for human osteoblasts to mineralize a collagen matrix and differentiate to osteocytes. A bone on a chip approach will be a useful strategy to investigate the effect of mechano-chemical stimuli on bone cells response.

METHODS: Microfluidic devices were made of PDMS as describe previously [2]. Primary human osteoblasts (PromoCell) were seeded in rat tail type I collagen hydrogel (Dow Corning) at the final concentrations of $2.5x10^5$ and 10^6 cells/ml and cultured up to 21 days with 10 mM β-glycerophosphate (Sigma Aldrich) and 50 μM ascorbic acid (Sigma Aldrich). DNA content, extracellular ALP activity assay, calcein staining, BSP2 and DMP1 immunofluorescent staining were conducted after 7, 14, 21 days. Live cell images were analyzed with a custom-made Python software to quantify protrusion length change over time, characteristic of osteocytic differentiation.

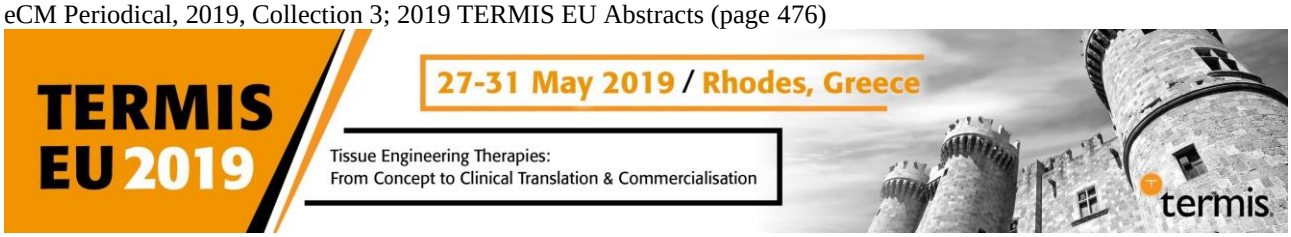
RESULTS & DISCUSSION: Osteoblasts expressed a mature phenotype during the 3D culture. They did not proliferate, expressed ALP, deposited calcium compounds on the matrix and produced BSP2. Mineral deposition was formed for both concentrations tested. For a cell density of 10^6 cells/ml, we measured longer protrusion length after 21 days of culture compared to day 7. In addition, other osteocytic markers increased as of the number of cell-cell connections and deposition of DMP1 on the extracellular matrix. On the other hand, cells cultured at $2.5x10^5$ did not produce DMP1, confirming that osteoblast-osteocyte transition is regulated by cell seeding density [3].

CONCLUSIONS: These data show how microfluidic platforms can be used as in vitro mature bone tissue models, inducing specific bone matrix production (DMP1) to mimic the cortical bone environment with the differentiation of osteoblasts to osteocytes. The system is applicable for investigating the effect of fluid flow on 3D-cultured human bone cells.

ACKNOWLEDGEMENTS: The CuraBone project received funding from ITN-MSCA, grant agreement No. 722535.

REFERENCES

[1] Huh D et al. Trends Cell Biol. 2011; 21:745-54.
[2] Del Amo C et al. Integr Biol. 2017; 9:339-49.
[3] McGarrigle M et al. Eur Cell Mater. 2016; 31:323-40.



Hierarchical bioassemblies of cartilage µtissues for scaffold-free osteochondral implants

G. N. Hall[1, 2], W. L. Tam[1, 2], L. Geris[1, 3, 4], F.P. Luyten[1, 2, *], I. Papantoniou[1, 2, *]
Presenting Author: Gabriella Nilsson Hall, gabriella.nilssonhall@kuleuven.be
[1]Prometheus Div. of Skeletal Tissue Engineering, KU Leuven, Belgium [2]Skeletal Biology and Engineering Research Center, KU Leuven, Belgium [3]GIGA in silico medicine, Université de Liège, Belgium [4]Biomechanics Section, KU Leuven, Belgium. * Shared senior authorship

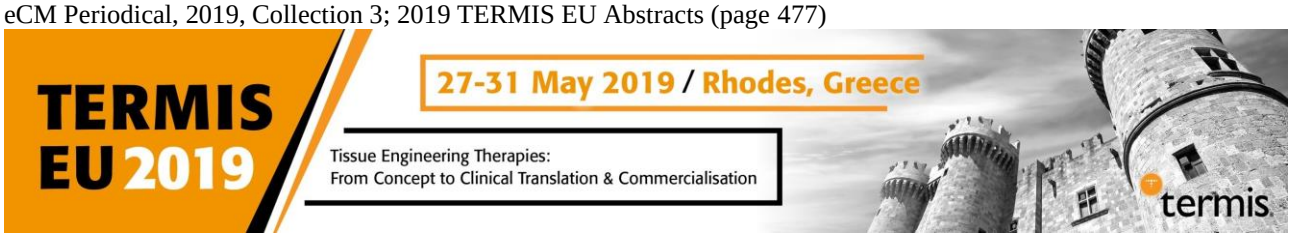
INTRODUCTION: Biofabrication of complex hierarchical tissue structures relies on the development and progressive fusion of µTissue modules. During chondrogenic differentiation, µ-spheroids mature and secrete increasing amounts of glycosaminoglycan rich extracellular matrix to form µTissues. In this study, we investigated in vitro µTissue fusion and demonstrated that bioassembled constructs preserved their spatial properties in vivo. This was shown for both adult progenitor as well as induced pluripotent stem cells (iPSC) as cell source.

METHODS: Human periosteum progenitor cells (hPDC) or chondrocytes derived from iPSC (ch-iPSC) were seeded onto non-adherent agarose micro-wells resulting in cellular aggregation and µ-spheroids. These µ-spheroids were differentiated up to 3 weeks to form either hypertrophic-cartilage µTissues (hPDC) or cartilage µTissues (ch-iPSC). The µTissues were characterized with RT-qPCR and histology. Finally, two different bi-layered constructs were created and implanted ectopically in nude mice for 4 weeks.

RESULTS & DISCUSSION: Chondrogenic differentiation was up-regulated after 14 days for hPDC (14D) followed by hypertrophic markers after 21 days (21D) in culture. Histology further demonstrated accumulation of cartilaginous extracellular matrix (ECM) over time and metachromatic Safranin O staining after 21 days. These findings suggest that the µTissue maturation mimicked the process of endochondral ossification, as seen during long bone development. Spontaneous fusion was detected for all conditions with day 21 µTissues demonstrating slower fusion, indicating that the ECM maturation may interfere with the fusion process. Finally, two µTissue populations of distinct maturation levels (14D and 21D) were fused into a bi-layered cartilage construct composed of 2000 µTissues. In addition, a second bi-layered construct was formed using day 21 µTissues and iPSC µTissues. Both constructs further matured in vivo with maintained pattern into one cartilaginous and one mineralized part with bone.

CONCLUSIONS: Bi-layered hierarchical constructs maintained their in vitro engineered spatial features even after 4 weeks ectopic in vivo implantation. A complex osteochondral tissue was formed with layers directly correlating with the implanted tissue properties. These defined µTissue modules possess excellent properties for biofabrication of complex tissues through bio-assembly and -printing.

ACKNOWLEDGEMENTS: (FP/2007-2013)/ ERC: n. 279100 and 249191, GOA/13/016 and FWO-SB: 1S05116N, RegMed XB



Polyurethane scaffold for cartilage tissue engineering

G. Lutzweiler[1,2], J. Barthes[3], P. Schaaf[1,2], W. Drenckhan[,2], E. N. Vrana[3]
Presenting Author: Gaëtan Lutzweiler: glutzweiler@unistra.fr
[1]Institut Nationnal de la Santé et de la Recherche Medicale (INSERM), Strasbourg, France [2]Institut Charles Sadron (ICS), Strasbourg, France [3]Protip Medical, Strasbourg, France

INTRODUCTION: Porous scaffolds are widely investigated for tissue engineering purposes. While the influence of pore size and porosity has been deeply studied [1], interconnections size (i.e the aperture connecting two pores) hasn't been so much investigated. Herein, we generated polyurethane scaffold with well controlled pore and interconnection sizes, and looked at the influence of the interconnections on Wharton's Jelly mesenchymal stem cells (WJMSCs) growth.

METHODS: Polyurethane scaffolds were generated using the so called "sphere templating" approach [2]; paraffin microbeads were generated by an emulsion, and subsequently sieved to select beads with diameter of 133±28µm. Beads were sintered at several temperatures and times to create a neck between adjacent beads. Polyurethane (FoamPartner, Swiss) was cast on the beads and filled the interstitial space between the beads and let to solidify. Paraffin was then dissolved in n-hexane to leave the porous structure. Scaffolds were then placed under plasma cleaner for 3min, 200mTorr in order to make the surface more hydrophilic. WJMSCs were seeded on each scaffold at a density of 1.10^6 cells/scaffold.

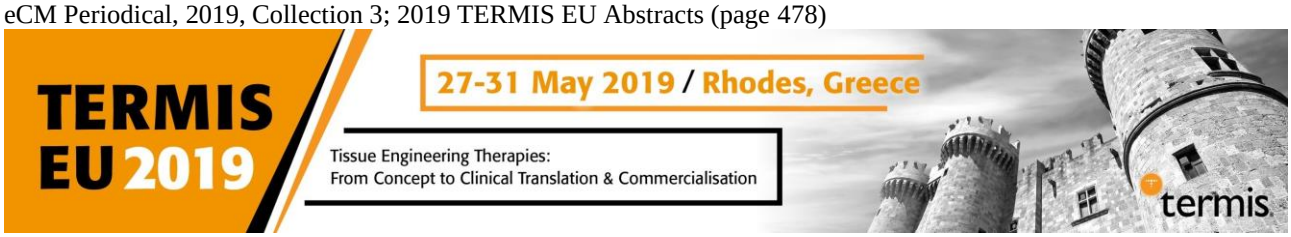
RESULTS & DISCUSSION: Porous scaffolds were obtained with highly spherical pore shapes. Interconnection diameter were controlled through the sintering process of the paraffin beads, one having interconnection diameters of 27±10µm named as SMALL, and 52±12µm for the other (named as LARGE). Pore diameter was 133±28µm. DNA concentration was found significantly higher in scaffolds with LARGE interconnections specially for day 14 and 21. WJMSCs had a higher ability to spread across the scaffold with LARGE interconnections while cells remained isolated and mostly on surface for SMALL ones which prevented a good colonization of the whole scaffold

CONCLUSIONS: These results underline that in interconnections diameter drastically influence the colonization of polyurethane scaffold by WJMSCs.

ACKNOWLEDGEMENTS: Financial support from the doctoral school of Strasbourg University, Horizon 2020 PANBioRA and Institut Carnot MICA (Dia Art).

REFERENCES

[1] Murphy et al. 2010. Biomaterials. 31.3: 461–466.
[2] Ma, Peter X, et al. 2001. Tissue Engineering 7.1: 23–33.



Porcine hepatocytes culture on biofunctionalized 3D hydrogels as an in vitro platform to predict drug hepatotoxicity

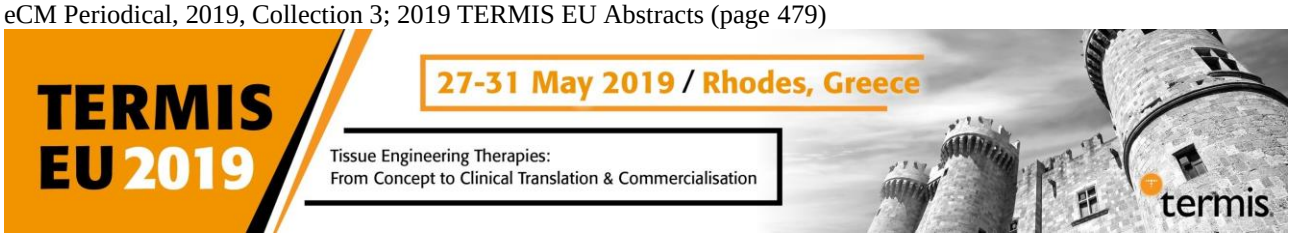
L. Wu[1], G. Ferracci[2,3,4], Y. Wang[3,4], T.F. Fan[3,4], N.J. Cho[3,4,5], P.K.H. Chow[1,6,7]
Presenting Author: Gaia Ferracci, gaia.ferracci@gmail.com
[1]Division of Surgical Oncology, National Cancer Centre Singapore, Singapore [2]HealthTech NTU, Interdisciplinary Graduate School, Nanyang Technological University, Singapore [3]School of Materials Science and Engineering, Nanyang Technological University, Singapore [4]Centre for Biomimetic Sensor Science, Nanyang Technological University, Singapore [5]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore [6]Duke-NUS Medical School, Singapore [7]Department of Hepatopancreatobiliary and Transplant Surgery, Singapore General Hospital, Singapore

Drug-induced liver injury is one the most frequent reasons for drug failure at the clinical and post-marketing phases and is also among the main causes of acute liver failure in the USA and Europe [1]. Pre-clinical in-vivo animal studies, performed to evaluate the efficacy and safety of new potential drug candidates, are highly costly and time-consuming [2]. Therefore, in-vitro liver platforms for drug testing based on hepatocytes from different animal species might be a potent tool for high throughput screening to reduce in-vivo animal studies during drug development and lessen drug attrition rates and costs. Towards this end, various researches have shown the importance of combining multiple characteristics of the in-vivo hepatic environment to develop artificial liver systems [3]. Hence, the aim of the present study is to establish a functional porcine hepatocyte culture using a biofunctionalized 3D inverted colloidal crystal (ICC) hydrogel platform. The performances of non-adhesive bare poly (ethylene glycol) diacrylate (PEGDA) ICCs and PEGDA ICCs coated with either collagen type I or fibronectin were investigated. Porcine hepatocytes viability, morphology, hepatic-specific functions and patterns of gene expression were evaluated over a period of two weeks in culture to test diclofenac, a well-known hepatotoxic drug. A 3D cell culture environment, mimicking the hepatic lobular organization, together with the presence of extracellular matrix (ECM) proteins, especially fibronectin, led to different aggregation patterns and facilitated hepatocyte viability and maintenance of the liver-specific phenotype in vitro, enabling to the prediction of hepatotoxicity.

ACKNOWLEDGEMENTS: This research was financially supported by the National Research Foundation (NRF) Singapore Competitive Research Programme (NRF-CRP10-2012-07) grant, the Nanyang Technological University (NTU) - Interdisciplinary Graduate School (IGS), the National Cancer Centre Singapore and the SingHealth Experimental Medicine Centre.

REFERENCES

[1] Björnsson ES. Arch Toxicol 2015; 89(3):327-34
[2] May JE et al. Brit J Biomed Sci 2009; 66(3):160-165
[3] Lee BH, Shirahama H et al. NPG Asia Mater 2017; 9:e412



Ferritic fibre networks for magnetically-induced bioactivation

G K. Levy[1], A. Symeonidou[1], R.A. Brooks[2], M.A. Birch[2], A.E. Markaki[1]
Presenting Author: Galit Katarivas Levy, gk406@cam.ac.uk
[1]Department of Engineering, University of Cambridge, Cambridge, UK [2]Division of Trauma and Orthopaedic Surgery, Addenbrooke's Hospital, Cambridge, UK

INTRODUCTION: Magneto-active coatings/scaffolds based on interconnected networks of ferromagnetic fibres have been proposed to promote the growth of peri-prosthetic bone [1-2]. When the scaffold is actuated in vivo by an external magnetic field, it deforms elastically as the ferromagnetic fibres tend to align along the field direction, imposing strains to in-growing bone tissue. Such deformation is known to promote bone cell growth provided the strains lie in the beneficial range [3]. The present work aims to generate in vitro proof of concept data of the osteoconductive potential of such magneto-mechanically actuated scaffolds.

METHODS: Human Osteoblasts (HObs) were seeded onto 85% porous 444 ferritic (magnetic) stainless steel networks and cultured in McCoy's medium. The magneto-mechanical actuation protocol, involved static culture for 7 days, followed by actuation for a period of 7 days using a sinusoidally varying magnetic field between 0.3 and 1.1 Tesla, at a frequency of 0.2 Hz for 4 hours (3000 cycles per day). Gene expression and protein expression were investigated using real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) and enzyme linked immunosorbent assay (ELISA).

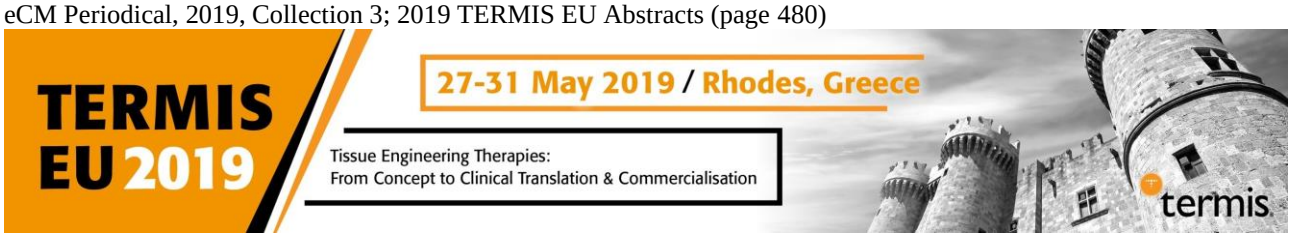
RESULTS & DISCUSSION: An increase in osteogenic gene expression has been observed following magnetic actuation of ferromagnetic networks compared to non-actuated networks (control baseline). In addition, an immunoassay showed ~2 times higher BMP2 concentrations in cell culture supernates of actuated networks. Bone morphometric proteins (BMPs), especially BMP2, play a pivotal role in adult skeletal development and bone formation. The results are the outcome of three independent studies, no statistical differences were observed across the studies.

CONCLUSIONS: The results show an actuation-mediated upregulation of specific genes involved in the osteogenic lineage. Collectively, the proposed magnetic actuation strategy has the potential to promote osteogenesis.

ACKNOWLEDGEMENTS: Financial support for GKL was received from Blavatnik Family Foundation and Reuben Foundation. Financial support for RAB has been provided via the National Institute for Health Research (NIHR).

REFERENCES

[1] Markaki AE et al. Biomaterials; 2004; 25:4805-4815.
[2] Malheiro VN et al. Biomaterials; 2011; 32:6883-6892.
[3] Frost HM. J Bone Miner Res; 1992; 7(3):253-261.



Wet spun PLA multifilaments with incorporated microgels

G.P. Paar[1,2], P. Duhr[2], C. Molano-López[3], S. Braun[3], A. Pich[3], T. Gries[2], S. Jockenhoevel[1], A. Blaeser[1,2]
Presenting Author: Georg-Philipp Paar, georg.paar@ita.rwth-aachen.de
[1]Dept. of Biohybrid & Medical Textile (BioTex), AME-Applied Biomedical Engineering, Helmholtz Institute Aachen, RWTH Aachen University, Aachen, Germany [2]Institut für Textiltechnik of RWTH Aachen University, Aachen, Germany [3]DWI-Leibniz Institute for interactive materials, RWTH Aachen University, Aachen, Germany

INTRODUCTION: Biodegradable materials, such as polyglycolic acid (PGA) and polylactic acid (PLA), are used commercially in medicine for sutures, osteosynthesis systems and drug delivery systems and in research for meshes and tissue engineering scaffolds. In vivo hydrolytic degradation of PGA and PLA results in release of degradation products, which can cause local acidosis and lead to dramatic clinical complications. Incorporation of various additives (e.g. caffeine, tricalcium phosphate, calcium hydroxide, calcium carbonate, magnesium hydroxide, magnesium oxide) into PGA filaments for pH buffering failed due to insufficient buffering capacity and instabilities in the spinning process [1]. However, amine based microgels were shown to be a successful buffering agent for acidic degradation products [2,3]. These pH-responsive colloidal polymer networks are heat sensitive and therefore have to be incorporated into filaments using solution spinning. Incorporation of microgels into filaments by solution spinning and buffering of the pH value was successful. The spun filaments are inferior because of low tensile strength and high variation of tensile strength.

METHODS: In wet spinning trials monofilaments and multifilaments were extruded from PLA/chloroform solution into isopropanol and ethanol. Process parameters such as the quantity of incorporated microgels, spinneret geometry and polymer concentration in the spinning dope are evaluated. Mechanical characteristics are evaluated by tensile testing and morphology by light microscopy. Furthermore, the incorporated quantity of microgels in the fiber was determined by TGA measurements.

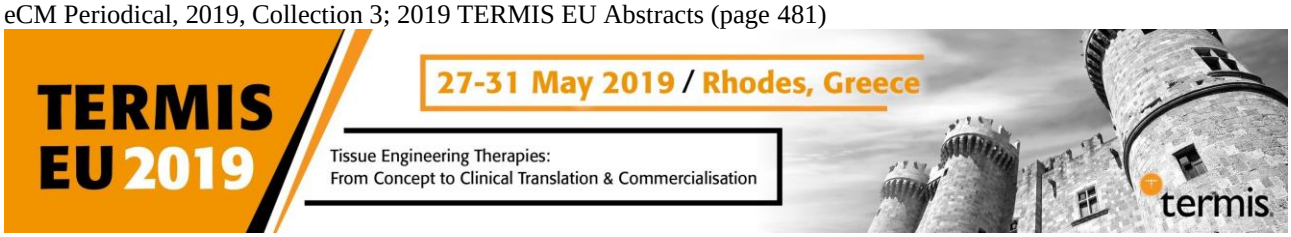
RESULTS & DISCUSSION: Tensile strength of wet spun PLA filaments is higher compared to dry spun PLA filaments. Round cross section filaments were produced. Wet spinning of PLA mono- and multifilament with 7 % incorporated microgels was successful, with a tensile strength of up to 14,9 cN/tex. A loss of 20 % of mass of microgels during the spinning process was observed. The effect of microgel incorporation on fiber morphology is small, however a significant decrease in tensile strength was noted.

CONCLUSIONS: Wet spinning showed to be a promising alternative solution spinning process, which allows production of microgel incorporated PLA filaments with round cross section and increased tensile strength.

ACKNOWLEDGEMENTS: The Project “pHMed” is supported by the European Regional Development Fund North Rhine-Westphalia (EFRE.NRW).

REFERENCES

[1] Schuster P. Shaker 2014; doctoral thesis
[2] Fehér K. et al. BioNanoMaterials, 2015; 16(4): 259–264
[3] Poggenburg B. et al. Kunststoffe in der Medizintechnik, 2014; 139-152



Therapeutic approach based on mesenchymal stromal cell secretome for spermatogenesis recovery

G.D. Sagaradze [1], N.A. Basalova [1,2], V.I. Kirpatovsky [1,3], D.A. Ohobotov [1,2], O.A. Grigorieva[1], P.P. Nimiritsky[1], P.I. Makarevich [1,2], Z.A. Akopyan[1,2], A.A. Kamalov [1,2], A.Y. Efimenko [1,2]
Presenting Author: Georgy Sagaradze, georgy.sagaradze@gmail.com
[1]Medical Research and Education Center, Lomonosov Moscow State University, Moscow, Russia [2]Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia [3]Research Institute of Urology named N.A. Lopatkin - branch FGBU "Federal medical research center named after P.A. Herzen", Moscow, Russia.

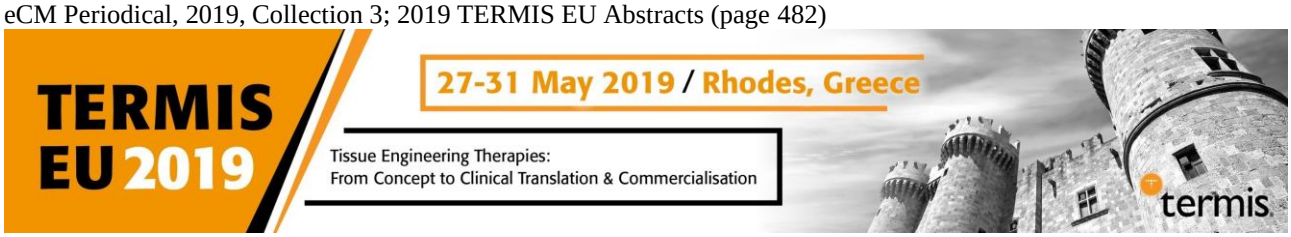
INTRODUCTION: Male infertility is a substantial medical problem worldwide, often idiopathic. Due to the possible multifactorial nature of this disease, there is lack of effective therapy for the majority of male infertility cases. Thus, the strategy to recover spermatogenesis by targeting both spermatogonial stem cell (SSC) and their microenvironment called niche might be effective. Mesenchymal stromal cells (MSC) could be a promising tool to regulate SSC niche as these cells were shown to secrete a wide spectrum of paracrine factors supporting proliferation, survival and differentiation of cells in different resident stem cell niches. What is more important, MSC secrete molecules supporting functions and viability of SSC, Leydig and Sertoli cells. Thus, the study of efficacy and mechanisms of spermatogenesis restoration stimulated by MSC secretome might help to apply novel approaches for male infertility treatment.

METHODS: We developed the combined drug based on human MSC conditioned medium (CM) and collagen as a protective depo for MSC secreted components. Using a model of rat bilateral abdominal cryptorchidism for reversible SSC niche injury we observed that MSC CM effectively restored spermatogenesis. We applied immunohistochemical methods as well as morphometric analysis of contents of epididymes and serum testosterone concentration analysis to evaluate spermatogenesis recovery.

RESULTS & DISCUSSION: We observed that the injection of either MSC secretome combined with the collagen or MSC themselves recovered injured SSC niche. The numbers of primary and secondary spermatocytes in seminiferous tubules were increased. The total and moving spermatozoa numbers presented in the epididymis were also increased after the injection. Besides the recovery of germinal epithelium cells, we also showed the increase in Sertoli cell number and restrain of Leydig cell population growth. This might indicate important mechanisms associated with spermatogenesis recovery as a direct correlation between number of tubules containing a large number of proliferating cells and spermatozoa number, and an inverse correlation between the level of interstitial cell proliferation and spermatozoa fraction were revealed.

CONCLUSIONS: The application of MSC secretome combined with the collagen might be a promising approach to treat male infertility. MSC secretory function might be principal for spermatogenesis recovery as the beneficial effects of MSC as well as MSC secretome were comparable.

ACKNOWLEDGEMENTS: The reported study was funded by RFBR according to the research project № 18-315-00403.



Development of an in vitro three-dimensional colorectal tumor model for drug screening

G. Rubí-Sans[1,2], I. Cano-Torres[1,2], S. Pérez-Amodio[2,1,3], M.A. Mateos-Timoneda[2,1,3], E. Engel[1,2,3]
Presenting Author: Gerard Rubí-Sans, grubi@ibecbarcelona.eu
[1]Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain [2]CIBER en Bioingeniería, Biomateriales y Nanotecnología (CIBER-BBN), Madrid, Spain [3]Dept. Materials Science and Engineering, EEBE, Technical University of Catalonia (UPC), Barcelona, Spain

INTRODUCTION: The use of cell-derived matrices (CDM) is a promising alternative to decellularized tissues/organs as these are bioactive and biocompatible materials consisting of a complex assembly of proteins, growth factors and matrix macromolecules. 3D cell-cultured microparticles combined with macromolecular crowding (MMC) effect, offers the possibility to tailor-made bioactive materials for tissue engineering applications. We propose CDMs as potential colorectal tumor models for personalized medicine by mimicking tissue microenvironment properties [1].

METHODS: Human adipose mesenchymal stem cells are seeded at 100,000 cells/mg of PLA microparticles [2] in 24 well plates and cultured for 10 days under stirring conditions. Obtained CDMs are characterized by quantifying total protein and DNA, immunofluorescence staining of key components of CDMs, topography by SEM and gene and protein expression by qRT-PCR and WB. Besides, tissues are decellularized and particles are removed to be then recellularized in a perfusion bioreactor with colon cancer cells and cancer associated fibroblasts (CAFs) [3] to further characterize how cells remodel initial CDMs.

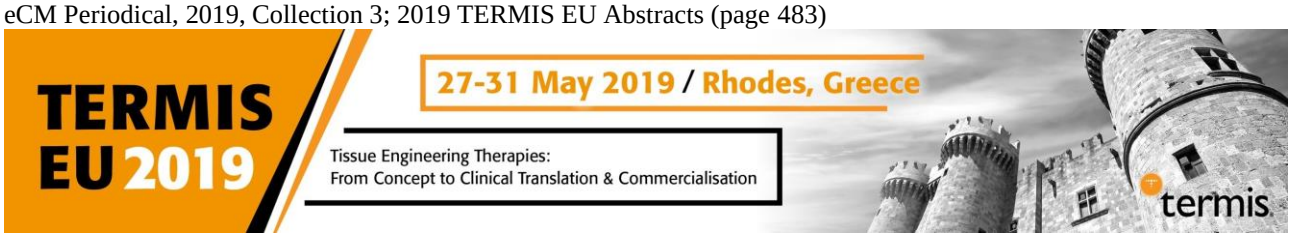
RESULTS & DISCUSSION: Addition of MMCs enhances protein deposition in CDMs. Fibrillary proteins collagen types I and III, which are highly present in colon tumor extracellular matrix (ECM), are over expressed after 10 days of culture. Tissues density and size is greater, and final tissue stiffness is increased. Cells and microparticles were successfully removed from CDMs, and their recellularization and cancer CDM characterization are taking place to finally produce an in vitro tumor model to understand cancer promoting mechanisms, to develop a patient-specific drug screening platform and to identify potential therapeutic targets.

CONCLUSIONS: CDMs composition, like expression of fibrillar proteins of the ECM, and the tunable matrix stiffness provides reproducible tissue microenvironment. By repopulating the tissue microenvironment with cancer and stroma cells, we have a promising platform for in vitro tumor model generation.

ACKNOWLEDGEMENTS: Authors would like to thank MINECO (MAT2015-68906-R) for funding.

REFERENCES

[1] Crotti S, et al. J Cell Phys, 2017, 232(5), 967–975
[2] Levato R, et al. Macromol Biosci, 2012, 12, 557–566
[3] Balkwill FR, et al. J Cell Sci, 2012, 125, 5591–5596



Nanocomposite bioink for novel fluid-gel cells printing for skeletal regeneration

G. Cidonio[1], M. Glinka[1], M. Cooke[2], J.I. Dawson[1], L. Grover[2], R.O.C. Oreffo[1]
Presenting Author: Gianluca Cidonio, g.cidonio@soton.ac.uk
[1]Bone and Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, University of Southampton, Southampton, United Kingdom [2]School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom

INTRODUCTION: Cell printing remains limited in the size and functionality of tissue that can be generated due to: (i) resolution, (ii) polymer content levels within bioinks and (iii) limitation in assembly of printed layers [1,2]. Herein, we detail a novel nanocomposite bioink based on Laponite nanoclay (LAP) and gellan gum (GG) printed in a fluid-gel bath that enables skeletal biofabrication.

METHODS: LAP and GG were blended at 60 °C. Agarose fluid-gel support rinsed off immediately after deposition and crosslinking with $CaCl_2$. C2C12 myoblasts were encapsulated at 5×10^6 cell ml^{-1} and printed in agarose. Viability (live/dead) and functionality (alkaline phosphatase staining) were investigated at 1, 7 and 21 days. Printed and VEGF-absorbed samples were implanted on the chick chorioallantoic membrane (CAM).

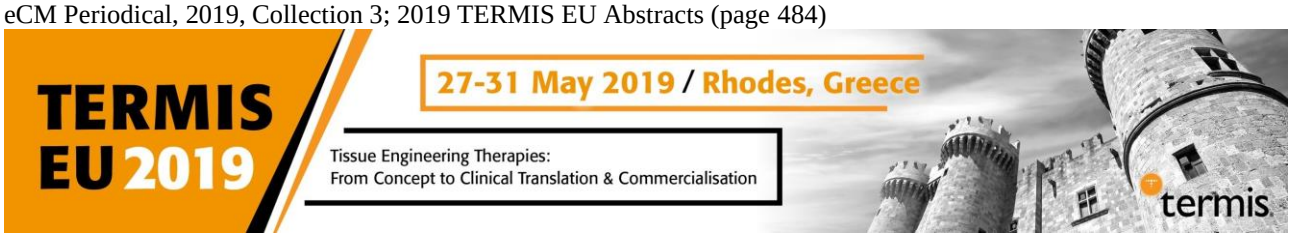
RESULTS & DISCUSSION: LAP-GG hydrogel printed in agarose was able to produce high shape fidelity constructs resulting in a significant ($p<0.0001$) difference (322 ± 9 µm) in deposition resolution compared to in-GG printing (488 ± 9 µm). An increase in resolution was observed following LAP-GG printing in agarose compared to GG alone ($p<0.0001$). C2C12 were printed and remained viable in GG and LAP-GG composites, with a significant ($p<0.0001$) increase in proliferation and functionality for C2C12 printed in LAP-GG. VEGF in agarose was absorbed by LAP-GG and GG while printing and the resulting constructs were implanted in the developing CAM. LAP-GG-VEGF samples analysed after 7 days showed significantly enhanced vascular infiltration ($p<0.01$) compared to VEGF-free controls and GG-VEGF

CONCLUSIONS: LAP addition to GG bioink combined with a fluid-gel support resulted in enhanced printing resolution, promoting cell proliferation and drug (VEGF) absorption and localisation. The current studies demonstrate the potential of the suspended manufacturing platform to augment vasculogenesis in 3D cell-laden scaffolds with therapeutic implications for hard and soft tissue engineering.

ACKNOWLEDGEMENTS: Funding to RO from the UK Regenerative Medicine Platform Hub (MR/K026682/1) and Acellular/Smart Materials - 3D Architecture (MR/R015651/1) & University of Southampton is acknowledged.

REFERENCES

[1] Ahlfeld T et al. Biofabrication 2017; 9(3):034103;
[2] Moxon S R et al. Adv Mater. 2017; 29(13):1605594.



Nano-composite scaffolds for bone tissue regeneration: Comparison between static and dynamic culturing conditions

G. Turco[1], M. Gruppuso[1], F. Vecchies[1], D. Porrelli[1], M. Mardirossian[1], E. Marsich[1]
Presenting Author: Gianluca Turco, gturco@units.it
[1]Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy

INTRODUCTION: In bone tissue engineering, scaffolds are conceived to support cell proliferation and colonization by mimic the structure and the morphology of the surrounding native tissue. [1-3] Beside the architectural properties of scaffolds, other features are paramount to achieve the correct integration of the construct as the biocompatibility of the scaffold constituents and its degradation rate. The colonization process on one hand, and the differentiation of mesenchymal cells on the other hand, are influenced by the kinetics of nutrients and fluid exchange in the system. In this study we compared two culturing conditions: static and dynamic. The latter condition was achieved by means of a bioreactor. Effects of the culturing conditions were assessed on the proliferation and differentiation of human dental pulp stem cells.

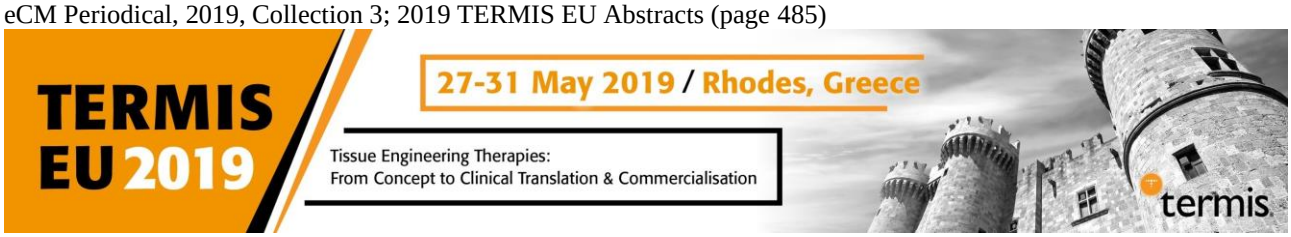
METHODS: Human dental pulp stem cells (hDPSCs) were successfully isolated from third molars teeth extracted for orthodontic reasons from healthy patients under age of 35. Alginate-Hydroxyapatite (Alg/HAp) scaffolds were obtained as previously reported by our group [1,2] and loaded with lactose modified chitosan (CTL). hDPSCs were seeded (70,000cells/scaffold) on Alg/HAp-CTL scaffolds. Subsequently, scaffolds were randomly divided in two groups: static and dynamic. Static samples were located in incubator at 37 °C, and 5% pCO_2. Dynamic samples were cultured in a bioreactor with a medium flow rate of 100µL/min. Proliferation of cells was assessed with an Alamar Blue assay at days: 0, 1, 3, 5, 7. Differentiation of cells was evaluated with an Alizarin Red staining protocol.

RESULTS & DISCUSSION: Cells exhibited a proliferation behavior which was function of the culturing conditions. The proliferation rate of cells cultured in the bioreactor was significantly lower than the one exhibited by the cells cultured in static conditions. On the other hand, results obtained from the Alizarin Red assays indicated a more rapid differentiation of cells cultured in dynamic conditions if compared with the ones cultured in static conditions.

CONCLUSIONS: This work helped to shed a light on the culturing conditions of undifferentiated stem cells and how these conditions influenced both cells proliferation and differentiation. The setting of the culturing conditions is essential to predict in vitro the subsequent behavior of the scaffold once implanted in vivo. Future work will involve characterization of the scaffold structural behavior when stressed with dynamic flow.

REFERENCES

[1] G. Turco, E. Marsich, F. Bellomo, S. Semeraro, I. Donati, F. Brun, M. Grandolfo, A. Accardo, S. Paoletti, Biomacromol. 10(6) (2009) 1575-83.
[2] E. Marsich, F. Bellomo, G. Turco, A. Travan, I. Donati, S. Paoletti, J. Mater Sci. Materials in Medicine 24(7) (2013) 1799-807.
[3] D. Porrelli, A. Travan, G. Turco, E. Marsich, M. Borgogna, S. Paoletti, I. Donati, Macromolecular Mater and Eng 300(10) (2015) 989-1000.



In vitro model of tendon inflammation - Comparison of different animal models in relation to human cells

G.L. Oreff [1], M. Fenu[1], S. Gültekin[1], I. Ribitsch[1], F. Jenner[1]
Presenting Author: Gil Oreff, gil.oreff@vetmeduni.ac.at
[1]Department for Companion Animals and Horses, Veterm, University Equine Hospital, Vetmeduni Vienna, Vienna, Austria

INTRUDUCTION: Tendinopathy represents a common and significant problem affecting people and animals alike [1-2]. While several animal models are used to study tendon pathologies [3], a comparison between different species in relation to human cells is lacking. The current study aims to report the differences and similarities of tenocytes response to inflammation in several in vitro animal models and human cells.

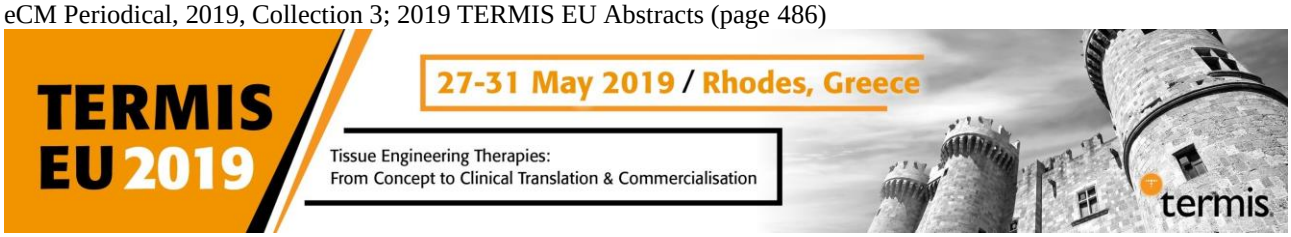
METHODS: Tenocytes from the Achilles tendon (mouse, rat and human) and the correspondent Superficial Digital Flexor tendon (horse and sheep) were used in the study. Primary cells from three donors per specie were included. Tenocytes were characterized upon acute (24 hours) and chronic (constant exposure) inflammation using 10 ng/µl IL1β and 10 ng/µl TNFα in comparison to unstimulated condition. Experiments were carried out to evaluate the proliferation capacity, gene expression profile and migratory properties of the cells. Proliferation was quantified by PicoGreen assay and proliferation rates were calculated in comparison to unstimulated condition after 24, 48 and 72 hours. Migration was evaluated through wound healing assay up to 96 hours. Finally, quantitative polymerase chain reaction (qPCR) was performed for several tendon and inflammation markers, as well as extracellular matrix degradation and production related genes.

RESULTS & DISCUSSION: The wound healing assay revealed similarity among all species. The migration capacity of the cells was reduced significantly upon inflammation, with stronger effect upon chronic inflammation. PicoGreen assays pointed out inherent differences between species. While proliferation rate decreased in sheep cells upon inflammation, in rats the opposite effect was observed. Moreover, the horse tenocytes increased proliferation upon acute inflammation while decreased upon chronic condition. Gene expression showed agreement between species in certain markers while other markers revealed altered trends in different animals. Similar trends in gene expression was observed in the small animals (mouse and rat) and in the large animal (sheep and horse) cells.

DISCUSSION AND CONCLUSIONS: It appears that tenocytes react to inflammation in a different manner in each species. In most aspects examined, small animals and large animals behave differently, however similarities could be seen between species inside each group. This work highlights the fact that none of the species behaves in the same manner as human cells in all aspects. However, it is also noted that each animal model is mimicking certain human tenocytes characteristics. Therefore, future studies should consider the differences shown in the current study, hence the animal model chosen to study inflamed human tenocytes should be selected with caution and in relation to the presented results.

REFERENCES

[1] Riley. Nat Clin Pract Rheumato. 2008; 4:82-9
[2] Dowling et al. EVJ. 2000; 32(5):369-78
[3] Mobasheri et al. Histol Histopathol. 2013; 28:955-64



Calcium-functionalized 3D silk gelatin bioink promotes osteogenesis of mesenchymal stromal cells: Perspectives for orthopedic surgery

G. Sharma [1], M. Desando[2], S. Petretta[2,3], I. Chawla[1], Bartolotti[2], C. Manferdini[4], F. Paolella[4], E. Gabusi[4], D. Trucco[4], S. Ghosh[1], G. Lisignoli[4]
Presenting Author: Gina Lisignoli, gina.lisignoli@ior.it
[1]Regenerative Engineering Laboratory Department of Textile Technology, Indian Institute of Technology, India [2]Laboratorio RAMSES, IRCCS Istituto Ortopedico Rizzoli (IOR), Italy [3]RegenHU CH, Switzerland [4]Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS IOR, Italy

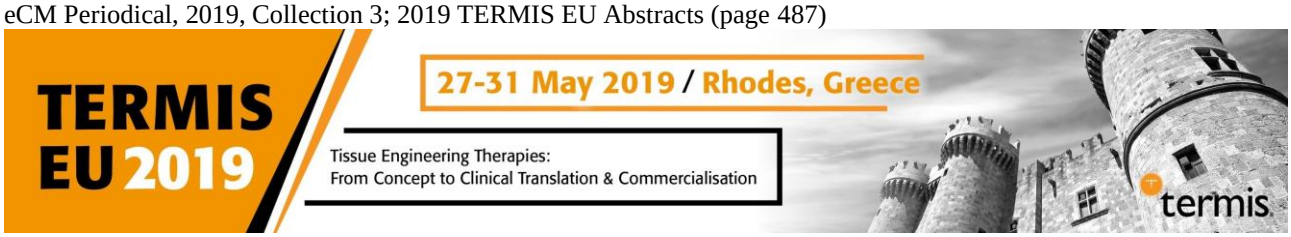
INTRODUCTION: Bone tissue engineering is aimed at developing innovative biomaterials capable of promoting tissue repair. Herein, we developed a calcium functionalized silk gelatin-based 3D (SF-G-$CaCl_2$) scaffold and evaluated the potential of enhancing the osteogenic differentiation of encapsulated human mesenchymal stromal cells (hMSCs).

METHODS: Silk gelatin-based 3D bioprinted constructs, composed of 5% bombyx mori silk fibroin (SF) and gelatin (G) were functionalized with 2.6 mM $CaCl_2$ and encapsulated with hMSCs through the bioprinting platform (3D Discovery, RegenHU). SF-G was used as experimental control. SF-G-$CaCl_2$ and SF-G constructs were cultured with D-MEM high glucose with and without osteogenic factors (GFs). Cell viability assessment and osteogenic differentiation through histology, gene expression analyses by Real Time PCR (RUNX2, COL1A1, ALP, OCN and SOST) and protein analyses by MALDI-TOF mass spectrometer (BMP-2, BMP-4, β catenin) were carried out. 1,14 and 21 days were chosen to monitor osteogenic differentiation.

RESULTS & DISCUSSION: SF-G-$CaCl_2$ resulted biocompatible ensuring high percentage of cell viability. Histological analysis with von Kossa staining showed significant increase of mineralization deposition in the presence of calcium. Gene expression analyses displayed a high expression of RUNX2 and COL1A1 in 3D bioprinted SF-G-$CaCl_2$ ($P<0.01$). The maximum level of ALP expression was observed for SF-G-$CaCl_2$ at day 14. Moreover, hMSCs-laden SF-G-$CaCl_2$ showed higher OCN and SOST expression compared to SF-G ($P<0.001$). Proteomic assessment gave evidence of an up-regulation of osteogenic signaling specific: BMP2 and BMP4 and β-catenin in SF-G-$CaCl_2$ constructs.

CONCLUSIONS: We developed a 3D osteogenic environment based on a gradual calcium release that promoted the osteogenic differentiation of hMSCs at both gene and protein levels with the involvement of in vivo like signaling pathways. The advantages of combining Ca^{2+}, SF-G bioink and 3D bioprinting technology opens potential insights for improving in vitro osteogenic potential of hMSCs for future BTE approaches.

ACKNOWLEDGEMENTS: Financial support was received from Istituto Ortopedico Rizzoli fund "5 per mille 2018".



Development of a novel pre-vascularized skin substitute using bottom-up approach

G. Imparato[1], C. Mazio[1], C. Casale[2], F. Urciuolo[2], C. Attanasio[3], F. Rescigno[1], P. Netti[1,2]
Presenting Author: Giorgia Imparato, giorgia.imparato@iit.it
[1] Center for Advanced Biomaterials for HealthCare@CRIB, Istituto Italiano di Tecnologia, Largo Barsanti e Matteucci 53, 80125 Naples, Italy [2]Interdisciplinary Research Centre on Biomaterials (CRIB), University of Naples Federico II P.le Tecchio 80, Naples, Italy [3]Department of Chemical, Materials and Industrial Production (DICMAPI) University of Naples Federico II, P.le Tecchio 80; Naples, Italy [3]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Italy

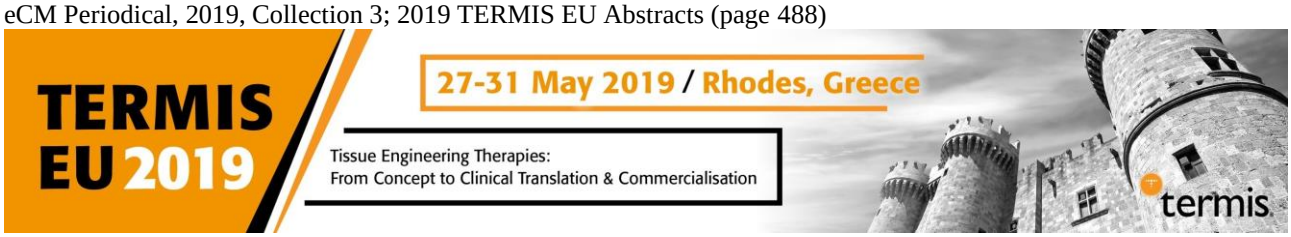
INTRODUCTION:
A pre-vascularized skin can accelerate the formation of anastomosis in vivo ensuring the survival of the implant. In the same time, in vitro, it can be a suitable model in view of fundamental research and industrial applications like drugs screening. Here, we vascularize a dermis equivalent model in vitro and prove its ability to anastomose with host vessels in mouse.

METHODS: Human dermal equivalent was produced as previously reported [1]. For producing pre-vascularized dermis (PVD) HUVECs were seeded on a surface of the samples [2]. Confocal Multiphoton imaging was used to monitor in vitro vessel formation and collagen deposition. Animal studies were performed following the guidelines of EU (2010/63/EU). Six immunoincompetent nu/nu mice 6/7 weeks old were implanted with the PVD and six with the non-vascularized ones. Animals were sacrificed 1 week after graft implantation and the explants were fixed in formalin (n=3) and paraformaldehyde (n=3) for immunofluorescence and histological analysis.

RESULTS & DISCUSSION: Human dermal equivalent was featured by the presence of primary Human Dermal Fibroblasts (HDFs) embedded into their own extracellular matrix (ECM). Confocal imaging showed that HUVECs were able to form capillary like structures (CLS) having an inner lumen in 1 week. The molecular analysis showed that the evolution of CLS in vitro was guided by VEGF-A. CLS were totally embedded into the ECM rich in collagen produced by HDFs, and were featured by bifurcation as it occurs physiologically. In order to study tissue integration in vivo, after 9 days of in vitro culture the PVD was implanted subcutaneously on the back of nu/nu mice. The results indicated that capillaries were perfused in vivo and in turn proved the existence of functional anastomosis between CLS and host vessels.

CONCLUSIONS: We obtained a pre-vascularized dermis model with CLS positive for maturation markers from 1 week after Huvec seeding. PVD was easy-to-handle and to suture. In vivo results showed that CLS were able to anastomose with host vessels in 1 week and be perfused by blood ensuring the sustenance of the graft. Ongoing experiments are carrying on to develop a vascularized full thickness skin model for wound healing application.

REFERENCES
[1] Imparato G. et al. Biomaterials 2013; 34:7851-61
[2] Mazio C. et al. Biomaterials 2019; 192:159-70



Micro-fragmented adipose tissue promotes long-term cell migration in a rabbit model of osteoarthritis: Biological insights for clinical translation

G. Desando[1], I. Bartolotti[1], L. Martini[2], N. Nicoli Aldini[2], M. Fini[2], A. Roffi[3], E. Kon[4], F. Perdisa[3], G. Filardo[3], B. Grigolo[1]
Presenting Author: Giovanna Desando, giovanna.desando@ior.it
[1]IRCCS Istituto Ortopedico Rizzoli, Laboratorio RAMSES, Italy [2]Laboratorio Studi Preclinici e Chirurgici [3]Applied and Translational Research Center, Bologna, Italy [4]Humanitas University Department of Biomedical Sciences, Milan, Italy

INTRODUCTION: Basic and translational research has enabled improvements in the clinical management of osteoarthritis (OA). Recently, the adipose "niche" has emerged as a promising therapy being a rich source of progenitor cells available with different procedures [1-3]. Herein, we investigated the migratory potential of micro-fragmented adipose tissue (MFAT), stromal vascular fraction (SVF) and expanded- adipose derived stromal cells (ASCs) in a rabbit OA model to promote joint tissue repair.

METHODS: OA was induced in rabbits by anterior cruciate ligament transection. ASCs (n=6) and SVF (n=6) were obtained by enzymatic treatment using collagenase NB4, whereas MFAT (n=6) by a mechanical treatment in a one-step closed system (Lipogems). Cell were evaluated for their viability and characterized for specific mesenchymal markers. For local biodistribution analysis, cells were labelled with PKH26, a fluorescent dye. After OA onset, $2x10^6$ ASCs, SVF and MFAT were intra-articularly injected. Analyses on cell distribution and co-localization for PKH26 and CD-146 were done at short and long-term follow-ups: 7 and 30 days. The Monte Carlo method and the general linear model for multiple comparisons were used for statistics.

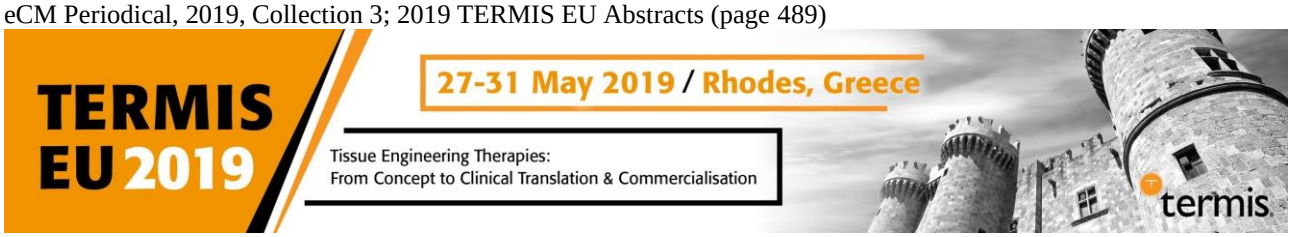
RESULTS & DISCUSSION: A good cell viability was found in all cell compounds showing high percentages for mesenchymal markers. Distinct and long-term migration was found for ASCs, SVF and MFAT. At day 7, ASCs and SVF showed a high tropism towards the synovium, whereas MFAT versus articular cartilage. Conversely, an opposite migration was found at day 30 for all cell compounds. Different percentages of CD-146 progenitors were found for all cell therapies up to day 30. Moreover, MFAT displayed a higher percentage for the M2 macrophage marker CD-163 when compared to SVF ($P<0.05$).

CONCLUSIONS: The long-term presence of adipose derived cells has a clinical significance, since it ensures a reservoir of progenitors capable of launching various repair processes on body's demands. Moreover, the highest percentage of CD-163 in MFAT poses some biological premises on its superior healing potential for OA treatment.

ACKNOWLEDGEMENTS: Financial support was received from the Italian ministry of Health (RF 2011-02352638).

REFERENCES

[1] Bourin P et al. Cytotherapy.2013; 15(6):641-8
[2] Pak J et al. J Biomed Sci. 2017; 24(1):1-12
[3] Pak J et al. Int J Mol Sci.2018; 19 (7):2416



Improvement of Langerhans islets function in three-dimensional collagen gel in vitro and in vivo

M. Salamone[1], S. Rigogliuso[1], S. Campora[1], C. Bruno[1], P. Cinà[1], G. Ghersi[1-2]
Presenting Author: Giulio Ghersi, giulio.ghersi@unipa.it
[1]ABIEL S.r.l., c/o ARCA Incubatore di Imprese, Viale delle Scienze, ed. 16 - 90128 Palermo, Italy
[2]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche; Viale delle Scienze, ed. 16 Università degli studi di Palermo, 90128 Palermo, Italy

INTRODUCTION: The transplantation of pancreatic islets currently represents a promising therapeutic option for the management of insulin dependent diabetes (1). During the isolation procedure, islets receive several stress that continue to occur during the pre-transplant culture period. The enzymatic digestion is a critical step to obtain functional islets (2). Moreover the culture of islets in Three-Dimensional Culture condition seems to maintain their organoid morphology; interesting results show also that the co-culture of islets with mesenchymal stem/stromal cells (MSC) improve viability and function and graft survival (3).

METHODS: Recombinant COL G (Class I) and COL H (Class II) blend are used to purify islets from rat. The Islet were cultured in Three-Dimensional (3D) type-I collagen gel in the presence or absence of stromal vascular fraction (SVF) isolated from rat adipose tissue. To assess insulin release the GSIS index insulin release from islets cultivated with glucose 16,7 and with 5,6 mM in different days were quantified. To follow the functionality of islets in vivo, we use the same collagen gel system in transplantation experiment into Streptozotocin (STZ)-induced diabetic rats.

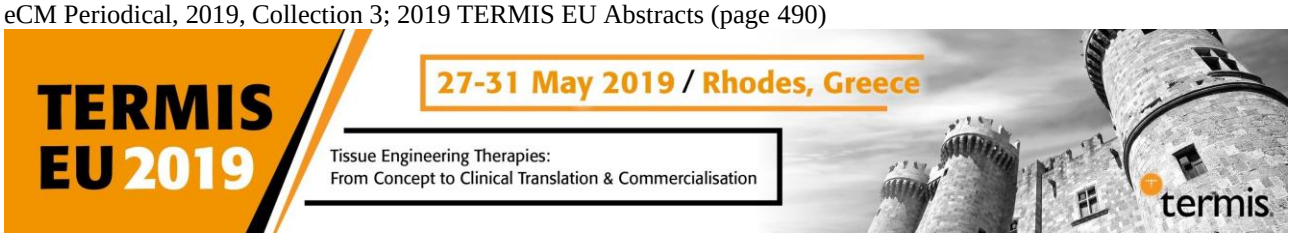
RESULTS & DISCUSSION: We observed islets morphology for several weeks and we follow their function using Elisa Test. The results showed that islets cultured in 3D condition maintain the functionality up to 15 days (GSIS index more of 1,2; after that time islets started to reduce the insulin secretion in vitro. In in vivo experiment we show a reversion of diabetes in rat transplanted into the omentum with about 2000 IEQ (islets equivalent). Moreover, SVF were co-culture in 3D collagen gel in order to study the interaction between the islet and the SVF. We observe an increase number of SVF surrounding the Islet probably stimulated by factor secreted by them. We do not observe an increase of insulin secretion in presence of SVF.

CONCLUSIONS: We are able to revert type 1 diabetes in rat model using islets purified with recombinant collagenase and transplanted into the omentum with collagen hydrogel. Moreover the co-colture of SVF and islet can be used to study of interaction between pancreatic islets and adipose tissue cells (for example by inducing hypoxia).

ACKNOWLEDGEMENTS: Financial support was received from EU H2020 Drive project, 645911 (www.drive-project.eu).

REFERENCES

[1] Shapiro AM, et al. N Engl J Med. 2000; 343:230-238;
[2] Heide Brandhorst et al. Cell Transplant. 2017. 26(11): 1733–1741.
[3] Bianca Marmontel de Souzaa et al ISLETS. 2017. 9, NO. 2, 30–42



Is the terminal complement complex a target for intervertebral disc degeneration therapeutics?

G.Q. Teixeira[1], Z. Yong[1], R. M. Goncalves[1,2], A. Kuhn[3], M. Ruf[4], A. Nerlich[5], U. Mauer[6], A. Ignatius[1], R. Brenner[3], C. Neidlinger-Wilke[1]
Presenting Author: Graciosa Q. Teixeira, graciosa.teixeira@uni-ulm.de
[1]Institute of Orthopaedic Research and Biomechanics, Ulm University, Ulm, Germany [2]Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal [3]Division for Biochemistry of Joint and Connective Tissue Diseases, Department of Orthopedics, Ulm University, Ulm, Germany, [4]Spine Center, Karlsbad-Langensteinbach, Germany [5]Institute of Pathology, Academic Hospital Munich-Bogenhausen, Munich, Germany [6]Department of Neurosurgery, German Armed Forces Hospital, Ulm, Germany

INTRODUCTION: Terminal complement complex (TCC) was previously identified in herniated human intervertebral disc (IVD) tissues [1]. Here, we characterize TCC deposition in the different IVD regions and its correlation with distinct disc pathologies.

METHODS: Disc tissues were collected from healthy donors (Healthy, n=6, age 12±7), patients with scoliosis but no signs of disc degeneration (Sc, n=10, age 16±5), or with disc degeneration (DD, n=36, age 60±13, Pfirrmann grade 3-5). TCC deposition was investigated in different IVD regions: annulus fibrosus (AF), nucleus pulposus (NP) and endplate (EP). Randomly selected Sc and DD cells (passage 2-5) were analyzed for gene expression of complement regulators CD46, CD55 and CD59, inflammation markers, matrix proteins and degrading enzymes. TCC deposition was induced in vitro by IVD cells culture with 5% human serum (HS), and analyzed by ELISA. Culture in serum-free medium served as control.

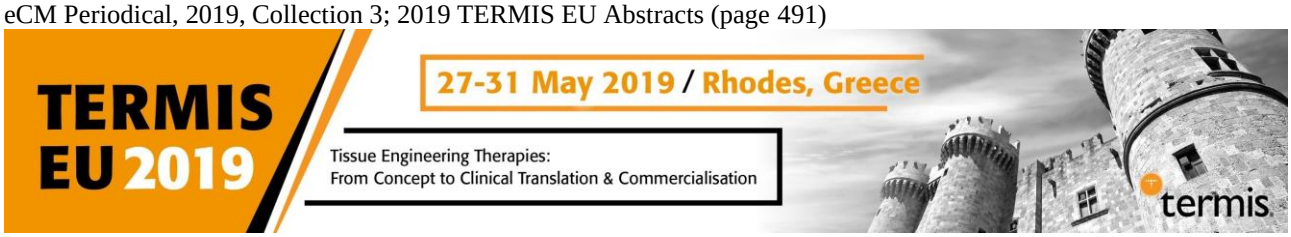
RESULTS & DISCUSSION: Although TCC immunopositivity was observed with high variability, a significantly higher frequency of TCC+ cells was found in Healthy (young) and DD compared to Sc group ($p<0.05$). After cell expansion, CD46 and aggrecan were significantly down-regulated in NP cells of DD compared to Sc ($p<0.05$). An increase in TCC deposition was observed in presence of HS ($p<0.05$), but no differences were observed between DD and Sc expanded cells.

CONCLUSIONS: The data suggests that TCC is formed in IVD cells both in very young or strongly degenerated discs, which might correlate with vascularization. TCC deposition could be induced in vitro, confirming that IVD cells are not completely protected. Ongoing studies explore the functional relevance of TCC in DD as possible target for new therapeutic approaches.

ACKNOWLEDGEMENTS: DFG (NE 549/6-1, BR 919/12-1) and Ulm University (L.SBN.0157).

REFERENCES

[1] Grönblad M et al. Spine. 2003; 28(2):114-8 28(2):114-8



Hyaluronan supplemented culture media significantly increases early chondrogenesis glycosaminoglycan synthesis and reduces the upregulation of collagen X in a stem cell-based implant

G. Monaco[1, 2], M. Alini[1], A. El Haj[2,3], M. J. Stoddart[1, 2]
Presenting Author: Graziana Monaco, graziana.monaco@aofoundation.org
[1]AO Research Institute, AO Foundation, Davos, CH [2]Institute for Science & Technology in Medicine, Keele University, UK [3]University of Birmingham, Healthcare Technology Institute, UK

INTRODUCTION: High molecular weight hyaluronan (hMwHA) is a key bioactive macromolecule of healthy synovial fluid (HSF) whilst also effecting osmolality[1]. We hypothesize that a hMwHA supplemented culture medium may better recapitulate the biological features of HSF present in the patients' intra-articular joints. Thus, our aim is to investigate the effect of physiological levels of hMwHA supplemented into culture media on the chondrogenesis of human mesenchymal stem cells (hMSCs) that would be present in a traumatic defect after marrow stimulation techniques such as microfracture.

METHODS: Bone marrow hMSCs at P3 were evenly seeded fibrin at $5x10^6$ cells/150µl and retained within macroporous Polyurethane (PU) scaffolds. Control medium was serum free DMEM plus 1% ITS+, 1% Pen/Strep, 1% non-essential amino acid, 50µg/ml ascorbate-2-phosphate, 5µM ε-amino-caproic acid, 10^{-7} M dexa (HA- TGFβ-). This media was further supplemented with 10 ng/mL TGFβ1 (HA- TGFβ+) or with 0.2% HA (HA+ TGFβ-) or both (HA+ TGFβ+). 1,800 kDa HA was added to simulate HSF concentration and viscosity (2.3 mg/ml)[2,3]. 1,000kDa HA was also added to the scaffolds of one group at 0.02% (HAS TGFβ+). Real time qPCR was done to investigate gene expression. Total DNA, sulphated GAG and Safranin O/Fast Green staining were evaluated.

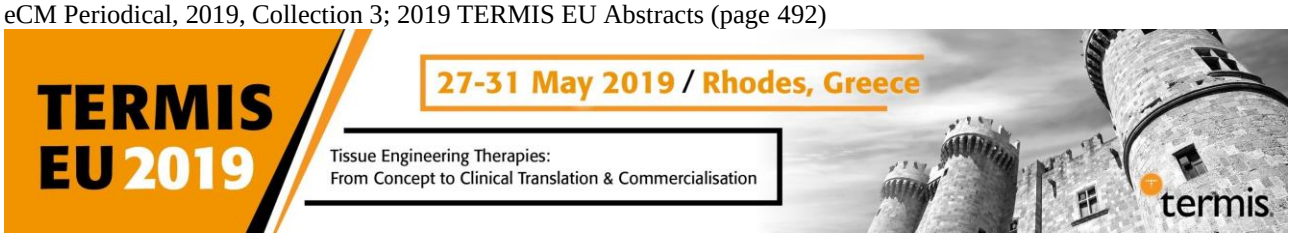
RESULTS & DISCUSSION: The level of GAG per media synthesized by HA+TGFβ- group was significantly and consistently higher if compared with the control medium TGFβ free (HA- TGFβ-) and the standard chondrogenic media containing active TGFβ (HA- TGFβ+) within the first week of chondrogenesis (**$p<0.005$; *$p<0.05$). Exogenous hMwHA (HA+ TGFβ+) reduced the upregulation of the hypertrophic cartilage marker collagen X, normally induced by active TGFβ.

CONCLUSIONS: Collectively, these data illustrate the beneficial effect of hMwHA supplemented media on 3D hMSC chondrogenic culture. In the current climate of animal welfare and "3Rs", HA media, by mimicking in-vivo environment, can be helpful to reduce in-vitro artefacts, enabling more accurate prescreening of potential cartilage repair therapies.

ACKNOWLEDGEMENTS: This project was funded by the AO Foundation, Switzerland.

REFERENCES

[1] Avenoso et al. Inflammation Research 2017; 1-16.
[2] Akmal et al. J Bone Joint Surg Br 2005; 87: 1143-1149.
[3] Balazs at al Arthritis Rheum 1967; 10: 357-376.



Self-assembling peptides with RGD motifs as scaffolds for tissue engineering

G. Deidda[1,2],* M. Farsari [1,2], A. Mitraki[1,2]
Presenting Author: Graziano Deidda, graz1976@iesl.forth.gr
[1]Department of Materials Science & Technology, University of Crete, Heraklion, Greece [2]Institute of Electronic Structure and Laser, IESL-FORTH, Heraklion, Greece

INTRODUCTION: We used a combination of theoretical and experimental approaches towards self-assembling (S.A.) peptide rational designs. [1] [2] The cell attachment motif RGD and a key-role residue were projected to be incorporated to the S.A. central motif GAITIG in order to allow respectively cell adhesion and metal nanoparticle binding. [3]

METHODS: We monitored the assembly of the peptide-derived networks and their morphology through: X-ray diffraction, TEM, FESEM and AFM. Cell-culture experiments used for viability were: MTT assay and LIVE/DEAD test. An ultraprecise femtosecond (220 fs) laser was used to fabricate 3D fully biocompatible scaffolds via Multi Photon Stereolithography (MPS).

RESULTS & DISCUSSION: The designed RGD S.A. peptides proved to be appropriate building blocks to form higher order assembled networks. (Fig. 1). [2] Cys-containing S.A. scaffolds could be efficiently attached to a hybrid zirconia-derived composite in a 3D fs laser fabrication named "scaffold-on-scaffold" strategy for cell attachment (Fig. 2a). In addition, preliminary results indicate that S.A. peptides containing Tyr and Trp responded positively to 1P- and 2P- irradiation (Fig. 2b).

CONCLUSIONS: These S.A. peptides will subsequently be studied for in vitro and in vivo models as they are amenable to offer open-ended possibilities towards multifunctional tissue engineering scaffolds of the future. Especially, these multi-functional scaffolds in the form of hydrogel have found important involvements in heart regenerative medicine applications.

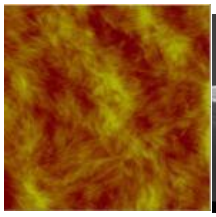
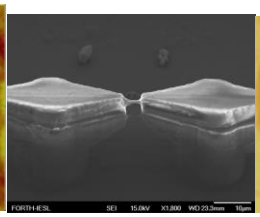
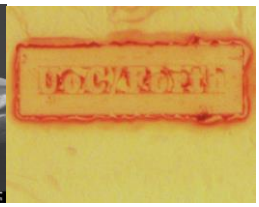
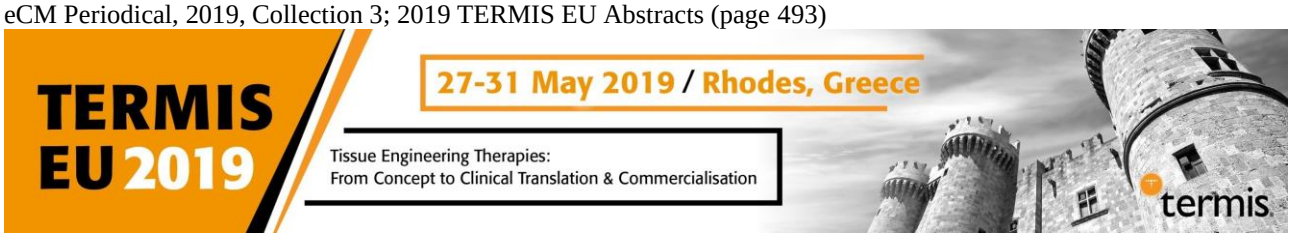




Figure 1 : (Left) AFM analysis showed clearly fibril formation of the peptides. **Figure 2:** (Middle) 3D structures produced by the 2PP technique. Zirconia-based «scaffold-on-scaffold» sandwich. **Figure 3:** (Right) fabrication for soft-tissue engineering.

ACKNOWLEDGEMENTS: Financial support was received from the European Union funding under the 7th Framework Programme under Grant Agreement Number 317304.

REFERENCES

[1]. Y. Loo, et al. Adv. Mater. 2015; 2557-86.
[2]. P. Tamamis, et al. J. Phys. 2009; Chem. B, 15639-47.
[3]. G. Deidda, & S.V.R. Jonnalagadda, et al. ACS Biomater Sci. Eng. 2017; 1404-16.



Self-assembled peptide hydrogel for accelerating the osseointegration period of dental implants

G. Onak[1], O. Gökmen[1], O. Karaman[1*]
[1]Department of Biomedical Engineering, Izmir Katip Celebi University, Izmir, Turkey
Presenting Author: Günnur Onak, gunnur.onak@ikc.edu.tr

INTRODUCTION: Osseointegration is described as formation of functional and structural connection between the surface of the dental implant and host bone [1]. Tissue grafts have widely been used to fill the defects between dental implant and host tissue to accelerate the osseointegration process. However, healing time after dental implant application would take several months. Self-assembled peptide hydrogels have been successfully used for regeneration of many tissues such as bone, cartilage, neural and blood vessel. One of the major limitations of using self-assembled peptide hydrogel on bone regeneration is their inadequate mechanical property and ability of inducing osteogenic and vasculogenic differentiation simultaneously. The objective of this study is to develop and characterize in situ multifunctional self-assembled peptide hydrogels in different concentrations and determine the efficacy of inducing osteogenic of mesenchymal stem cells with their functional epitopes to use in dental implant osseointegration process.

METHODS: KLD 12(KLDLKLDLKLDL), KLD 12- EEEEE and KLD 12-EEGGC were manually synthesized manually on 4-Methylbenzhydrylamine (MBHA) resin. KLD-12 powder was dissolved in PBS in a peptide concentration of 1%, 2% and 4% (w/v). MSCs were re-suspended in a complete culture medium, and the peptide solution was added to prepare a peptide/cells suspension, with a final peptide concentration of 0.5 %, 1% and 2% by diluting with the cell density of 10^6 cells/ml. Cell encapsulated peptide hydrogels were incubated in osteogenic medium. Calcium Assay, ALP Assay and DNA quantification assay were evaluated on KLD 12-EEEEE and KLD 12-EEGGC groups by 7d, 14d, 21d and 28d to compare the effect on calcium phosphate nucleation and osteogenic differentiation of human mesenchymal stem cells. Atomic force microscope (AFM) was employed to examine the inner structure of self-assembled peptide hydrogel. The viscoelastic behavior of the hydrogels was characterized by rheology measurement.

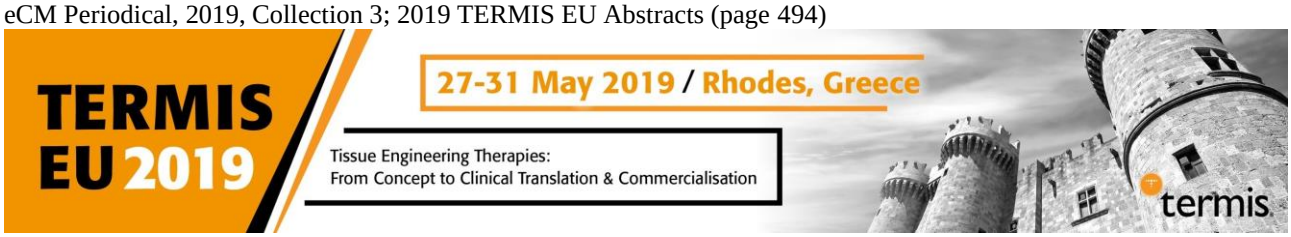
RESULTS & DISCUSSION: AFM results revealed that self-assembled KLD-12 peptide resembled nanofibril formation. It was observed that when the concentration of peptides was increased, mechanical properties such as compressive and shear modulus was improved. No significant difference in osteogenic differentiating parameters was observed between different epitope containing groups.

CONCLUSIONS: Self-assembled KLD-12 peptide hydrogels in different concentration was successfully produced. After determining ideal concentration in terms of mechanical properties and osteogenic differentiation capacity, modified KLD 12 peptide with osteogenic motifs will induced to support both bone tissue as well as taking the shape of defect by injection. This injectable multifunctional scaffold that will be developed could be used in clinics to accelerate dental implant osseointegration.

ACKNOWLEDGEMENTS: This study was financially supported by BAP (Research Projects Fund of Izmir Katip Celebi University Scientific) through the research project of 2016-ONP-MUMF-0022.

REFERENCES

[1] Alghamdi H. et al. J Funct Biomater, 2018, 9.1:7



Cerium doped zinc oxide (ZnO) for improved cell viability & craniofacial vascularization

H. Akhtar [1], A.A. Chaudhry [2], G. Reilly [1], J.A. Darr [3], T. Ashton [3], I.U. Rehman [1]
Presenting Author: Hafsah Akhtar, hakhtar2@sheffield.ac.uk
[1]Kroto Research Institute, University of Sheffield, UK [2]Interdiscplinary Research Center in Biomedical Materials (IRCBM), Pakistan, [3] Clean Materials Technology Group, University College London (UCL), UK

INTRODUCTION: Craniofacial bone is highly vascularized tissue and its repair and regeneration is challenging especially when the network of blood vessels is disrupted due to any pathology or fracture, which makes the healing extremely difficult. The traditional scaffold implantation does not induce angiogenesis (formation of blood vessels), instead it relies on inflammatory healing response that results in insufficient oxygen and nutrient supply, hence, resulting in non-uniform cell differentiation and cell death [1,2]. This project investigated the tailored chemistry approach to obtain nano ceramics with added functionality. Therefore, the aim of this project ventured to assess the potential of cerium doped ZnO for improved cell viability & vascularisation.

METHODS: Continuous Hydrothermal Flow System was used to synthesize cerium doped ZnO. Raman Spectroscopy, X-ray Diffraction, Transmission Electron Microscopy, Brunauer–Emmett–Teller (BET) was performed to analyze nanoparticle properties. Human Osteosarcoma Cells MG63 were used for Cell viability and VEGF release assay. 10,000 cells/cm^2 were seeded in 24 well plates and were allowed to attach for 24 h, followed by introduction of nanoparticle suspensions. Moreover, supernatant was collected for VEGF release analysis and 7-day study was performed, and cell viability was analyzed at Day 1, Day 3, Day 5 and Day 7, respectively.

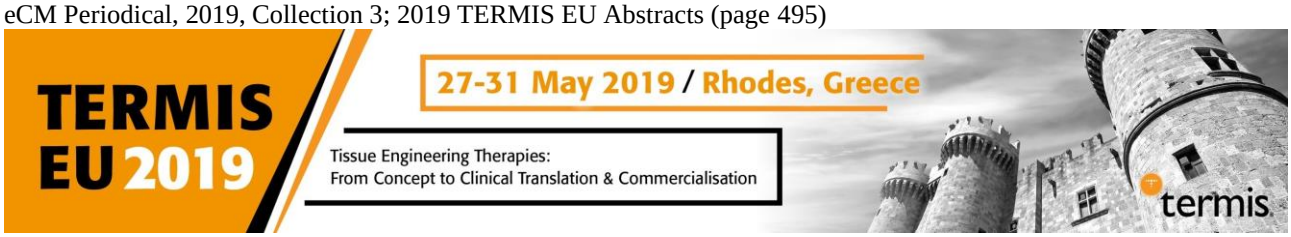
RESULTS & DISCUSSION: Chemical characterization suggested successful synthesis of cerium doped ZnO. Transmission Electron microscopy and Brunauer–Emmett–Teller (BET) demonstrated that the particle size ranged from 60-90nm and possess rod shaped morphology. 7-day cell viability study revealed that the cerium doped ZnO are non-toxic. Furthermore, increased cellular activity was observed in presence of Cerium Doped ZnO.

CONCLUSIONS: The preliminary data illustrated the non-cytotoxic nature of cerium doped ZnO. It also revealed promising results to initiate vascularization, which is the pre-requisite for bone regeneration. Further investigation on release mechanisms and biocompatibility are in progress which will lead to additional understanding of future potential for craniofacial bone regeneration and vascularization.

ACKNOWLEDGEMENTS: Financial support was received from Doctoral Academy, University of Sheffield; Nanoparticle synthesis process was funded by Clean Materials Technology, University College London.

REFERENCES

[1] Shahzadi, et al. International journal of biological macromolecules, (2018), 120, 721-728.
[2] Ahtzaz, S. et al. ‘Materials & Design. Elsevier, (2017), 132, pp. 409–418.



Regulating paracrine crosstalk of mesenchymal stem cells with endothelial cells and osteoblasts by bone-mimetic material

H. Rammal[1], L. Entz[1], S.C. Gangloff[1], C. Mauprivez[1], H. Kerdjoudj[1]
Presenting Author: Halima Kerdjoudj, Halima.Kerdjoudj@univ-reims.fr
[1]EA 4691 « Biomatériaux et inflammation en site osseux » (BIOS), URCA, Reims, France

INTRODUCTION: The capacity to secrete protective and biologically active mediators places MSCs among the most suitable tools for tissue regeneration. Studying mesenchymal stem cell (MSCs) responses to biomaterials provides evidences that their chemical, biophysical and/or mechanical cues have a deep impact on MSCs fate[1]. However, current strategies neglect the effect of materials on MSCs paracrine activities. The present study investigates whether bone-mimetic material (B-MM) could promote MSCs pro-regenerative secretome; especially on the production of angiogenic and osteogenic factors

METHODS: Bone-mimetic material (B-MM) made from inorganic calcium phosphate supplemented with chitosan and hyaluronic acid biopolymers was built-up using an automated spraying device[2]. Human MSCs were seeded in 24 well plates at 24 × 10^3 cells/cm^2 on UV-decontaminated B-MM and cultured for 21 days. Between 19th and 21st day of culture, MSCs conditioned media (MSCs-CM) were collected, and stored at -80°C. Cytokines, chemokines and growth factors release was quantified by ELISA and their activities were evaluated on human endothelial cells (ECs) and osteoblasts (OBs).

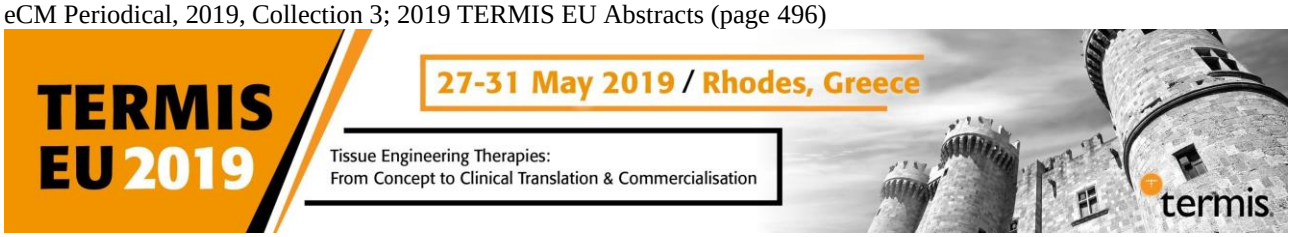
RESULTS & DISCUSSION: MSCs cultured on B-MM, composed of carbonated apatite, chitosan and hyaluronic acid biopolymers for 21 days, showed the formation of bone-like nodules. However, bone-like nodule arising from MSCs fibroblastic layers occupied only 8 % of cultured area. The secretome analysis showed that B-MM significantly affects the production of soluble mediators required for bone healing and homeostasis. MSCs on B-MM decreased the production of IL-1□, IL-6 and IL-8 inflammatory mediators and increased the release of PGE2, HGF, b-FGF and VEGF angiogenic growth factors, enhancing thus ECs chemotaxis, inducing ECs inflammatory profile, regulating neutrophils recruitment through an up-regulation of ICAM-1 and interestingly promoting ECs osteogenesis properties by up-regulating BMP-2. IL-1□, IL-6, IL-8 and VEGF are reported to mediate, in dose-dependent manner, bone regeneration by promoting pre-osteoblasts proliferation and differentiation[1]. As for OBs cultured in osteogenic media, qRT-PCR experiments showed that MSCs-CMs up-regulate the expression levels of RUNX2, COL1A1 and ALPL over the time, however no boosting effect of OBs differentiation was observed as BGLAP, late bone specific marker, was not detected.

CONCLUSIONS: Our data provide, herein, evidences that the indirect crosstalk between MSCs and various cell types involved in bone regeneration might be finely regulated by B-MM.

ACKNOWLEDGEMENTS: This work was partially supported by “Interreg V France-Wallonie-Vlaanderen program-TEXTOS” and “Geules Cassées” fundation

REFERENCES

[1] Jin et al., Clin. Orthop. Surg. 2018, 10, 271.
[2] Rammal, et al., ACS Appl. Mater. Interfaces 2017, 9, 12791.



Label-free intrinsic cathodoluminescence resolves spectral signatures of highly heterogeneous tissue isolated collagen scaffolds

H.M. Larsson[1], M. Cantoni[1], M.S. Zielinski[1]
Presenting Author: Hans Mattias Larsson, mattiashans.larsson@epfl.ch
[1]CIME, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

INTRODUCTION: Resolving complex tissue structures in situ become extremely challenging since different types of cross-linked collagens and interacting extracellular matrix proteins might be involved, and typical characterization techniques, such as histology, immunohistochemistry, scanning electron microscopy (SEM) or diffraction-limited optical methods based on fluorescence or second-harmonic generation (SHG) cannot provide unambiguous compositional information. Recent examples of applying material science techniques, such as SEM correlated with energy-dispersive X-ray spectroscopy (EDS), showed interesting results in characterization of critical insights in pathological processes in calcified lesions in cardiovascular tissues, and helped to identify typical amino-acid fragments of collagen fibrils in preserved prehistoric specimens. Here we show a similar approach based on the intrinsic cathodoluminescence (CL) imaging contrast.

METHODS: A state of the art quantitative CL-SEM microscope was used in label-free characterization of tissue-engineered collagen scaffolds. Mass-spectrometry was used to confirm the identified extracellular matrix proteins of the scaffolds in the CL-SEM analysis.

RESULTS & DISCUSSION: Presented SEM (Fig. 1a) and hyperspectral intrinsic CL (Fig. 1b) images of bovine collagen gel were obtained with 40 and sub-10 nm spatial resolution, respectively. High accuracy in localization of spectral auto-CL signatures specific to molecular composition of isolated collagen fibrils (Fig. 1c) was possible due to the excitation with a sub-4 nm electron beam probe size, and confined carrier diffusion lengths within the bioorganic material. Deconvolution-based analysis of such hyperspectral auto-CL images allowed to differentiate between bovine, rat and humanized collagens, as well as localize thin layers of human fibrinogen complex over a 200 μm field of view (FOV), confirmed by correlation with a mass spectroscopy.

CONCLUSIONS: Overall, this spectrally resolved label-free technique provides quantitative information about material heterogeneity with a sub-40 nm localization accuracy, and appears to be a powerful tool in monitoring molecular distributions. Label-free intrinsic cathodoluminescence technology with its resolution close to an average size of a protein and molecular identification capacity should be of interest to the tissue engineering community.

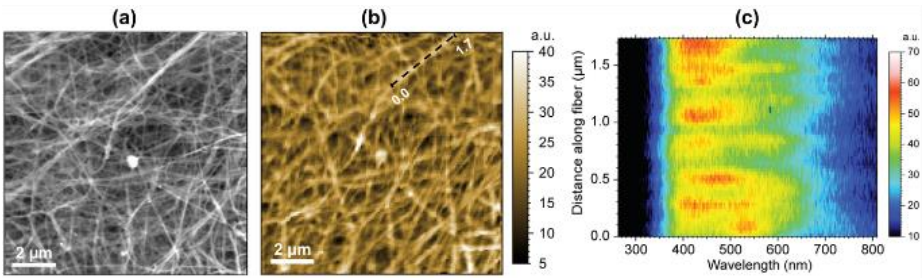
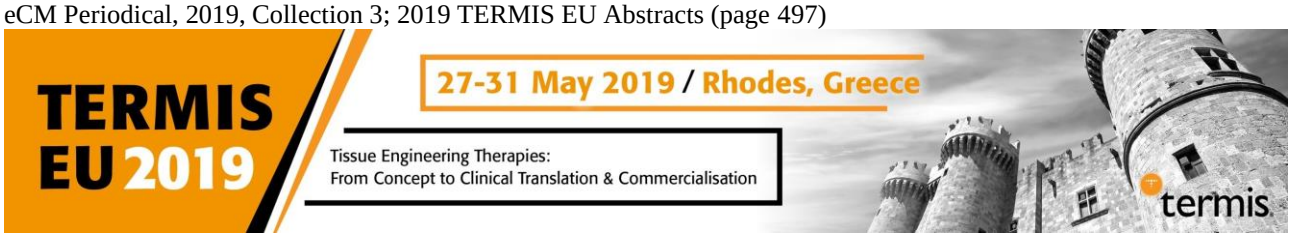


Figure 1: a) SEM image of a bovine collagen gel sample, and (b) corresponding panchromatic auto-CL image, obtained with 550 nm spectral bandwidth. (c) Auto-CL spectra projection along a thick collagen fibril, marked with the black dashed line in (b). Each spectrum corresponds to a single pixel within the auto-CL image.



Quantitative imaging of the cell mechanical environment of 3D multicellular systems

M.M. Vaeyens[1], J.B. Fano[1], J.A.S. Herrera[2], A.J. Peñas[1], C. Steuwe[3], M. Roeffaers[3], H. Van Oosterwyck[1,4]
Presenting Author: Hans Van Oosterwyck, hans.vanoosterwyck@kuleuven.be
[1]Mechanical Engineering dept., KU Leuven, Belgium [2]Continuum Mechanics dept., University of Seville, Spain [3]Center for Surface Chemistry and Catalysis, KU Leuven, Belgium, [4]Prometheus, div. Skeletal Tissue Engineering, KU Leuven, Belgium

INTRODUCTION: Cell-matrix mechanical interactions modulate cell fate decisions and multicellular organization and as such are important for tissue engineering. We have developed optical imaging procedures and computational algorithms to quantify cellular forces in 3D multicellular culture systems relevant for tissue engineering. Here we show their importance for the study of angiogenesis.

METHODS: 3D in vitro assays of vascular endothelial invasion in collagen hydrogels were optimized for live cell optical imaging by means of either confocal fluorescence microscopy or selective plane illumination microscopy (SPIM). SPIM provides fast 3D imaging of relatively large, multicellular systems with reduced phototoxicity, making it ideal for live cell imaging. Cell-matrix mechanical interactions during vascular invasion were quantified by means of novel traction force microscopy (TFM) algorithms. First, non-rigid image registration was performed to resolve 3D matrix deformations around invading sprouts. Second, cellular forces that were responsible for the measured deformations were quantified using an iterative, finite element based solution scheme that makes use of a novel physics-based regularization method.

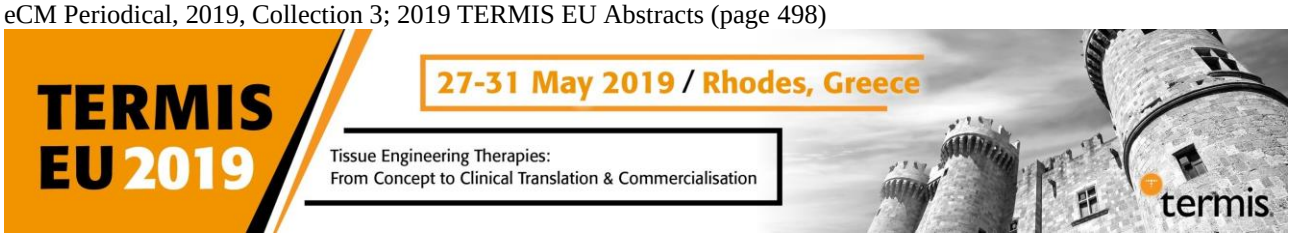
RESULTS & DISCUSSION: We previously demonstrated the superior accuracy and robustness of non-rigid image registration for 3D matrix deformation calculation, based on the use of fiducial markers as well as collagen network imaging (by means of Second Harmonic Generation) [1]. Applying our novel TFM methods to invading angiogenic sprouts, we observe robust patterns of radially pulling forces around sprout protrusions. When interfering with actin turnover (by adding cytochalasin D), forces are diminished, in turn inhibiting vascular invasion. Time lapse imaging data revealed strong correlations between matrix deformations and protrusion dynamics.

CONCLUSIONS: Our 3D TFM methods enable to study cellular forces in 3D multicellular systems relevant for tissue engineering.

ACKNOWLEDGEMENTS: ERC StG n°308223, FWO (G.0821.13, G0B9615N, G087018N) and KU Leuven (C14/17/111).

REFERENCES

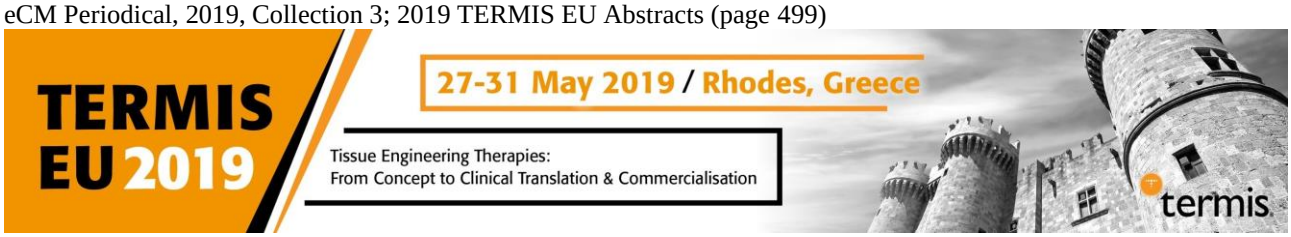
[1] Jorge-Peñas A et al. Biomaterials. 2017; 136: 86-97



Development of magnetic control systems for orthopedic regenerative medicine

H. Markides[1], R.H. Morris[2], A. Spicer[2] A.J. El Haj[1]
Presenting Author: Hareklea Markides, H.markides@bham.ac.uk
[1]Healthcare Technologies Institute, Department of Chemical Engineering, Birmingham University, UK [2]School of Science and Technology, Nottingham Trent University, Nottingham, UK

Despite advances in the regenerative medicine field, few therapies have progressed to routine clinical application, with a number of translational challenges to be addressed before therapies can be approved. Superparamagnetic Iron Oxide Nanoparticles (SPIONs) have a multifunctional role to play in addressing such challenges to develop and translate stem-cell based treatments. One of the main translational challenges we currently face is to understand and control cell fate after implantation. SPION labelling allows for magnetic manipulation of therapeutic cells to remotely activate, track and target cells in vivo by external magnetic field to address these challenges. Here, we aim to introduce novel magnetic devices and describe how such devices can be used to enable cell therapy translation.



Translation of synthetic electrospun patch to the clinic

H.L. Morris[1], A.A. Lach[1], J.A. Martins[1], P.A. Mouthuy, A.J. Carr
Presenting Author: Hayley Morris, hayley.morris@ndorms.ox.ac.uk
[1]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, OX3 7LD, United Kingdom

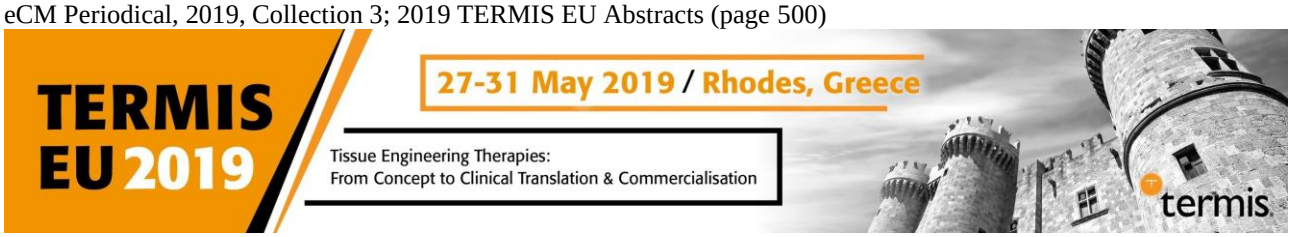
INTRODUCTION: Rotator cuff tears are a common and often debilitating problem. Despite technical improvements in surgical procedures, post-operative re-rupture rates occur in around 40% of cases. A clear need exists for materials that are both mechanically strong, and also stimulate the endogenous repair process of tissue. An electrospun product has been developed to fulfill this need; a multi-component patch comprised of bioactive electrospun layers, bonded to a mechanically strong woven backing. Preliminary testing has shown a good safety profile and improved cellular responses both in vitro and in vivo. This biodegradable product is now in preparation for the first in-man safety and feasibility trials.

METHODS: The patch development process will be used as a case study to detail the transition of tendon biomaterials from academic research, to GMP (Good Manufacturing Practice) cleanroom manufacture, along with discussion of the gathering of preclinical evidence of safety and performance of the device. Adoption of ISO 13485:2016 Quality Management Systems: Requirements for Medical Devices, provides a practical foundation from which to address the regulations and responsibilities involved in medical device manufacture.

RESULTS & DISCUSSION: The patch is a class III medical device, made using novel production techniques - as a result, the regulatory burden is high. In the UK, applications to initiate the first in-man clinical trial are made to the MHRA (Medicines and Healthcare Regulatory Agency). Preclinical data supporting evidence of biological safety must be gathered into an Investigator's Brochure for review by the regulatory authority. This will include information on: biological evaluation of the device, risk management, sterilization compatibility, device traceability, design control and evidence of manufacturing quality and consistency.

CONCLUSIONS: Novel biomaterials are frequently invented within an academic setting, however, translation of these materials to the clinic is often slow and problematic. Through dissemination of this case study, we intend to show some of the methods, and requirements for translating implantable electrospun biomaterials towards human applications.

ACKNOWLEDGEMENTS: This research was funded by the National Institute for Health Research (NIHR), the Medical Research Council the Wellcome Trust and the NIHR Oxford Biomedical Research Centre.



Decellularized porcine matrix as a multifunctional biomaterial for regenerative medicine

H. Capella-Monsonís[1,2], D.I. Zeugolis[1,2]
Presenting Author: Héctor Capella-Monsonis, H.CAPELLAMONSONIS1@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland [2] Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Decellularized xenografts represent an alternative in regenerative medicine due to their preserved biofunctional cues and mechanical properties, which represent an advantage over polymeric scaffolds. Herein we hypothesized that a decellularized porcine graft would possess advantageous properties for tendon and soft tissue regenerative medicine.

METHODS: The biophysical, biochemical and biological properties of a decellularized porcine peritoneum (DPP) were compared to commercially available products. These included a collagen-GAG matrix (CGM), a collagen-cellulose matrix (CCM) and a decellularized ovine forestomach (OFM). Characterization was carried out as previously described [1] and cell response was assessed employing human tenocytes, macrophages, stem cells (ADSC), fibroblasts (ADF) and endothelial cells (HUVECs), depending on the clinical field of study.

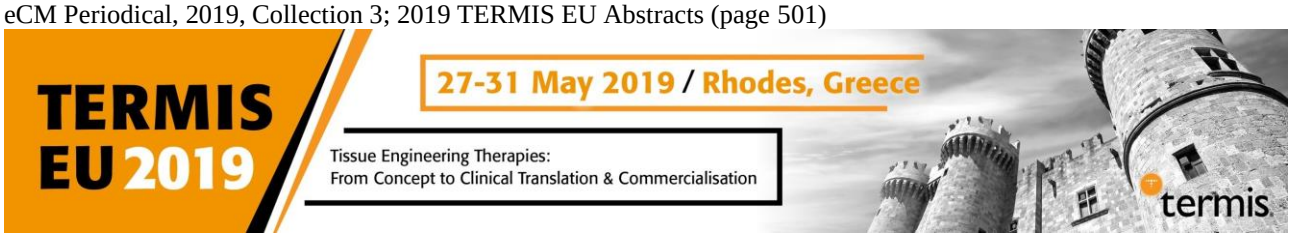
RESULTS & DISCUSSION: DPP was compared to CGM, which is employed clinically in tendon procedures. DPP showed lower crosslinking ratio ($p<0.05$) and coefficient of friction ($p<0.01$), but higher mechanical properties ($p<0.01$). Tenocyte response in vitro showed cytocompatibility of DPP in means of cell proliferation and metabolic activity, which in the CGM were lower than the control ($p<0.01$). This was also observed in macrophages, which also presented a lower proliferation in response to CGM ($p<0.05$). CGM also triggered a higher shift to M1 phenotype as demonstrated by a higher proportion of elongated cells ($p<0.01$) and a higher level of TNFα ($p<0.01$). ADSC response showed a higher proliferation of the cells when seeded on DPP ($p<0.01$), where a change of morphology of the cells was observed. However, flow cytometry analysis with markers $CD73^+$, $CD90^+$, $CD44^+$ and $CD45^-$ showed a maintenance of the stemness of ADSC after 21 days on both materials. DPP was also compared to CCM and OFM, commercially available wound dressings. SDS-PAGE showed DPP and OFM were shown to preserve soluble collagen type I. ELISA growth factors analysis also showed both OFM and DPP to contain FGFb, TGFβ and VEGF, although DPP with higher levels ($p<0.05$). The angiogenic effect of all three materials was observed in vitro with a HUVECs scratch assay employing conditioned media from each material. However, only media conditioned with DPP showed an angiogenic effect in an ex vivo aortic ring model ($p<0.05$).

CONCLUSIONS: DPP presents a lower crosslinking and preserves biofunctional cues that provide higher cytocompatibility than CGM, granting it with potential for tendon tissue engineering. In addition, it also could represent a stem cell delivery vehicle. The preserved biofunctional cues also grant DPP with potential for soft tissue applications.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland, (15/CDA/3629 and 13/RC/2073) and Viscus Biologics LLC (Ohio, USA).

REFERENCES

[1] Capella-Monsonis H et al. Nat Protoc, 2018 (**13**) 507-529.



Engineering the tumour microenvironment in vitro using self-assembling peptide hydrogels

H. Clough[1], A. Saiani[2], A. Miller[1, 3], O. Tsigkou[2]
Presenting Author: Helen Clough helen.clough@postgrad.manchester.ac.uk
[1]School of Chemical Engineering & Analytical Sciences, The University of Manchester, Manchester, UK [2]School of Materials, The University of Manchester, Manchester, UK [3]Manchester BIOGEL, Alderley Park, Alderley Edge, UK

INTRODUCTION: The tumour microenvironment (TME) is a diverse interplay between cancer cells, other cell types, extracellular matrix (ECM) and vasculature [1]. Self-assembling peptide hydrogels (SAPH) are a class of biomaterials gaining popularity due to their nanofibrous structure, tuneable properties and chemical definition [2]. This project aims to validate SAPH as a potential platform to support cancer cell growth and vasculature formation, mimicking many features of the in vivo TME.

METHODS: MCF-7 breast adenocarcinoma cells were encapsulated within PeptiGelAlpha-1 (Manchester BIOGEL, UK) at a density of $1x10^6$ cells/mL and cultured for up to 14 days. Zymography and ELISA of MMP-2 were used to investigate cell-mediated remodeling and migration within the hydrogel. Oscillatory rheology was used to determine the mechanical properties of the hydrogels. Fluorescence imaging was used to determine the presence of a hypoxic core, while histological sectioning and Ki67 staining were carried out to investigate cell proliferation. Human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) were encapsulated within PeptiGelAlpha-1 alone and functionalized with 1%, 10% & 40% RGD to assess vascular tube formation capacity.

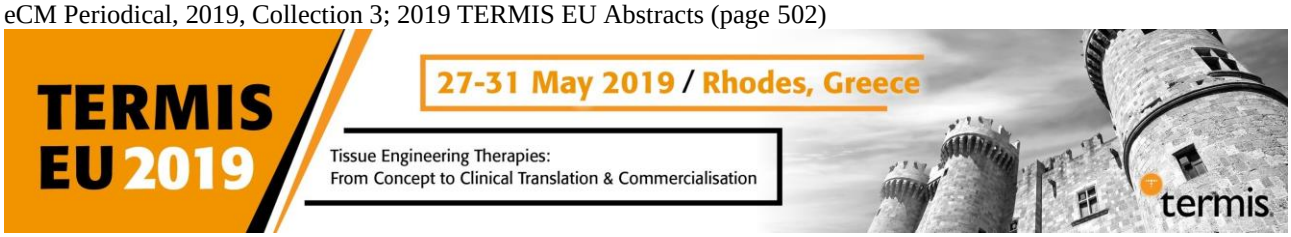
RESULTS & DISCUSSION: PeptiGelAlpha-1 alone was shown to support the viability and growth of MCF-7 cells up to 14 days. H&E staining of hydrogel sections showed the cells were dispersed throughout the hydrogel. Ki67 staining showing proliferation of the cells will also be presented. Oscillatory rheology and an MMP-2 ELISA assay were used to determine if MCF-7 cells were actively remodeling the matrix. Compared with PeptiGelAlpha-1 alone, functionalized SAPH stimulated the elongation and aligning of HUVECs and MSCs. Data on hypoxia will also be presented.

CONCLUSIONS: SAPH are able to support MCF-7 cell phenotype and viability and mimic features of the TME. The RGD binding domain within SAPH appears to enhance tube formation of HUVECs and MSCs.

ACKNOWLEDGEMENTS: The authors acknowledge financial support via the EPSRC-MRC CDT at The University of Manchester (grant no. EP/L014904/1) and Henry Royce Institute funded equipment.

REFERENCES

[1] Balkwill F et al. J Cell Sci. 2012; 125: 5591-5596.
[2] Koutsopoulos S. J Biomed Mater Res Part A. 2016; 104:1002-1016.



IL-23 inactivation by targeted liposomes to mediate the regression of autoimmune diseases

A.C. Lima[1,2], C.F. Campos[2,3], C. Cunha[2,3], A. Carvalho[2,3], R.L. Reis[1,2,4], H. Ferreira[1,2], N.M. Neves[1,2,4]

Presenting Author: Helena Ferreira, helenaferreira@i3bs.uminho.pt

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal [4]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

INTRODUCTION: Biologic drugs revolutionized the treatment of autoimmune diseases (ADs). However, they present limited efficacy mainly due to their short half-life. This work used liposomes, the most successful nanodelivery systems in the clinic, to neutralize interleukin (IL)-23. IL-23 has a crucial role in the regulation of the cellular mechanisms involved in inflammation. Indeed, this pro-inflammatory cytokine potently enhances the generation of pathogenic T_H17 cells that are central drivers of ADs [1]. Furthermore, liposomes can incorporate imaging agents, such as gold nanoparticles (AuNPs), conferring them monitoring functionality. Therefore, a theranostic nanomedicine strategy was developed to reduce the deleterious inflammation observed in ADs.

METHODS: Unilamellar liposomes (LUVs) of EPC, DSPE-PEG-Mal, Chol (1.85:0.15:1) and vitamin E (200:1) incorporating 20 nm AuNPs were functionalized with the anti-IL-23 antibody [2]. LUVs characterization included the determination of their size distribution (DLS), zeta potential (LDE), stability (DLS and LDE), morphology (TEM) and cytocompatibility in the presence of human articular chondrocytes (hACs) and human macrophages (THP-1 cell line). The maximum immobilization of antibody and the capture of IL-23 presented in the macrophage conditioned medium was assessed using secondary antibody unbound quantification and ELISA, respectively. To examine the effect of LUVs on the reduction of IL-17 production it was used PBMCs stimulated with CD3/CD28 beads, IL-1β and IL-23.

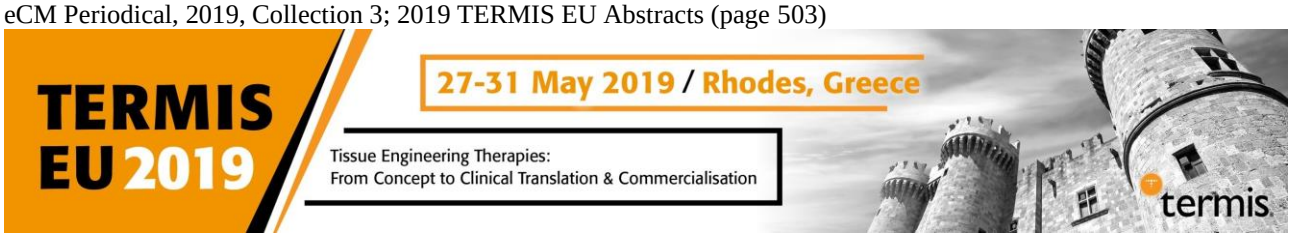
RESULTS & DISCUSSION: Liposomes were able to efficiently integrate AuNPs into their interior, displaying a homogeneous average size of 137 nm, a negative surface charge and a spherical shape. Moreover, the vesicles were stable for six months at pH 7.4 and 4 ºC. Cellular assays with hACs and human macrophages showed that LUVs are cytocompatible for the tested concentrations. The anti-IL-23 antibody was efficiently immobilized at the liposomes surface (82.1 + 8.4%) and captured 53.5 + 9.6% of the cytokine produced by activated macrophages. Finally, LUVs were able to neutralize IL-23 since a reduction on IL-17 production by activated PBMCs was observed.

CONCLUSIONS: The developed formulation was able to inhibit IL-23 effects, namely the production of IL-17 that shows the value of this strategy for ADs treatment.

ACKNOWLEDGEMENTS: This work was supported by NORTE 2020 Structured Project, co funded by Norte2020 and FCT (PTDC/CTM-BIO/4388/2014- SPARTAN).

REFERENCES

[1] Lee et al. Nat Immunol. 2012; 13: 991–999
[2] Manjappa AS et al. J Control Release. 2011;150(1):2-22



ECM functionalization of stem cell-derived cardiac 3D spheroids

H.V. Almeida[1,2,3], M. Tenreiro[1,2], A.F. Louro [1,2], M. Serra[1,2]
Presenting Author: Henrique V. Almeida, henrique.almeida@ibet.pt
[1]iBET, Oeiras, Portugal [2]ITQB-NOVA, Oeiras, Portugal [3]CNC, Coimbra, Portugal

INTRODUCTION: Human induced pluripotent stem cells (hiPSC) enable the generation of cardiomyocytes (CM) that can be used in the development of cardiac models for drug screening and new therapy development. However, these CMs present immaturity limiting its applications [1]. Several methods have been used in order to induce maturation of iPSC-derived CMs. There is evidence that cardiac extracellular matrix (ECM) can affect positively cell maturation and cell-derived ECM deposition, particularly when included in 3D tissue engineered cultures [1]. Hence, we aimed to induce ECM deposition and CM maturation by including cardiac ECM particles in iPSC-derived bioengineered cardiac 3D spheroids (previously developed in our lab [2]).

METHODS: iPSCs were differentiated into CM using an established protocol [2]. At day 6 of differentiation cardiac progenitors were aggregated using AggreWells (STEMCELL Tech.). Cell aggregates (with or without ECM particles) were in dynamic culture for 20 days. Briefly, biomaterial derived from cardiac ECM was pulverized into microparticles and incorporated in the aggregates in the aggregation step (1%w/v), after suspension in culture media and centrifugation. Beating cell aggregates were characterized after 20 days in culture.

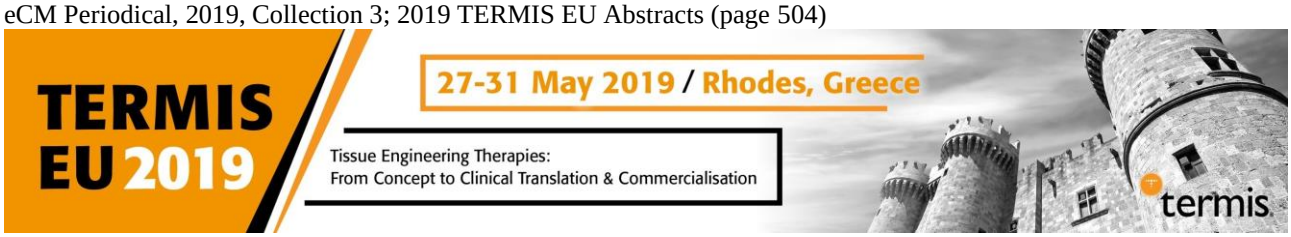
RESULTS & DISCUSSION: CM only and CM+ECM after aggregation demonstrated viability and stability throughout culture period. Morphologically, CM+ECM aggregates presented ECM elements in the surface of the spheroids. Histological assessment demonstrated that CM+ECM aggregates are more cellularized and with superior ECM deposition of GAG (glycosaminoglycans) and Collagen per DNA. Immunofluorescence confocal microscopy corroborated this CM-derived ECM deposition, which was validated with GAG and collagen quantification, higher for the CM+ECM group.

CONCLUSIONS: ECM biomaterial functionalization was successful, with superior cardiac-specific ECM being deposited in the CM+ECM groups. This fact indicates that ECM with specific characteristics can be included in these relevant 3D spheroids and generate more representative and mature cardiac models.

ACKNOWLEDGEMENTS: Financial support was received from FCT (SFRH/BPD/120595/2016). Technical support (AFM and SEM): T. Calmeiro and D. Gomes from CENIMAT, FCT/UNL.

REFERENCES

[1] Fong A et al. TE A. 2016; 22(15-16):1016-25;
[2] Correira C et al. Biotech. & Bioeng. 2018; 115(3): 630-44



Improvement of three-dimensional liver tissue models through the development of a novel perfusion culture system

H. Hoyle[1], S. Przyborski[1,2]
Presenting Author: Henry Hoyle, h.w.hoyle@durham.ac.uk
[1]Department of Biosciences, Stockton Road, Durham University, Durham, UK [2]Reprocell Europe, Sedgefield, UK

INTRODUCTION: The growth of cultured cells is governed by factors in the surrounding microenvironment including the physical space in which cells occupy [1]. We have utilised three-dimensional (3D) and perfusion techniques to create a novel bioreactor system which aims to bring culture conditions closer to those found in vivo. The liver has been chosen for testing of the system as it is a complex and highly perfused organ and therefore models of the liver should benefit greatly from this method [2, 3]. The system is used as a platform for hepatic co-culture models using immortalised cell lines with the aim to allow such cells to have more physiological relevance and increase their value in drug development.

METHODS: The HepG2 line is a hepatocellular carcinoma cell line which is used extensively for studying liver metabolism and protein trafficking. In an attempt to enhance cell structure and function, we have developed a novel yet simple perfusion system which can be used for 3D culture of HepG2 cells in a more physiologically relevant environment. Further complexity is created through use of a supportive layer of fibroblasts and the addition of non-parenchymal cells such as endothelial cells.

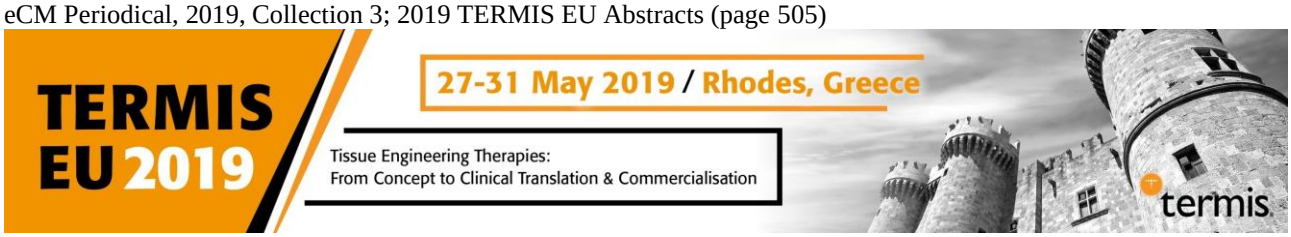
RESULTS & DISCUSSION: Cultures of HepG2 cells in perfused Alvetex® culture show enhanced proliferation and viability compared to both 2D and 3D Alvetex® static cultures. An altered morphology is also seen with changes in the levels of cell-cell contacts and hepatocyte polarity. Growth on a fibroblast layer minimises cell invasion into the substrate and helps to minimise effects of shear stress on the hepatocytes, providing a homogeneous 3D tissue disc. This system was also found to be successful for maintaining the viability of precision-cut liver slices over prolonged periods, another technique which can be used for the modelling of the liver.

CONCLUSIONS: Introducing perfusion to 3D Alvetex® based cultures illustrates the potential for more physiologically relevant in vitro cell culture. Through this methodology we aim to create models which have improved structure and enhanced function compared to current assays, increasing the accuracy and predictive capacity of such models in drug testing.

ACKNOWLEDGEMENTS: Financial support was received from the EPSRC and the Faculty of Science, Durham University.

REFERENCES

[1] Cukierman E. et al. Science 2001; 294(5547):1708-12
[2] V. Soldatow et al. Toxicol. Res. 2013 2(1): 23-39
[3] C. Lin and S. Khetani, Biomed. Res. Int. 2016 (14): 1-20



Osteoclast differentiation of murine precursor cells on microgroove patterned substrate

H. Jeong[1], D. Kim[1], T. Ushida[1], K.S. Furukawa[1]
Presenting Author: Heonuk Jeong, jeong@biomed.t.u-tokyo.ac.jp
[1]Tissue Engineering Laboratory, University of Tokyo, Bunkyo-ku, Tokyo, Japan

INTRODUCTION: Osteoclast, which is differentiated from hematopoietic stem cells plays important roles in maintaining bone homeostasis through bone resorption. In the process of osteoclast differentiation, the topographic cue is one of the critical factors on the interaction between bone surface and cell [1]. Osteoclast precursor cell reacts sensitively to the surface which has rough topography through development of podosome, integrin-mediated adhesion structure [2]. However, for application to tissue engineering, well-defined topographic parameters are needed to study the control of differentiation of osteoclasts. In this study, we have examined the effect of microgroove patterns to induce osteoclast differentiation using murine precursor cells and the mechanisms involved in modulation of differentiation by surface topography.

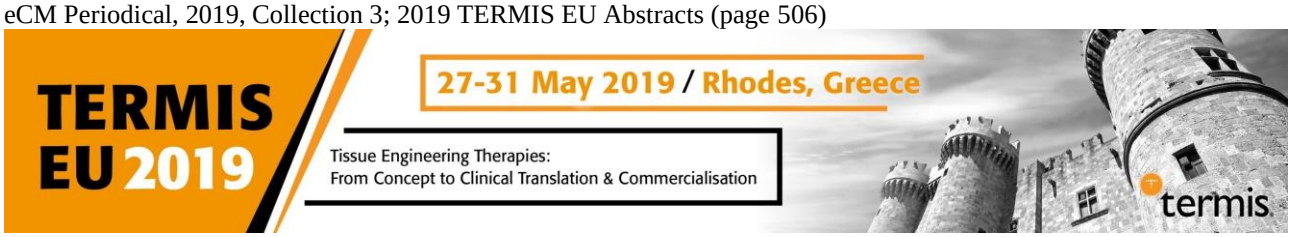
METHODS: Microgroove patterned substrates were designed in line-shape with 2μm of width, 1μm of height and 1, 5, and 10μm of spacing respectively and were made of polydimethyl-siloxane (SILPOT 184, Dow Corning Toray) by soft lithography. Osteoclast precursor cells, isolated from bone marrow of Sprague Dawley rats were cultured on the substrates with vitronectin-coating (100 ng/cm^2) in osteoclast differentiation medium at 37°C and 5% CO_2. Furthermore, to inhibit the activation of ROCK, MyosinII, and Rac1, cells were treated with 10μM of blebbistatin, Y-27632, and Rac1 inhibitor, respectively. After 4 days of culture, differentiated osteoclasts were determined via TRAP (Tartrate-resistant acid phosphatase) staining.

RESULTS & DISCUSSION: In differentiation medium, osteoclasts were observed in all substrates including flat surface without groove pattern by TRAP staining. As counting TRAP-positive cells stained in purple, the proportion of differentiated osteoclasts were most on the pattern spacing 1μm ($p < 0.05$ when compared to other patterns and $p < 0.01$ when compared to flat surface). In the presence of inhibitors, the osteoclast differentiation decreased significantly on 1μm ($p < 0.01$).

CONCLUSIONS: From this study, the effect of microgroove pattern, particularly spacing 1μm, was shown to promote osteoclast differentiation. Moreover, inhibitor experiments provided the possibility that precursor cell could sense the surface topographic while controlling the contractile stress via signal pathways that regulate actin stress fibers in the process of osteoclast differentiation. It is expected that these results will help to understand the process of cell differentiation by sensing the topography of bone surface, and furthermore, will be applied effectively to bone tissue regeneration.

REFERENCES

[1] Geblinger D et al. J Cell Sci. 2010;123(9): 1503-10.
[2] Vanderoost J et al. Calcif Tissue Int. 2016;92(3):240-50



The impact of Vero Clear, Vero Pure White and Med610 on L929 cells and human oral fibroblasts

L. Handermann[1, 2], G. Oberoi[1, 2, 5], K.H. Schneider[2, 3, 4], E. Unger[5], F. Moscato[4, 5], A. Schedle [1, 2], A. Moritz [1, 2], H. Agis [1, 2]
Presenting Author: Hermann Agis, hermann.agis@meduniwien.ac.at
[1]Department of Conservative Dentistry and Periodontology, School of Dentistry, Medical University of Vienna, Vienna, Austria [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria [3]Center for Biomedical Research Medical University of Vienna, Vienna, Austria [4]Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, Austria [5]Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria

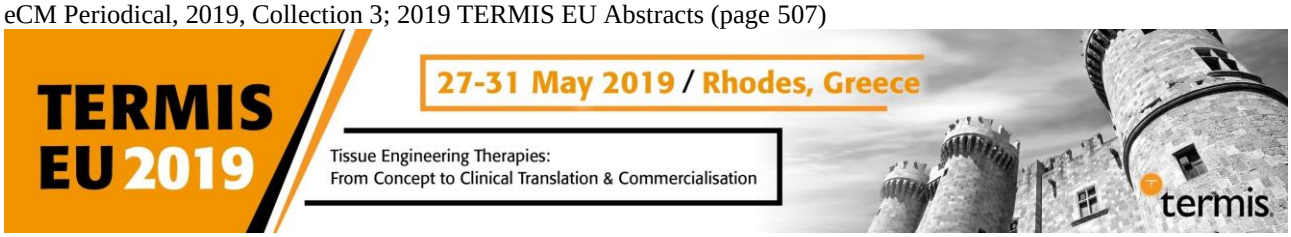
INTRODUCTION: Additive manufacturing also known as 3D printing is about to revolutionize the field of dentistry. It allows the production of personalized surgical guides for oral surgery and endodontics. Furthermore, 3D printing is used to generate orthodontic devices including aligners, hard splints, retainers, and more. A key aspect for the clinical application of these devices is biocompatibility including the aspect of cytotoxicity. As part of the M3dRES consortium we evaluated the cytotoxicity of 3D printed Vero Clear, Vero Pure White, and the clinically approved Med610 printed in vertical and horizontal orientation.

METHODS: Specimens of Vero Clear, Vero Pure White, and Med610 were printed in vertical and horizontal orientation using the Stratasys Objet500-Connex3 system. The samples were then evaluated using scanning electron microscopy to reveal the surface structure. L929 cells, fibroblasts of the gingiva and dental pulp-derived cells were exposed to specimens of Vero Clear, Vero Pure White, and Med610 printed in vertical and horizontal orientation. The MTT and Toxdent assays were used to investigate the cytotoxicity of the material. Glass discs served as a negative control and carboxylate cement Durelon™ was used as a toxic positive control.

RESULTS & DISCUSSION: Scanning electron microscopy revealed surface structure differences between vertically and horizontally printed specimens. We found that Vero Clear, Vero Pure White, and Med610 did have a similar impact on viability as the not toxic negative control. The toxic positive control dramatically reduced viability. Vero Clear, Vero Pure White, and Med610 printed in vertical and horizontal orientation behaved similarly. This was found in L929 cells, fibroblasts from the gingiva, and dental pulp-derived cells. Interestingly, dental pulp-derived cells showed a higher variance with regard to their capacity of formazan formation than the fibroblasts from the gingiva or L929 cells.

CONCLUSIONS: Our results show that all three 3D printing materials Vero Clear, Vero Pure White, and Med610 do not show cytotoxic activity in our system with regard to L929 cells, fibroblasts from the gingiva or dental pulp-derived cells.

ACKNOWLEDGEMENTS: Research of the authors on 3D-printing within the M3dRES project (858060) is supported by the Austrian Research Promoting Agency (FFG).



Enzyme-controlled, starch-based hydrogels for mesenchymal stromal cell survival and paracrine functions

C. Denoeud[1], J. Paquet[1], J. Boisselier[2], A. Moya[1], A. Diallo[1], G. Luo[1], S. Marinesco[3], A. Meiller[3], V. Larreta-Garde[2], E. Pauthe[2], E. Potier[1], H. Petite[1]
Presenting Author: Hervé Petit, herve.petite@univ-paris-diderot.fr
[1]Laboratory of Osteo-Articular Bioengineering and Bioimaging, University of Paris Diderot, Paris, France [2]Biomaterial for Health Group, ERRMECe, Department of Biology, University of Cergy-Pontoise, France [3]Neuroscience Research Center, University of Lyon 1, Lyon, France

INTRODUCTION: In the context of cell-based regenerative medicine, exogenously administered mesenchymal stromal cells (MSCs) exhibited a poor survival rate. A possible explanation for this limited cell survival is that, upon implantation, MSCs encounter a harsh ischemic microenvironment characterized by low oxygen tension and nutrient deprivation. This issue can be overcome by in situ supplying glucose that acts as the main metabolic fuel for MSCs in hypoxia and enhances their survival. The objective of the study is to engineer a tissue-construct that provides sufficient level of glucose to MSCs and enhances their survival when transplanted in vivo.

MATERIALS & METHODS: To this aim, hydrogels containing fibrin, starch (a polymer of glucose) and AMG (an enzyme that release glucose from starch) were formulated. These injectable, self-supported hydrogels released glucose amounts in accordance with that required by hMSCs for their survival.

RESULTS & DISCUSSION: In vitro, under near anoxia, MSCs loaded in fibrin/starch/AMG hydrogels exhibited a survival rate 115 times higher than the one loaded in fibrin hydrogels, after 14 days. Moreover, when ectopically implanted in nude mice, luciferase-labelled hMSCs loaded in fibrin/starch/AMG hydrogels exhibited a significant improvement of their viability (x4 after 14 days) in comparison to hMSCs loaded in fibrin gels as demonstrated by the follow-up of the luciferase activity by bioluminescence imaging. These data were further substantiated by monitoring the number of hMSCs remaining in the hydrogels implanted ectopically in mice. At day 14 days, fibrin / AMG / starch scaffolds contained 7.5 times more viable hMSCs than fibrin hydrogels.

CONCLUSIONS: This work establishes for the first time that a construct based on a fibrin/starch/AMG hydrogel delivers glucose over time and enhances the survival of hMSCs. Most interestingly, the data obtained with hMSCs are now extended to adipose-derived MSCs and myoblasts.

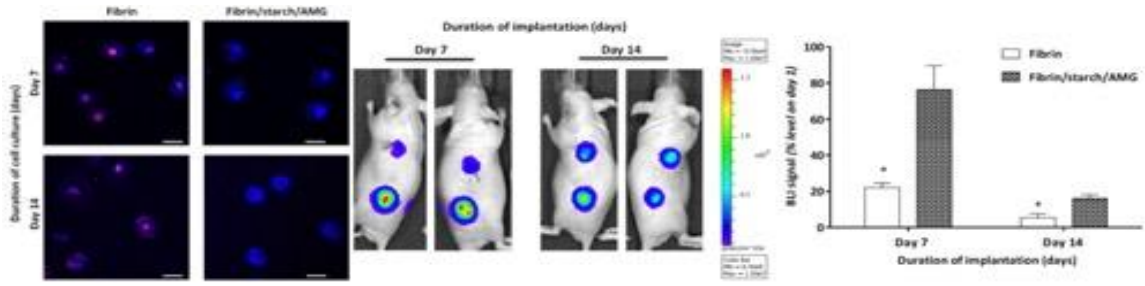
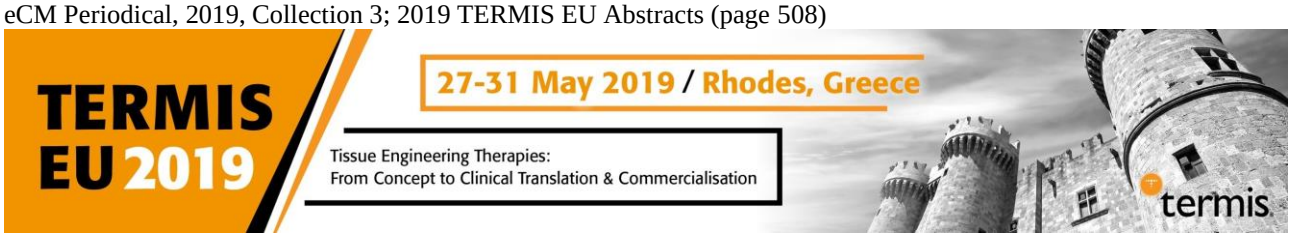


Figure 1: (left) Hoescht and propidium iodide stained hMSCs maintained in hydrogels under near-anoxia. **Figure 2:** (right) Bioluminescence imaging and quantification of luciferase-labelled hMSCs loaded in fibrin and in fibrin/starch/AMG hydrogels, after subcutaneous implantation in nude mice.



A new formulation of porous injectable calcium phosphate cement foam for bone repair

H. Moussi [a,b], K. Flégeau[a], J. Véziers [a], T. Rouillon[a], F. Tancret[b], S. Quillard[b], H. Terrisse[b], V. Geoffroy[a], P. Weiss[a], J. Le Bideau[b], H. Gautier[a,c]
Presenting Author: Hilel Moussi, hilel.moussi@etu.univ-nantes.fr
[a]Regenerative Medicine and Skeleton, Université de Nantes, INSERM, UMR 1229, 1 Place Alexis Ricordeau BP 84215, 44000 Nantes, France [b]Institut des Matériaux Jean Rouxel, Université de Nantes, CNRS UMR 6502, 2 rue de la Houssinière, BP 32229, 44322 Nantes, France [c]Faculté de Pharmacie, Université de Nantes, 9 rue Bias 44035 Nantes, France

INTRODUCTION: One of the challenges in bone tissue engineering with calcium phosphate cements is to create macroporous, degradable, injectable and bioactive materials[1]. In this approach, the challenge is to incorporate encapsulated human mesenchymal stem cells (hMSCs) in a degradable cement to functionalize it. The goal is to maintain cells alive as long as possible to secrete bioactive factors for bone repair. Previously, a foam composite of calcium deficient hydroxyapatite (CDHA) and silanized hydroxypropyl methylcellulose (Si-HPMC)[2] hydrogel with appropriate mechanical properties, injectability and porosity was developed. This previously used Si-HPMC hydrogel, that is poorly resorbable, was replaced here by silanized hyaluronic acid (Si-Hya Ac) hydrogel, that is biocompatible and biodegradable. All these materials were characterized. The thus obtained injectable cement foams are studied regarding their porosity, phase identification and mechanical behaviour.

METHODS: For the formulation of the cement, α-TCP and Na_2HPO_4 are mixed to form the cement's phase of CDHA. α-TCP is synthetized at high temperature (1365°C) followed by very fast tempering. The hydrogel and the NaH_2PO_4 (with air) solutions are initially sealed in two commercial syringes, being ready for use. Directly after the mix, these two components (CDHA cement and Si-Hya Ac hydrogel) are mixed in the syringe and create the foam material. α-TCP is characterized by X-ray diffraction. The behaviour of different formulations of Si-Hya Ac are compared to Si-HPMC by rheology with the elastic modulus (G'), the maximum strength fracture and the resistance energy. Then the Si-Hya Ac is tested in the composite (Si-Hya Ac/CDHA) to find the same mechanical properties than Si-HPMC/CDHA. For this, the strength compression and flexion are compared. Finally, porosity with microCT are observed, and ionic interactions are investigated. Additionally, the biocompatibility and the cytotoxicity of all the component and the different composite formulations is studied.

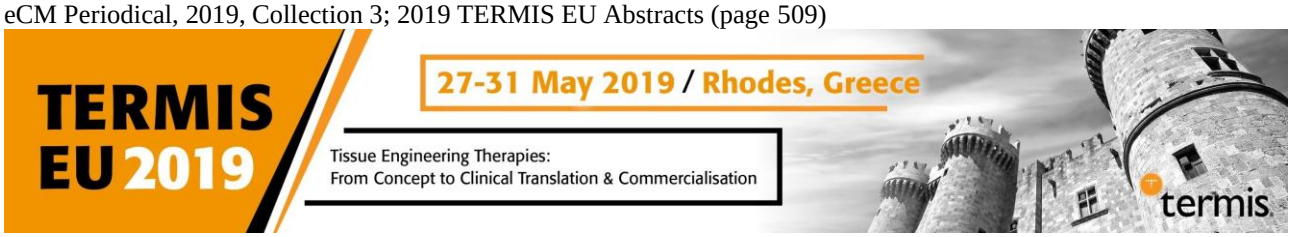
RESULTS & DISCUSSION: This new formulation CDHA/Si-Hya Ac shows interesting biocompatibility and good mechanical properties compared to the reference CDHA/Si-HPMC.

CONCLUSIONS: The present study is a new route towards formulation of different and functional hierarchically porous cement using silanized polymers as foaming agent. The next step will be to compare the biological behaviours of the different formulations with and without encapsulated cells.

ACKNOWLEDGEMENTS: Financial support was received from Bioregate Nantes, Loire Atlantique.

REFERENCES

[1] J. Zhang et al., Acta Biomater., vol. 31, pp. 326 -338, 2016
[2] W. Liu et al., Acta Biomater., vol. 9, no. 3, pp. 5740 -5750, 2013.



Beneficial effect of adipose tissue mesenchymal stem cell supernatants in in vitro model of chronic skin wound

H. Kraśkiewicz, M. Paprocka, J. Kaczyńska, K. Panek, A. Szyposzyńska, A. Klimczak
Presenting Author: Honorata Kraśkiewicz, honorata.kraskiewicz@hirszfeld.pl
The Laboratory of Biology of Stem and Neoplastic Cells, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy of Polish Academy of Science, Wrocław, Poland

INTRODUCTION: Mesenchymal stem cells **(**MSCs) secrete a cocktail of growth factors and cytokines, which might be involved in the wound healing [1-2]. Herein, we compare paracrine profile of immortalised adipose tissue (at) MSC derived from chronic wound patient vs healthy donor and assess the influence of MSCs supernatants in in vitro model of chronic wound.

METHODS: Human atMSC were transfected with SV40 and hTERT plasmids to generate stable cell lines. Supernatants were produced by 24 h culture of MSCs in serum free media, (1% O_2). Human skin cell lines: fibroblasts (MSU-1), keratinocytes (HaCaT) and endothelial cells (HSkMEC) were seeded in 96 well plates in serum free medium in hypoxic conditions (1% O_2) that mimics chronic wound microenvironment. Cells were treated with different ratios of atMSC-supernatants (0, 10, 25, 50 and 100%) and serum free medium. Biological factors were assessed by human Cytokine Antibody Array. Cell morphology and metabolic activity (MTT) were assessed at day 0, 1, and 3.

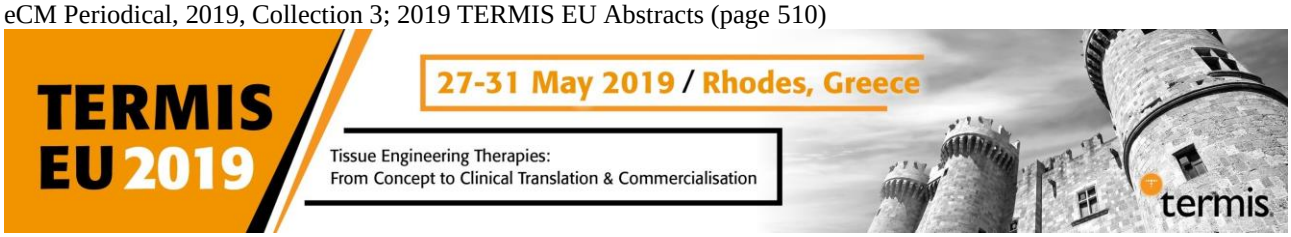
RESULTS & DISCUSSION: Following immortalisation primary atMSC derived from chronic wound patient and healthy donor maintained their MSC phenotype which was confirmed by the expression of surface markers CD90, CD105, and CD73. Moreover, two clones of MSC of healthy donor were obtained. Analysis of the secretion profile of all four MSC lines confirmed presence of many biological factors involved in wound healing process such as VEGF, FGF, IL-8, MMP, GRO. Biological activity of atMSC supernatants was confirmed in vitro on fibroblasts, keratinocytes and endothelial cell lines. Microscopic evaluation and MTT analysis showed increase in all skin-derived cell survival in the presence of atMSC supernatants. Significant increase ($p < 0.001$) in metabolic activity of endothelial cells in the presence of supernatant from atMSC derived from chronic wound patient was observed as early as day 1 and was maintained during the next two days.

CONCLUSIONS: atMSC supernatants have beneficial effect on metabolic activity of different types of skin-derived cells involved in wound healing. Analysis of biological efficiency of all four supernatants revealed that concentration of 50% is the most effective on target cells metabolic activity.

ACKNOWLEDGEMENTS: Financial support was received from Foundation for Polish Science (FNP) under grant no POIR.04.00-00-3024/17-00.

REFERENCES

[1] Wu Y et al. Stem Cells 2007; 25:2648-265
[2] Walter MN et al. Exp Cell Res. 2010; 316:1271-1281



Differentiation of human pluripotent stem cells into cardiomyocytes cultured on thermo-responsive polymer coated with extracellular matrix

H.C. Su[1], T.C. Sung[1], A. Higuchi[1,2]
Presenting Author: Huan Chiao Su, joe10311031@icloud.com
[1]Department of Chemical and Materials Engineering, National Central University (NCU), Zhongli, Taiwan [2]Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama, Japan

INTRODUCTION: The human pluripotent stem cell is ideal source for tissue regeneration medicine. However, it is difficult to isolate differentiated/progenitor cells from undifferentiat-ed cells on culture dishes. In this study, we used thermo-responsive polymers such as poly-N-isopropylacrylamide (polyNIPAAm), Poly(N-isopropylacrylamide-co-butylacrylate) (poly-NIPAAm-co-BA), which have low critical solution temperature (LCST) around 20 degrees and are selected as the coating polymers on the cell culture dishes conjugated with different extracellular matrices (ECMs). With this smart biomaterials, we developed sorting dishes to isolate/purify human pluripotent stem cells-derived cardiomyocytes (hPSCs-derived-CMs) on the cell culture surface [1].

METHODS: PolyNIPAAm or polyNIPAAM copolymer were coated on 6 wells cell culture dishes conjugated with different ECMs (recombinant vitronectin (rVN) and Laminin-521 (LN-521). Cell culture dishes conjugated with ECMs were used as controls. Human pluripotent stem cells (hPSCs) were seeded and attach at first 24 h. Essential 8^{TM} (Gibco) medium was changed every day. After hPSCs became confluence, the cell was induced to differentiate into CMs by using the JoVE and induction protocols developed in our lab. The CMs differentiation efficiency was evaluated by immunocytochemistry, beating colonies number, sarcomere length, and cardiac troponin T (cTnT) expression after 10 days.

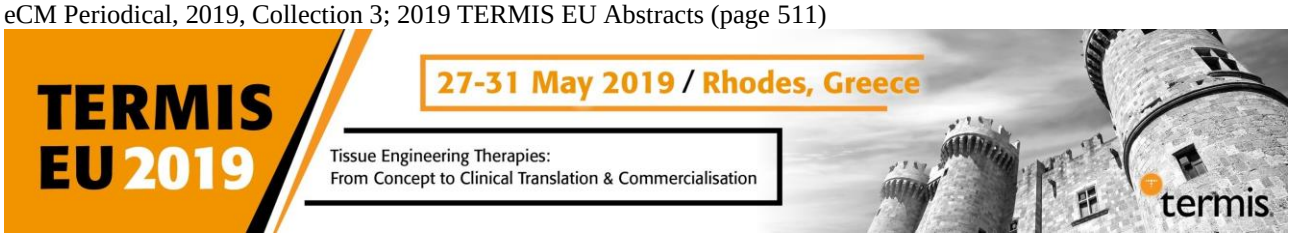
RESULTS & DISCUSSION: We found on the polyNIPPAm surface coated with LN-521 (10μg/ml) has stronger affinity to CMs than coated with other ECMs. Surface marker cTnT was expressed higher for detached cells collected below LCST than non-detached cell. Flow cytometry assay shows cTnT expressions are higher than 90% after resorting. Furthermore, thermo-responsive polymer dishes coated with ECMs showed better cell attachment than TCPS dishes coated with ECMs directly. Our results also showed that hPSC-derived-CMs had higher survival rate on polyNIPAAm copolymer surface coated with rVN compared to dishes directly coated rVN on differentiation stage.

CONCLUSIONS: These data indicated the thermo-responsive polymer conjugated with LN-521 has potential to develop a sorting dish for purified CMs in hPSCs-derived-CMs cell culture.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Science and Technology (MOST 107-2119-M-008-002).

REFERENCES

[1] Higuchi A et al. Laboratory Investigation. 2017; 97(10):1167.



Photoreactive electrospinning of elastomers for cardiac tissue engineering applications

H.M. Younes[1,4], H. Ismail[1], S. Zamani[1], M. A. Elrayess[2], W. Kafienah[3]
Presenting Author: Husam Younes, husamy@qu.edu.qa
[1]Pharmaceutics & Polymeric Drug Delivery Research Laboratory, College of Pharmacy, Qatar University, Doha, Qatar [2]Anti-Doping Lab Qatar, Sports City Road, Doha, Qatar [3]Faculty of Medical and Veterinary Sciences, School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom [4]Office of Vice President for Research & Graduate Studies, Qatar University, Doha, Qatar

INTRODUCTION: Reactive electrospinning is capable of producing in situ crosslinked scaffolds resembling the natural extracellular matrix efficiently with tunable characteristics. In this study, we aimed to synthesize, characterize and investigate the in vitro cytocompatibility of electrospun fibers of acrylated poly (1,10-decanediol-co-tricarballylate) (APDT) copolymer prepared utilizing photoreactive electrospinning process using ultraviolet radiation for crosslinking[1], to be used for cardiac tissue engineering applications.

METHODS: The APDT pre-polymer was synthesized and characterized as reported by our research group[1,2]. The 3D elastomeric mesh was fabricated by means of assisted photo-reactive electrospinning (ES) using polyvinyl pyrrolidone (PVP) as chain entanglement enhancer. APDT with high molecular weight PVP in ethanol solution was prepared and stirred until a clear solution was obtained. The prepared solution was transferred to a syringe and was electrospun to produce an elastomeric mat via optimized electrospinning parameters. The polymer jet was crosslinked in situ using ultraviolet (UV) lamp that lead to the deposition of elastomeric electrospun matt on the collector. At the end of the ES process, the deposited fibers were left under UV for 10 minutes for complete crosslinking of any un-crosslinked residues. The produced scaffolds were fully characterized using different methods. Cell viability and cytotoxicity of the fibers on cultured cardiomyoblasts and cell-scaffolds interaction studies were conducted.

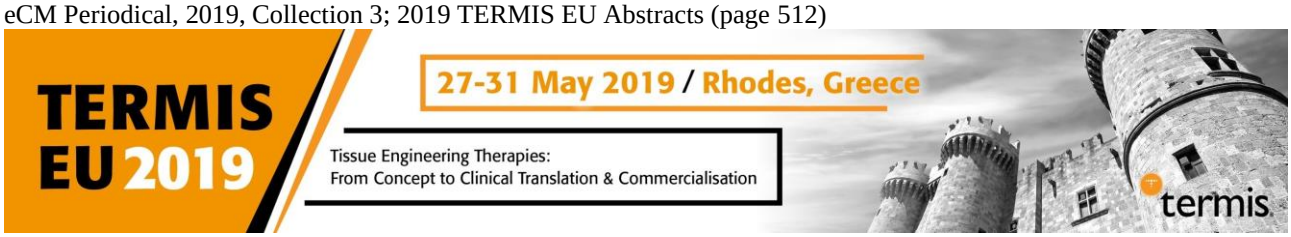
RESULTS & DISCUSSION: Chemical, thermal and morphological characterization confirmed successful synthesis of the polymer used for production of the electrospun fibrous scaffolds with more than 70% porosity. Mechanical testing confirmed the elastomeric nature of the fibers required to withstand cardiac contraction and relaxation. Cell viability assay showed no significant cytotoxicity of the fibers on cultured cardiomyoblasts and cell-scaffolds interaction study showed a significant increase in cell attachment and growth on the electrospun fibers compared to reference.

CONCLUSIONS: The data support the successful preparation of photocrosslinked electrospun APDT based elastomers as promising scaffolds for cardiac tissue engineering applications.

ACKNOWLEDGEMENTS: This project was made possible by NPRP grant # NPRP 09-969-3-251 from Qatar National Research Foundation (a member of Qatar Foundation) through its National Priorities Research Program granted to Dr. H. Younes. The statement made herein are solely the responsibility of the authors.

REFERENCES

[1] M. Shaker, J. Doré, H.M. Younes. Journal of Biomaterials Science. 2010; 21: 507-528.
[2] H. Ismail et al. Polymers 2018; 10 :455



Magnetically-responsive smart microparticles based on mussel adhesive protein for site-specific and prolonged drug delivery

H. Choi[1], Y. Jo[1], and H. Cha*
Presenting Author: HyunSun Choi, gustjs0419@postech.ac.kr
[1]Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, Korea

INTRODUCTION: Mussel Adhesive Protein (MAP) has superior adhesive property and biocompatibility, being exploited as a promising biomaterial. Magnetic force could be utilized for the targeted delivery of therapeutic drugs [1]. Therefore, we present magnetically-guidable sticky microferrogels in which therapeutic drugs could be site-specifically delivered and sustainably released during prolonged periods.

METHODS: MAP ferrogels were fabricated through microfluidic device-based water in oil (W/O) emulsion formulation [2]. In case of water phase, 15% (w/v) MAP in D.W, 1% (w/v) Fe_3O_4 and 30 mM genipin as a crosslinker, were utilized and for oil phase, FC-oil with 2% (w/v) surfactant was used. For the fabrication of the most monodisperse microparticles, flow rates of water and oil phase were optimized. In vitro cellular behavior analyses such as morphology and viability, MC3T3 fibroblasts were deposited on coated surfaces with MAP, genipin, Fe_3O_4, and their combination, respectively.

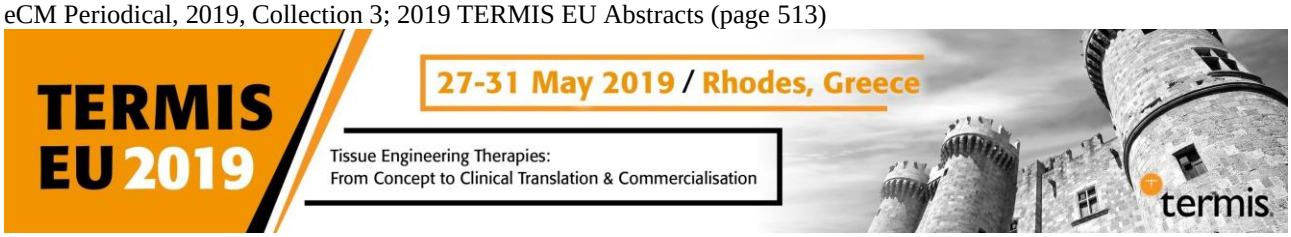
RESULTS & DISCUSSION: Genipin-crosslinked MAP ferrogels showed highly monodisperse shape and size, and robust mechanical property. The most monodisperse microgels were fabricated through flow rates of 5 ml/hr water phase and 20 ml/hr oil phase, and polydispersity index (PDI) of these ferrogels were 1.19%, which is much lower than 32.86% of conventionally fabricated microparticles. These uniform and monodisperse properties are beneficial for controlled and sustainable drug delivery according to target application. Particles size decreased as they were crosslinked from 123.1 μm to 53.6 μm. Due to magnetic force of Fe_3O_4 incorporated in particles, these particles could be guided toward permanent magnet, indicating these ferrogels could precisely deliver payload to specific location. In addition, MC3T3 showed spread morphology similar to negative control in all groups and cell viability also increased consistently with time. Therefore, we demonstrated that the MAP ferrogel system is biocompatible.

CONCLUSIONS: These magnetic-responsive sticky MAP ferrogels can be successfully exploited as promising drug carrier platform with enhanced controllability and robustness for localized and sustainable drug delivery.

ACKNOWLEDGEMENTS: Financial support was provided by the Marine Biomaterials Research Center grand from Marine Biotechnology Program of the Korea Institute of Marine Science & Technology Promotion funded by the Ministry of Oceans and Fisheries, Korea.

REFERENCES

[1] Gwangjun Go et al. Adv Healthcare Mater. 2017, 6, 1601378
[2] Dongfei Liu et al. Lab on a chip. 2017; 17, 1856-1883



The synergistic effect of topography and substrate rigidity in the development of a collagen scaffold for tendon tissue engineering

I. Sallent[1,2], D.I. Zeugolis[1,2]
Presenting Author: Ignacio Sallent, ignacio.sallent@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Biomaterial-based tendon implants are designed to withstand high physiological loads but often lack the appropriate biochemical, biophysical and biological structure to drive tendon regeneration by populating cells [1-2]. Herein, we ventured to create collagen I scaffolds that reproduce tendon natural anisotropy and rigidity, in an effort to engineer tendon tissue with native organization and strength.

METHODS: Porcine collagen I in solution was cross-linked with 4 arm PEG (4SP), poured on micro-grooved or planar PDMS moulds and dried to obtain imprinted collagen films. SEM/AFM and AFM indentation were used to analyse the surface topography and the rigidity of the scaffolds, respectively. Human tendon-derived cells were cultured up to 10 days and their morphology and ECM production was assessed by immunocytochemistry.

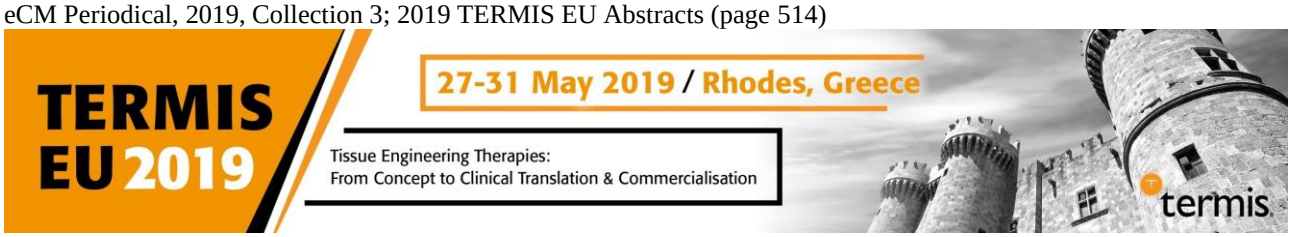
RESULTS & DISCUSSION: Precise control over the micro-grooved topography and the rigidity of the scaffolds was achieved by cross-linking the collagen with varying concentrations of 4SP at low pH and temperature. Human tenocytes cultured on the cross-linked collagen films aligned in the direction of the anisotropy for 10 days in culture. Synthesis, deposition and alignment of collagen III and tenascin C, two important tenogenic markers, were up regulated selectively on micro-grooved and rigid scaffolds, respectively.

CONCLUSIONS: Collectively, these results highlight the synergistic effect of anisotropy and matrix rigidity in the maintenance of the tenogenic phenotype in vitro. A precise combination of these biophysical cues in a collagen I scaffold will potentially lead to the design of smart biomaterials capable of driving cellular synthesis of organized tendon-specific matrix upon implantation.

ACKNOWLEDGEMENTS: Financial support was received from Marie Skłodowska-Curie Actions, Tendon Therapy Train (H2020-MSCA-ITN-2015-ETN), Grant Number: 676338.

REFERENCES

[1] Gaspar D et al. Adv Drug Deliv Rev. 2015; 84:240-56
[2] Lomas AJ et al. Adv Drug Deliv Rev. 2015; 84:257-77



Osteochondral tissue engineering: Stem cell culture in PLDLA scaffold

I.O. Roversi[1], V.L. Gervásio[1], M.A. Hausen[1], A.C. Motta[2], E.A.R. Duek[1,3]
Presenting Author: Igor de Oliveira Roversi, iroversi00@gmail.com
[1]Laboratory of Biomaterials, Faculty of Medical Sciences and Health, Pontifical Catholic University of São Paulo, Brazil [2]National Institute of Technology, Rio de Janeiro, Brazil [3]Faculty of Mechanical Engineering, University of Campinas, UNICAMP, Brazil

INTRODUCTION: A scaffold directed to cell proliferation must have interconnected pores for molecular communication and spaces that allow the matrix expression. Tissue engineering in bone and cartilage are essential to provide regenerative therapies for osteochondral defects in osteoarthritis, a public health issue [1-2].

METHODS: The PLDLA was obtained by bulk polymerization reaction in the ratio 70:30 of the monomers L-lactide and D, L-lactide under vacuum at 130° C. Subsequently the pores of the scaffold were obtained by solubilizing PLDLA with 1,4-Dioxane at 40% (m/v) followed by freezing the solution at -20° C for 12 hours and sublimation of the solvent in Liotop® K105 Freeze-dryer at -90° C for 48 hours. The scaffold was submitted to morphological analysis by Scanning Electron Microscopy (SEM). Human Mesenchymal Stem Cells (hMSC) were cultured in the scaffold in Gibco® Dullbecco's Modified Eagle's Medium (DMEM) supplemented with fetal bovine serum (FBS) and Antibiotic Antimycotic Solution Sigma® at $1x10^4$ hMSC/cm^3 for 7, 14 and 21 days. The hMSCs were submitted to differentiation with Gibco® StemPro Chondrogenesis Differentiation Kit (SPCD) and StemPro Osteogenesis Differentiation Kit (SPOD) at initial cell density of $4x10^4$ hMSC/cm^3 both groups for 21 days. The samples were fixed in paraformaldehyde (PFA) and processed for analysis by Confocal Laser Scanning Microscopy (CLSM). The hMSCs nuclei were stained with Fluoroshield-DAPI, while the chondrogenesis differentiation group were identified for aggrecan by indirect labeling using Alexa-Fluor®647 as fluorescent dye and osteogenesis differentiation group were indentified for osteopontin by indirect labeling with the same fluorophore dye.

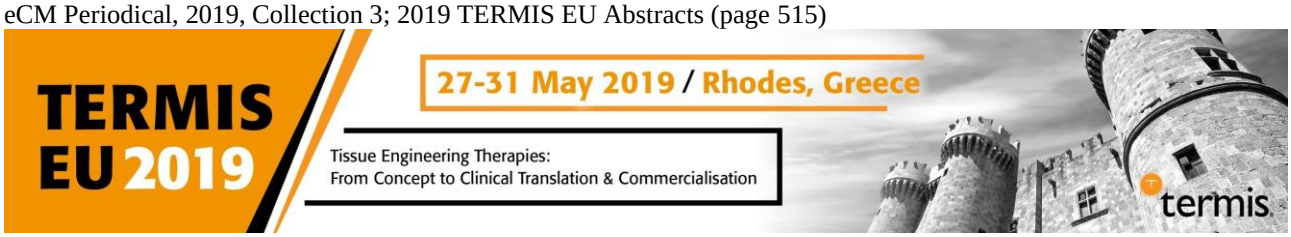
RESULTS & DISCUSSION: The SEM microarchitecture obtained in scaffolds was composed of ellipsoid pores measuring 43.87 ± 68.49μm (minimum diameter) and 75.80 ± 116.95μm (maximum diameter) interconnected by canaliculi. The hMSC cultured with DMEM showed cell migration and proliferation forming a net inside the scaffold pores and the interconnected canaliculi. In SPCD culture was evidenced after 21 days high density cells in deeper areas in scaffold and strong aggrecan labeling. In SPOD culture after 21 days was visualized cell integration in scaffold and deposition of osteopontin and calcium nodes, as white points, typical of bone matrix.

CONCLUSIONS: The microarquitecture of the PLDLA scaffold has remarkable characteristics to allow cell proliferation, differentiation and matrix deposition. The results of cell culture and differentiation corroborate the applicability of this scaffold in the tissue engineering for osteochondral regeneration.

ACKNOWLEDGEMENTS: PIBIC/CNPq and FAPESP (2015/06883-7).

REFERENCES

[1] Vinatier C et al. Ann Phys Rehabil Med. 2016; 59(3):139-44.
[2] Zhang Y et al. J Cell Physiol. 2018; 233(3):1940-51.



Gene-activated matrix based on octacalcium phosphate and plasmid DNA carrying VEGF-A gene in clinical trial: safe and effective for bone grafting

I.Y. Bozo[1,2], A.Y. Drobyshev[3], V.S. Komlev[4], A.A. Isaev[5], R.V. Deev[1,5,7]
Presenting Author: Ilia Bozo, bozo.ilya@gmail.commailto:diana.gaspar@nuigalway.ie
[1]"Histograft" LLC, Moscow [2]Federal Medical Biophysical Center of FMBA of Russia, Moscow, Russia [3]A.I. Evdokimov Moscow State University of Medicine and Dentistry, Moscow, Russia [4]A.A. Baikov Institute of Metallurgy and Materials Science, Moscow, Russia [5]PJSC "Human Stem Cells Institute", Moscow, Russia [6]I.P. Pavlov Ryazan State Medical University, Ryazan, Russia

INTRODUCTION: Gene-activated bone substitute (GABS) is a complex of biocompatible scaffold and gene constructs encoding osteoinductive factor(s) [1]. We developed GABS based on octacalcium phosphate and plasmid DNA carrying gene of vascular endothelial growth factor (VEGF), called "Histograft", and completed world's first clinical trial (NCT03076138).

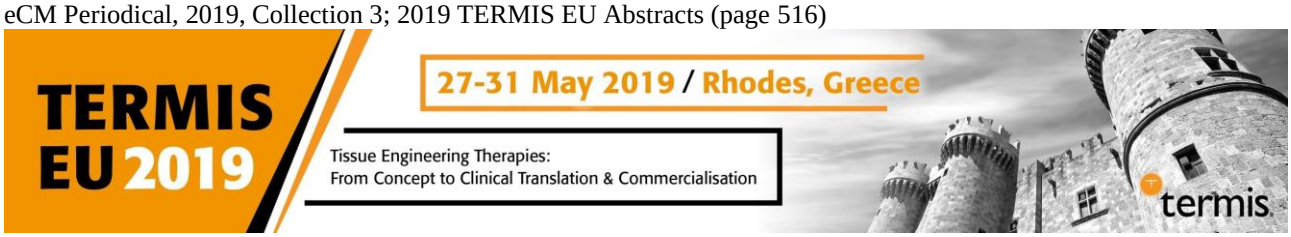
METHODS: The purpose of this study was to evaluate the safety and efficacy of the GABS "Histograft" (granular form) in treatment of patients needed bone grafting procedures. 20 patients aged 18±65 with acquired maxillofacial bone defects and alveolar ridge atrophy were enrolled in the clinical trial with the follow-up 6 months. As a primary outcome measure, we determined the quantity of newly formed bone tissue on CT scan using special tools ("ROI", region of interest, etc.): average density (in HU); size (length, width, height) and volume.

RESULTS & DISCUSSION: All 20 patients (6 men, 14 women, 46.6 ± 11.9 years) completed the study. The indications for bone grafting with "Histograft" included upper jaw alveolar ridge atrophy (16), periodontitis with teeth removal (2), radicular cyst (1), peri-implantitis (1). GABS implantation was as simple as any other bone substitutes in granular form. Neither adverse nor serious adverse events were detected in the clinical trial. The postoperative period in all patients was uneventful; clinicians removed the sutures in 10 days after surgery. According to CT, the tissues with an average density of 908.13 ± 114.40 HU filled the bone grafting area. The volume of augmentation corresponded to the volume of the GABS implanted. 6-8 months after surgery, when the patients completed the clinical trial, majority of them underwent dental implants placement in the bone grafting area, and prosthetics were performed. In all cases trephine biopsies were harvested during dental implants placement and newly formed bone tissue was detected around partially resorbed GABS granules.

CONCLUSIONS: The clinical trial proofed the safety and efficacy of the GABS "Histograft" for bone grafting. However, more clinical data including comparative studies are needed to define the range of possible medical indications where the GABS could show a beneficial effect.

REFERENCES

[1] Deev R et al. Biomed Res Int. 2015; 2015:365050.



Computational analysis of intrastriatal delivery of collagen

I.M. Syntouka[1,2], P.E. Riches[1], A. McDonald[2], A. Kazakidi[1]
Presenting Author: Ioanna Marina Syntouka, ioanna.syntouka@strath.ac.uk
[1]Biomedical Engineering Department, University of Strathclyde, Glasgow, UK [2]Collagen Solutions Plc, Glasgow, UK

INTRODUCTION: Parkinson's disease (PD) is a degenerative disorder that affects dopaminergic neurons in substantia nigra. Recently, cell therapy has emerged as a promising therapeutic strategy, with biomaterials being used to facilitate the cell deposition through intrastriatal injection. However, the existing delivery approaches have shown limited success in clinical translation. This study aims to develop a device for the delivery of a cell-embedded in situ forming collagen hydrogel. Here, computational approaches on the delivery of collagen to the striatum are presented, to gain insight into different parameters affecting the delivery.

METHODS: The delivery of collagen was modelled computationally in the two-dimensional space. The striatum was modelled as a circular space, with an area of 3.98 cm^2 corresponding to the mean volume of putamen in PD patients. Within the finite volume method framework, the Volume of Fluid (VOF) method was used, assuming two isothermal and immiscible fluids. The collagen flow was considered incompressible, with non-Newtonian fluid behavior characterized experimentally, and constant inlet velocity corresponding to a maximum delivery volume.

RESULTS & DISCUSSION: The interaction between the collagen and the brain tissue phases was analyzed, using two types of needle tips, a blunt needle tip and bevel needle tip (Fig. 1A, 1B). Alpha indicates the phase distribution, with a=1 indicating collagen, a=0, brain tissue and 0<a<1 indicating the interface. The effects of collagen injection on the pressure fields within the striatum were also examined (Fig. 1C, 1D). A difference in the pressure between the two needle tips was observed, with the bevel tip showing higher pressure on the site of the delivery.

CONCLUSIONS: The intrastriatal injection of a hydrogel is a complex process and computational analysis of the delivery can help identify the obstacles facing clinical translation. Further analysis is required including 3D reconstruction from MRI images and modelling in the three-dimensional space.

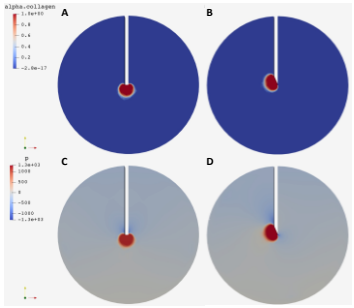
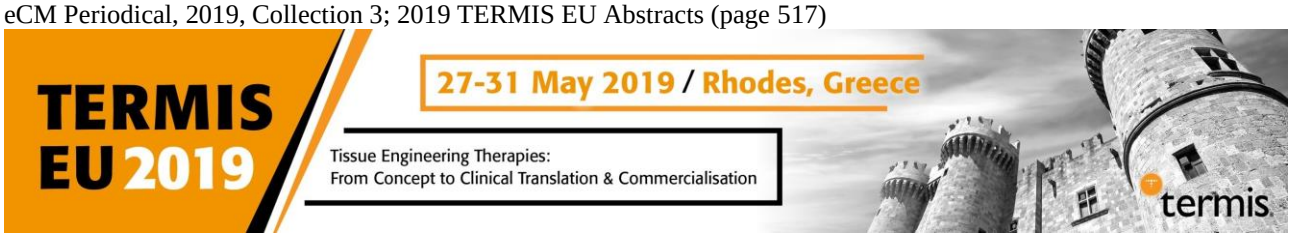


Figure 1: Two-dimensional model of collagen delivery to the striatum. (A) Interaction between collagen and brain tissue when injected with a blunt tip and (B) a bevel tip. (C) Pressure distribution for collagen injected with a blunt tip and (D) a bevel tip.

ACKNOWLEDGEMENTS: European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Innovative Training Network grant agreement No 676408



Bone by design: Functional bio-assemblies of cartilage intermediate micro-tissues

G.N. Hall[1, 2], L.F. Mendes[1, 2], C. Gklava[1, 2], L. Geris[1, 3], F. P. Luyten[1, 2,*], I. Papantoniou[1, 2,*]
Presenting Author: Ioannis Papantoniou, ioannis.papantoniou@kuleuven.be
[1]Prometheus the division of Skeletal Tissue Engineering, KU Leuven, Belgium [2]Skeletal Biology and Engineering Research Centre, KU Leuven, Belgium [3]Biomechanics Research Unit, Université de Liège, Liège, Belgium, * Shared senior authorship

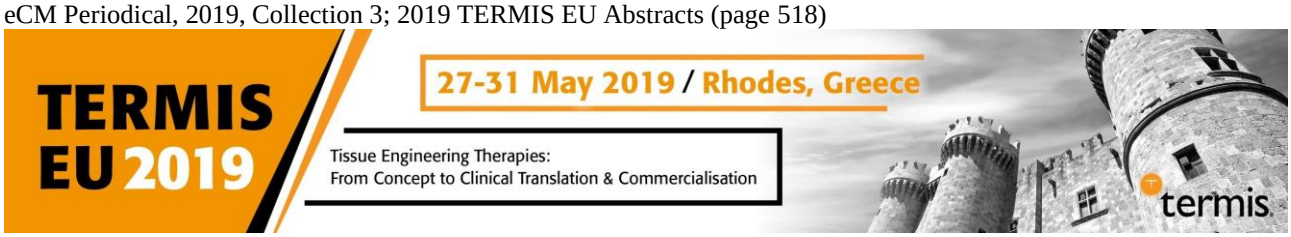
INTRODUCTION: The field of Regenerative Medicine and Tissue Engineering seeks to build functional tissues ultimately replacing failing organs, thereby curing the patient. The ability to produce large populations of small functional tissue niches that could be used for bottom-up assemblies of larger tissues would constitute a major step towards addressing this bottleneck. This could be achieved by providing a living engineering medium able to yield predictive results upon implantation.

METHODS: Human periost derived cells were seeded on non-adherent agarose microwells resulting in controlled cell aggregate populations containing 200 cells per unit. These were differentiated chondrogenically for 3 weeks. Media were changed every 3 days. Upon maturation these modules were fused into larger scaffold free implants and evaluated for bone formation in nude mice both ectopically and orthotopically. RNA seq analysis was carried for 7,14, 21 days as well as immunohistologic analysis.

RESULTS & DISCUSSION: We produced, in a scalable manner, thousands "cartilage intermediate" microtissues. Cells in these microtissues appeared to undergo developmental processes in vitro mimicking those encountered in the embryonic growth plate and during fracture healing in vivo. Upon reaching a degree of "autonomy", as defined by genomic comparison to developmental controls, these microtissues were able to continue their biological program upon implantation, resulting in the formation of bone organs without inappropriate contaminating tissue structures. This capacity was robustly exhibited independent of the implantation site. Strikingly critical size murine long bone defects (5 mm) were healed within natural physiological time scales with the development of a de novo bone marrow compartment while in all cases abundant implanted cells were present, demonstrating their critical role in the regenerative process.

CONCLUSIONS: With these advancements, we believe that we contribute towards establishing 'design specifications' scalable engineered skeletal implants. This paves the way for the mitigation of unmet clinical challenges of large non healing tissue defects such as critical size bone non-unions. The µtissues could be viewed as a living 'bioink' allowing bottom up manufacturing of multimodular tissues with intricate geometric features and inbuilt quality attributes.

ACKNOWLEDGEMENTS: (FP/2007-2013)/ERC: n. 279100 and 249191, GOA/13/016 and FWO-SB: 1S05116N



Hyperbranched cationic polymers as non-viral vectors using different strategies for potential genetic rare diseases treatment

I. Lara-Sáez[1], J.O. Ahern[1], F. Alshehri[2] M. Zeng[1], R. Murrillas[3], F. Larcher[3], J. Bonafont[3], B. Duarte[3], D. Zhou[1], W. Wang[1]
Presenting author: Irene Lara-Sáez, irene.lara-saez@ucd.ie
[1]Charles Institute of Dermatology, University College Dublin, Dublin 4, Ireland [2]Princess Nourah bint Abdulrahman University, Saudi Arabia [3]Epithelial Biomedicine Division, Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain

INTRODUCTION: The HPAE-EB cationic polymer family can electrostatically bind DNA, RNA and proteins to formulate <250 nm polyplexes showing high cellular uptake and release of the condensed nucleic acids and proteins in a triggered manner. Degradation via hydrolysis of ester groups in the HPAE-EB backbone allows release of nucleic acids and minimizes potential cytotoxicity due to intracellular accumulation post transfection. HPAE-EB has been proved to efficiently transfect GFP DNA in a wide variety of cell lines showing higher viability than other commercial reagents (jet-PEI and Lipofectamine 2000) [1]. Here we confirm efficient transfection using different systems for the treatment of Recessive Dystrophic Epidermolysis Bullosa (RDEB), an uncurable rare skin disorder caused by mutations in the COL7A1 gene which encodes collagen VII.

METHODS: The HPAE-EB polymer was complexed with non-integrative plasmids containing the full 8.9 kb COL7A1 cDNA including a) pcDNA3.1-COL7A1 (14.2 kb), and b) minicircle-COL7A1 (11.9kb), both using different promoters, and gene editing systems such c) CRISPR/Cas9 (9.4 kb) and d RNP CRISPR (2 sgRNA plus Cas9) systems, both encompassing a double guide RNA strategy to mediate an exon skipping approach [2] over pathogenic mutations, and assessed for toxicity, efficiency and collagen VII synthesis/correction in RDEB Keratinocytes (RDEBK). Viability and efficiency were assessed 48hrs post transfection by AlamarBlue assay and GFP detection. Cells for flow cytometry were harvested, RNA for RT-qPCR, genomic DNA for PCR and sanger sequencing and protein for Western Blot. RDEB human skin graft mouse model was treated topically with HPAE-EB pcDNA3.1-COL7A1 to confirm in vivo efficiency.

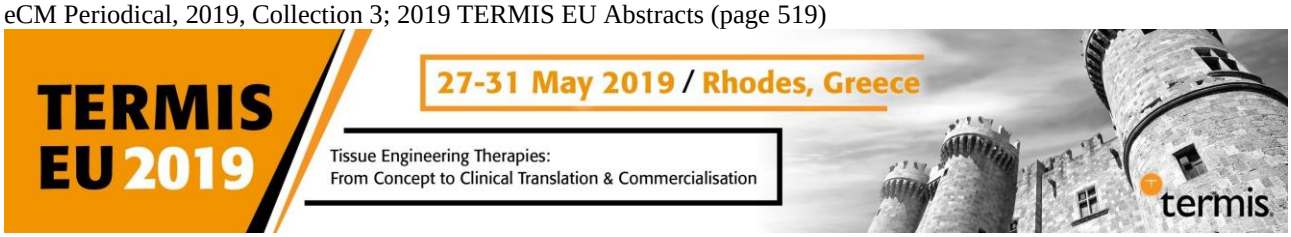
RESULTS & DISCUSSION: GFP signal using gWIZ GFP, minicircle-COL7A1 or CRISPR/Cas9 at 30:1 ratio (polymer:DNA) shows the efficiency and cytocompatibility of the transfections, achieving in vitro genomic correction mediating exon 80 skipping and in vivo recombinant collagen VII.

CONCLUSIONS: The results show that the HPAE-EB polymer is a suitable gene delivery vector that can successfully work with very different systems, condensing and efficiently delivering topically large plasmids as well as in vivo for RDEB gene therapy.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland grant number (13/IA/1962).

REFERENCES

[1] Zhou D et al. ACS Applied Materials & Interfaces. 2016;8(50): 34218-34226.
[2] Hainzl S et al. Mol. Therapies. 2017;25(11) 2573-2584.



In vitro characterization of keratin added fibers for cornea regeneration

I. Cruz-Maya[1,2], M.A. Álvarez-Pérez[3*], C. Varesano[4], C. Vineis[4], V. Guarino[1]
Presenting Author: Iriczalli Cruz Maya, cdiriczalli@gmail.com
*Corresponding author: Marco Antonio Alvarez Perez; marcoalv@unam.mx
[1]Institute of Polymers, Composites and Biomaterials, National Research Council, Naples, Italy [2]Department of Chemical, Materials and Industrial Production Engineering, University of Naples Federico II, Naples, Italy [3]Tissue Bioengineering Laboratory, DEPeI-FO, Universidad Nacional Autónoma de México, Mexico [4]Institute for Macromolecular Studies, Biella, Italy

INTRODUCTION: The use of fibrous proteins as keratin has been recently explored to improve the biological properties of scaffolds in tissue regeneration [1]. Electrospinning is one of the most recognized technologies able to confine - at the level of single fiber - a self-adapting pattern of morphological, chemical or physical signals to reproduce a functional ECM-like micro-environment. Herein, fibers made of Poly ε-caprolactone (PCL) and wool keratin will be investigated to evaluate the contribution of chemical and morphological features at in vitro epithelial cell behavior.

METHODS: Bicomponent fibers were processed via electrospinning by combining keratin - extracted from wool by sulphitolysis- and PCL into a unique solution. Electrospinning conditions were optimized in order to fabricate keratin added fibers without beads and/or defects. Morphological features i.e., average diameters and fibre distribution was investigated via SEM supported by image analysis. In order to in vitro validate the scaffolds, hMSC and HaCat cells were seeded onto PCL and keratin/PCL fibers to compare the viability until 14 days. Cell morphology was also evaluated by SEM microscopy, while new collagen formation was estimated by Sirius red dye at 14 and 21 days. The presence of cytokeratin markers was finally evaluated by immunofluorescence.

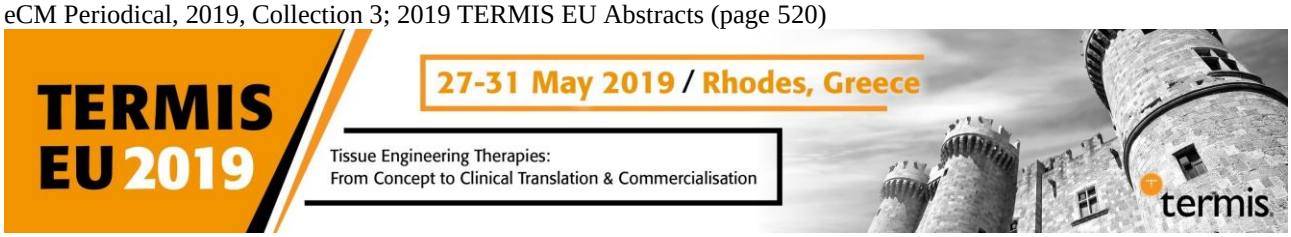
RESULTS & DISCUSSION: Electrospinning was optimized to form wool keratin and PCL composite fibres. The feasibility of the process was basically proved by ATR-FTIR analysis. Clear peaks for amide groups into the spectra confirm the presence of keratin into the fibers, without any alteration due to the electrostatic forces interactions during the process. The presence of keratin also induced an increase of the average diameter -144.1 ± 43.9 nm- respect to those of PCL fibers -81.7 ± 26.7 nm-. The increase in fibre wettability due to the presence of the hydrophilic protein, explained the improvement in cell adhesion in comparison to PCL fibres, used as control. SEM images on cell-seeded samples underlined a higher tendency of hMSC to spread in the presence of keratin along the fibers corroborated by roliferation progressively increased until 14 days. The bioactive effect of keratin was also confirmed by the higher formation of ex novo collagen in vitro after 14 and 21 days while the presence of cytokeratins was confirmed by fluorescence microscopy.

CONCLUSIONS: In this work, fabrication of PCL/keratin nanofibers was optimized to explore the potential use as cell-instructive scaffolds, with improved biological properties, due to the mutual effect of physical and biochemical cues. They promise to be a good candidate to regenerate epithelial tissues such as cornea.

ACKNOWLEDGEMENTS: H2020, Marie Skłodowska-Curie Actions (Grant n. 665403) and DGAPA-UNAM: PAPIIT IT203618.

REFERENCES

[1] Cruz-Maya I et al. AIMS Mater Sci. 2018; 5(2):156-170



hMSC derived mechanically-induced extracellular vesicles (EVs) induce potent regenerative effect in vivo in local or IV injection in a model of chronic heart failure

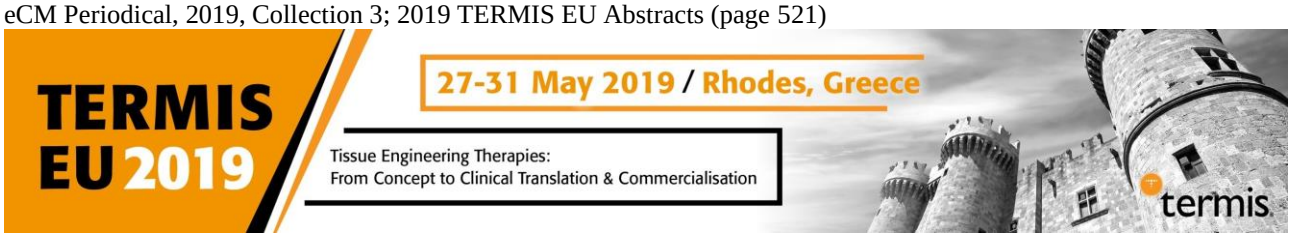
I. Marangon*[1], M. Piffoux*[1], N. Mougenot[2], C. Wilhelm[1], F. Gazeau[1], O. Abgulut[3], A. Silva[1]
Presenting Author: Iris Marangon: iris.marangon@univ-paris-diderot.fr
[1]Laboratoire Matière et Systèmes Complexes, CNRS UMR 7047 Univ. Paris Diderot, France [2]Sorbonne Universités, Univ. Pierre et Marie Curie Paris 6, Plateforme PECMV, UMS28, Paris, France [3]Laboratoire Adaptation biologique et vieillissement, UMR8256, CNRS, Université Pierre et Marie Curie Paris 6, France

INTRODUCTION: On the road toward the use of extracellular vesicles (EVs) for regenerative medicine, technological hurdles remain unsolved: high-yield, high purity and cost-effective production of EVs.

METHODS: Pursuing the analogy with shear-stress induced EV release in blood, we are developing a mechanical-stress EV triggering cell culture approach in scalable and GMP-compliant bioreactors for cost-effective and high yield EV production. The 3rd generation setup allows the production of up to 300 000 EVs per MSC, a 100-fold increase compared to classical methods, i.e physiological spontaneous release in depleted media (around 2000 EVs/cell), with a high purity ratio $1x10^{10}$ p/µg.

RESULTS & DISCUSSION: We investigated in vitro the regenerative potential of high yield mechanically-induced MSC-EVs by demonstrating an equal or increased efficiency compared to classical EVs with the same amount of EVs. The regenerative properties of mechanically-induced MSC-EVs was confirmed in vivo in a murine model of chronic heart failure demonstrating that high, medium shear stress EVs and serum starvation EVs or mMSCs had the same effect using local injection. We later tested the effect of the injection route and the use of xenogenic hMSC-EVs on their efficiency in the same model of murine chronic heart failure. Heart functional parameters were analyzed by ultrasound 2 months post infarction (1 month post EV injection).
Interestingly, hMSC-EVs had the same effect compared to mMSC-EVs in local injection, showing that xeno-EVs in immunocompetent mices were well tolerated. Moreover, hMSC-EV IV injection was as efficient as local intra-myocardium muscle injection with an increase in the left ventricular ejection fraction of 26% compared to pre-treatment values, whereas PBS injected controls lost 13%. High yield hMSC derived mechanically-induced EVs are therefore interesting candidates for simple IV injected regenerative therapies.

CONCLUSIONS: We demonstrated an equal or superior regenerative effect of high yield mechanically-produced EVs compared to spontaneously-released EVs or parental cells in vitro and in vivo, and good tolerance and efficacy of hMSC EV both with IV and local injection. This unique technology for EV production combines decisive assets for clinical translation of EV-based regenerative medicine: a GMP-compliant setup, high density cell culture, high yield release of EVs per cell, high purity EVs.



Differentiation of endothelial cells and smooth muscle cells in bi-layered vascular grafts under flow perfusion

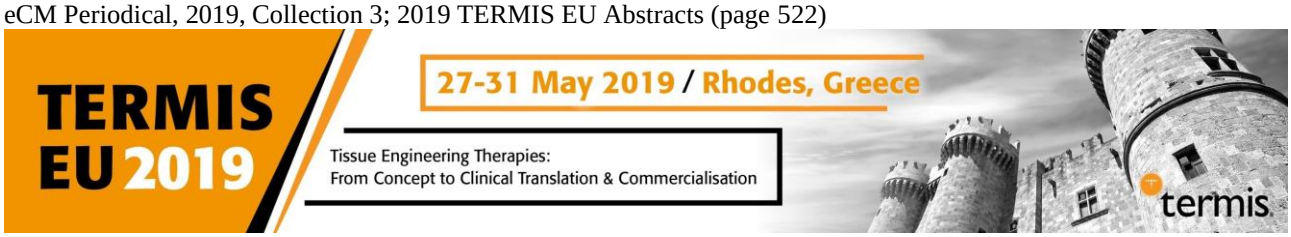
I. Pennings[1,2], E. van Haaften[3,4], T. Jungst[5], J. Bulsink[6], A. Rosenberg[1], C. Bouten[3,4], A. Smits[3,4], J. Groll[5], D. Gawlitta[1,2]
Presenting Author: Iris Pennings, i.pennings-2@umcutrecht.nl
[1] Department of Oral and Maxillofacial Surgery & Special Dental Care, UMC Utrecht, Utrecht University, The Netherlands [2]Regenerative Medicine Center Utrecht, Utrecht, The Netherlands [3]Soft Tissue Engineering and Mechanobiology, Eindhoven University of Technology, Eindhoven, The Netherlands [4]Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands [5]Department for Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, University of Würzburg, Germany [6]Department of Biomedical Engineering, Eindhoven University of Technology, the Netherlands

INTRODUCTION: Here we present a fabrication approach for bi-layered vascular grafts, inspired on the native architecture of small diameter vessels with a confluent endothelium on the luminal side and circumferentially oriented vascular smooth muscle cells in the thicker outer layer. A two-compartment bioreactor culture chamber was designed to emulate the *in vivo* physiological environment of the blood flow on the scaffold's luminal side and to maintain the two cell layers in their preferred culture media with the ultimate aim to improve their respective differentiation.

METHODS: Scaffold fabrication: A nonwoven luminal layer was created with solution electrospinning (solES) after which a second, oriented layer with big pores was deposited with melt electrowriting (MEW) (3 mm Ø, 2 cm length). Poly-ε-caprolactone (PCL) was used to create both layers. Bench-top experiment: Human Endothelial Colony Forming Cells (cb-ECFCs) and Multipotent Mesenchymal Stromal Cells (bm-MSCs) were seeded on the inside and onto the outer scaffold layer, respectively. Constructs were mounted into the custom-built culture chamber and perfused (physiological shear rate 115 s^{-1}) with EGM on the luminal side for 3 days, while the outside was maintained in vSMC differentiation medium. Analysis: The grafts were analyzed for the expression of a.o. vSMC, extracellular matrix (ECM) and endothelial markers (gene expression and immuno/histochemical staining) and graft permeability by measuring the leakage of inulin (2-5 kDa) from the flow loop to the outer compartment.

RESULTS & DISCUSSION: The bi-layered tubular scaffolds promoted organization of the vSM-like cells in a circumferential and multi-layered fashion, and expressed the vSMC markers calponin, αSMA, SM22α and the ECM components (tropo)elastin and laminin subunits α4 and α5. On the luminal side, endothelialization was supported; the endothelial monolayer, positive for VE-cadherin/ Von Willebrand factor/ CD31, was still present after perfusion and responded to shear stress, according to the upregulation of the stress responsive genes Krüppel-like factor 2 (Klf2), endothelial nitric oxide synthase (eNOS) and Cyclooxygenase-2 (COX-2). Inulin was retained in the perfusion loop, demonstrating the sealing capacity of the cells.

CONCLUSIONS: The feasibility was shown of combining solES and MEW to engineer bi-layered constructs, fostering the differentiation and stacking of the vSM-like cells, while also supporting endothelialization. The bioreactor system with the two media compartments allowed for perfusion of leak-free cell-seeded grafts, with the confluent endothelium responding to the imposed shear rate. The vSMC differentiation medium in the outer compartment stimulated the vSMC phenotype in the medial layer, improving the differentiation of the tissue engineered vascular grafts.



Incorporation of hair follicles into reconstructed human skin

I. Vahav[1,3], L. van den Broek[3,4], M. Thon[3], H. Monsuur[3], S.W. Spiekstra[3], B. Atac[1,2], R.J. Scheper[4], G. Lindner[1,2], U. Marx[1], S. Gibbs[3, 5]
Presenting Author: Irit Vahav i.vahav@vumc.nl
[1]TissUse GmbH, Berlin, Germany [2]Technische Universität Berlin, Department of Biotechnology, Berlin, Germany [3]Department of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands [4]A-Skin BV, Amsterdam, The Netherlands [5]Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

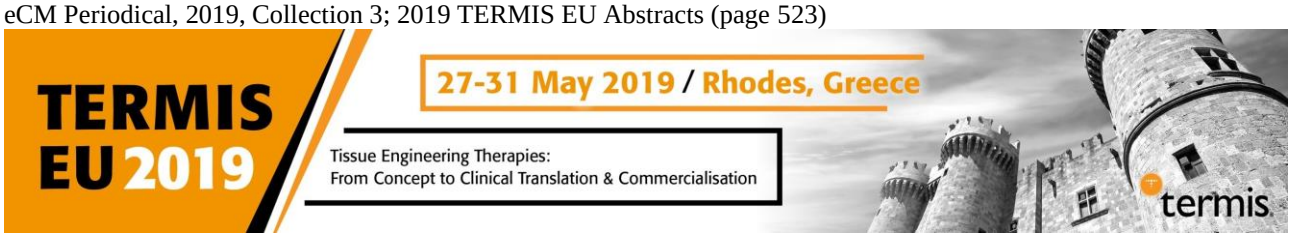
INTRODUCTION: The hair follicle plays a critical role in thermal regulation, physical protection, dispersion of sweat and sebum, sensory and tactile functions and social interactions. Although human skin models have shown to be a promising tool for in vitro studies with the potential to replace animal testing, there is no model that includes the hair follicle. Skin models that closely represent human skin physiology and incorporate the hair follicle are required for basic research, modeling skin disease and in vitro drug testing. The aim of this study was to determine whether neopapilla spheroids, mimicking follicular papillae, can be incorporated into reconstructed human skin and to determine whether they have hair follicle-inductive potential in vitro.

METHODS: Dermal papilla cells were isolated from human hair follicles, expanded and used to reconstruct self-aggregated neopapilla spheroids. Neopapillae were incorporated into a bilayered skin substitute (reconstructed epidermis on a fibroblast-populated hydrogel) and cultured air-exposed to promote epidermal stratification.

RESULTS & DISCUSSION: Notably, neopapillae within the collagen hydrogel stimulated epidermal downgrowth resulting in engulfment of the neopapilla spheres within 10 days of air-exposed culture. Our results indicate that epidermal invagination might be a response to a local chemotactic gradient generated by underlying neopapillae, demonstrating the native behavioural characteristics of epidermal-dermal interactions in the initial stages of hair follicle morphogenesis.

CONCLUSIONS: Reconstructed human skin containing neopapillae with hair follicle-inductive properties can be constructed in vitro thus providing a promising new tool for investigating human hair follicle formation and related hair diseases in vitro.

ACKNOWLEDGEMENTS: This study was supported by a EuroStars project (grant: TESHI 8855) and a Dutch TTW project (grant: NextSkin 15581). Contributions to Gerd Lindner were made possible by DFG funding project LA 1028/7-2 and a ProTUTec grant from the Technische Universität Berlin.



Patterned discoidal microfilms modulate osteogenic differentiation

I.M. Bjørge[1], C.R. Correia[1], T.B. Yesil[1], J.F. Mano[1]
Presenting Author: Isabel M. Bjørge, isabelbjorge@ua.pt
[1]Department of Chemistry, CICECO, University of Aveiro, Aveiro, Portugal

INTRODUCTION: At its core, bone is composed of highly aligned collagen fibres. Thus, when directing the differentiation of stem cells towards the osteogenic lineage, an option is to use nano/micro-grooved substrates to control cell alignment and consequently the deposition of extracellular matrix [1]. Previous works using patterned surface topography are limited to 2D approaches, not fully mimicking the 3D in vivo environment of bone tissue. In order to bring surface patterning into a 3D context, we have developed patterned microfilms (PFs). Through a bottom-up approach, cell-PF aggregation forms 3D structures that can be injected, independently or encapsulated, through minimally invasive procedures. PFs act as cell carriers while delivering topographical cues via surface patterning.

METHODS: PFs were produced by nanoimprinting using CDs as nanopatterned templates. Polyvinyl alcohol counter-moulds of CDs were used to nanoimprint polycaprolactone microparticles at an optimised time, temperature, and load. The osteogenic potential of PFs was assessed by culturing pre-osteoblastic MC3T3-E1 cell line or human adipose-derived stem cells (hASCs) with plasma-treated PFs. Additionally, hASCs were cultured within a previously developed liquefied and multilayered encapsulation system [2,3]. Both systems were cultured up to 21 days in basal or osteogenic medium.

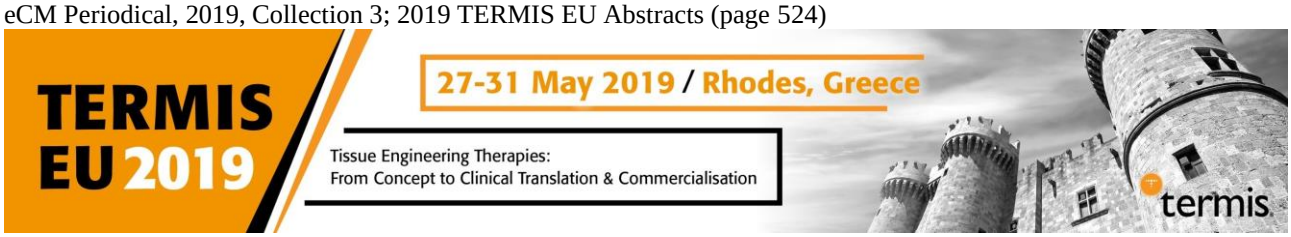
RESULTS & DISCUSSION: As aimed, PFs presented a marked grooved surface patterning. Cells adhered to PFs and proliferated, which was evaluated by an increase in DNA content for MC3T3-E1 cells aggregates. For hASCs cultured within liquefied capsules, both osteopontin and hydroxyapatite nodules were identified at 21 days for both basal (BAS) and osteogenic (OST) medium.

CONCLUSIONS: The ability of PFs to support cell adhesion, proliferation and differentiation was demonstrated. PFs have been shown to be interesting platforms for osteogenic differentiation and should be submitted to further tests for confirmation.

ACKNOWLEDGEMENTS: I. M. Bjørge acknowledges financial support by the Portuguese Foundation for Science and Technology with doctoral grant (SFRH/BD/129224/2017) and the European Research Council (ERC-2014-ADG-669858-ATLAS).

REFERENCES

[1] Watari et al. Biomaterials. 2012;33(1):128-136
[2] Correia et al. Sci Rep. 2016;6:21883
[3] Correia et al. Acta Biomater. 2017;53:483-494



Oxygen conditioning effect on an in vitro co-culture model of tendon-to-bone interface

I. Calejo[1,2], R. Costa-Almeida[1,2], R.L. Reis[1,2,3], M.E. Gomes[1,2,3]
Presenting Author: Isabel Calejo, isabel.calejo@i3bs.uminho.pt
[1]3B's Research Group, I3Bs, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [2]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

INTRODUCTION: Tendon-to-bone interface comprises a heterotypic cellular niche. The native interface is hypovascular, suggesting that the junction is physiologically hypoxic. As it bridges tendon and bone, which require different oxygen concentrations, a tight coordination of different oxygen concentrations along the junction must be considered when trying to mimic and understand biological events occurring within the tissue. Herein, an optimized in vitro co-culture model of tendon-derived cells (hTDCs) and pre-osteoblasts (pre-OBs) [1] was used to study the effect of a restricted oxygen environment on cell behavior.

METHODS: Single cultures of hTDCs or pre-OBs and direct contact co-cultures (1:1 cell ratio) were maintained for 14 days in a 5% oxygen (O_2) tension (hypoxia) using three medium conditions containing different osteogenic supplementation ratios (OM, 0%, 50%, 100%). Controls were performed under normoxia. Cell proliferation and protein synthesis, alkaline phosphatase (ALP) activity and mineral deposition (alizarin red, AZ) were quantified. Gene expression of tendon-, bone and interface-related was assessed by RT-PCR.

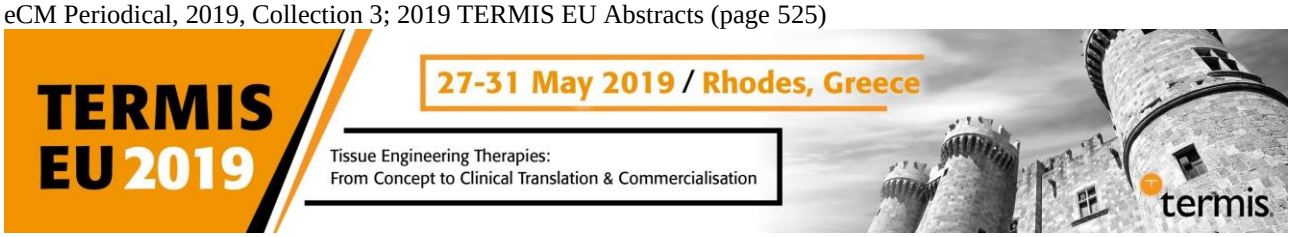
RESULTS & DISCUSSION: Hypoxia reduced cell proliferation, independently of OM supplementation, in comparison with normoxia for all cultures ($p<0.0001$). An overall increase in matrix mineralization and ALP activity was observed at 14 days in co-cultures independently of OM supplementation, compared to pre-OBs alone ($p<0.0001$). Interestingly, oppositely to co-cultures under normoxia, increasing OM concentration in 5% O_2 led to a reduction in matrix mineralization in co-cultures (50%OM, $p<0.009$; 100%OM, $p<0.0001$). In terms of total protein synthesis, hypoxia led to an overall reduction in synthesis, particularly in hTDCs. A synergistic effect between heterotypic cellular interactions, osteogenic medium and hypoxia was observed in the transcription levels of interface-related markers in co-cultures (COMP, ACAN, co-culture D14, $p<0.05$; versus single cultures D14, $p<0.0001$)

CONCLUSIONS: Overall, 5% O_2 diminished proliferation and protein synthesis. Combining osteogenic supplementation and hypoxia reduced matrix mineralization by cells in co-culture. Nevertheless, studying the expression of specific markers, such as HIF-1 alpha will allow a better assessment of the hypoxic response of cells in both single and co-cultures, toward identifying the role of cell-cell interactions and OM on the expression of bone, tendon and interface-related markers.

ACKNOWLEDGEMENTS: Hospital da Prelada (Porto, Portugal) for lipoaspirates and tendon samples; ERC Grant CoG MagTendon nr 772817, the Project NORTE-01-0145-FEDER-000021, HORIZON 2020 under the TEAMING Grant agreement No 739572 - The Discoveries CTR and FCT - PhD grant of IC (PB/DB/128088/2016).

REFERENCES

[1] Calejo, I. et al Cell Prolif. 2018 51; 1–15.



Advances in angiogenesis control on ELRs-based scaffolds for tissue engineering applications

I.G. de Torre[1, 2], T. Flora[1], M. Alonso[1], J. C. Rodríguez-Cabello[1]
Presenting Author: Israel González de Torre, igonzalez@tpnbt.com
[1]BIOFORGE, CIBER-BBN, Edificio Lucia, Universidad de Valladolid, Paseo Belén 19, 47011, Valladolid, Spain [2]technical Proteins Nanobiotechnology (TPNBT S.L.) Paseo Belén 9 A, 47011, Valladolid, Spain

INTRODUCTION: The development of new capillary networks in engineered constructs is essential for their survival and their integration with the host tissue.

METHODS: ELRs encoding different bioactivities (RGD, REDV, QK, DRIR, GTAR) have been designed, produced characterized following well-established methodologies previously described. These ELRs were further chemically functionalized to form hydrogels. These hydrogels have been in vitro and in vivo tested to assess their angiogenic potential.

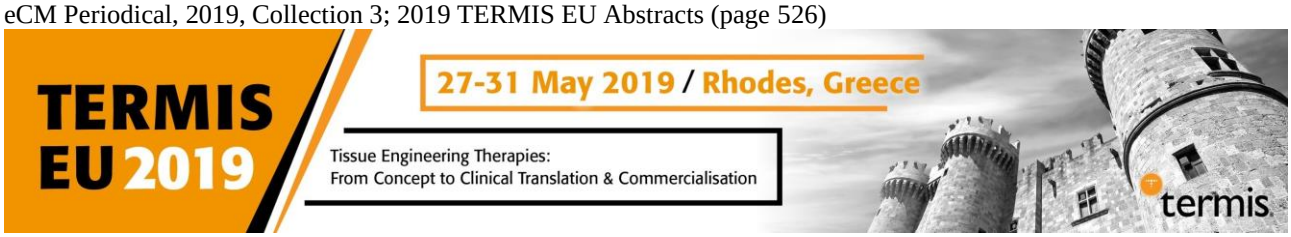
RESULTS & DISCUSSION: Selectivity provided by REDV sequences demonstrate that adhesion of endothelial cells is markedly enhanced on those substrates functionalized with 75% REDV and 25% RGD [1]. The adhesion ratio of endothelial cells was significantly higher than for HFF1 cells, thus indicating a selective adhesion with respect to endothelial cells. RGD-REDV-bioactivated or unmodified ELR-hydrogels were subcutaneously implanted. While in vivo vascularization and host cell infiltration within the bioactivated gels were highly enhanced, the two processes were strongly inhibited in non-functionalized [2]. VEGF mimetic peptide (QK peptide), tethered chemically into ELR-based hydrogels and injected into a hind limb region in mice, enhances the formation of new capillaries within the constructs. The in vivo findings showed that a functional microvasculature was obtained in QK hydrogels, thus providing a pro-angiogenic environment for cell survival and tissue growth [3]. ELRs bearing sequences with different degradation rate (DRIR, GTAR) have proven that the control over the degradation rate of the scaffolds gives us an extra control over the formation of new functional capillaries [4].

CONCLUSIONS: Our study showed how angiogenesis and host integration of the engineered tissues can easily be controlled by modulating the functionalization of the hydrogel alone.

ACKNOWLEDGEMENTS: Financial support was received from “ELASTISLET” (grant 646075) and the “ANGIOMAT TRAIN” project (grant 317304).

REFERENCES

[1] Flora T et al. Eur. Polym. J. 2018; 106:19-29
[2] Staubli SM et al. Biomaterials. 2017;135:30-41
[3] Flora T et al. J. Mater. Sci. Mater. Med.. 2019; Just accepted
[4] Flora T et al. Biofabrication. 2019; Just accepted



An in vitro platform for the quantitative evaluation of cell-mediated contraction and alignment

I.V. Roberts[1], A. Tirella[1], C.Y.L. Valdivieso[2], G. Cossu[3], N. Tirelli[1,4*]
Presenting Author: Iwan Vaughan Roberts, iwan.roberts@postgrad.manchester.ac.uk
[1]Division of Pharmacy and Optometry, School of Health Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK [2]School of Materials, University of Manchester, Manchester, M13 9PL, UK [3] Division of Cell Matrix Biology and Regenerative Medicine, University of Manchester, Manchester, UK [4] Laboratory of Polymers and Biomaterials, Fondazione Istituto Italiano di Tecnologia, via Morego 30, 16163 Genova, Italy

INTRODUCTION: Cell-mediated matrix contraction naturally occurs in tissue remodeling e.g. in scarring, as well as in engineered systems. These processes can be desirable (e.g. formation of hard connective tissue in a tendon or closure of a wound) or not (fibrotic reactions, hard capsule formation). We propose a fibrin-based platform for the quantification of cell-mediated biomaterial contraction; this allows to assess the efficacy of pro- or anti-fibrotic factors, but also illustrates the practical limits of artificial matrices, for which massive contraction during cell colonization is often not desirable.

METHODS: Fibrinogen and thrombin were mixed (along with human dermal fibroblasts for 3D-seeded constructs) and pipetted into indented poly(dimethylsiloxane) (PDMS) molds (final concentrations: 3.125, 6.25, 12.5 mg/mL fibrinogen, 1 U/mL thrombin, 20 mM $CaCl_2$, $2x10^5$ cells/mL for 3D seeding and 5,000 cells/cm^2 for 2D seeding). The resulting anchored constructs exposed to ± 10 ng/mL TGF-β1 for 48h and cultured for additional 5 days.

RESULTS & DISCUSSION: 2D or 3D cell seeding resulted in markedly different contraction modalities; respectively, they produce elongated structures by "wrapping" gels along the contraction axis, or through a bulk reduction in volume. The contraction speed was inversely related to the gel stiffness, and increased with a typical fibrotic agent, TGF-β1. The latter significantly upregulated ($p<0.01$) both collagen 1 and α-smooth muscle actin (RT-qPCR), suggesting a myofibroblastic evolution parallel to increased contraction.

CONCLUSIONS: Collectively, these data demonstrate the applicability of this platform to evaluate aligned biomaterial contraction, to inform both anti-scarring treatments and the development of aligned engineered tissues.

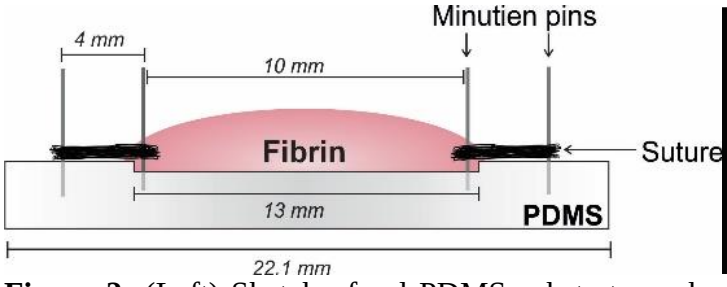


Figure 2: (Left) Sketch of gel-PDMS substrate anchoring.

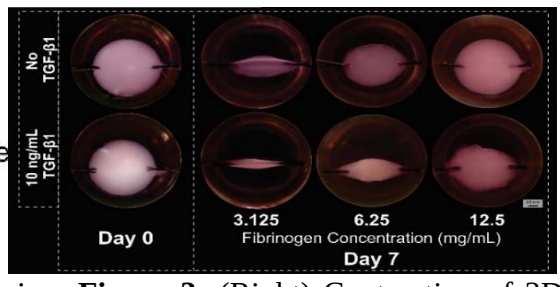
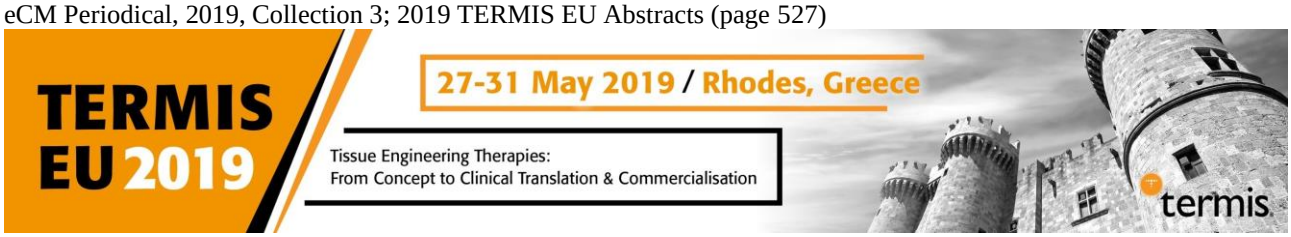


Figure 3: (Right) Contraction of 3D-seeded constructs at day 0 and 7 as a function of fibrinogen concentration, ±TGF-β1.

ACKNOWLEDGEMENTS: The authors wish to acknowledge the funding provided by the Engineering and Physical Sciences Research Council (EPSRC) and Medical Research Council (MRC) Centre for Doctoral Training in Regenerative Medicine (EP/L014904/1).



Culturing neural tissue on biocompatible graphene oxide thin films in development of tissue engineering scaffolds

J Brewster[1], K.S Lim[2], X Yang[3], and L.H Jiang[4]
Presenting Author: Jack Brewster, mnjrb@leeds.ac.uk
[1]Institute of Medical and Biological Engineering, UK [2]Christchurch Regenerative Medicine and Tissue Engineering University of Otago, NZ [3]Division of Oral Biology [4]Faculty of Biological Science University of Leeds, UK

INTRODUCTION: Graphene oxide (GO) is a two dimensional nanomaterial with promise as a biomaterial. Though biologically inert, GO is physically strong, and flexible; it comprises planar lattice of sp2 bonded carbon atoms and features varied oxygen containing functional groups, which are promising targets for chemical modification [1]. GO features increased hydrophilicity and superior reported cell attachment characteristics compared to pristine graphene and artificial polymers [2]. Graphene materials have highly desirable properties such as electrical conductivity [3], and therefore GO is of interest as a scaffold for neural tissue regeneration. Aim: To examine the compatibility of growing cells of the central nervous system with GO surfaces.

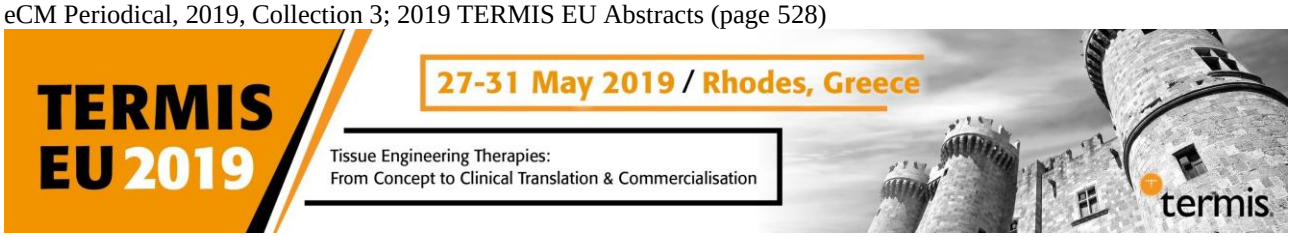
METHODS: GO was assembled into homogenous, unbroken plasticized coatings onto glass or plastic disks via spin coating. Primary neurons prepared from 3 day postnatal mice or SH-SY5Y human neuroblastoma cells were seeded on surfaces at 20,000 cells per surface. Primary neurons were subsequently maintained in Neurobasal Plus media. Differentiation of SH-SY5Y cells was induced with 3 μM retinoic acid. Live cell cultures were imaged and cells assessed for morphology and viability. Fixed cells were imaged for immunoreactivity.

RESULTS & DISCUSSION: GO surfaces facilitated cell attachment and supported typical morphology and proliferation. When induced to differentiate, SH-SY5Y cells on GO surfaces developed cholinergic phenotypes with neurite-like projections arranged as intercellular networks and remained viable for more than 14 days in vitro (>95% viability). Primary Neurons on GO surfaces developed expansive TAU positive dendritic morphologies by 25 days in vitro, and synaptophysin positive vesicles at dendritic termini, when co cultured with astrocytes. Overall, cell cultures attached to GO surfaces displayed superior morphology, maturity and viability than controls.

CONCLUSIONS: Our results indicate GO is a compatible cell-supporting and instructive substrate for neural tissue scaffolds and is suited to culture of neural tissues. Spin coating provides a robust method to prepare batches of substrates.

REFERENCES

[1] Dreyer, D.R., A.D. Todd, and C.W. Bielawski, Harnessing the chemistry of graphene oxide. Chemical Society Reviews, 2014. **43**(15): p. 5288-5301.
[2] Zhang, K.H., et al., Aligned PLLA nanofibrous scaffolds coated with graphene oxide for promoting neural cell growth. Acta Biomaterialia, 2016. **37**: p. 131-142.
[3] Ryan Alan, J., et al., Electroconductive Biohybrid Collagen/Pristine Graphene Composite Biomaterials with Enhanced Biological Activity. Advanced Materials, 2018. **30**(15): p. 1706442.



A new nanoindentation method for local dynamic mechanical analysis (micro-DMA) of heterogenous silicon elastomers (PDMS) and other viscoelastic biomaterials

J. Pyszkowski [1]
Presenting Author: Jakob Pyszkowski jakob.pyszkowski@optics11.com
[1]Optics11, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

INTRODUCTION: The micro-mechanical properties of biological tissue are highly diverse and play an important role in tissue engineering and regenerative medicine, by regulating a wide range of physiological processes. Local dynamic mechanical analysis of complex tissues or heterogenous polymers while immersed in liquids proves to be very difficult to perform. Based on previous experiments on PDMS using a ferrule-top indentation method, we present here our results on similar sample using a modified nanoindentation setup, the Piuma Nano-indenter, for local viscoelastic characterization.

METHODS: Silicon elastomers were prepared in a 35mm petri dish, with a Sylgard 184 ratio of 50:1 and 20:1, respectively, resulting in a mix of concentrations to create a stiffness gradient within the sample in range of stiffnesses representative for biological tissues. We demonstrate that our micromechanical testing technique can accurately determine moduli in the range between 10 and 100kPa and over a frequency range of 0.1–10 Hz. This method is featured with a fiber-optical ferrule-top micro-machined force transducer (Figure 2), enabling a wide range of mechanical tests: from quasi-static experiments to derive elastic moduli, to step-response tests (e.g. creep, stress-relaxation), dynamic mechanical analysis (micro-DMA) and constant strain rate tests to characterize sample viscoelastic behavior.

RESULTS & DISCUSSION: Dynamic nanoindentation measurements show increased storage modulus E' and loss modulus E", ranging from 15kPa to 89kPa, and from 1.5kPa to 78 kPa, respectively. Single measurement points of a total number of 100 were collected across a grid of 1cm x 1cm for oscillation frequencies of 0.1, 1 and 10 Hz.

CONCLUSIONS: The collected micro-DMA data on PDMS demonstrate the ability of the featured nanoindentation system to extract very localized viscoelastic properties of tissue-like materials, in agreement with macroscopic rheology measurements [2].

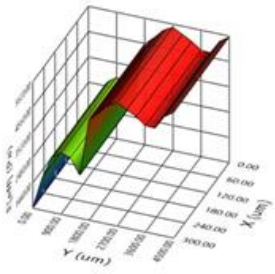
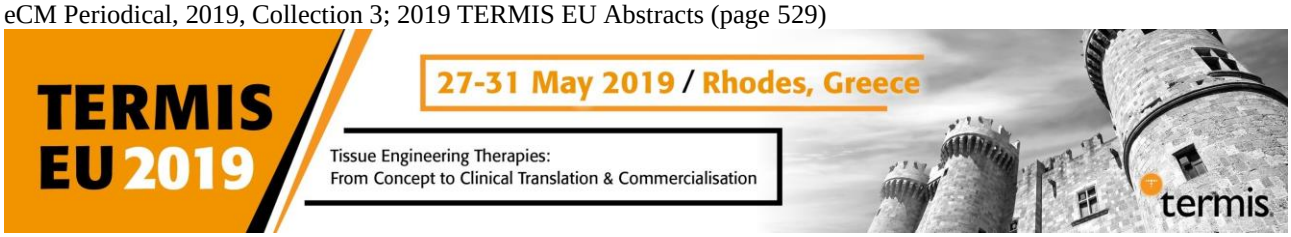


Figure 1: (left): Section of the measured grid showing an increase of E' over a length of 4.5mm.
Figure 2: (right): Ferrule-top nanoindentation probe, including a cantilever and optical fiber for force readout [2].

ACKNOWLEDGEMENTS: Financial support as well as the Piuma Nanoindenter setup was received and sponsored by Optics11, Amsterdam, NL



Optimization of 3D bioprinting of human neuroblastoma cells using sodium alginate hydrogel

J. Lewicki[1], J. Bergman[1], C. Kerins[1], O. Hermanson[1]
Presenting Author: Jakub Lewicki, jakub.lewicki@ki.se
[1]Department of Neuroscience, Karolinska Institutet, Biomedicum, Stockholm, Sweden

INTRODUCTION: There are many parameters in extrusion-based three-dimensional (3D) bioprinting of different materials that require fine-tuning to obtain the optimal print resolution and cell viability. To standardize this process, methods such as parameter optimization index (POI) have been introduced [1]. The POI aims at pinpointing the optimal printing speed and pressure to achieve the highest accuracy keeping theoretical shear stress low.

METHODS: $1 \cdot 10^7$/ml SK-N-BE(2) human neuroblastoma cells were encapsulated in 2% sodium alginate (SA) and 3D printed using freeform reversible embedding of suspended hydrogels (FRESH) method [2] and Allevi 2 3D bioprinter. Set of four different extrusion pressures (5, 7.5, 10, 12.5 psi) and four speeds (2, 4, 6, 8 mm/s) were used for the material deposition. POI method was used to determine the optimal printing parameters based on printed line width analysis. Printed constructs were also analyzed in the context of cell viability and aggregation up to 7 days in cell culture.

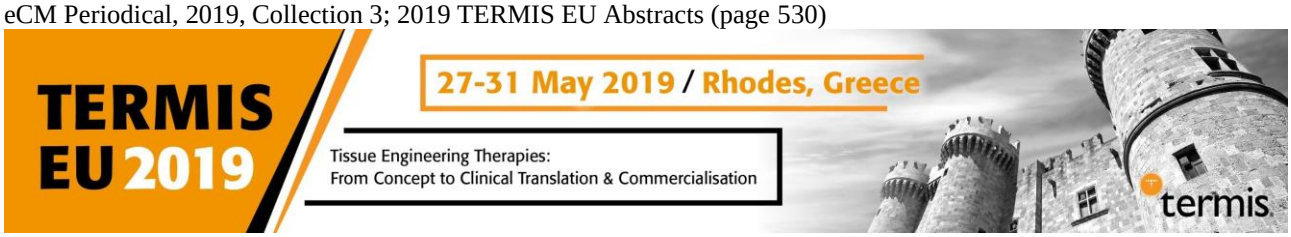
RESULTS & DISCUSSION: Our results demonstrate a notable difference between optimal parameters for printing 2% SA with and without cells in the hydrogel. We also detected a significant influence of long-term cell culture on the printed constructs. When taking these conditions into consideration, we could define a set of parameters that resulted in good quality prints maintaining high neuroblastoma cell viability (83% viable cells) during 7 days of cell culture using 2% SA and FRESH bioprinting. The highest POI value was achieved for 7.5 psi extrusion pressure and 8 mm/s of deposition speed.

CONCLUSIONS: This observation suggests that the POI has to be evaluated in the perspective of the final application, including presence of cells and cell culture conditions.

ACKNOWLEDGEMENTS: This study was supported by project grants from the Swedish Research Council (VR-MH), the Swedish Cancer Society (Cancerfonden), and the Swedish Childhood Cancer Foundation (Barncancerfonden) to O.H.

REFERENCES

[1] Webb B, Doyle B Bioprinting. 8 (2017) 8–12
[2] Hinton T et al. Sci. Adv. 1 (2015) e1500758–e1500758



Engineering anisotropic tissues using ultrasound cell patterning

J.P.K. Armstrong[1], J.L. Puetzer[1], S.C. Skaalure[1], O. King[2], C. Meinert[3,4], A.C. Franklin[5], P.G. Bassindale[5], C.M. Terracciano[2], D.W. Hutmacher[3,4], T.J. Klein[3,4], B.W. Drinkwater[5], A.W Perriman[6], M.M. Stevens[1]

Presenting author: James Armstrong, james.armstrong@imperial.ac.uk

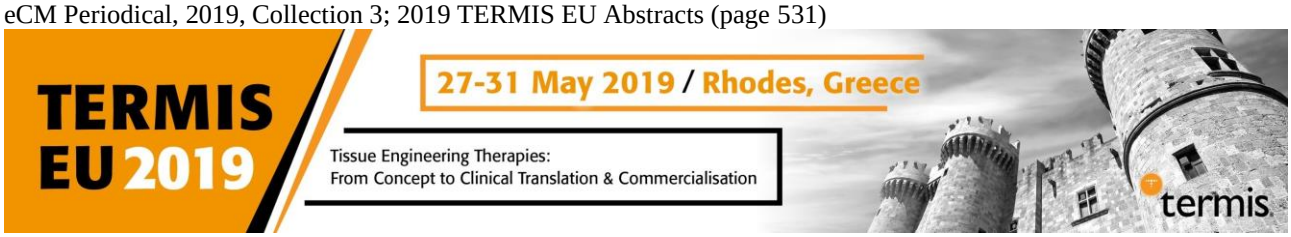
[1]Department of Materials, Department of Bioengineering, and Institute for Biomedical Engineering, Imperial College London, London, U.K [2]National Heart and Lung Institute, Imperial College London, London, U.K [3]Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia [4]Australian Research Council Training Centre in Additive Biomanufacturing, Queensland University of Technology, Brisbane, Queensland, Australia [5]Department of Mechanical Engineering, University of Bristol, Bristol, U.K [6]School of Cellular and Molecular Medicine, University of Bristol, Bristol, U.K

INTRODUCTION: Tissue engineering strategies must seek to reproduce native cellular organization to ensure synchronous function, mechanical anisotropy and correct tissue maturation.

METHODS: We employed an acoustic patterning device with integrated piezotransducers to deliver ultrasound standing waves (<30 min, 2.0-2.1 MHz) that could pattern cells into various geometries (e.g. lines, clusters). Hydrogels were used to encapsulate the cell arrays for tissue engineering. For instance, C2C12 myoblasts patterned in lines were used to engineer aligned skeletal muscle.

RESULTS & DISCUSSION: Here we demonstrate, for the first time, that acoustic fields can be used to produce aligned cell fibers in an engineered tissue. For instance, we show that a brief application of ultrasound standing waves can be used to pattern myoblasts into collagen-based hydrogels for the engineering of muscle tissue with dense, aligned fibers. This strategy offered great flexibility across different bioengineering protocols, addressing several key limitations facing in vitro muscle tissue engineering. Myoblasts patterned in a type I collagen hydrogel contracted the surrounding matrix to produce high-density muscle fibers, anisotropic tensile mechanics and a remodeled extracellular matrix. Indeed, the cell-mediated contraction was used in combination with collagen clamping to generate, to the best of our knowledge, the first instance of a tissue construct exhibiting cell alignment on both an individual cell and population-wide level. A third protocol, utilizing photo-crosslinked GelMA hydrogels, was used to demonstrate that acoustic patterning can significantly enhance myofibrilliogenesis, compared to unpatterned controls. Negligible myoblast fusion was observed in the unpatterned tissue after seven days, however, the acoustically-patterned muscle exhibited upregulated MRF4, a key marker of myotube maturation, and large, multinucleated myotubes expressing α-myosin skeletal fast and tropomyosin. The myotubes were aligned within the acoustically-patterned muscle fibers, with the patterning extending across entire tissue constructs (cf. 350 cell widths / 7 mm). We have extended this method to engineer other anisotropic soft tissues using 2D ultrasound fields to pattern stacked fibres in full 3D volumes.

CONCLUSIONS: The ability to fabricate anisotropic tissue constructs with ordering over large length scales will be a critical factor in the engineering of functional tissue grafts and physiologically-relevant disease models. Indeed, the ability to rapidly and dynamically align label-free cells en masse using generic hydrogels and cell culture apparatus makes this platform technology highly advantageous for a host of next-generation bioengineering strategies.



Development and characterization of a photo-crosslinkable hyaluronic acid bioink for neural tissue engineering applications

J.J. Britton[1, 2], V.K. Kanala[1], A. Loffredo[1], A. Pandit[1], M.J. Biggs[1]
Presenting author: James Britton, j.britton3@nuigalway.ie
[1]Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]National University of Ireland Galway (NUI Galway) Hardiman scholarship, Ireland

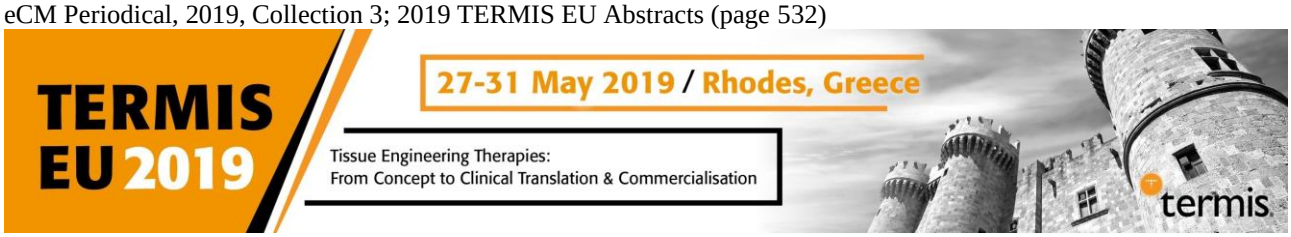
INTRODUCTION: Regeneration of the spinal cord after injury remains a great challenge due to the complexity of this organ. Inflammation and gliosis at the injury site hinder the outgrowth of axons and hence prevent synaptic reconnection and reinnervation of paralysed tissue. Implantable biomimetic biomaterials have proven to be an exciting tissue engineering approach to promote axon outgrowth across the injury site. Hydrogels provide a substrate for cell adhesion and migration while also reducing inflammation after injury. Hyaluronic acid (HA) is the main component of the spinal cord extracellular matrix and plays a vital role in cell proliferation and axonal guidance. In this study, we have characterised a photocrosslinkable HA-tyramine hydrogel from a chemical, mechanical, electrical, and biological perspective.

METHODS: Using Rheology, the mechanical properties of HA-tyr in response to incremental increases in UV exposure was measured and compared to that of isolated rodent spinal cord tissue. Using potentiometry, the electrical conductivity of HA-tyr was examined. Using the Nanoscribe two-photon polymerisation (2-PP) 3D printer, HA-tyr was photocrosslinked according to computer aided Design (CAD) created geometries. The laser power and scan speed was optimised to create reproducible 3D printed hydrogels. The cytocompatibility of photocrosslinking HA-tyr was assessed using dorsal root ganglion explants and immunohistological staining.

RESULTS & DISCUSSION: From our experimentation, we have examined the degree of tyramine functionalisation of HA via nuclear magnetic resonance spectroscopy. This parameter is important in determining the crosslinking efficiency of any photocrosslinkable bioink. We have found that the mechanical properties of HA-tyr can be tuned to mimic that of native spinal cord via optimization of the photo-initiator concentration and UV exposure. Using potentiometry, the electrical conductivity of photocrosslinked HA-tyr was assessed and compared to that of native spinal cord tissue at physiologically relevant voltages. Spinal cord tissue has greater conductivity which could be correlated with the isotropic structure and myelin presence. Using dorsal root ganglion explants, the tissue compatibility of photocrosslinked HA-tyr was assessed using immunohistochemistry. The laser power and scan speed of the 3D printer was optimised to facilitate hydrogel patterning.

CONCLUSIONS: This generated data clearly illustrated the feasibility of using tyramine functionalized Hyaluronic acid as a bioink for neural engineering.

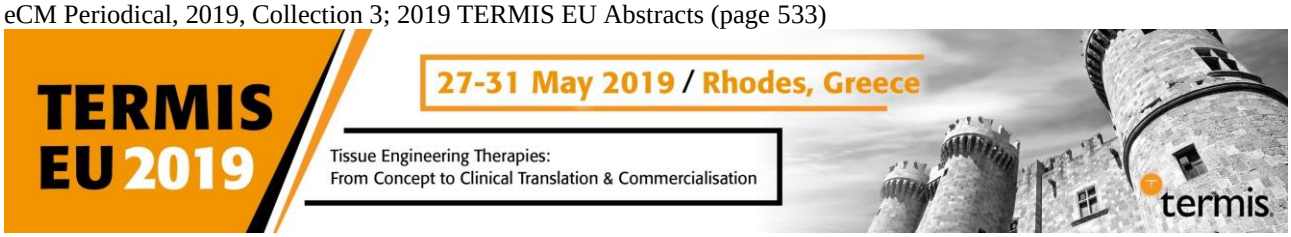
ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland, (Grant RSF 3757) and the National University of Ireland Galway (NUI Galway) Hardiman scholarship.



Is rich always good? Extra-cellular matrix in 3D neural cultures

H. Renner, M. Grabos, H.R. Schöler, J.M. Bruder
Presenting Author: Jan Bruder, jan.bruder@mpi-muenster.mpg.de
Max Planck Institute for Molecular Biomedicine, Roentgenstrasse 20, 48149 Muenster, Germany

Recent advances in stem cell technology have led to the development of three-dimensional (3D) culture systems, which have fueled hopes to augment the physiological relevance of high throughput screens (HTS). However, current protocols based on extracellular matrices such as matrigel either yield complex but highly heterogeneous aggregates (“organoids”). Here, we present a fully HTS-compatible workflow combining automated seeding, culture, and analysis of neural precursor cells resulting in highly homogenous automated midbrain-like neural microtissues (ANTs). The ANTs display a zonal 3D architecture and mimic relevant organ function in the form of spontaneous neural activity synchronized across the entire microtissue. We explore the effects of different extracellular matrices on neural outgrowth in 3D, comparing defined and non-defined matrices. Overall, our techniques solve three main challenges that have prevented 3D cell culture from use in HTS: Intra- and inter batch variation, scale up, and automated cost-efficient single-cell readout. We utilize this standardized technology to assess the effect of extracellular matrix on neural outgrowth in 3D.



Crucial geometrical factors in engineering cell shape for controlling type I and II collagen mRNA expression of human chondrocytes

J.C. Lauer[1], S. Hermann[2], M. Erb[1], K. Strobel[1], M. Selig[1], B. Rolauffs[1]
Presenting Author: Jasmin C. Lauer, jasmin.lauer@uniklinik-freiburg.de
[1]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Dept. of Orthopedics and Trauma Surgery, Germany [2]Dept. of General and Visceral Surgery; Albert-Ludwigs-University of Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany

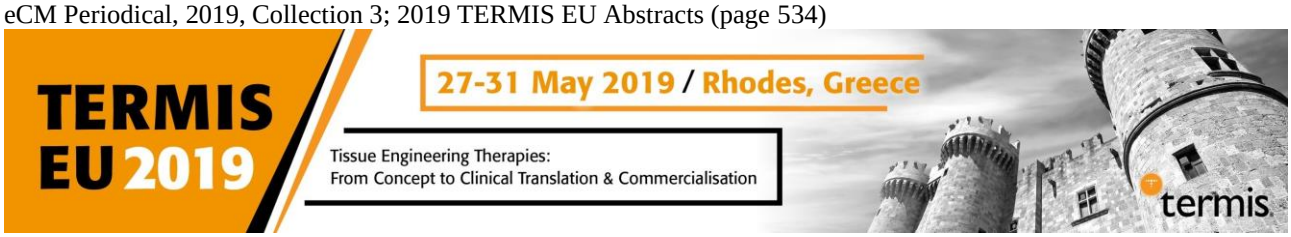
INTRODUCTION: Chondrocytes (CHs) that dedifferentiate display changes in cell morphology that are associated with changes in the expression levels of type I and II collagen (COL I,II). Utilizing this association of form and function, we aimed to engineer specific CH shapes for controlling CH mRNA expression through micro-patterned adhesion sites (MPs). The goal was to identify geometric aspects of cell shape that are crucial for controlling COL I and II.

METHODS: Human primary CHs from osteo-arthritic articular cartilage were cultured on fibronectin-coated MPs for 24 h or 7 days. The customized MP geometries were H-shaped or designed as circles either with increasing area, aspect ratio (AR) or number of prongs while keeping the other parameters constant. F-actin, β-tubulin and the nuclei were IF-stained. The recorded images were used for calculating a panel of 7 quantitative shape descriptors (cell area, AR, roundness, length, width, circularity, solidity) and for generating image heatmaps. ddPCR was used to measure the mRNA expression of COL2A1 and COL1A2. Partial least square (PLS) regression was performed to identify the relative impact of MP-engineered CH shape on mRNA expression.

RESULTS & DISCUSSION: Almost all shape descriptors of CHs on MPs were significantly different from CHs on non-patterned controls ($p<0.05$). Comparing CHs on MPs among MPs with varying area, AR, and prongs revealed significantly different cell shapes ($p<0.05$), predominantly for cell area and solidity. Thus, MPs can be used for engineering CH shape but MP and cell geometries did not express a one-to-one relationship. In CHs with two nuclei, a CH AR from 1.2 to 1.5 directed the nuclei locations towards the longitudinal MP axis. This was more pronounced in CHs with a higher AR and demonstrated that nucleus distribution and, in turn, the direction of CH division can be controlled through MP AR. COL2A1 and COL1A2 mRNA expression levels of CHs on MPs were significantly different from CHs on non-patterned control areas ($p<0.05$). The range of fold-change in expression levels that we achieved through using MPs was 0.1-0.7 for COL1A2 and 0.4-3.9 for COL2A1, relative to non-patterned control CHs. Investigating the effects of all 7 shape descriptors on mRNA expression, PLS uncovered that COL1A2 mRNA expression was correlated highest to MP-engineered cell solidity (PLS loading coefficient: -0.467), whereas COL2A1 expression was correlated highest to cell AR (PLS loading coefficient: -0.649). Testing for significance revealed COL1A2 expression fold-change correlated significantly with cell solidity (cc: -0.796, $p<0.0001$) and COL2A1 correlated significantly with cell AR (cc: -0.362, $p<0.05$).

CONCLUSIONS: CH shape can be engineered by MPs and can be used for controlling the orientation of cell division and type I and II collagen expression modulation. The factors most crucial were cell AR and solidity.

ACKNOWLEDGEMENTS: Volkswagenstiftung (92701) and the Forschungskommission of the Medical Faculty Freiburg (ROL 1153/17).



Optimisation of microparticle formulations for cytokine delivery for macrophage modulation in spinal cord injury

J.Z. Stening[1], F.R.A.J. Rose[1], L.J. White[1]
Presenting Author: Jasmine Stening, paxjzs@nottingham.ac.uk
[1]Regenerative Medicine and Cellular Therapies, School of Pharmacy, University of Nottingham, UK

INTRODUCTION: Currently spinal cord injury lacks treatment capable of restoring limb function and sensation. Pharmaceutical strategies focus on alleviating the triggered high inflammatory environment. Understanding macrophage behavior and roles of their sub phenotypes has suggested a method for controlling inflammation by modulation towards a pro-immunoregulatory subgroup (M2) using cytokine IL-4. Microparticles are widely reported as drug delivery methods for controlled and sustained release. We aim to determine suitable release profiles for IL-4 delivery.

METHODS: Particles were manufactured using double emulsion [1,2] with 50:50 or 85:15 (lactide:glycolide ratio) PLGA (52kDa). Release kinetics were tailored by: (i) incorporation of a PLGA-PEG-PLGA triblock modifier (TB) [1] and (ii) changing the total polymer (TP) percentage. Total model protein (Lysozyme) loaded was 10mg/ml for 1g polymer and 5mg/ml for 500mg polymer. Release results were reported as µg protein/ mg particles with a maximum of 10µg/mg encapsulated. Protein encapsulation efficiencies and release were analysed using a micro BCA assay to detect total protein content.

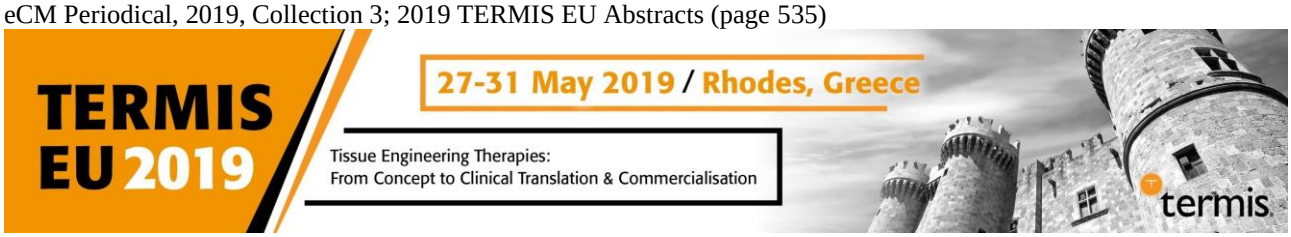
RESULTS & DISCUSSION: Microparticles fabricated were 20-50µm in size and had a smooth morphology. In a comparison of surface thickness the fastest release was observed for 10%TP 85:15 PLGA with a burst release of 2.5µg/mg on day 1. The slowest release occurred for 10%TP 50:50 PLGA with 1µg/mg released by day 20. In a comparison of triblock percentages for 20%TP 50:50 PLGA, 30%TB showed the fastest release with 10µg/mg released by day 20 and 0% and 20%TB showed the slowest release. 10%TB showed a burst release of 3µg/mg at day 1 and promising sustained and continuous release to 6µg/mg.

CONCLUSIONS: Particles prepared from 10% or 15%TP were unsuitable for controlled release with a fast burst followed by minimal daily release. Particles manufactured from 50:50 and 85:15 PLGA alone displayed release too slow for IL-4 delivery. Addition of a PLGA-PEG-PLGA TB enabled release to be controlled with less initial burst release and accelerated overall release. Microparticles fabricated from 20%TP, 50:50 PLGA with 10%TB showed a release profile most suited to controlled release.

ACKNOWLEDGEMENTS: Supported by the International Foundation for Research in Paraplegia, Switzerland ‘Combining stem cell and drug delivery to modulate macrophage phenotype toward M2 in spinal cord injury’, grant no. P155.

REFERENCES

[1] White LJ et al. Mater Sci Eng C. 2013; 33(5):2578–83
[2] Abu-Awwad HADM et al. Acta Biomater. 2017; 57:225–37



Gelatin-polyester membranes for regenerating the corneal endothelium

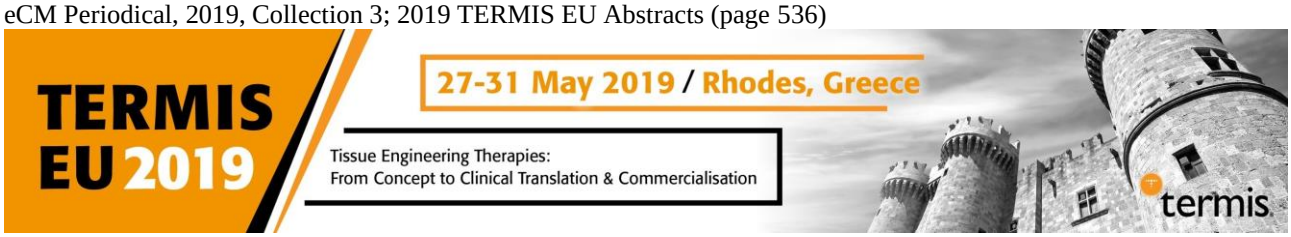
J. Van Hoorick[1,2], B. Van den Bogerd[3], J. Delaey[1] , J. Van Erps[2], H. Thienpont[1,2], C. Koppen[3], N. Zakaria[3], P. Dubruel[1], S. Van Vlierberghe[1,2]
Presenting Author: Jasper Van Hoorick, Jasper.vanhoorick@ugent.be
[1]Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium [2]Brussels Photonics, Department of Applied Physics and Photonics, Vrije Universiteit Brussel, Brussels, Belgium [3]Department of Ophthalmology, Visual Optics and Visual Rehabilitation, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium

INTRODUCTION: Over 10 million people worldwide suffer from corneal blindness with a yearly prevalence rate of 1.5 million. One important cause of corneal blindness is related to dysfunction of the corneal endothelium. This monolayer of cells maintains the cornea in a state of physiological deturgescence via a pump-and-leak mechanism.[2] Human corneal endothelial cells cannot undergo mitosis and therefore cannot regenerate. Following ageing, trauma or disease, the cell density will drop below a critical threshold thereby impairing the efficient pumping of excessive fluid from the cornea, leading to edema. Concomitant loss of the organized collagen structure results in opacity, thereby impairing vision. Currently, the only treatment consists of transplantation of healthy cadaveric corneal tissue. Unfortunately, donor supply does not meet the demand, thereby underlining the need for tailored synthetic scaffolds to develop ex vivo manufactured biomimetic grafts.

METHODS: Transparent membranes were developed by spincoating onto glass slides (ø = 12 mm) using a multi-layer approach combining: a sacrificial gelatin layer, a poly(D,L-lactide) (PDLLA) layer (Corbion) for structural integrity and a UV-crosslinkable, functionalized gelatin as extracellular matrix (ECM) mimic. Different gelatin derivatives were evaluated including methacryloyl and norbornene derivatives. An Argon plasma treatment prior to gelatin deposition ensured covalent attachment of gelatin. The membranes were characterized for glucose diffusion, membrane thickness, transparency, surface composition and in vitro biocompatibility using immortalized corneal endothelial cells.

RESULTS & DISCUSSION: PDLLA was selected as starting material to provide mechanical integrity. In vitro biological assays indicated that PDLLA as such does not provide cell interactivity. Therefore, a crosslinked gelatin coating was applied. The produced membranes were transparent throughout the visual spectrum (> 98%) for thicknesses ranging from 0.8 to 1.5 µm thereby complying to the dimensions of the natural Descemet's membrane (<10 µm). Furthermore, the membranes exhibited sufficient glucose diffusion both in the presence and absence of a gelatin coating, which is an important prerequisite for membrane function. Finally, upon cell seeding, the primary corneal endothelial cells retained their characteristic hexagonal shape. Immunocytochemistry showed the expression of Na^+/K^+ ATPase and ZO-1, the hallmark proteins to identify healthy functional endothelial cells. Finally, the number of focal adhesion points of cells seeded onto the membranes showed no significant difference compared to the positive control.

CONCLUSIONS: Ultrathin (< 1.5 µm) membranes were constructed using PDLLA for mechanical integrity and gelatin as ECM mimic. The membranes were transparent, permeable to glucose and supported cell culture rendering them interesting towards microsurgery thereby alleviating the current donor shortage.



Collagen-hydroxyapatite nano-scaffold as a novel bone void filler

J. Milosevic[1], D. Troppa[1], A. Ludwig[1], C. Heine[1], T. Ganey[1], H.J. Meisel[1]
Presenting Author: Javorina Milosevic, javorina.milosevic@spinplant.de
[1]SpinPlant GmbH, Halle (Saale), Germany

INTRODUCTION: The next generation of bone graft substitutes shall provide a handable product for an efficient bone ingrowth and regeneration causing neglectable side-effects. They should primarily demonstrate high patient safety, therapeutic efficacy, unlimited availability, but also cost efficiency. In this study, a biomimetic scaffold that mimics the natural bone tissue microenvironment based on collagen-nanoceramics has been designed.

METHODS: To produce extracellular matrix (ECM)-mimicking nano-scaffold for bone repair with preserved native collagen structure, a unique electrospinning technology was used. By means of different techniques including SEM analysis, MTT proliferation assay, ALP (alkaline phosphatase) activity and immunocytochemistry, the spun matrices were comprehensively characterized. Mesenchymal stem cells from human adipose tissue (hASCs) have been used to characterize the developed matrices (designated as SpinFill). In addition to biocompatibility studies, the scaffolds were compared for their performance in vitro. For the comparison, two similar products already existing on the market were used as reference devices (Cerasorb Foam and Vitoss Scaffold Foam). To assess adhesion and proliferation, the colorimetric raw data (OD-values) of the respective MTT-assay normalized to product volume were used in order to compare the analyzed products. The osteogenic potential of the different products was compared using normalized ALP-values. The finding was confirmed by immunocytochemical staining for osteogenesis marker osteopontin (OPN, Fig. 1D).

RESULTS & DISCUSSION: SpinPlant technology relies on the preservation of the collagen nativity, assuring stability of the product, which is a prerequisite for an efficient osseointegration and new bone formation. SpinFill consists of biomaterials shown to be non-toxic, biodegradable and at the same time stable and porous, promoting cell adhesion and spreading. The in-vitro performance of SpinFill was in several aspects significantly better than that of both reference devices.

CONCLUSIONS: These findings collectively support the therapeutic potential of SpinPlant's biodegradable collagen-hydroxyapatite nano-scaffold for bone regeneration.

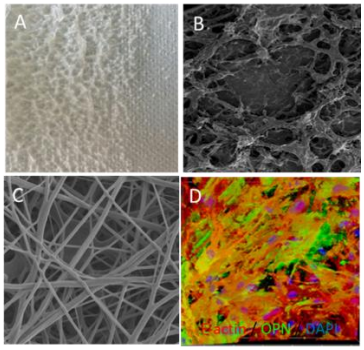
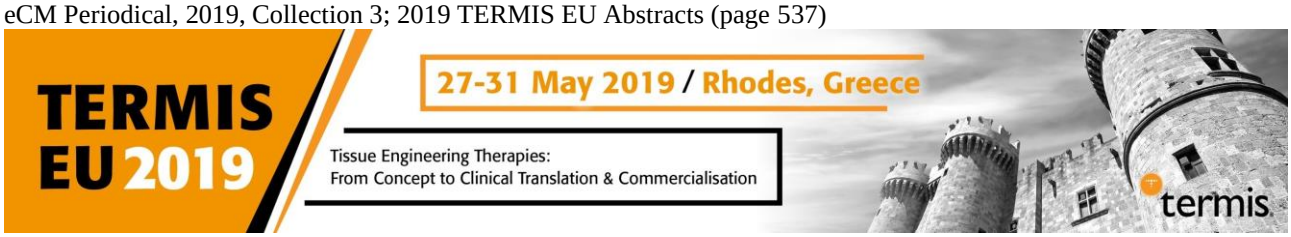


Figure 1: Macroscopic (A) and microscopic (B- 100x, C-50,000x) view of the collagen-hydroxyapatite nano-scaffold, when seeded with human ASCs showed prominent staining for F-actin (red) and osteogenesis marker osteopontin (green, OPN in D), two weeks after differentiation in vitro. SEM image reveals a complex organization of the nano-scaffold (C).



Long term fate of iron oxide nanoparticles in the organism

J. Volatron[1], G. Charron[1], M. Tharaud[2], F. Gazeau[1]
Presenting Author: Jeanne Volatron, jvolatron@gmail.com
[1]Matière et Systèmes Complexes (MSC), Paris Diderot University, Paris, France [2]Institut de Physique du Globe de Paris (IPGP), Paris, France

INTRODUCTION: Iron oxide nanoparticles (IONPs) are of particular interest in tissue engineering, regenerative medicine and cancer therapy due to unique magnetic properties allowing MRI monitoring, magnetic stimulation of cells and local hyperthermia tissues [1]. In this context the evaluation of IONPs life cycle is a major challenge, to optimize upstream their properties and develop nano-object that are robust and safe to use. Our group have previously shown that IONPs injected intravenously in mice underwent local degradation within lysosomes of macrophages in spleen and liver [2] with local iron recycling by endogenous protein [3]. Here we propose a multi-scale approach to follow the six months' fate of all the constituents of the IONPs: the magnetic core, the coating (ensuring their stability) and the non-magnetic byproducts (resulting from the degradation of the magnetic core).

METHODS: At the organism scale, the main difficulty is to distinguish the exogenous elements (intravenously injected IONPs and their byproducts) from endogenous iron. Our strategy was thus to enrich the inorganic core of IONPs with a minor iron isotope, ^{57}Fe, and to double-label the coating with two lanthanides. IONPs were followed-up by MRI, Transmission Electron Microscopy (TEM) and their magnetic properties were quantified by Ferromagnetic Resonance (FMR). Their byproducts (nonmagnetic ^{57}Fe species) and the lanthanide coating were quantified by High resolution ICP-MS. The fate of these labelled IONPs were follow up in the mice over a period of 6 months.

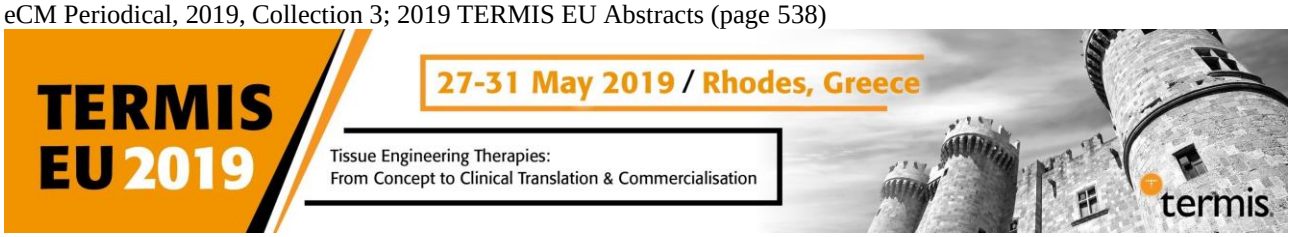
RESULTS & DISCUSSION: Surprisingly, during the first hour after intravenous injection, there is a partial dissociation of the coating from IONPs. The coating is then directed to the kidneys and eliminated from the body. One hour after injection, IONPs are mostly located in liver and spleen. Rapid and complete degradation is observed in liver during the first month after injection, while the degradation is slower in spleen. Finally, from three months after injection, we observed a transfer of the non-magnetic byproducts from liver to the spleen via red blood cells, testifying the recycling of exogenous iron by the organism.

CONCLUSIONS: These data illustrate the fate, the remediation and the recycling of exogenous IONPs in the organism over a period of six months.

ACKNOWLEDGEMENTS: Financial support was received from Dim Nano K NanoSciences, C'Nano IDF and from H2020 European Project NoCanTher number 685795.

REFERENCES

[1] N. Luciani et al. Acta Biomater. 2016; 37:101-110
[2] L. Lartigue et al. ACS Nano. 2013; 7(5):3939-52
[3] J. Volatron et al. Small 2017; 13(2)



Ionic release from cotton wool-like bioactive glass fibres promotes early onset of mesenchymal stem cell differentiation and mineralization in vitro

J. Maksimcuka[1], G. Poologasundarampillai[2], P.J. Withers[1,3], O. Tsigkou[1]
Presenting Author: Jekaterina Maksimcuka, jekaterina.maksimcuka@manchester.ac.uk
[1]School of Materials, University of Manchester, Oxford Road, Manchester, UK [2]School of Dentistry, University of Birmingham, 5 Mill Pool Way, Edgbaston, UK [3]Manchester X-ray Imaging Facility and BP International Centre for Advanced Materials, UK

INTRODUCTION: Bioactive glass (BG) is known for its ability to bond to soft and hard tissues and form hydroxyapatite via ion exchange. BG ions have been shown to upregulate osteogenic differentiation of mesenchymal stem cells. Here, ability of Silica and Calcium ions released from BG cotton wool-like fibres to enhance osteogenesis and true apatite formation as early as day 4, when compared to standard procedures, is showcased.

METHODS: Bioactive glass (BG) with composition 70% SiO and 30% CaO was prepared through sol-gel process and electrospun into fibres. Conditioned medium (BGCM) was prepared by incubating 5 mg of fibres per 1 ml of neat media for 24 h at 37□C. Final ionic concentration was measured through Inductive Coupled Plasma (ICP) spectroscopy. Human bone marrow MSCs were cultured with osteogenic differentiation media (Promocell) alone or enriched with bone morphogenic protein 2 or BGCM. To evaluate osteogenic differentiation and mineral deposition alkaline phosphatase (ALP) enzymatic activity, collagen type 1, osteocalcin, calcium (alizarin red) and collagen deposition (Sirius red) were investigated. Gene expression profile was assessed using the Human Osteogenesis RT² Profiler™ PCR Array. SEM, EDX and XRD mapping was performed to evaluate and characterize the bone apatite formation.

RESULTS & DISCUSSION: BGCM supplemented osteogenic media stimulated significant calcium phosphate deposition, as verified by EDX. Alizarin red staining demonstrated significant ($p<0.005$) calcium deposition in BGCM supplemented osteogenic media as early as day 4. A sharp increase of ALPase enzymatic activity at day 4 and decline afterwards was also observed indicating an early onset of osteogenic differentiation of MSCs. EDX and XRD map have confirmed that the produced deposits are made of calcium and phosphorus. Effect on osteoclasts will also be presented.

CONCLUSIONS: Our data demonstrate the impact of inorganic ions on osteogenic differentiation of MSCs. This could potentially be used alongside standard osteoporosis treatment to enhance localized bone formation.

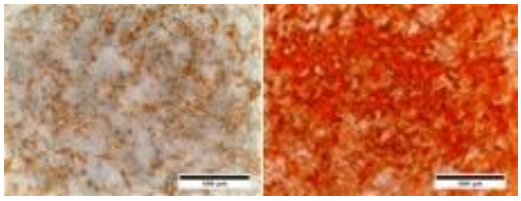
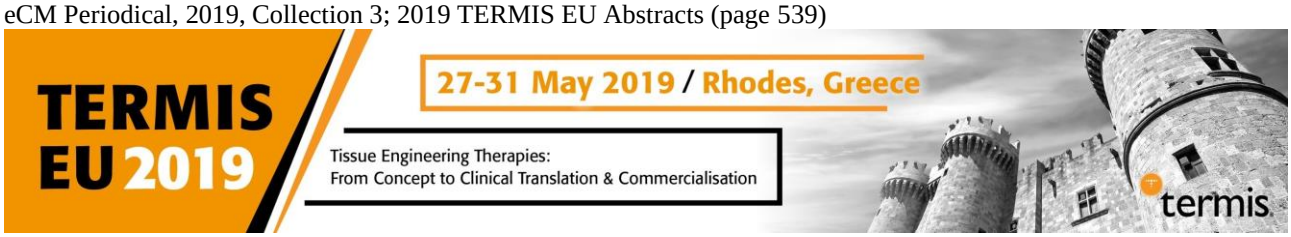


Figure 1: Alizarin red staining of MSCs after 7 days in culture with Osteogenic media (left) or osteogenic media enriched with BG ions (right). Red indicates calcium phosphate deposits. Scalebar-500 µm.

ACKNOWLEDGEMENTS: We thank EPSRC and MRC for funding (EP/L014904/1) and facilities and support provided by the School of Materials, University of Manchester.



A novel biofabrication approach using ultrasound-guided particle manipulation

J. Shapiro[1], B. Drinkwater[2], A. Perriman[3], M. Fraser[1]
Presenting Author: Jenna Shapiro, jenna.shapiro@bristol.ac.uk
[1]Bristol Interaction Group, University of Bristol, Bristol, UK [2]Department of Mechanical Engineering, University of Bristol, Bristol, UK [3]School of Cellular and Molecular Medicine, University of Bristol, Bristol, UK

INTRODUCTION: The formation of a vascular network in a tissue engineered construct in vitro remains an open problem [1]. Acoustic radiation forces are gaining attention as an attractive method of quickly patterning microparticles, including cells, within a support medium, with little to no cellular damage [2, 3]. Here, we introduce a proof-of-concept design for a modular ultrasonic patterning system, capable of rapidly patterning cell-scale aqueous droplets.

METHODS: Arrays of ultrasonic transducers operating at a frequency of 40 kHz were positioned in 3D printed PLA supports, following computational modelling of predicted acoustic pressure fields. Medical nebulisers (Healthcare World) and a piezoelectrically-actuated droplet-on-demand (DOD) generator (Microfab), were used to produce droplets of 1–5 µm and 80 µm, respectively. Water-sensitive paper (Syngenta), which turns blue upon contact with aqueous solutions, was used to visualise deposition patterns. Alginate microbeads were formed by depositing a 0.5% aqueous sodium alginate solution via the DOD generator into a bath containing 102 mM $CaCl_2$.

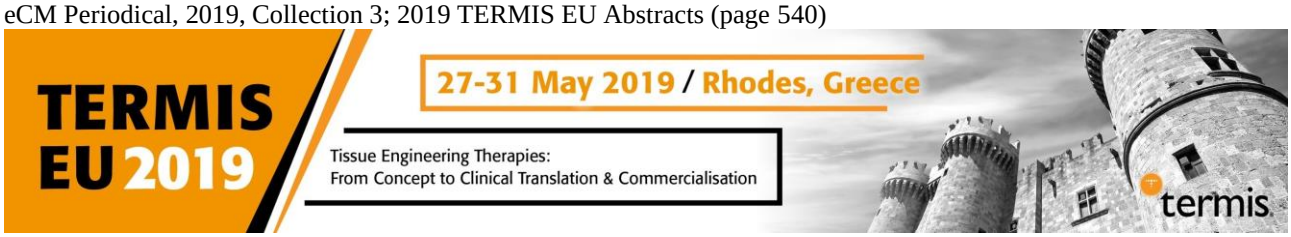
RESULTS & DISCUSSION: Water droplets produced by a medical nebuliser (< 5 µm diameter) and DOD generator (80 µm) were patterned within seconds of exposure to the acoustic field, as were the larger calcium alginate microbreads. Spatial resolution is determined by a combination of the initial droplet size distribution and the ultrasonic standing wave pattern. Droplet drying kinetics indicate a significant reduction in droplet radius over time, dependent upon polymer concentration and ambient humidity, with effective polymer concentration increasing over the drying period. These findings indicate that droplets can initially be larger and more dilute than the desired final size and concentration.

CONCLUSIONS: The patterning we demonstrate forms a first step towards the generation of larger, high-resolution 3D structures, including vasculature. Crucially, the resolution of this technique has the potential to approach cellular length scales.

ACKNOWLEDGEMENTS: Financial support was received from the UK Engineering and Physical Sciences Research Council (EPSRC, EP/M021882/1).

REFERENCES

[1] Duan, B. Ann Biomed Eng. 2017;45(1):195-209.
[2] Drinkwater, B. Lab Chip. 2016;16:(2360-75).
[3] Armstrong, JP, et al. Adv Mater. 2018; 30(43):1802649.



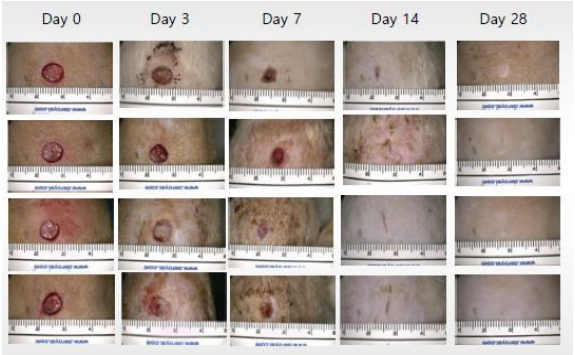
Novel hydrogel complex for enhanced skin regeneration

S. Oh[1], J. Suh[1], S. An[2], J. Kim[2], J. Huh[1], J. Lim[1]
Presenting Author: Jeong Ok Lim, jolim@knu.ac.kr
[1]Joint Institute for Regenerative Medicine, Kyungpook National University, School of Medicine, Kyungpook National University Hosipital, Daegu, South Korea [2] Daegu Gyeongbuk Medical Innovation Foundation (DGMIF) Daegu, South Korea

INTRODUCTION: Current challenge in 3D printing in biological fields lie in lack of proper printing materials. Therefore, development of hydrogel materials to enable successful structured printing of biological substitute is on high demand. Our study aimed to formulate a hydrogel complex having proper rheological, chemical and biological properties to be useful for printing tissue scaffolds, especially for skin tissue regeneration. We characterized the complex formulation in vitro and in vivo for its potential for enhanced skin regeneration.

METHODS: 1**.** Hydrogel printing: The gelatin based hydrogel complex was structured by printing under 40 % fill density, 250 % input flow and 5 mm/s print speed. The temperature of dispenser and bed were 20 ℃ and 15 ℃ respectively. After printing, UV light was irradiated for 4 minutes. 2. Biocompatibility study: C_2C_{12} and 3T3 cells ($1*10^6$/ml) were respectively mixed with hydrogel complex before it print. Mixed cell and hydrogel complex were printed on chamber slide. Live/Dead assays were conducted at day1, day3, day5 and day7. 4. Structural Stability **:**$(L_2-L_1) / L_1$ where L_1: Length of 3D printed nose with day 0 / L_2 : Length of 3D printed nose after immersion. Structured hydrogel was immersed in PBS for 21 days. 5. Water absorbency: $(W_2-W_1) / W_1$ where W_1 : Weight of 3D printed hydrogel before immersing / W_2 : Weight of hydrogel after swelling. 6. Mechanical properties**:** Universal Test Machine (OTT-003, Oriental TM, South Korea), Tested with 3 kgf load cell and 30 mm gauge length. 7. In vivo study: Safety and efficacy were investigated with mice for 4 weeks.

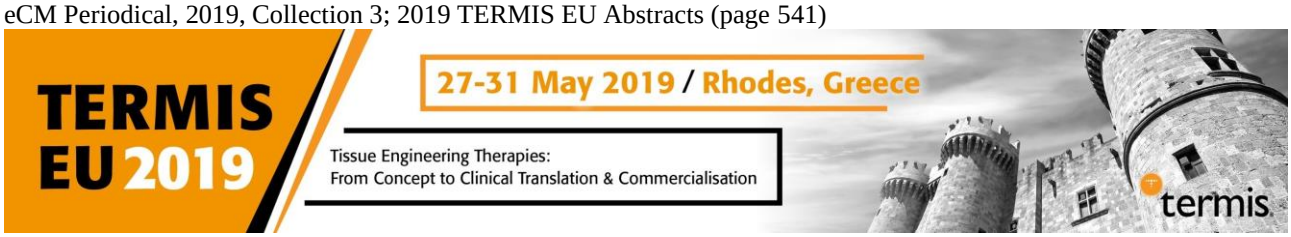
CONCLUSIONS: The newly hydrogel complex has a great potential as a material for pattering 3D structure for soft tissue reconstruction since they are easy to print, gelling, not toxic, stable, and cost effective.



	Average	Standard deviation
Tensile strength (MPa)	0.175	0.007
Elongation At break (%)	43.58	1.58

Figure 1: (left) Observation of wound healing after hydrogel application (top: control. 2nd, 3rd, 4th lines are treated with various hydrogel complex. **Table 1:** (right) Mechanical properties of structured hydrogel

ACKNOWLEDGEMENTS: 2017-2019 Medical Cluster R&D Support Project, Daegu Gyeongbuk Medical Innovation Foundation, Ministry of Health & Welfare, the Republic of Korea (HI15C2505).



An in vitro model for the treatment of intervertebral disc degeneration using nasal chondrocytes

J. Kasamkattil[1,2], M.H.P. Gay[1,2], A. Mehrkens[1], A. Barbero[2], I. Martin[2], S. Schären[1]
Presenting Author: Jesil Kasamkattil, jesil.kasamkattil@usb.ch
[1]Department of Spinal Surgery, University Hospital Basel, Switzerland [2]Tissue Engineering, Department of Biomedicine, University of Basel & University Hospital Basel, Switzerland

INTRODUCTION: Intervertebral disc (IVD) degeneration is the most common cause of specific low back pain [1]. To treat the IVD degeneration, human nasal chondrocytes (hNCs) were identified as a possible alternative cell source due to their ability to survive and produce extracellular matrix in an IVD environment. hNCs were shown to possess high proliferation capacity and are capable to adapt to heterotopic transplantation sites [2]. This study aims to investigate the interaction of hNCs with human nucleus pulposus cells (hNPs) in a 3D in vitro co-culture model.

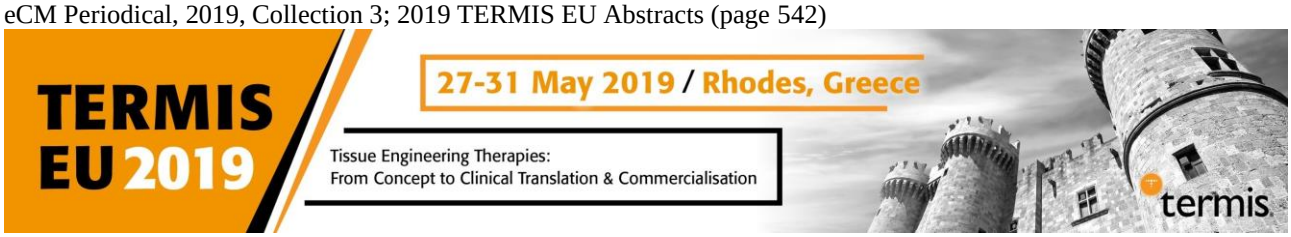
METHODS: In vitro nucleus pulposus micro-tissue was fabricated by pooling eight hNPs micro-aggregates (25'000 cells per micro-aggregate, cultured for two weeks) together. GFP-labelled hNCs were then added to the hNP micro-tissue either as single cell suspension (200'000 cells per micro-tissue) or as 16 micro-aggregates (12'500 per micro-aggregate, cultured for two days). Monoculture hNPs and co-culture hNPs-hNCs were cultivated for two weeks. Preliminary analysis was conducted upon macroscopic investigations during co-culture time.

RESULTS & DISCUSSION: Macroscopic investigations showed that: (i) control hNPs micro-aggregates in monoculture did not fuse into a stable construct, (ii) hNPs micro-aggregates in co-culture with hNCs suspension or with hNCs micro-aggregates merged to create one accumulative mass (indicating positive interactions between the two cell types), (iii) metabolic activities was high and similar in the two co-culture groups (indicating new matrix formation).

CONCLUSIONS: Preliminary results indicate that the experimental approach to investigate the interaction of hNCs with hNPs is feasible. Visual evaluation suggests that co-culture of hNCs and hNPs has an advantageous effect on matrix production. Histological and biochemical analysis will be performed to validate and quantify the impact of hNC cells on the viability, proliferation and extracellular matrix production of hNPs and vis-versa.

REFERENCES

[1] de Schepper, E.I. et al. (2010) Spine (Phila Pa 1976) 35 (5), 531-6.
[2] Pelttari, K. et al. (2014) Sci Transl Med 6 (251), 251ra119.



3D bioprinting of vascularized composite constructs for bone repair

J. Nulty[1], F.E. Freeman[1], D.J. Kelly[1]
Presenting Author: Jessica Nulty, jnulty@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

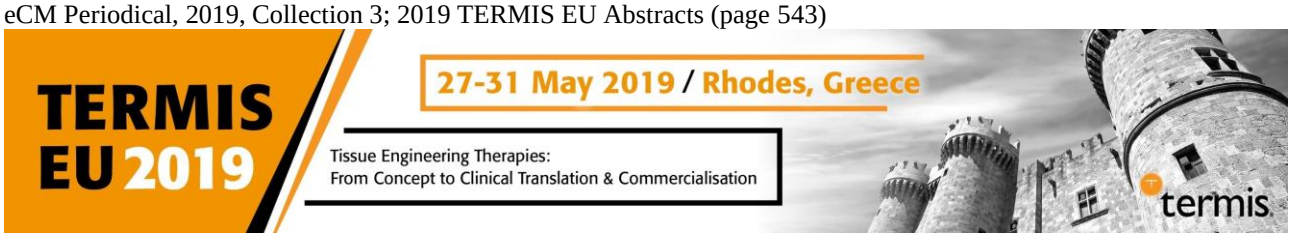
INTRODUCTION: Without a functional vascular network, large engineered tissues will ultimately fail. The overall aim of this study is to 3D bioprint a prevascularised construct and to investigate its capacity for bone regeneration. The first aim of this study was to compare different bioink formulations, cell combinations and culture periods for their potential to establish stable microvessels both in vitro and in vivo. The second aim of this study was to incorporate vessel forming bioinks into a 3D bioprinted cartilage template and to investigate the capacity of this construct to support endochondral bone formation in vivo.

METHODS: In vitro vessel study: Three bioink hydrogels (alginate, GelMA and fibrin) were seeded with human umbilical vein endothelial cells (HUVECs) cultured in EGM-2 media with 5ng/mL of VEGF for 10 days in vitro. Groups were evaluated for average vessel length using ImageJ. In vivo vessel study: Bioprinted fibrin constructs containing MSCs, HUVECs or HUVECs & MSCs were cultured for either 12h or 7 days in EGM-2 with VEGF and subcutaneously implanted into balb/c mice for 1 & 2 weeks. Constructs were evaluated for vessel number using histology (H&E). In vivo Bone formation: Cartilage templates were engineered by bioprinting fibrin laden with MSCs (10E6 cells/ml) with or without an inner channel. Constructs were cultured in chondrogenic media for 2 weeks at 5% O_2 and a further 1 week at 20% O_2. At this point, using the fibrin bioink a co-culture of HUVECs:MSCs was 3D printed into the inner channel of one the groups, creating 3 distinct bioprinted cartilage templates: Solid, Empty channel & Vasco channel. All groups were cultured in EGM-2 media with 5ng/mL of VEGF for 7 days and implanted subcutaneously into balb/c mice for 4 & 8 weeks. Bone formation was determined by µCT analysis.

RESULTS & DISCUSSION: In vitro vessel analysis: Over the course of 7 days, HUVECs had formed microvessels in both GelMA and fibrin, however there was no endothelial sprouting observed in the Alginate. Fibrin bioinks supported the most consistent growth of microvessels over time. From this point on Fibrin was chosen as the optimum bioink for all future studies. In vivo vessel analysis: At day 7, only the cultured co-culture constructs (containing an in vitro engineered vasculature) contained perfused vessels. This beneficial difference was maintained over 14 days in vivo. In vivo Bone Analysis: All bioprinted cartilage templates supported robust endochondral bone formation in vivo. Although prevascularizing constructs lead to a higher percentage of area vascularized within the engineered tissue, implant architecture appeared to play a more dominant role in determining the levels of mineralization in vivo compared to prevascularizing the tissue prior to implantation.

CONCLUSIONS: These results show that fibrin is an ideal bioink to support HUVEC sprouting and microvessel formation. Bioinks of fibrin containing a HUVEC:MSC co-culture support the development of a stable primitive vascular network in vitro. Furthermore, prevascularizing 3D printed constructs in vitro using this novel bioink led to increased anastomosis when implanted in vivo. Future studies will investigate if incorporating microvessels into 3D bioprinted templates will lead to enhanced bone regeneration in large bone defects.

ACKNOWLEDGEMENTS: Financial support was received from the ERC 204144.



Lactate-induced cardiac tissue regeneration

J. Ordoño[1,2], S. Pérez-Amodio[1,2,3], A. Aguirre[4], E. Engel[1,2,3]
Presenting Author: Jesús Ordoño, jordono@ibecbarcelona.eu
[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology, Spain [2]CIBER Bioengineering, Biomaterials and Nanotechnology, Spain [3]Dpt. Material Science and Metallurgical Engineering, UPC, Spain [4]Institute for Quantitative Health Sciences and Engineering (IQ), Michigan State University, USA

INTRODUCTION: Lactate plays key roles in multiple cellular processes, including angiogenesis, wound healing and cancer growth and metastasis [1,2]. Herein we explored the potential role of lactate on cardiac regeneration and iPSC differentiation.

METHODS: Neonatal mice cardiac cells were isolated and incubated with L-lactate solution. Whole neonatal mice hearts were also maintained ex vivo in Matrigel and cultured with or without lactate. Human iPSCs were differentiated to cardiomyocytes by GSK3 and Wnt inhibition. Immunostaining, FACS, RT-qPCR and TEM analysis were conducted.

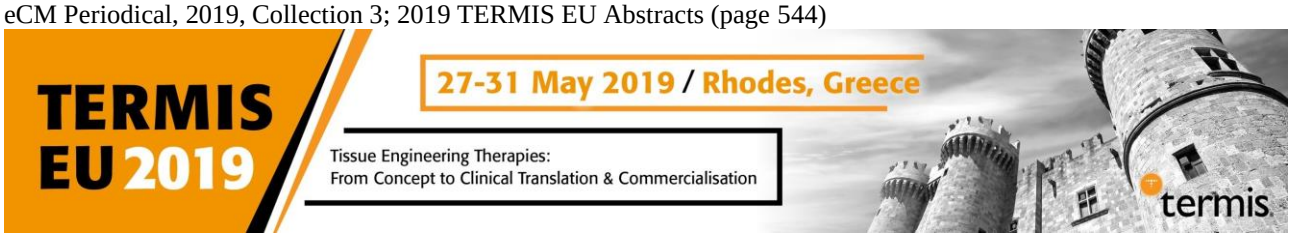
RESULTS & DISCUSSION: Immunostaining analysis with ki67 revealed an increase in the number of proliferating mice cardiomyocytes in the presence of lactate after 4 days of treatment. Moreover, transcription factor p63, which is involved in stem and progenitor cell regulation, was highly overexpressed in the presence of lactate, as RT-qPCR data revealed. Ex vivo culture of neonatal mice hearts showed a better maintenance of the integrity of the heart tissue up to 8 days in lactate, as well as an enhanced beating ability compared to control without lactate. This result was correlated with higher sarcomere width in cardiac tissue from hearts incubated in lactate, as confirmed by TEM image analysis. Therefore, the effect of lactate on human iPSCs-derived cardiomyocytes was also evaluated. MTS assay was used to assess the viability of these cells in the presence of different concentrations of lactate. Immunofluorescence imaging revealed the localization of p63 protein associated to the cellular membrane and cytoplasm. No significant DNA damage was observed on iPSCs-derived cardiomycytes after incubation with lactate, as it was shown by FACS analysis on γH2A.X. Additionally, both proliferation and dedifferentiation markers were evaluated by FACS and RT-qPCR analysis.

CONCLUSIONS: Together, these data supports the use of lactate as a modulating signal for cardiac tissue engineering.

ACKNOWLEDGEMENTS: Financial support was received from MINECO (MAT2015-62725-ERC and MAT2015-68906-R) and EUIN73. Research in the Aguirre Laboratory was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award number K01HL135464 and it was possible thanks to Fundacio Privada Daniel Bravo Andreu.

REFERENCES

[1] Sun S, et al. Physiol. 2017;32:453-463.
[2] Dhup S, et al. Curr. Pharm. Des. 2012;18:1319-13130.



Motored PLGA microspheres for localized inflammation inhibition

J. Wang[1], J. Toebes[2], J. A. Jansen[1], F. Yang[1], D. A. Wilson[2]
Presenting Author: J. Wang, jiamian.wang@radboudumc.nl
[1]Department of Biomaterials, Radboud University Medical Centre, The Netherlands [2]Department of system chemistry, Radboud university, Nijmegen, The Netherlands

INTRODUCTION: Periodontitis is chronic inflammation of the periodontal tissues, caused by microbial pathogens in the periodontal pocket. It is the major cause of tooth loss in adult humans. The disease is treated by scaling and root planing. However, this process is not efficient enough to produce a contamination free environment, thus adjunctive therapies are often included, e.g. local administration of antimicrobials. The antimicrobials can be delivered inside the periodontal pocket via chips, gels or microspheres. However, those passive delivery methods cannot ensure the drugs to reach the inflamed spots. Therefore, an active delivery method is advantageous for the periodontal treatment. At the site of infection, reactive oxygen species (ROS) are generated by immune cells, such as macrophages, to kill the pathogens. H_2O_2 is the precursor for the production of most ROS and is therefore found at increased levels in inflamed tissues. The aim of this study was to develop enzyme-grafted PLGA microspheres, which can move towards high gradients of H_2O_2 for the treatment of periodontitis.

METHODS: The enzyme grafted PLGA microspheres were prepared by electrospraying process followed by a 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide coupling modification. The morphology and compositions of PLGA microspheres before and after functionalization were detected by scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS). Release profile of doxycycline, the incorporated drug in the microspheres was determined by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) analysis. A cell model (Raw 264.7) were used to track the movement of PLGA microspheres.

RESULTS & DISCUSSION: The SEM micrographs before and after functionalized with catalase were shown in Figure 1. After functionalization, the asymmetry structure of catalase was built on the surface of PLGA microspheres. The EDS results displayed the element of Nitrogen on the functionalised microspheres, indicating the presence of catalase. The drug release profiles showed the identical patterns for the PLGA microspheres before and after functionalization, suggesting that the functionalization process did not affect drug release. The microsphere tracking experiment showed that the enzyme-grafted PLGA microspheres were sensitive to the gradient of H_2O_2 produced by Raw 264.7.

CONCLUSIONS: The enzyme-grafted PLGA microspheres perform a directional movement in response to the gradient of H2O2. The functionalization process did not affect the drug release from the PLGA microspheres. The developed delivery system is promising for periodontal treatment.

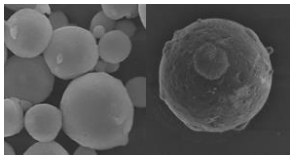
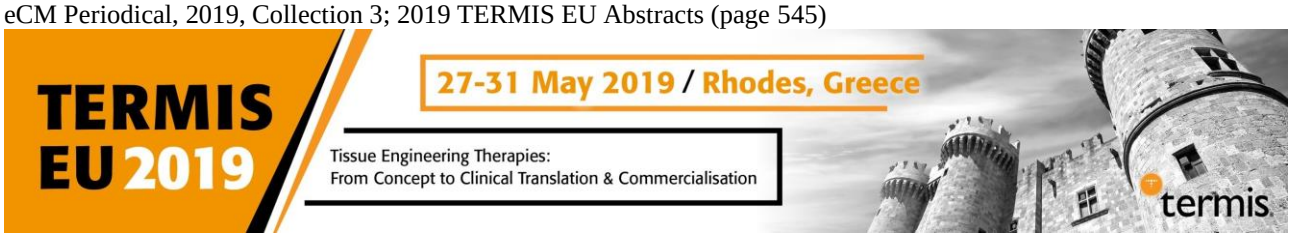


Figure 1: Morphology of doxycycline loaded PLGA microspheres before (Left) and after (Right) functionalization (scale bar: 1μm).



3D bioprinting of cell-laden bone constructs with advanced bioinks

J.H. Zhang[1], M. Rubert[1], H. Eyisoylu[1], H.J. Huang[2], X.H. Qin[1], R. Müller[1]
Presenting Author: Jianhua Zhang, Jianhua.zhang@hest.ethz.ch
[1]Institute for Biomechanics, ETH Zürich, 8093 Zürich, Switzerland [2]Laboratory for Multifunctional Materials, ETH Zürich, 8093 Zürich, Switzerland

INTRODUCTION: 3D bioprinting of cell-laden scaffolds becomes more prevalent in bone tissue engineering for applications in personalized medicine. However, existing hydrogel-based bioinks often result in insufficient mechanical strength and poor scaffold fidelity because of swelling or shrinking [1]. Here, we incorporated a 2D nanomaterial, graphene oxide (GO), into a alginate/gelatin bioink system for improving printability and scaffold performance. The influence of GO on cell viability, cell proliferation, osteogenic differentiation and mineral formation was investigated.

METHODS: Four different GO concentration (0, 0.5, 1, 2 mg/ml) were prepared by adding different amounts of GO in glycerol/PBS (9.1% v/v) solution. The same amount of alginate (0.8% w/v) and gelatin (4.1% w/v) was dissolved in the solution to prepare GO composite inks. Shear-thinning behavior of all inks was characterized rheometrically. Bioinks were prepared by mixing ink solution with human mesenchymal stem cells at a concentration of 5 million cells/ml. 3D cell-laden scaffolds were bioprinted layer-by-layer using a INKREDIBLE$^+$ cell bioprinter. Scaffold morphology, mechanical properties, cell viability and cell morphology were investigated at day 1, day 7 and day 42 of cell culture in osteogenic media. Moreover, mineral formation of the 3D bioprinted cell-laden bone scaffolds was assessed by weekly micro-CT scans.

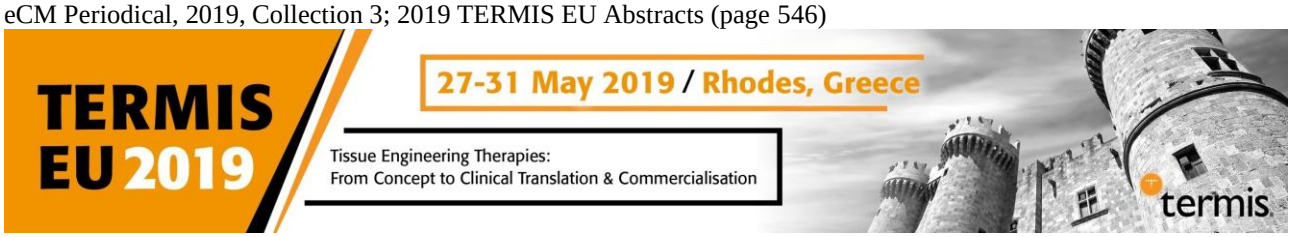
RESULTS & DISCUSSION: The GO ink solutions had higher viscosity and improved shear-thinning properties with increasing GO content. Higher compressive moduli were obtained at day 1 with higher GO concentrations. Meanwhile, scaffolds with higher GO showed improved scaffold morphology with less shrinkage and higher level of cell viability at day 1 and day 7 as compared to the lower GO and no GO groups. At day 42, 2 mg/ml GO group exhibited the lowest compressive modulus due to swelling; 1 mg/ml GO group had the best scaffold morphology with similar compressive modulus in comparison with the 0.5 and 0 mg/ml GO groups. The highest mineral volume was found in the 1 mg/ml GO group (95.56 ± 5.5 mm^3), which was significantly higher than 0 mg/ml GO. Cell morphology exhibited a remarkable difference between no GO and GO groups at day 42.

CONCLUSIONS: We have demonstrated that the incorporation of GO significantly improved printability, scaffold morphology and mechanical properties while keeping excellent cell viability and cell spreading morphology. An optimal GO concentration at 1 mg/ml had the highest mineral formation at day 42.

ACKNOWLEDGEMENTS: J. Zhang gratefully acknowledges financial support from CSC.

REFERENCES

[1] Caliari S et al. Nat. Methods. 2016; 13(5):405.



Osteochondral scaffold with gradients for interfacial tissue regeneration

J. Y. Yun[1], H.J. Cha[1*]
Presenting Author: Jin Young Yun, jinyoung1421@postech.ac.kr
[1]Department of Chemical Engineering, Pohang University of Science and Technology, Pohang 37673, Korea

INTRODUCTION: The complex interfacial tissue regeneration between soft and hard tissues is one of the most challenging fields in tissue engineering. Herein, we developed a model for an osteochondral scaffold consisted of bio-silicified organic-inorganic composite with mechanical and biochemical gradients for tissue reconstruction at interface between bone and cartilage. Furthermore, its functionalities can be improved through encapsulation of cells or growth factors related to osteochondral tissue repair.

METHODS: Mussel adhesive protein (MAP) was modified with silica-precipitating peptide to fabricate organic-inorganic composite. The modified MAP proteins were mixed with polycaprolactone (PLC; Sigma Aldrich) at various proportions to improve its mechanical properties. The scaffolds were fabricated by electrospinning of the mixtures. The nanofibers of the each mixture were stacked layer-by-layer to form gradient scaffolds. As the scaffolds were treated with 1 M tetramethly orthosilicate (TMOS), biosilica was rapidly precipitated within 5 min.

RESULTS & DISCUSSION: The nanofibers were successfully fabricated from each mixture of the modified MAP and PCL by electrospinning (Figure 1). Each layer has different properties including biodegradability and mechanical strength depending on the proportion of constituent. It indicates that the scaffold with mechanical and biochemical gradients can be manufactured owing to superior adhesive property of MAP. Additionally, it can be developed into organic-inorganic hybrid composite via bio-inspired reaction under ambient condition.

CONCLUSIONS: It was shown that hybrid composite scaffolds with gradients can be successfully fabricated. The model for interfacial tissue reconstruction will be completed through collaboration with cells or growth factors.

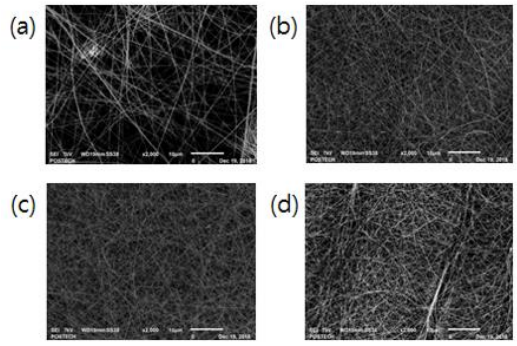
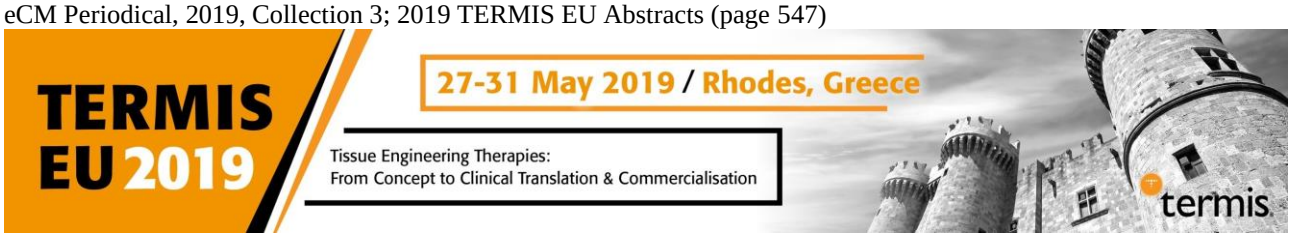


Figure 1: SEM images of the mixture of the modified MAP and PCL with various ratios; (a) 1:9, (b) 2:8, (c) 3:7, (d) 5:5.

ACKNOWLEDGEMENTS: Financial support was provided by the Marine Biomaterials Research Center grand from Marine Biotechnology Program of the Korea Institute of Marine Science & Technology Promotion funded by the Ministry of Oceans and Fisheries, Korea.



A hybrid injectable hydrogel from hyperbranched PEG macromer as a stem cell delivery and retention platform for diabetic wound healing

Q. Xu[1], Jing Lyu[1], W. Wang[1]
Presenting Author: Jing Lyu, jing.lyu@ucdconnect.iemailto:diana.gaspar@nuigalway.ie
Charles Institute of Dermatology, School of Medicine, University College Dublin, Ireland

INTRODUCTION: The injectable hydrogel with desirable biocompatibility and tunable properties can improve the efficacy of stem cell-based therapy [1-2] However, the development of injectable hydrogel remains a great challenge due to the restriction of crosslinking efficiency, mechanical properties, and potential toxicity. Herein, we generated an in situ formed injectable hydrogel system fabricated by biocompatible reagents for effective stem cell retention and delivery for diabetic wound healing.

METHODS: The new HP-PEGs were synthesized via in situ reversible addition fragmentation chain transfer (RAFT) polymerization using an FDA approved anti-alcoholic drug-Disulfiram (DS) as the RAFT agent precursor. Functionalised hyaluronic acid (HA-SH) was used to crosslink with HP-PEG to form injectable hydrogels. Mechanical strength was tested by rheometer and degradation profile were tested in PBS. Adipose-derived stem cells were encapsulated in the hydrogel to detect the cytocompatibility and stemness. In in vivo study, humanized diabetic wounds in rats were generated and treated by hydrogel, hydrogel with ADSCs. Histological assay was used to analyse the wound healing efficacy, inflammation, angiogenesis, and re-epithelialisation.

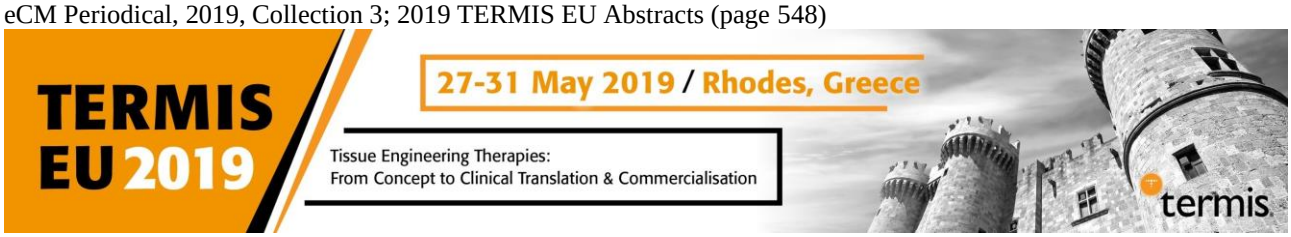
RESULTS & DISCUSSION: The in situ RAFT polymerization of PEGDA using DS as a RAFT agent precursor endows the resultant hyperbranched PEG polymers good biocompatibility and abundant pendent acrylate groups, which can be utilized to form an injectable hydrogel with a rapid gelation rate (less than 2 min) and a tunable mechanical property. The HP-PEG/HA-SH hydrogels also demonstrated non-swelling and antifouling properties. Both in vitro and in vivo biocompatibility tests showed non-toxicity of the hydrogels. ADSCs encapsulated in the hydrogels also demonstrated the maintenance of the multi-potency and secretion capacity. Hydrogel/ADSCs also exhibited accelerated diabetic wound healing and improved wound environment in a humanized diabetic wound model in rats, such as faster wound closure rate, reduced inflammation response, promoted vascularization and angiogenesis and re-epithelialisation.

CONCLUSIONS: This versatile hydrogel system encapsulated with ADSCs exhibited an accelerated diabetic wound healing process through inhibiting inflammation, promoting angiogenesis and re-epithelialization.

ACKNOWLEDGEMENTS: Financial support was received from Science Foundation Ireland (No 13/IA/1962, 10/IN.1/B2981(T), 15/IFA/3037), Health Research Board of Ireland (HRA-POR-2013-412).

REFERENCES

[1] Dong, Y et al. Adv Funct Mater. 2017; 27(24):1606619-30
[2] Waqar, H et al. Wound Rep Reg, 2014; 22(3):313-25



Interplay between Lamin A/C and Zyxin shapes actin network in 3D

J. Zonderland, I. Lorenzo Moldero, C. Mota, L. Moroni
Presenting Author: Jip Zonderland, J.Zonderland@maastrichtuniversity.nl
Maastricht university, MERLN Institute for Technology-Inspired Regeneration Medicine, Complex Tissue Regeneration department, Maastricht, The Netherlands

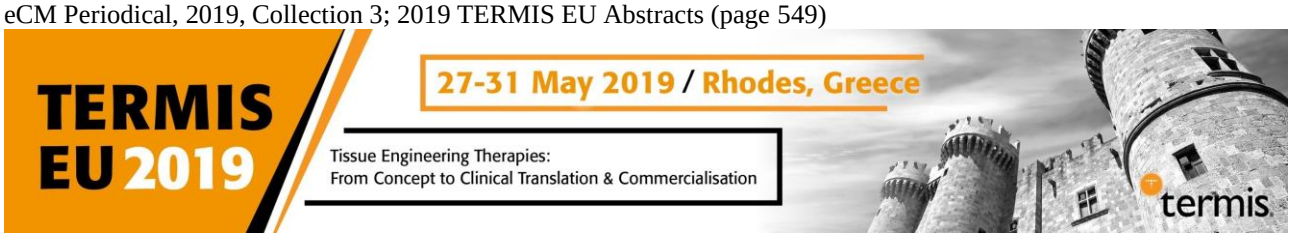
INTRODUCTION: Mesenchymal stromal cells (MSCs) change their phenotype in response to the environment using complex mechanosensitive signal transduction chains. Environmental signals from the ECM are translated to the nucleus through focal adhesions and the dynamic cytoskeletal modifications. Lamin A/C forms a protein meshwork under the nuclear membrane and plays a key role in transducing mechanical signals from the cytoskeleton to gene regulatory machinery in the nucleus. However, the regulation of lamin A/C expression and its role in cells' ability to respond to a 3D environment is not well known.

METHODS: Human bone marrow-derived mesenchymal stromal cells (hMSC) were seeded on additive manufactured- and electrospun scaffolds and compared to a flat film of the same material. Lamin A/C, focal adhesion proteins and F-actin were investigated using western blot and immunofluorescent imaging. Knock downs of zyxin, lamin A/C, paxillin and YAP1 and lamin A/C overexpression were performed to study the role of these proteins in cells in a 3D environment.

RESULTS & DISCUSSION: In 2D, hMSCs form thick actin stress-fibers and focal adhesions. We have shown that in a 3D environment, additive manufactured or electrospun scaffolds, focal adhesions are few and faint and no actin stress fibers are formed (Figure 1A). Together with this, we observed a strong decrease of lamin A/C expression in both 3D environments. This change was functional, as the lower lamin A/C expression lead to improved migration through small pores, due to a more flexible nucleus. Cell density influenced focal adhesion and lamin A/C expression, but this did not account for the observed differences between 2D and 3D cell culture systems. Knock down of Zyxin abolished the focal adhesions and the actin stress fibers, and also the Lamin A/C expression. Similar to Zyxin knock down, Lamin A/C changed the actin cytoskeleton, where it only polymerized in the cell perimeter (Figure 1B). Combinations of lamin A/C overexpression and zyxin knockdown, and overexpression of lamin A/C in scaffold cultures revealed an interplay between zyxin and lamin A/C that shaped the actin network in 3D.

CONCLUSIONS: Zyxin and lamin A/C work together to regulate the actin network in 2D and in 3D constructs, dictating cell shape and phenotype. This data can be used to better understand mechanosensitive signaling and cell behavior in a 3D environment and potentially advance regenerative medicine approaches using 3D constructs.

ACKNOWLEDGEMENTS: We are grateful to the European Research Council starting grant "Cell Hybridge" for financial support under the Horizon2020 framework program (Grant # 637308)



Identification and validation of a novel immunophenotype for quality assessment in mesenchymal stem manufacturing

J. Fitzgerald[1], G. Shaw[1], F. Barry[1]
Presenting Author: Joan Fitzgerald, j.fitzgerald23@nuigalway.ie
[1]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Translation of MSC therapies to the clinic has been slower than anticipated with mixed clinical outcomes reported. One considerable barrier to progress in the field is a lack of standardised and validated testing protocols for quality assurance leading to cell products of uncertain quality. Although minimal criteria have been proposed [1], it is apparent that these are insufficient predictors of clinical efficacy.

METHODS: Human bone marrow derived MSCs from two donors were cultured using three specified expansion conditions. The surface immunophenotype of each cell preparation were probed by high throughput flow cytometry using a combinatorial antibody profiling tool comprising of 230 antibodies.

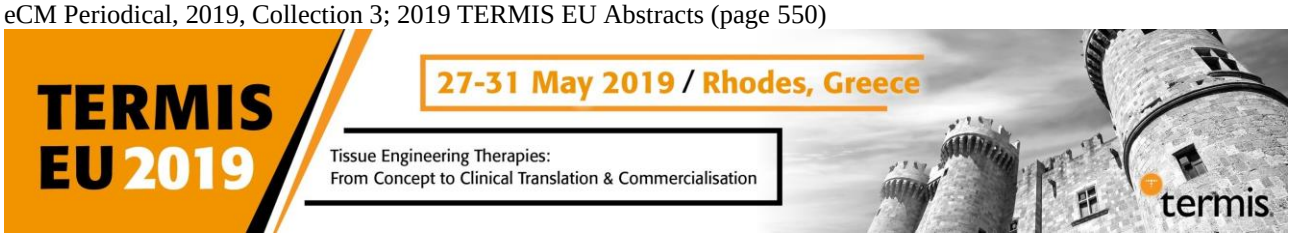
RESULTS & DISCUSSION: Analysis of expression of the surface proteins revealed significant variation in response to culture conditions and considerably less variation between donors. Of the panel of 230 markers 107 were negative, 24 had high expression in all samples, 1 had low expression and 98 displayed significant differences between cell preparations, of which 78 were culture condition dependent. Cluster analyses revealed that marker expression in one culture condition varied considerably from the other two. Phenotypic characterization of the cell preparations, assessed by morphology and growth kinetics, showed similar patterns of variability between the culture conditions. Statistical and pathway analysis revealed a panel of markers which were highly differentially expressed between the culture conditions and could be used as indicators of cell quality.

CONCLUSIONS: These data reveal a panel of MSC markers which may be used as phenotypic indicators for enhanced in-process and product release quality testing in MSC manufacturing. Ongoing work involves the complete validation of these markers and correlation of their expression to functional attributes including angiogenic, immunosuppressive and immune modulatory capacities, differentiation and colony forming propensity.

ACKNOWLEDGEMENTS: Financial support was received from Celtic Advanced Life Science Innovation Network (80885).

REFERENCES

[1] Dominici M et al. Cytotherapy. 2006;8(4):315-317



Fetal-inspired strategies for intervertebral disc regeneration

J. Caldeira[1,2], M. Fiordalisi[1,2,3], C. Pereira[1,2,4], A.J. Silva[1,2], M. Pinto[1,2], R. Gonçalves[1,2], M.A. Barbosa[1,2,3]

Presenting Author: Joana Caldeira, joana.caldeira@ineb.up.pt

[1]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal [2]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal [3]ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal [4]FEUP – Faculdade de Engenharia da Universidade do Porto, Portugal

INTRODUCTION: Low back pain (LBP), for which existing therapies are inadequate, affects over 70% of the world population, having a tremendous social and economic impact due to the enormous costs associated to treatment and work absenteeism. The years lived with this disability rank in front of those from AIDS and lung cancer among others. LBP is often caused by intervertebral disc (IVD) degeneration, which occurs with age and is accompanied by extracellular matrix (ECM) depletion [1]. As so, novel therapeutic solutions that modulate the disc microenvironment need to be developed. We have recently demonstrated the ECM changes that occur in the IVD with development and ageing, and reported that pro-regenerative molecules like COLXII and COLXIV, are almost exclusively expressed at this developmental stage [2]. As so, we propose revolutionary strategies to treat IVD degeneration by recreate a microenvironment similar to pre-natal stages.

METHODS: On one hand we have tested different solutions and treatment times for fetal NP decellularization. Decellularized NPs were assessed to evaluate biochemical, structural histological and biomechanical properties. Repopulation of the scaffolds with adult NP cells in also understudy. The acellular materials were also used to develop a hydrogel by varying pH and temperature, among others. ECM-based hydrogels were characterized by SEM and gelation kinetics. On the other hand, we have established a lentiviral system for CRISPR-mediated activation of fetal ECM genes.

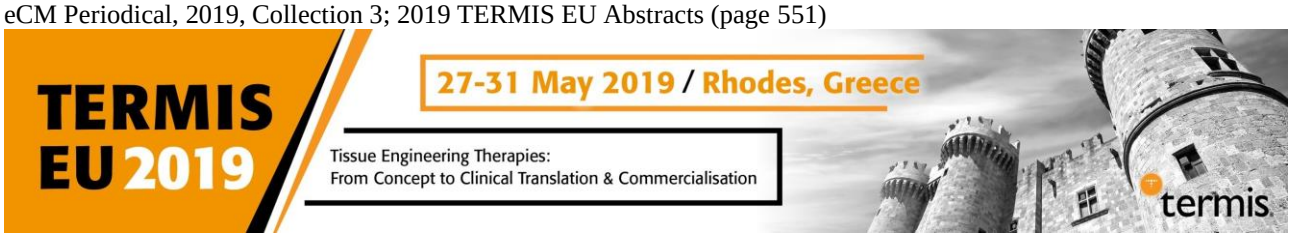
RESULTS & DISCUSSION: We have identified an SDS-based protocol that is effective at removing cells from different aged NPs while preserving glycosaminoglycans and collagen content. Topographical and biomechanical analysis have shown differences among the different biomaterials which consequently impacted on NP cell phenotype. Using the CRISPR system, we were able to transduce and select clonal MSC populations homogeneously expressing the target genes and corresponding proteins.

CONCLUSIONS: This original study will provide the foundations for the establishment of a pioneer treatment based on cutting edge technology with potential to improve the already promising stem cell based therapies.

ACKNOWLEDGEMENTS: Financial support was obtained from EUROSPINE, L'Oréal Portugal Medals of Honor for Women in Science 2018 and FCT (Fundação para a Ciência e a Tecnologia).

REFERENCES

[1] Colombier P et al. Joint Bone Spine. 2014; 81:125-129.
[2] Caldeira J et al, Sci Rep. 2017, 7:11629-11644.



Safety and performance of biomaterials in patients: A case study of polydioxanone implants

J. Martins[1], A. Lach[1], H. Morris[1], A. Carr[1], P.A. Mouthuy[1]
Presenting Author: Joana Martins, joana.martins@ndorms.ox.ac.uk
[1]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, OX3 7LD, United Kingdom

INTRODUCTION: Biomaterials have been used in the development of medical implants for several decades. Polydioxanone (PDO) is a biodegradable synthetic polymer used in the manufacturing of commonly used medical implants, namely PDS™ II suture (Ethicon Inc.). However, no review has yet been performed assessing the effect of PDO implants on safety and performance in patients.

METHODS: Authors performed a search for commercially approved PDO implants in FDA's databases and also a literature search for their use in clinical trials or in clinic. Safety and performance was assessed based on a scoring system developed by authors and included surgical site infection (SSI) rates, inflammatory reaction rates, foreign body response and postoperative pain and fever rates. Clinical outcomes of PDO implants were also compared to clinical outcomes of implants manufactured with different materials.

RESULTS & DISCUSSION: A number of 43 PDO implants were approved by the FDA since 1981, with different shapes and sizes. In the last decade, FDA reported 1278 adverse reactions or product malfunctions and 16 recalls were found. The literature search revealed 46 publications involving the use of PDO implants in patients, covering different medical specialties. PDO sutures and meshes/plates presented low rates of SSI, inflammatory reaction, foreign body response and postoperative fever whereas PDO clips/staples showed high rates of SSI, postoperative pain and fever, with reports of failure in function. As a result, PDO clips/staples were scored low for safety and performance. The remaining types of PDO implants (sutures, plates/meshes and screws/pins) resulted in a high score for safety and performance. Results also showed that, when comparing PDO implants with non PDO alternatives, PDO sutures performed better than non PDO sutures, with higher safety score. Additionally, PDO plates/meshes and PDO screws/pins have the same performance levels as non PDO implants.

CONCLUSIONS: This review suggests that PDO medical devices are generally safe and perform well upon implantation in patients. However, it remains important to design future PDO implants (namely weight, shape, structure, and mechanical properties) with careful consideration of the host tissue properties.

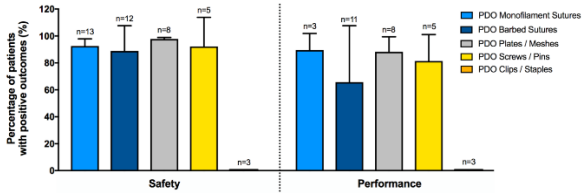
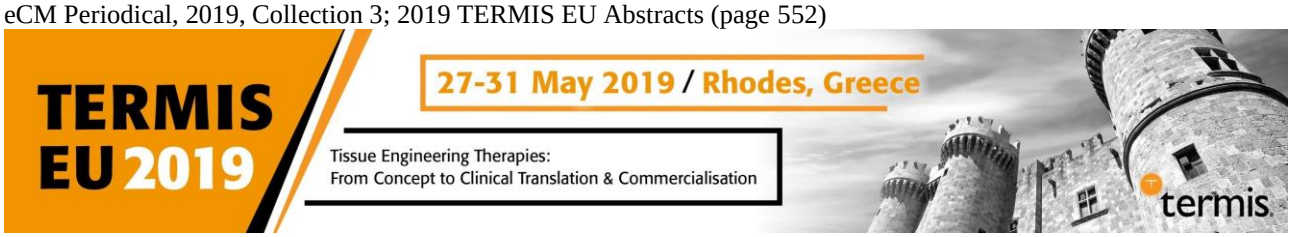


Figure 1: Safety and performance of PDO implants according to type of implant. n=number of publications.

ACKNOWLEDGEMENTS: This research was funded by the National Institute for Health Research (NIHR) Invention for Innovation (i4i) [II-LB-0715-20001], the Wellcome Trust [106946] and by the NIHR Oxford Biomedical Research Centre



Evaluation of the angiogenic effect of elastin incorporation on silk fibroin 3D printed scaffolds

L. Costa[1,2], J.B. Costa[1,2,3], J.M. Oliveira[1,2,3], R.L. Reis[1,2,3], J. Silva-Correia[1,2]
Presenting Author: Joana Silva-Correia, joana.correia@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: A novel enzymatically crosslinkable silk fibroin (SF) bioink has been developed for printing high-resolution reproducible 3D structures [1]. The potentiality/versatility of SF bioinks to be combined with different biomolecules for better mimicking native tissues has been recently investigated by introducing elastin for replicating the outer intervertebral disc [2]. In this work, the effect of elastin on the angiogenic response of SF bioprinted scaffolds was explored by using the chick chorioallantoic membrane (CAM) assay [3].

METHODS: The 16 wt% silk fibroin (SF) and silk fibroin/elastin (S/E) (ratio of 90:10, respectively) bioprinted scaffolds were cut into 4 mm diameter cylindrical discs and implanted on CAM at day 10 of embryonic development. Filter paper (FP) and gelatin sponge (GSp) discs were used as positive and negative controls, respectively. At day 14, the excised sections were prepared for histology after acquisition of in ovo and ex ovo stereomicrophotographs. The angiogenic response was characterized by quantification of blood vessels convergence, H&E staining and immunohistochemistry for SNA-lectin.

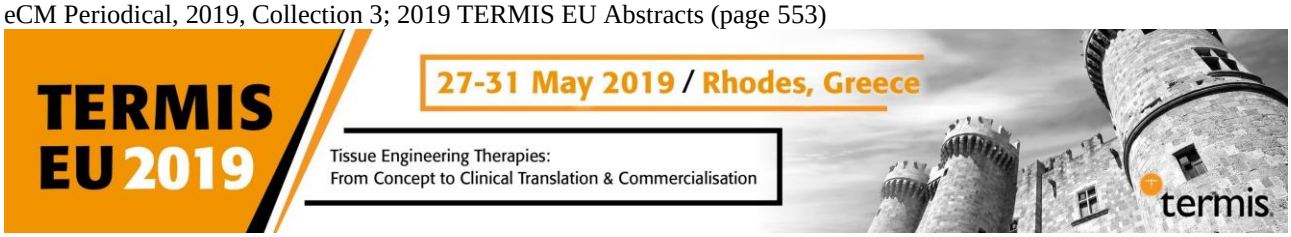
RESULTS & DISCUSSION: The analysis of blood vessels convergence suggests that both SF and S/E scaffolds stimulated an angiogenic response as supported by the significantly higher number of blood vessels converging to the implanted scaffolds, when compared to GSp. A small increase on the number of blood vessels converging towards the S/E scaffolds was noticed, which may result from the incorporation of elastin on SF bioink. The histological characterization has shown endothelial cells infiltration and a massive CAM tissue infiltration/ingrowth through the porous structure of both SF and S/E scaffolds.

CONCLUSIONS: The data presented herein suggests that the presence of elastin has a positive effect on the angiogenic performance of the bioprinted scaffolds. Further studies are needed to confirm this effect.

ACKNOWLEDGEMENTS: Financial support was received from Foundation of Science and Technology (PTDC/BBB-ECT/2690/2014, IF/00115/2015, IF/01285/2015).

REFERENCES

[1] Costa JB et al. Adv Healthc Mater. 2017; 6:1701021
[2] Costa JB et al. Mater Today Commun. 2018; doi:10.1016/j.mtcomm.2018.01.011
[3] Silva-Correia J et al. Tissue Eng Part A. 2012; 18(11-12):1203-12



Alginate-based bioinks for bioprinting of pancreatic islets and blood vessels with a coaxial needle setup

J. Idaszek[1], M. Nguyen Quoc[1], M. Volpi[1,2], M. Klak[3], M. Constantini[1,4], N. Contessi[2], S. Farè[2], M. Wszoła[3], W. Święszkowski[1]
Presenting Author: Joanna Idaszek, joanna.idaszek@pw.edu.pl
[1]Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland [2]Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy [3]Foundation of Research and Science Development, Warsaw, Poland [4]Department of Soft Condensed Matter, Institute of Physical Chemistry of Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Type 1 diabetes arises due to destruction of insulin-producing beta cells in pancreas. Transplantation of pancreatic islets is performed as one of the treatment strategies. High post-transplantation loss of the islets motivated development of various encapsulation procedures. However, the ability of the islets to secrete insulin is impaired by lack of sufficient supply of oxygen and nutrients by dense vasculature network [1]. Biofabrication of islets-laden constructs together with a dense vasculature network might enhance islets functionality. The aim of this work was to develop bioinks for simultaneous extrusion-based bioprinting of pancreatic islets and blood vessels.

METHODS: Bioprinting of alginate blended with fibrinogen (AlgFib; Alg 4 wt/v %, Fib 5 w/v%) and alginate blended with pancreatic extracellular matrix (AlgECM; 3 w/v% alginate, 15 wt/v % ECM) bioinks was performed using Bioplotter (EnvisionTEC) equipped with a coaxial needle and microfluidic pumps after optimization of the printing parameters using $CaCl_2$ as a primary crosslinking agent. The as made 3D hydrogel scaffolds were crosslinked with thrombin or/and $SrCl_2$ and characterized with respect to shape fidelity, swelling and stability in physiological conditions. Human umbilical vein cells (HUVEC) with or without addition of human mesenchymal stem cells (HMSC) were encapsulated in AlgFib bioink and porcine pancreatic islets in the AlgECM bioink. Viability was investigated after printing and during in the vitro culture by means of live/dead assay. Islet functionality was assessed using GSIS assay.

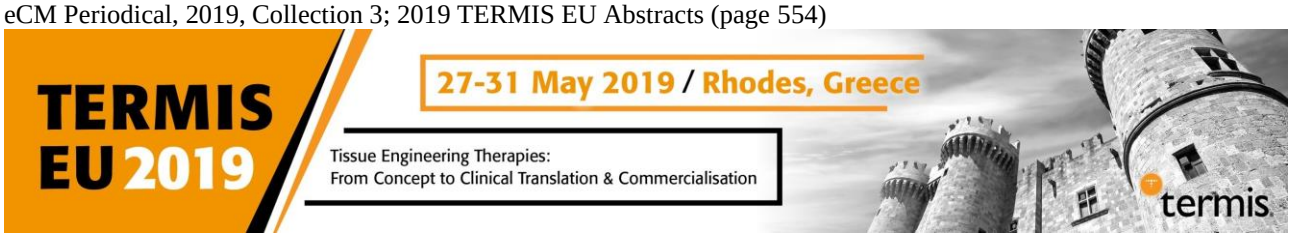
RESULTS & DISCUSSION: The bioprinting did not affect islets viability but reduced HUVEC and HMSC viability to approximately 80%. During culture, the HUVEC spread on the surface of the fibers and created dense layer of cells. Moreover, we observed proliferation of the HUVEC/MSC in the AlgFib scaffolds crosslinked only with thrombin (less stable than the AlgFib scaffolds crosslinked with thrombin and $SrCl_2$. The islets sustained their functionality; moreover, it was higher than in the case of free floating islets.

CONCLUSIONS: Application of alginate-based bioinks and coaxial needle system allows for fabrication of 3D hydrogel scaffolds laden with functional islets and vessel-like structures.

ACKNOWLEDGEMENTS: This work was supported the STRATEGMED programme (contract no. STRATEGMED3/305813/2/NCBR/ 2017)

REFERENCES

[1] Brissova M et al. Diabetes 2008; 57(9):2269-71



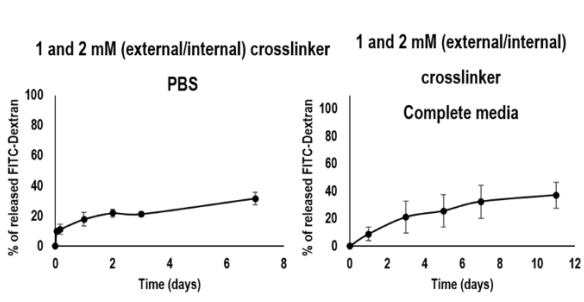
Replicating and modulating skin fibrosis in vitro: Multi-compartment collagen devices as dual drug delivery vehicles

J. Q. Coentro[1,2], D.I. Zeugolis[1,2]
Presenting Author: João Coentro, j.quintascoentro1@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Complex pathophysiologies, including skin fibrosis, involve different signalling mechanisms, with a multitude of interconnected potential therapeutic targets [1]. Therefore, there is a need for the development of multi-compartment delivery vehicles for combinatorial and synergistic therapeutic approaches [2]. In this study it was hypothesized that multi-compartment crosslinked collagen type I systems can deliver multiple bioactive agents in a controlled manner in an in vitro model of skin fibrosis.

METHODS: Multi-compartment collagen-based systems were made by mixing dialyzed type I collagen with 10x PBS, followed by neutralisation and crosslinking with 1 and 2.0 mM 4 arm-succinimidyl glutarate ester PEG, respectively, and incubated at 37ºC. The release of encapsulated drugs from the hydrogels was studied by fluorimetry and ELISA and the effect of the delivered bioactive agents was assessed through imaging and quantification for fibrotic markers in a macromolecular crowding induced in vitro model.

CONCLUSIONS: In summary, this indicates that this system is suitable for dual delivery of multiple bioactive agents, resulting in a controlled release in vitro and illustrating its potential in therapy.



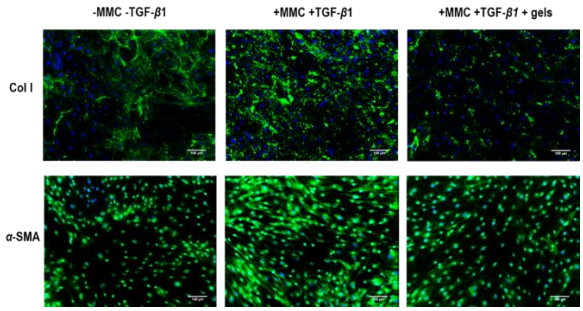
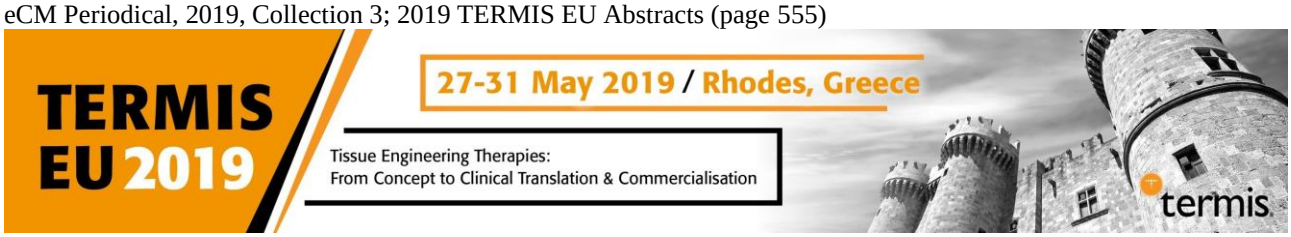


Figure 1: (left) A pilot study using FITC-dextran proved that the inner compartment was capable of promoting a sustained release over a long period of time (7 days), which was further confirmed with drug release assays using Trichostatin A, fitting the intended therapeutic release profile. **Figure 2:** (right) The studied in vitro model showed increased collagen I deposition and α-smooth muscle actin expression, mimicking a fibrotic condition, which was reverted with the use of the designed multi-compartment systems, indicating amelioration of fibrosis.

ACKNOWLEDGEMENTS: Science Foundation Ireland, co-funded under the European Regional Development Fund (Grant Number 13/RC/2073).

REFERENCES

[1] Coentro et al, Adv Drug Deliv Rev. 2018; in press.
[2] Pugliese et al, Adv Mater. 2018; in press.



Sustained PDGF-BB delivery improves strength of rabbit Achilles tendons after full transection

O. Evrova[1,2], G. Meier Bürgisser[1], C. Ebnöther[1], A. Adathala[1], M. Calcagni[1], E. Bachmann[3], J. G. Snedeker[3], C. Scalera[4], P. Giovanoli[1], V. Vogel[2], J. Buschmann[1]
Presenting Author: Johanna Buschmann, johanna.buschmann@usz.ch
[1]Plastic Surgery and Hand Surgery, University Hospital Zurich, Zurich, Switzerland [2]Laboratory of Applied Mechanobiology, ETH Zürich, Vladimir-Prelog-Weg 1-5/10, 8093 Zurich, Switzerland [3]Uniklinik Balgrist, Department of Orthopedics, Forchstrasse 340, 8008 Zurich, Switzerland [4]ab medica, Via Nerviano, 31, 20020 Lainate (Milan), Italy

INTRODUCTION: Tendon rupture repair suffers from the fact that very often mechanically inferior scar tissue is formed during the healing process. This may end up in re-rupture, causing pain and high costs because people cannot go back to work for months. Acceleration of the healing process could counteract the formation of scar tissue and strengthen the tendon at earlier time points post-operation.

METHODS: A full transection rabbit Achilles tendon model was used. A bi-layered electrospun DegraPol® polymer tube, with one layer containing PDGF-BB, was implanted around the conventionally sutured tendon. Emulsion electrospun tubes and coaxially electrospun tubes were fabricated and characterized by SEM, FTIR, and DCS. Their in vitro release kinetics of PDGF-BB were assessed and their in vivo performance was compared with respect to biomechanical, histological and immunohistochemical characteristics three weeks post-operation.

RESULTS & DISCUSSION: PDGF-BB was released in a sustained way (Fig. 1) and was bioactive after release in vitro. Significantly higher load until failure values were obtained for tendons treated with the growth factor PDGF-BB, for both, growth factor released from emulsion electrospun as well as from coaxially electrospun polymer tubes, respectively, when compared to tendons treated with the growth factor-free tube. Cellular distribution in PDGF-BB treated tendons was more homogenous compared to PDGF-BB-free samples. Moreover, the sustained release of PDGF-BB to wound site increased the proteoglycan content and decreased the collagen III intensity as assessed by immunohistochemistry, suggesting an advanced stage of tendon healing. The alpha-SMA expression, acting as a marker for fibrosis, was significantly lower in tendons receiving a sustained PDGF-BB delivery compared to samples without this growth factor.

CONCLUSIONS: Collectively, these data clearly illustrate the beneficial effect of a sustained PDGF-BB delivery to the healing Achilles tendon after full transection.

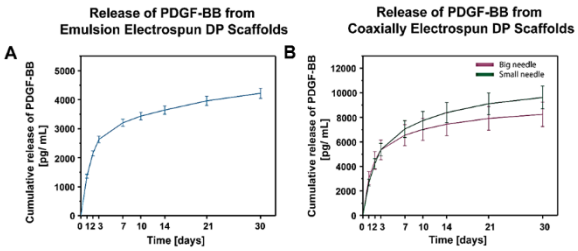
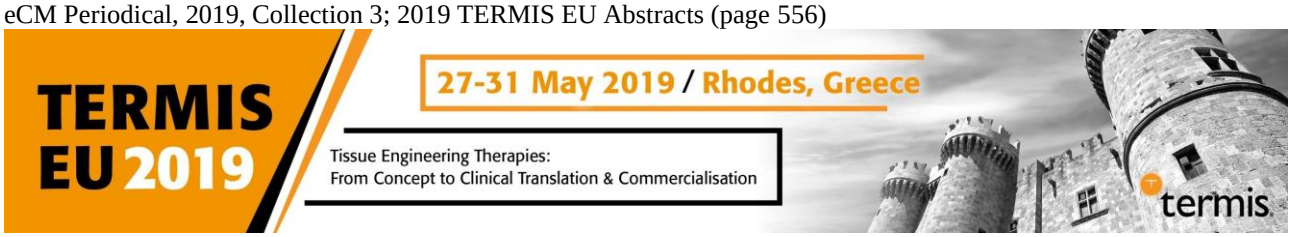


Figure 1: Release kinetics of PDGF-BB in vitro from emulsion electrospun tubes (A) and coaxially electrospun tubes (B).

ACKNOWLEDGEMENTS: We are thankful for the financial support by the Hartmann-Müller foundation. Moreover, we thank ab medica, Italy, for financial support of O.E.



Novel human placenta-based extract for vascularization strategies in tissue engineering

J. Hackethal[1,2], Anna Maria Weihs[2,3], Alexandra Hofer[4], Lisa Karner[1,2], Magdalena Metzger[1,2], Peter Dungel[1,2], Simone Hennerbichler[2,5], Heinz Redl[1,2], Andreas Herbert Teuschl[2,3]
Presenting Author: Johannes Hackethal, johannes.hackethal@trauma.lbg.ac.at
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in AUVA Trauma Research Center, Vienna, Austria [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria [3]Department of Life Science Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria [4]Research Area Biochemical Engineering, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria [5]Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria.

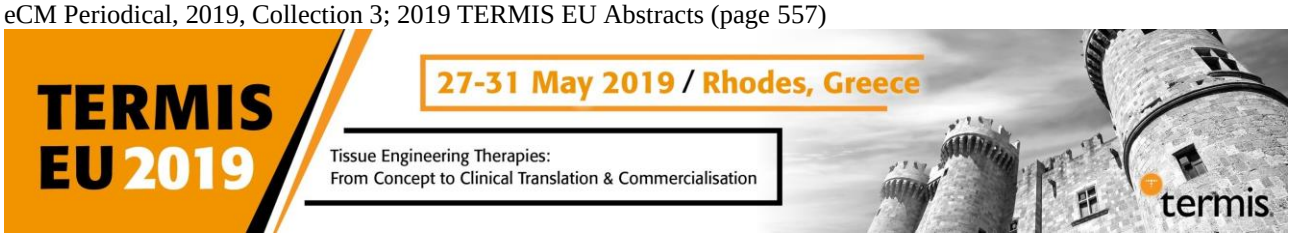
INTRODUCTION: There is critical unmet need for new vascularized tissues to support or replace injured tissues and organs. Various synthetic and natural materials were already established for use of 2D and 3D in vitro neovascularization assays, however, they still cannot mimic the complex functions of the sum of the extracellular matrix (ECM) in native, intact tissue. Here, we describe an effective method of isolating a human placenta substrate (hpS) that induces the spontaneous formation of an interconnected network of green fluorescence labeled human umbilical vein endothelial cells (gfpHUVEC) in vitro.

METHODS: The substrate was biochemically characterized by using a combination of BCA, DNA and GAG content assays, SDS-PAGE analysis and Western blot, angiogenesis arrays and chromatographic thrombin detection. 2D in vitro cell culture experiments have been performed using HUVEC and NIH3T3 fibroblasts to determine the vasculogenic potential of hpS. Then, 3D in vitro vasculogenesis assays have been performed by seeding gfpHUVEC in a hpS-fibrinogen clot.

RESULTS & DISCUSSION: The total protein content of the substrate was calculated as 1.74±0.26 mg/mL (mean±SD). The DNA remnants were significantly reduced whereas the DNA content was not significantly reduced after extraction when compared to native tissue. On SDS PAGE and angiogenesis array, multiple proteins with angiogenesis-inductive properties were assessed. In 2D in vitro experiments, HUVEC spontaneously differentiate into cell networks when grown on hpS-coated well plates with a significantly higher degree of complexity (number of tubules/junctions; total/mean tube length) when compared to Matrigel™. In 3D in vitro studies using a mix of hpS, fibrinogen, and HUVEC, randomly-oriented 3D cell networks were formed after approximately one week of culture.

CONCLUSIONS: We have established an effective method to isolate multiple proteins with angiogenesis-inductive properties from healthy human placenta tissue (hpS) with various potential applications for TERM. This material could be used as a novel platform for a human-material-based technology, for various 2D and 3D in vitro assays, as a medium supplementation, 3D bio printing, and probably also for clinical applications.

ACKNOWLEDGEMENTS: Financial support was received from the Austrian Research Promotion Agency FFG (# 849755) and the City of Vienna Competence Team Project Signal Tissue (MA23, #18-08).



Bisphosphonate nanoclay complexation for hyaluronic acid hydrogel self-assembly and growth factor localization

Y.H. Kim[1], X. Yang[2], S.A. Lanham[1], J. Hilborn[2], R.O.C. Oreffo[1], D. Ossipov[2], J.I. Dawson[1]
Presenting Author: Jonathan I. Dawson, jid@soton.ac.uk
[1]Bone & Joint Research Group, Centre for Human Development, Stem Cells & Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton, SO16 6YD United Kingdom [2]Department of Chemistry, Ångström Laboratory, Polymer Chemistry, Uppsala University, 751 21 Uppsala, Sweden

INTRODUCTION: Nanoclays have generated interest in biomaterial design for their ability to enhance the mechanics of biomedical materials such as hydrogels and impart biological function [1]. As well as functioning as physical cross-linkers via interactions with certain polymers, clay interactions with proteins have been applied for sustained localization to promote in vivo tissue regeneration. To date, both clay-biomolecule and polymer-clay nanocomposite strategies have typically sought to harness the negatively charged clay particle surface via exchange or intercalation. As such, biomolecule-clay and polymer-clay interactions are set in competition, potentially limiting the functional enhancements achieved. Here, we apply specific bisphosphonate interactions with clay particle edge sites to develop a class of self-assembling hydrogels and functionalized clay nanoparticles with preserved surface functionality for enhanced BMP mediated bone induction.

METHODS: Physical hydrogels were prepared by interaction of HA derivatized with bisphosphonate (BP) groups and Laponite nanoparticles. Two types of HA-BP derivatives were used in which BP groups were linked either through disulfide bonds (HA-SS-BP) or by thiol-ene addition to HA-SH derivative resulting to multiple BP ligand attachment (HA-BPn) [2]. Gels were formed via mixing with predispersed Laponite nanoparticles (LAP) and tested for mechanical properties via rheology. Release of Cy9 labelled model protein lysozyme was tested in vivo via an in vivo imaging system (iVis) and BMP-2 ectopic bone induction confirmed via micro CT and histology.

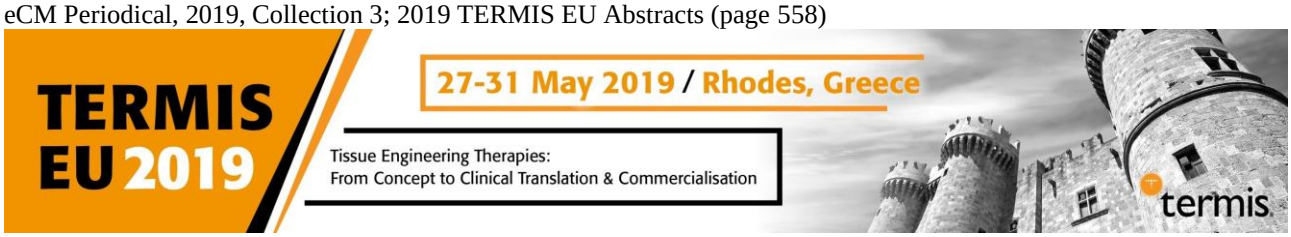
RESULTS & DISCUSSION: Laponite bisphosphonate interactions allow physical crosslinking of high water content hyaluronic acid hydrogels. Laponite addition (2% wt. vol.) to a BP functionalized HA yields a stiff gel, while no gel is formed with addition of Laponite to HA alone. Hydrogels incorporating Cy7-lysozyme were implanted into back sub

CONCLUSIONS: Clay-bisphosphonate complexes can generate self-assembling nanocomposite hydrogels that preserve clay surface sites for protein binding.

ACKNOWLEDGEMENTS: Work funded by EPSRC (EP/L010259/1) and EC 7th Framework Grant, BioDesign (262948).

REFERENCES

[1] Mousa M et al. Biomaterials 2018; 159: 204-214
[2] Yang X et al. Chem. Mat. 2012; 24(9):1690-7.



CRISPR/Cas9 based COL7A1 genomic editing in RDEB via non-viral polymer delivery system

J. O'Keeffe Ahern[a], I. Lara-Sáez[a], R. Murrillas[b], F. Larcher[b], J. Bonafont[b], B. Duarte[b], D. Zhou[a] and W. Wang[a]

Presenting author**:** Jonathan O'Keeffe Ahern, jonathan.o-keeffe-ahern@ucdconnect.ie

[a]Charles Institute of Dermatology, University College Dublin, Dublin 4, Ireland [b]Epithelial Biomedicine Division, Centro de Investigaciones Energéticas Medioambientales y Tecnológicas, Madrid, Spain

INTRODUCTION: Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a rare skin fragility and blistering disorder characterized by mutations in the COL7A1 gene which encodes Collagen VII, a major component of the anchoring fibrils which act to provide structural integrity to the skin. To date there is no curative treatment, with only palliative measures in existence. Current treatments for RDEB are focussed on ex-vivo usage of CRISPR/Cas9 genomic editing to permanently correct specific mutations in COL7A1. Here we present the establishment of a non-viral polymer-based gene delivery platform for delivering a CRISPR/Cas9 system encompassing a double guide RNA strategy to mediate an exon skipping approach over pathogenic mutations leading to functional recovery of COL7A1[1,2].

METHODS: The HPAE-EB polymer was complexed with CRISPR/Cas9 plasmid encoding a double guide RNA and assessed for biophysical properties (size, surface charge, encapsulation efficiency) which confer advantages to mediate efficient and cytocompatible gene delivery. Optimized polyplex conditions were identified and used to transfect HEK293 and RDEB keratinocytes. Transfection efficiency was assessed by 48 hrs post transfection with GFP and cell viability was measured using AlamarBlue assay. Genomic DNA was harvested 72 hrs post transfection for PCR and Sanger Sequencing confirmation of successful genomic editing.

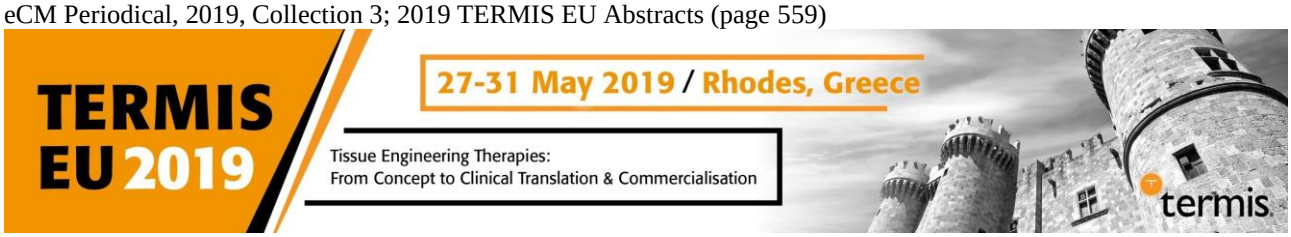
RESULTS & DISCUSSION: Picogreen encapsulation, Zetasizer and Zetapotential analysis show that the HPAE-EB polymer can bind with the CRISPR/Cas9 plasmid effectively forming small uniform nanoparticles with desired cationic surface charge. RDEB keratinocyte transfections demonstrate the cytocompatible nature of the HPAE-EB and PCR (Fig 2F) displays the desired genomic correction mediating exon 80 skipping over the pathogenic mutation location in COL7A1.

CONCLUSIONS: The data here demonstrates the feasibility of using the HPAE-EB polymer for delivering a CRISPR/Cas9 system to treat mutations in exon 80 for RDEB and highlights the potential for further development towards clinical application.

ACKNOWLEDGEMENTS: Science Foundation Ireland grant number (13/IA/1962).

REFERENCES

[1] Zhou D et al. ACS Applied Materials & Interfaces. 2016;8(50): 34218-34226.
[2] Hainzl S et al. Mol. Therapies. 2017;25(11) 2573-2584.



RGD-mutation of the heparin binding II fragment of fibronectin for guiding soft tissue integration of titanium implants

J. Guillem-Marti[1,2], A. Heras-Parets[1,2], M.P. Ginebra[1,2,3], J.M. Manero[1,2]
Presenting Author: Jordi Guillem-Marti, jordi.guillem.marti@upc.edu
[1]Biomaterials, Biomechanics and Tissue Engineering, Department of Materials Science and Metallurgical Engineering, Universitat Politècnica de Catalunya (UPC), Barcelona, Spain [2]Barcelona Research Center in Multiscale Science and Engineering, UPC, Barcelona, Spain [3]Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

INTRODUCTION: Titanium is the most widely used material for dental implants due to its good biomechanical properties and osseointegration capacity. However, the integration with soft tissues is crucial to ensure good sealing and avoid bacterial colonisation. The use of protein fragments from the extracellular matrix (ECM) obtained by DNA recombinant techniques can be used to improve cell-material interactions mimicking the natural environment [1]. In this regard, the heparin binding (HBII) fragment from fibronectin (FN) has the capacity to bind several growth factors including TGF-β [2], which is important for fibroblast activation. However, this fragment lacks the capacity to attract cells. The aim of this study was to mutate the DNA sequence of the HBII domain of FN, in order to introduce an RGD motif, thereby functionalizing the Ti surface with this adhesion and cell activation dual activity molecule in order to improve the soft tissue integration.

METHODS: Titanium discs (10 mm diameter) were polished using Al_2O_3 suspension (0.05 µm). Then, surfaces were activated by plasma treatment and silanized using APTES aminosilane. HBII fragment containing the type $III_{12\text{-}14}$ domains of FN was synthesized using DNA recombinant methods. The HBII DNA sequence was mutated using the Quick Change Mutagenesis Kit to generate an RGD sequence in the type III_{14} domain. Plasma FN and native HBII fragment were used as control. The growth factor binding capacity of functionalized surfaces was evaluated by ELISA. Human foreskin fibroblasts (HFFs) were seeded in serum-free conditions on the different studied samples and cell adhesion (LDH quantification and immunofluorescence staining), proliferation (LDH quantification), activation (α-SMA, qPCR, zymography) and migration were evaluated after 4h and 1, 3 and 7 days.

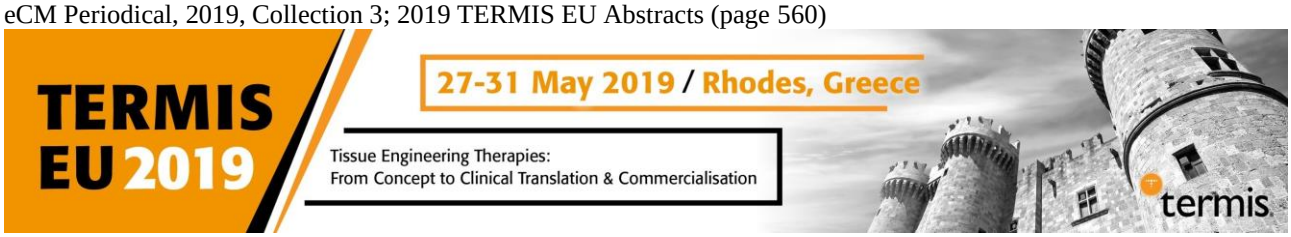
RESULTS & DISCUSSION: Cells were more spread on RGD-mutated HBII functionalized surfaces compared to native HBII. In addition, fibroblast activation observed by α-SMA staining was improved in RGD-mutated HBII (16.25% of cells) compared to native HBII (8.43%) and full-length FN (2.32%) due to a higher TGF-β binding capacity. Higher secretion of ECM molecules and ECM remodeling enzymes was also observed in RGD-mutated HBII functionalized surfaces, where cells migrated faster compared to native HBII and FN functionalized surfaces.

CONCLUSIONS: Collectively, the results demonstrate the potential of the novel molecule for soft tissue integration of Titanium implants.

ACKNOWLEDGEMENTS: Spanish Government for financial support MAT 2015-67183-R.

REFERENCES

[1] Herranz-Diez C et al. ACS Appl Mater Interfaces. 2016;8(4):2517-25
[2] Martino MM et al. FASEB J. 2010;24:4711-21



Dimensionality dictates substrate mechanics effects on adipogenesis by controlling Yap phosphorylation through cell spreading

J. Oliver-De La Cruz[1,2], J. Vrbsky[1], A.R. Perestrelo[1], V. Vinarský[1,2], F. Martino[1,2], S. Pagliari[1], P. Mozetic[1], E. Ergir[1], G. Forte[1,2,3]
Presenting Author: Jorge Oliver-De La Cruz, Oliver.jorge@fnusa.cz
[1]International Clinical Research Center (FNUSA-ICRC), Brno, Czechia [2]Competence Center for Mechanobiology in Regenerative Medicine, INTERREG ATCZ133, Brno, Czechia [3]Department of Biomaterials Science, Institute of Dentistry, University of Turku, Turku, Finland.

INTRODUCTION: Although mechanoregulated protein YAP is known to be involved in the adipogenic/osteogenic switch of mesenchymal stem cells (MSCs) [1,2], whether the mechanics of the matrix drives MSC commitment towards one given phenotype by regulating its shuttling, phosphorylation and turnover is not fully understood yet.

METHODS: Immortalized adipose tissue-derived mesenchymal stem cells (AD-MSCs) were exploited to produce cell lines expressing the mutant hyperactive form of YAP (YAP-5SA) and/or a reporter construct which results in the production of mCherry protein under the control of YAP-TEAD binding sites. These cells were challenged for adipogenesis in environments with defined mechanical properties through different bioengineering tools, including micropatterned surfaces and fibrin gels with different stiffnesses.

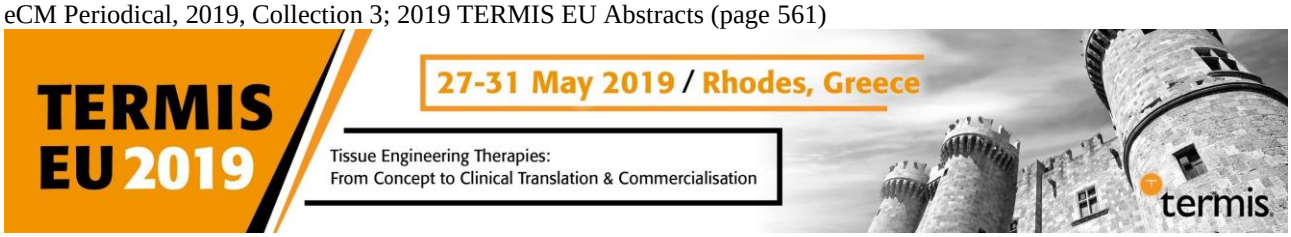
RESULTS & DISCUSSION: Mechanical regulation of MSC adipogenesis requires YAP repression through phosphorylation, regardless of the presence of a permissive biological environment. This mechanically-driven regulation of MSC fate is mediated by a combinatorial effect of F-actin remodeling and the tension produced by Myosin II motor protein on YAP localization. This phenomenon occurs independently of focal adhesion stabilization and TEAD transcriptional activity and is purely mediated by cell spreading downstream of substrate mechanics, as dictated by dimensionality

CONCLUSIONS: YAP transcriptional activity, as determined by cell ability to spread over or within a permissive environment, regulates MSC differentiation.

ACKNOWLEDGEMENTS: The work was supported from the European Regional Development Fund in frame of the project Kompetenzzentrum Mechanobiologie (ATCZ133) in the Interreg V-A Austria- Czech Republic programmme

REFERENCES

[1] Dupont et al. Nature. 2011. 474:179–83
[2] Nardone et al. Nat Comm. 2017. 15;8:1532



A method to create functional tissues in vitro after bioprinting

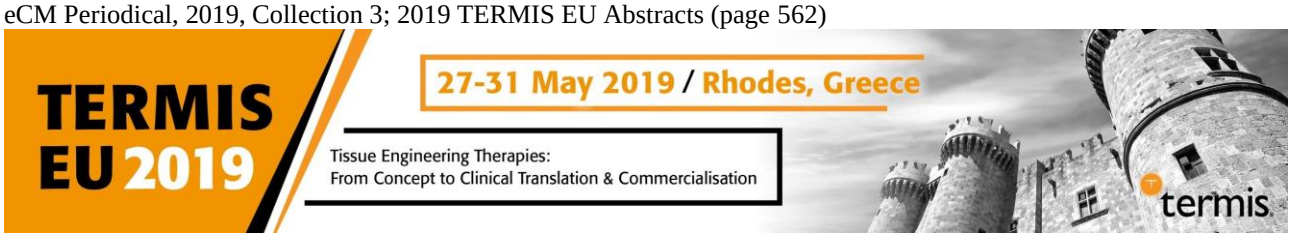
J.M. Baena[1,2,3]
Presenting Author: Jose Manuel Baena josbaema@gmail.com
[1]Tissue Engineering and 3D Printing Platform (PITI3D), IDIPAZ, Hospital Universitario de La Paz, Madrid, Spain [2]Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research, University of Granada, Granada E-18100, Spain [3]REGEMAT 3D S.L., Avenida de la innovación 1, 18100, Armilla, Granada, Spain

INTRODUCTION: A lot of efforts have been directed to the creation of functional tissues and organs in the lab. The lack of tissue regeneration in human beings and the deficiency of allogenic transplants in addition to the increasing of life expectancy make this problem to be considered as one of the most important ones of humanity in the current era. However, the results obtained are still far away from the desired. Many groups are working on improving the resolution of the systems to be able to directly print something with the same structure of a living tissue. We think that this approach is not going to be successful. For the creation of a living tissue it is crucial the bioprinting process but also the maturation of the construct. Replicating the human being adult conditions in vivo in the lab or the stimuli that occur in embryogenesis could improve the results of tissue engineering towards the clinical application of the technology.

METHODS: Here we propose a unique approach to create functional tissues starting from bioprinted constructs (fabricated using bioprinting) and a device that mimic the physiology and apply the right mechanical conditions of the structure to be replaced and through the maturation procedure, applying the right stimuli, creates a functional tissues. The stress distribution generator is based on the principle of Finite Element Method (FEM) in which loads are transferred in 3D using equations to represent the conditions that happens in reality. We think that the best stress distribution is the real one, and other approaches fail as do not mimic the real conditions happening in nature.

RESULTS & DISCUSSION: In the present work we show a method that helps to create functional tissues after bioprinting. For the creation of a living tissue it is crucial the bioprinting process and the ingredients selected to achieve the objective to create a functional specific tissue. But also the maturation procedure applied to the 3D cell laden constructs, that is even more important. If we think about bioprinting as a technology to recreate all the structure in the same form as shown in a living tissue, we are going to fail. We have to think on bioprinting as a way of creating cell laden 3D constructs as a precursor of a functional tissue. The maturation and tissue formation process will be as important or even more than the bioprinting one. Considering the strategies of both blocks in the diagram will be crucial to obtain the desired functional tissue. Here we present the results of the extracellular matrix created by different types of cells and scaffold structures and applying different mechanical stimuli and physiological conditions.

CONCLUSIONS: The stress distribution is crucial as stimuli to create the right tissue. Also, the scaffold architecture as it will affect the stimuli distribution and other important parameters as the biodegradation time. The selection of the right ingredients and the bioprinting procedure is very important in the success of the creation of functional living tissues, as well as the maturation procedure applied to the 3D cell laden constructs is even more important. This approach opens a wide research area for tissue engineers to develop protocols with different stimuli to create functional tissues after bioprinting.



Tunable collagen-PEG microgels synthesized by 3D printing and microfluidics

J.M. Rey[1,2], A. McDonald[1], M. Salmerón-Sánchez[2], C. González-García[2]
Presenting Author: Jose Manuel Rey, jose.manuelrey@collagensolutions.com
[1]Collagen Solutions, PLC., Glasgow, United Kingdom [2]Centre for the Cellular Microenvironment (CeMi), University of Glasgow, Glasgow, United Kingdom

INTRODUCTION: During recent years, microgels have emerged as an effective type of drug delivery system (DDS), showing advantages such as tuneable size, increased surface area and injectability[1]. The current study focuses on the adaptation of two scalable technologies, microfluidics and bioprinting, for the generation of monodisperse type-I collagen microgels crosslinked with PEG-4S, for regenerative therapies in Parkinson's Disease and bone repair by encapsulation of different growth factors.

METHODS: Type-I collagen (Collagen Solutions, UK) was neutralized and crosslinked with 4S-Star-PEG succinimide glutarate (Jenkem, USA) to form microgels in a PDMS microfluidic device with flow focusing configuration; and in a 3D Discovery® commercial 3D-printer by successive deposition of collagen and crosslinker solution on a hydrophobic Teflon® surface. Microgel size and mechanical properties were tuned varying the inlet channel/syringe diametre, flow speeds/pressure, pH and concentration of collagen and crosslinker. Microgels were characterized with rheological measurements, TNBSA assay and their cytotoxicity with LIVE-DEAD ® assay using Neu-7 astrocytes (n=3).

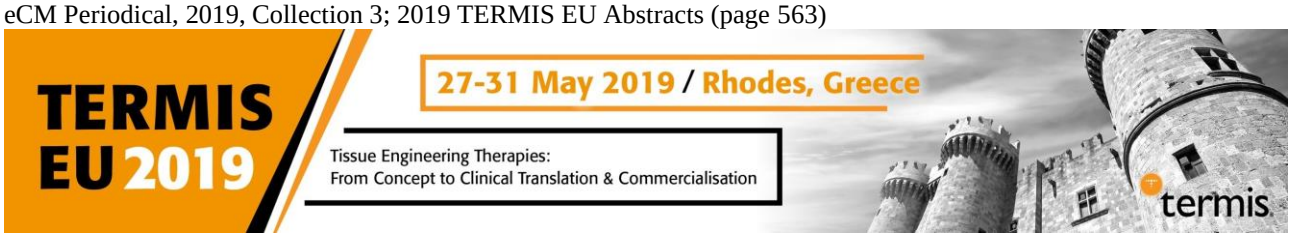
RESULTS & DISCUSSION: Microgels with different size and stiffness were successfully synthetized in a PDMS microfluidic device and by 3D-printing. The microgels are non-cytotoxic to cells and foster cell growth at different crosslinker concentrations. The typical output of each tecnique is 30 particles/min by 3D printing versus 600 particles/min using microfluidics, though these values can be increased for instance by parallelization.

CONCLUSIONS: We demonstrate that microfluidics and 3D-printing are adequate techniques for automatically generating monodisperse collagen microgels and provide a reliable tool for the posterior encapsulation of nanospheres and cells. The combination of microgels encapsulating nanometer-sized liposomes is expected to provide a controlled and sustained delivery system of different therapeutic factors.

ACKNOWLEDGEMENTS: The authors would like to thank European Union Horizon 2020 Programme (H2020-MSCA-ITN-2015) and Grant Agreement No. 676408 for providing financial support to this project.

REFERENCES

[1] Oh JK, et al. 2008;33(4):448–77.



Differentiation of patient matched adipose and bone marrow derived mesenchymal stem cells in an injectable hydrogel for intervertebral disc and bone repair

J Snuggs[1], A Thorpe[1], S Partridge[1], C Sammon[1], C Le Maitre[1]
Presenting Author: Snuggs, Joseph, J.Snuggs@shu.ac.uk
[1]Sheffield Hallam University, Sheffield, UK

INTRODUCTION: We have previously reported the development of an injectable synthetic Laponite® crosslinked pNIPAM-co-DMAc hydrogel that can deliver and induce nucleus pulposus (NP) differentiation[1] or, with the addition of hydroxyapatite nanoparticles (HAPna), osteogenic differentiation[2] of human mesenchymal stem cells (hMSCs) without the need for any additional growth factors in vitro. This synthetic hydrogel addresses several clinical requirements in one biomaterial, it is biocompatible in the liquid state and enables the safe and homogenous encapsulation of cells and HAPna, the fabrication technique does not require additional chemicals to be added for gelation or clean up and the hydrogel undergoes gelation at body temperature and therefore could use patients' own MSCs for injection directly into the target site to provide a simplified treatment strategy for intervertebral disc (IVD) degeneration or bone repair and augmentation. As there are multiple sources of hMSCs, this study investigated the incorporation of patient matched hMSCs derived from adipose tissue (AD) and bone marrow (BM) to determine their ability to differentiate within both hydrogel systems under different culture conditions.

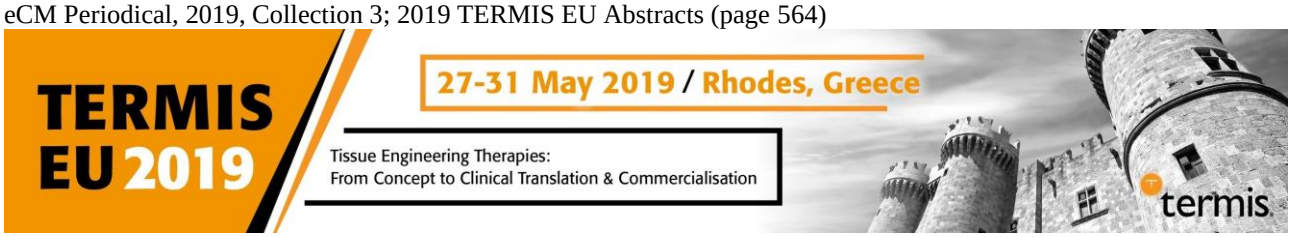
METHODS: Human FP and BM derived MSCs were isolated from femoral heads of patients undergoing hip replacement surgery for osteoarthritis with informed consent. MSCs were expanded in monolayer before being encapsulated into either NPgel or Bgel (containing 0.5mg/mL HAPna) and cultured for up to 6 weeks in 5% (NPgel) or 21% (Bgel) oxygen concentration. Samples were then fixed and processed before histological staining and immunohistochemistry were utilized to determine extracellular matrix production and phenotypic protein expression.

RESULTS & DISCUSSION: Both AD and BM derived MSCs were able to differentiate into both cell lineages. NPgel culture conditions increased expression of matrix components such as collagen II and aggrecan and NP phenotypic markers FOXF1 and PAX1, whereas when hMSCs from both sources were cultured under Bgel conditions, the expression of collagen I and osteopontin was increased, indicative of osteogenic differentiation.

CONCLUSIONS: In agreement with previous findings [1,2] our hydrogel system was able to induce NP and osteogenic differentiation of MSCs, dependant on the addition of compounds and culture conditions. Further to this we were able to show that both AD and BM derived MSCs can differentiate into both NP and osteogenic lineages, highlighting the versatility of this hydrogel when specific cells are cultured under precise conditions. Both NPgel and Bgel have the ability to differentiate patient derived MSCs from different sources into both NP and osteogenic lineages, which may give rise to novel treatment strategies for IVD degeneration and bone repair and may also tailor such strategies to patients' circumstances and needs.

REFERENCES

[1] Thorpe AA et al., (2016). Acta Biomater. 36:99-111.
[2] Thorpe AA et al., (2016). European Cells and Materials. 32, 1-23.



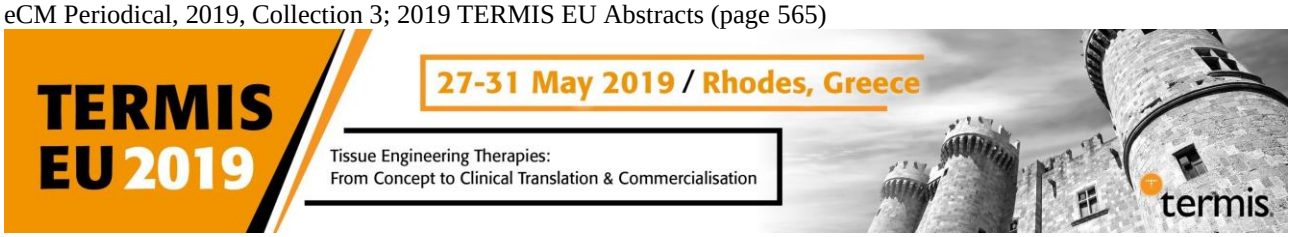
3D printed tissue engineered dermis using adipose tissue derived minimally manipulated autologous extracellular matrix (MA-ECM) for wound healing and skin regeneration

H. Jo[1], J. Jaeyun Kim[1]
[1]ROKIT HEALTHCARE, Inc, Seoul, Korea

Accidental skin injury requiring immediate or continuous intervention to restore skin and avoid future complications is ever-increasing worldwide[1]. In the U.S alone, 486,000 people received medical treatment for minor burns and 70,000 people were hospitalized for severe skin burn requiring acute care in 2015 according to the American Burn Association[1]. Another factor affecting engineered skin market growth is increasing number of patients with skin diseases. Each year over 5.4 million cases of non-melanoma skin cancer are reported and over 3.3 million people are surgically treated in the U.S. in 2014[2]. Numerous wound healing aid products in market help healing process but cannot provide fundamental solutions for severe degree wounds. Although there are several tissue engineered xenogeneic acellular dermal matrix or small intestine submucosa matrix product such as Cytal™ (pig urinary bladder) and Oasis® Wound Matrix respectively, average cost for the treatment is over $6,000 and over $11,000 if living cells are incorporated. Furthermore, average 12 weeks of long healing period to reach 50% healing rate demands significant standard care cost at home and hospital. In case of living cell product either cell seeded matrix or cell injection, extra 2 weeks for cell expansion at the beginning of treatment make these products not applicable to severely wound patients such as soldiers at combat fields who need urgent medical intervention. Therefore, the need for an engineered skin equivalent with improved wound healing efficacy still persists. Recently, adipose tissue gains more attention for potential autologous cell and extracellular matrix (ECM) source that can be used as an engineered skin equivalent material. Stromal vascular fraction (SVF) is a multi-cell complex containing ADSCs (adipose-derived stem cells), fibroblasts, endothelial cells, epithelial cells, macrophages. In addition, as a whole ECM, adipose derived ECM, not treated with any chemical process consists of more than 300 proteins and associated cytokines, provides an optimal 3D biomechanical environment and promotes cell-cell and cell-ECM interaction to stimulate ECM production and vascularization for wound healing/skin regeneration. We developed a technology/procedure that easily separates MA-ECM (minimally manipulated autologous-extracellular matrix), cluster of SVF and whole ECM, from lipoaspirate and turns those into a custom shaped tissue engineered dermis using 3D bio-printer ‘INVIVO’ (ROKIT HEALTHCARE, Inc, Korea) within 0.5hrs after liposuction. INVIVO 3D bio-printing enables cell printing with uniform density, suture free lamination depending on wound dermis depth, and area depending on wound shape. Large animal (pig) in vivo study confirmed that our skin equivalent consists of MA-ECM showed significantly higher healing rate during the first 2 weeks of post treatment period in full skin defect pig model. Furthermore, nevus removal cases showed a visible scar less healing within 2 week of MA-ECM operation. In conclusion, 3D cell-printed autologous MA-ECM tissue engineered dermis significantly reduced wound closure time and scar formation. We expect this technology opens up new opportunities to provide prompt care in a customized but cost effective way.

REFERENCES

[1] https://ameriburn.org/who-we-are/media/burn-incidence-fact-sheet/
[2] https://www.skincancer.org/skin-cancer-information/skin-cancer-facts
[3] Carter J. et al, Value in health 17 (2014) 801-813



Droplet microfluidics for generation of multicellular aggregates for cartilage tissue engineering

J. Aviles Milan[1,2], J. West[2], X. Niu[2], R.S. Tare[1,2], J.I. Dawson[1]
Presenting Author: Juan Aviles Milan, J.Aviles-Milan@soton.ac.uk
[1]Bone and Joint Research Group, Centre for Human Development, Stem Cells & Regeneration, University of Southampton, Southampton, SO16 6YD, UK [2]Engineering and the Environment and Institute for Life Sciences, University of Southampton, Southampton SO17 1BJ, UK

INTRODUCTION: Musculoskeletal disorders have a global outreach in the ageing population worldwide and many of them require articular cartilage repair [1]. Human bone marrow stromal stems cells (hBMSCs) are a promising cell source but they pose a serious limitation: subpopulation heterogeneity [2]. We hereby present a proof of concept for high-throughput aggregate formation in microdroplets simulating early chondrogenic condensation, envisioning sorting of hBMSC chondroprogenitor subpopulations.

METHODS: qPCR and immunofluorescence were conducted to study in vitro SOX9 expression in hBMSCs for various cell numbers at the gene and protein level, respectively, as an early chondrogenic functional marker. Human articular chondrocytes (HACs) were included as a human positive control. ATDC5 chondroprogenitor cells in chondrogenic differentiation medium were used for on-chip optimisation purposes, together with hBMSCs. Cells were and injected through the inlets of a PDMS chip with continuous gentle stirring. QX200™ generation oil (Bio-Rad) was used for droplet stabilisation. Droplets were imaged onto a haemocytometer after 6, 24, 48 and 72 h to visualise cell aggregation and viability.

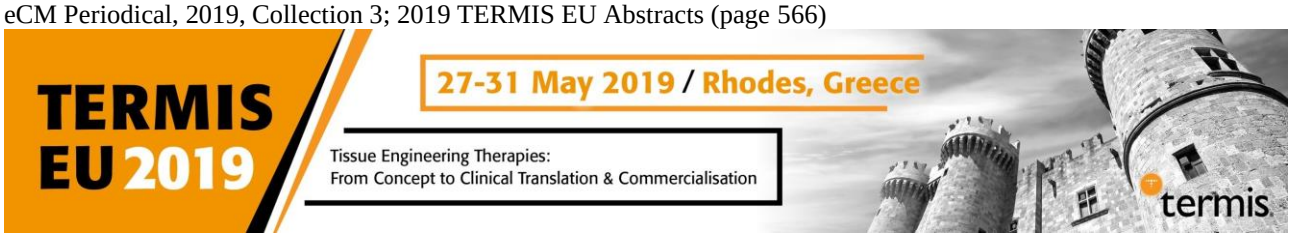
RESULTS & DISCUSSION: Immunofluorescence (IF) assays revealed heterogeneous SOX9 downregulation in hBMSCs from day 7 to day 21 for all cell numbers, whereas HACs maintained SOX9 protein expression. No significant changes were detected in SOX9 expression at early time points set on day 1, 3 and 7 following hBMSC aggregation ($p < 0.001$), as assessed by qPCR and IF on 500 and 5000 small cell aggregates ($n = 3$). The droplets generated on the microfluidic platform had volumes ranging from 0.5 to 4 nL (COV < 0.5%). Both ATDC5 and HBMSCs formed aggregates 24 h following encapsulation that remained viable over 72 h of incubation, suggesting the stability of the culture system for early SOX9 screening.

CONCLUSIONS: This work illustrates the high-throughput potential of droplet microfluidics to yield stable aggregates. The absence of fluctuations in SOX9 expression up to one week after aggregation and the droplet volumes attainable accentuate the suitability of this approach with a view to harnessing the hBMSC chondroprogenitor stem cell fraction.

ACKNOWLEDGEMENTS: Financial support was received from Versus Arthritis as part of a PhD Studentship for Juan Aviles Milan.

REFERENCES

[1] Briggs A et al. Gerontologist. 2016; 56(2):243-55
[2] Bianco P et al. Stem Cells. 2001; 19(3):180-92



Importance of decellularization method in corneal ECM hydrogels

J. Fernández-Pérez[1,2], M. Ahearne[1,2]
Presenting Author: J. Fernández-Pérez, fernanju@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin
[2]Department of Mechanical and Manufacturing Engineering, Trinity College Dublin

INTRODUCTION: Decellularized tissues can be used to fabricate hydrogels for corneal tissue engineering. However, the effect of the decellularization method on the gel characteristics has not previously been investigated. This study aims to examine the impact of 3 different decellularization protocols on the properties of corneal ECM-derived hydrogels.

METHODS: Three decellularization protocols were compared: SDS (S), Triton X-100 (T), and freeze-thawing cycles (F/T), all followed by incubation with DNAse. These were compared to a control group with no treatment (N) and a rat tail collagen type I gel (C). The parameters analyzed were decellularization efficiency, presence of ECM components, gelation kinetics, mechanical properties, ultrastructure and cytocompatibility using human corneal stromal cells.

RESULTS & DISCUSSION: All treatments removed significant amounts of DNA but only S removed sGAG significantly (Fig 1A). Collagen levels remained constant in all treatments. This was visually confirmed by histological staining. SDS-PAGE analysis showed the presence of collagen and other smaller proteins. Western blotting determined the presence of Keratocan, a typical component of the corneal ECM (Fig 1B). Gelation kinetics was determined by turbidimetric analysis. All treatments yielded slower gelling materials than commercially available collagen. F/T produced the fastest gelling material of all treatments, while S was the slowest (Fig 1C). Corneal stromal cells embedded in the hydrogels presented high cell viability as seen by the gel contraction activity, except for the S treatment, which rendered cytotoxic gels, presumably due to inefficient washing (Fig 1D).

CONCLUSIONS: This study highlights the importance of the decellularization method used to make ECM-derived hydrogels. From our results, F/T is the best method as the gelation time is the shortest, DNA removal is acceptable and loss of sGAG is minimal. Further research will focus on the use of these gels for the repair of small wounds on the ocular surface caused by trauma such as splintering.

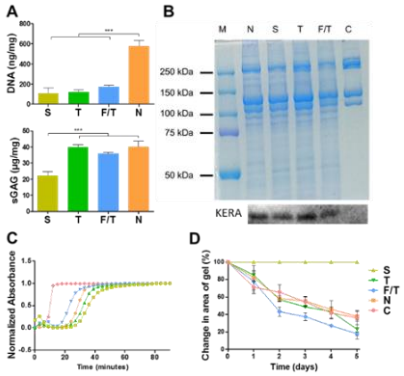
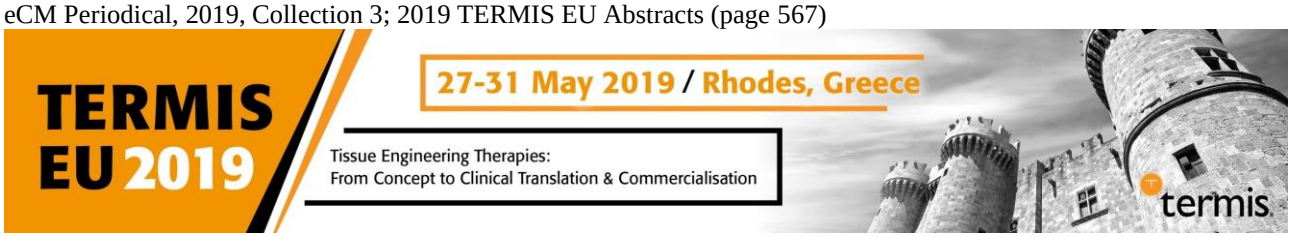


Figure 1: A) Biochemical analysis of DNA content (top) and sGAG content (bottom); B) Protein composition via SDS-PAGE (top) and Western Blot analysis of keratocan (bottom); C) Gelation kinetics; and D) Contraction assay

ACKNOWLEDGEMENTS: Research supported by European Research Council starting grant (EyeRegen 637460).



Gelatin hydrogel as surface coating for immunomodulation and tissue engineering

J. Barthès[1,2], H. Knopf-Marques[1,2], V. Ryabov[3], C. Muller[1,2], J. Kzhyskowska[3], P. Lavalle[2], N.E Vrana[1,2]
Presenting Author: Julien Barthès, j.barthes@protipmedical.com
[1]PROTiP Medical, 8 Place de l'Hôpital, 67000 Strasbourg, France [2]INSERM UMR 1121, 11 rue Humann, 67085 Strasbourg, France [3]University of Heidelberg, Department of Immunology, Mannheim, Germany

INTRODUCTION: Once a medical implant is implanted into the body, the host reactions can delay or even prevent the integration resulting in implant failure. The main reasons for implant failure are excessive inflammation, infection and lack of integration. In tissue engineering, there is a constant need for the development of surface coatings that can be applied on the implants with bioactive properties. Natural materials with their intrinsic properties close to the ECM microenvironment such as gelatin, hyaluronic acid are good candidates to develop biomimetic hydrogel. These hydrogels can be then functionalized with cytokines, growth factors and bioactive nanoparticles to give them improved bioactive properties. In the current study, we demonstrate the immunomodulatory properties of functionalized gelatin hydrogels in vitro and in vivo

METHODS: Gelatin, Gelatin/HA derivatives hydrogels enzymatically crosslinked have been developed to coat implant surfaces by spin-coating or dip-coating. Hydrogels were characterized using confocal microscopy, SEM. Inflammation and angiogenic markers were quantified with ELISA tests and PCR. An in vivo study was also performed on rats to study the effect of gelatin immunomodulatory coating applied at the surface of 3D printed tracheal patches and the inflammation was checked with histology, immunohistochemistry and cytokine readouts.

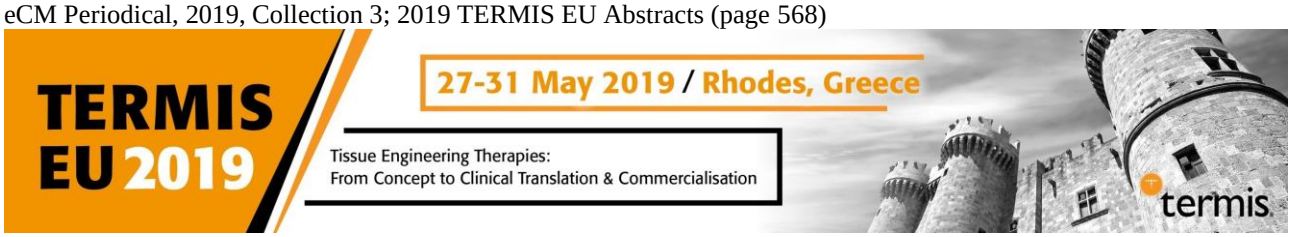
RESULTS & DISCUSSION: It was shown that Gelatin/HA-tyramine surface coating can act as a reservoir for anti-inflammatory (IL-4) and pro-angiogenic (VEGF) and then release these molecules to modulate the immune response and promote vascularization[1]. In an in vivo study on 24 rats, gelatin hydrogel loaded with anti-inflammatory cocktails (IL-10, PGE-2) was applied on the surface of tracheal patches. It was shown that this immunomodulatory hydrogel has significant effect on rat survival, upregulation of anti-inflammatory macrophages (M2) and reduction of inflammatory response.

CONCLUSIONS: Collectively, these data illustrate the possibility of conferring a multitude of beneficial biological properties to gelatin hydrogels to use them as an interface to promote implant integration.

ACKNOWLEDGEMENTS: This project received funding from the European Union's, FP7 (IMMODGEL, 602694) and H2020 (PANBioRA, 760921)

REFERENCES

[1] Knopf-Marques*, Barthes* et al. *ACS Omega*, 2017, 2 (3), pp 918–929.



Tissue specific maturation approaches enable biomimetic integration of multiple tissues on a chip to enhance preclinical models of toxicity and disease

K. Ronaldson-Bouchard[1], D. Teles[1], Alan Chramiec[1], K. Yeager[1], K. Hirschi[2], C. Chen[3], A. Christiano[4], and G. Vunjak-Novakovic[1]
Presenting Author: Kacey Ronaldson-Bouchard, kr2411@columbia.edu
[1]Laboratory for Stem Cells and Tissue Engineering, Department of Biomedical Engineering, Columbia University, USA [2]Yale Cardiovascular Research Center, Departments of Internal Medicine, Genetics, and Biomedical Engineering, New Haven, CT, USA [3]The Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA [4]Department of Dermatology, Columbia University, New York, NY 10032, USA

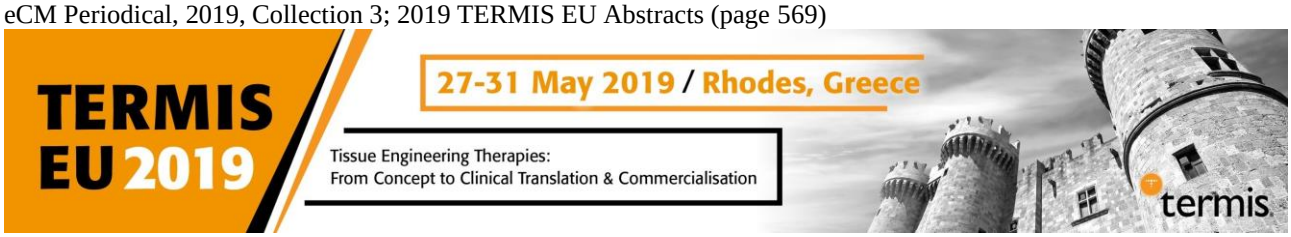
INTRODUCTION: To combat the rising costs and timelines of drug development, human engineered tissues can be used as miniature models of their respective organs to prescreen a drug's safety and efficacy in-vitro, providing human data before the drug is actually at the clinical stages of development. These physiologically relevant organ-on-a-chip platforms require the development of additional methodologies to functionally integrate the tissues, for studies of the human-on-a-chip, in a way that preserves their individual biological fidelity.

MATERIALS AND METHODS: We designed a series of custom bioreactors to first develop and mature each of the five tissues separately, using a single human induced pluripotent stem (iPS) cell line to develop all tissues, enabling patient-specific human-on-a-chip studies for personalized medicine approaches and the development of disease models. Upon tissue maturation, the engineered tissues were combined into an integrated platform that was designed to connect each tissue via culture above a vascularized transwell insert, while maintaining the homeostatic tissue specific niche in the tissue culture compartment above.

RESULTS AND DISCUSSION: We demonstrate the advantages of integrating mature engineered tissues in a way that preserves the homeostasis of each tissue while enabling communication between organ systems. The optimization of iPS differentiation protocols to achieve all cell types needed for engineering all of the included tissues will be detailed, with an emphasis on obtaining increased efficiency and cell numbers. To demonstrate the utility of the human-on-a-chip platform, the multi-tissue systemic response to drugs is shown and compared to the individual tissue specific responses. We further characterize the effects of flow versus static culture within the integrated system, where the inclusion of vascular flow within a specified shear stress range promotes endothelial barrier functionality and maintains the separation of tissue specific media. The vascular transwell inserts were optimized to facilitate endothelial barrier establishment while also enabling the transport of immune cells between compartments.

CONCLUSIONS: The development of methods to functionally integrate mature organ-on-a-chip systems in a way that promotes physiological relevance will enable future studies of the human-on-a-chip. By using a single patient iPS cell line to derive all tissue systems, patient-specific studies can be investigated. These methods can be further utilized as tools to mechanistically understand the variations in population specific responses to drugs and disease.

ACKNOWLEDGEMENTS: We gratefully acknowledge the NIH support of this work (1UG3EB025765-01).



Factor VIII-dependent coagulation events are required for the fibrotic response to implanted materials

K Sadtler[1,2], C MacIsaac[1,2], F Zepeda[1], RS Langer[1,2], DG Anderson[1,2]
Presenting Author: Kaitlyn Sadtler, ksadtler@mit.edu
[1]Koch Institute for Integrative Cancer Research, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge MA USA [2]Department of Anesthesia and Critical Care Medicine, Boston Children's Hospital, Harvard Medical School, Boston MA, USA

INTRODUCTION: The foreign body response (FBR) is characterized by the deposition of proteins around the surface of an implanted device followed by neutrophil and macrophage infiltration, formation of foreign body giant cells and subsequent deposition of a thick collagen capsule around the material. Current literature has focused on the later stages of the FBR, specifically macrophage and fibroblast activation and collagen deposition [1]. To understand how materials are detected as foreign, we evaluated the foreign body protein corona to investigate which proteins are adsorbing to a material and inducing the FBR.

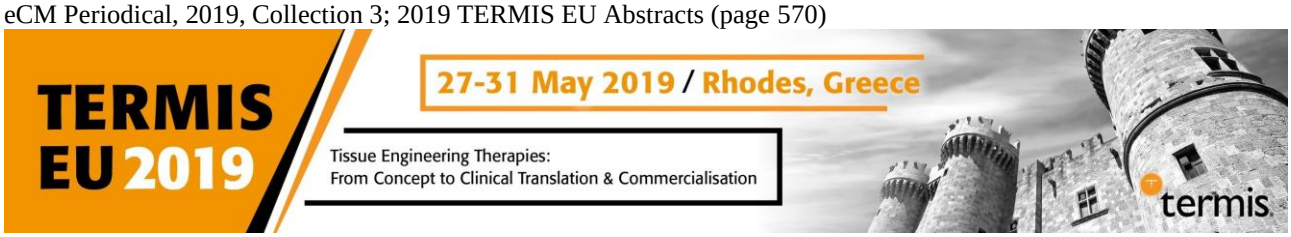
RESULTS & DISCUSSION: We detected four classes of proteins on the surface of polyethylene microparticles: complement, acute-phase proteins, clotting factors and apolipoproteins. As we previously described an increase in the FBR to materials in the context of traumatic muscle injury as opposed to a non-traumatic subcutaneous implantation [2], we analyzed the role of the clotting cascade in induction of the FBR. In wild type (WT) mice, after 4 weeks post-implantation, subcutaneous alginate microparticles are encased in a thick collagen matrix with dense cellular infiltrate as detected by hematoxylin and eosin, and picrosirius red staining (Fig. 1). In clotting-deficient mice (F8-/-) there is no development of a strong FBR, with a thin collagen capsule around the implant and minimal cellular infiltration. Compared to $F8^{-/-}$ mice, WT mice recruit 50 times as many cells (5.5 million vs 0.1 million, $p < 0.0001$). Furthermore, by flow cytometry, we detected a lower fraction of macrophages in $F8^{-/-}$ mice compared to WT mice. These data suggest that the clotting cascade is a major mediator of the foreign body response.

CONCLUSIONS: We propose that upon material implantation, the implant acts as a seed for protein deposition that increases local concentration of clotting factors thereby activating the cascade and resulting in the deposition of a fibrin clot around the material. This then signals to macrophages and fibroblasts to be recruited and deposit a dense collagen capsule around the material. We are currently evaluating the chemotactic agents responsible for inducing recruitment of macrophages by the deposited clot on the material surface.

ACKNOWLEDGEMENTS: This study was funded by a grant from the Juvenile Diabetes Research Foundation (JDRF). KS acknowledges support from the NIH NIBIB NRSA Fellowship # 1F32EB025688-01A1.

REFERENCES

[1] Anderson et al. Seminars in Immunol 2008. 20(2): 86-100.
[2] Sadtler et al. Biomaterials 2019. 192: 405-415.



Raman trapping microscopy for label-free and fast cell analysis in 2D-cell cultures, 3D tissue models and within liquid blood products

K. Schütze[1], B. Vaes[2], M. Steinke[2], R. Kronstein-Wiedemann[4], T. Tonn[4]
Presenting author: Karin Schütze, karin.schuetze@celltool.de
[1]CellTool GmbH, Tutzing, Germany [2]Regenesys, Bio-Incubator Leuven, Belgium [3]University Hospital Würzburg, Tissue Engineering and Regenerative Medicine and Fraunhofer Institute for Silicate Research ISC Würzburg, Germany [4]Experimental Transfusion Medicine – Medical Faculty Carl Gustav Carus, TU Dresden, Germany

INTRODUCTION: Increasingly there is a need to test functionality, integrity and sterility of cell-based products during manufacture and of the final product prior to transplantation. Raman trapping microscopy (RTM) is a non-invasive, label-free, highly sensitive analytical method for fast identification and monitoring of single cells in solution, within 2D or 3D-tissue. Here, we present RTM as a novel tool for gentle yet highly precise cell analysis in three independent experiments, providing an overview about the large versatility of this method. First, the influence of culture conditions on bone marrow stem cells was analyzed. Second we investigated the characteristics of primary human tracheo-bronchial epithelial cells (hTEC) used to build an engineered 3D human airway mucosa tissue model. And third we used RTM to monitor condition of blood products.

METHODS: First bone marrow stem cells were grown in different media for several days, fixed in 3% PFA and analyzed using RTM. Second Raman spectra were collected within cytoplama of human airway adenocarcinoma Calu-3 cells and isolated hTEC cells seeded on cell culture dishes with glass bottom. Third a few microliters of erythrocyte and thrombocyte concentrates, respcetively were diluted in PBS buffer and Raman spectra were taken at different time points. In all experiments, Principal Component Analysis (PCA) was used for analysis of spectral data.

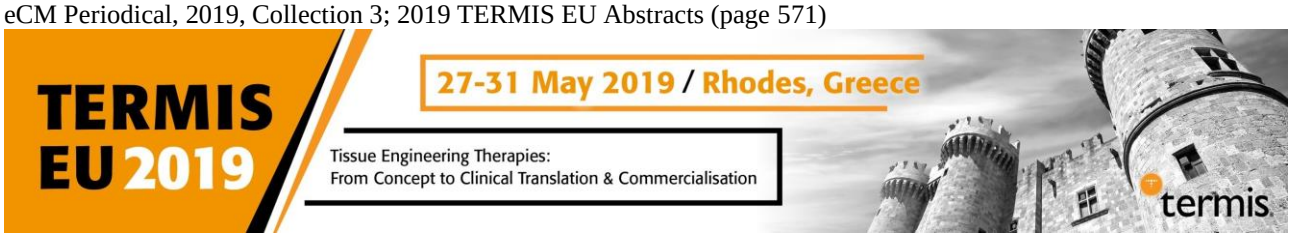
RESULTS & DISCUSSION: Using bone marrow stem cells grown in different culture media, RTM was able to detect variances between the differently cultured samples and group them into several subgroups although samples were blinded. Second: Comparing Raman spectra of living hTEC and adenocarcinoma Calu-3 cells relevant differences were identified in distinct wave number ranges. Furthermore, we could show that both erythrocytes and thrombocytes have their own Raman profile. In addition, change of Raman spectra with time was consistent with routine quality control studies of decrease in platelet activation capacity as well as with the correlation in metabolic consumption.

CONCLUSIONS: RTM is a fast and non-invasive method requiring less than 500 cells for analysis. Thus, RTM has the potential to become a standard for quality control of cell products and may lead to essential improvements in the field of transfusion medicine.

ACKNOWLEDGEMENTS: Financial support was received from EU7th Health Program grant agreement No.279288 as well as from the German Federal Ministry of Education and Research “KMU-innovative: Medizintechnik: HämatoRam” 13GW0112A.

REFERENCES

[1] Maria Steinke, Roy Gross, Heike Walles, Rainer Gangnus, Karin Schütze, Thorsten Walles: An engineered 3D human airway mucosa model based on an SIS scaffold; Biomaterials 35 (2014) 7355-7362



Human placenta ECM hydrogels for vascular graft surface coating to improve cell seeding efficiency in a bioreactor system

K.H. Schneider[1,2], S. Rohringer[1], G. Eder[1], R. Lieber[1], H. Kiss[3], U. Windberger[1], B.K. Podesser[1,2], H. Bergmeister[1,2]
Presenting Author: Karl Heinrich Schneider; karl.schneider@meduniwien.ac.at
[1]Ludwig Boltzmann Cluster for Cardiovascular Research at the Center for Biomedical Research, Medical University of Vienna, Vienna, Austria [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria [3]Department of Obstetrics and Gynecology, Division of Fetomaternal Medicine, Medical University of Vienna, Austria

INTRODUCTION: Efficient reendothelialization of acellular vascular grafts is crucial for graft patency and function. Therefore, a hydrogel for graft coating was developed to create additional anchoring-points for cells and support cell adhesion and migration in a pulsatile bioreactor system. Human placental tissue has major advantages to be used as tissue source in tissue engineering, due to its origin, distinctive vasculature, protein composition and its wide availability as clinical waste product [1,2]. In this study the vasculature from the placental chorion was used as raw material to create placental extracellular matrix hydrogels (pECM-HG) for vascular graft coating and tissue engineering applications.

MATERIAL AND METHODS: pECM-HG were produced by tissue decellularization followed by an enzymatic digestion using acidic pepsin solution. The pECM-HG were biochemically analyzed for DNA residuals and collagen and proteoglycan content was quantified. Rheological tests with different hydrogel concentrations were performed to examine the polymerization behavior of the material. Scanning electron microscopy (SEM) was used to visualize pECM-HG fibrous structures. Cytotoxicity, cell adherence and migration assays were performed. pECM-HG coated acellular vascular grafts were analyzed in a perfusion bioreactor system with regard to endothelial cell attachment during reseeding.

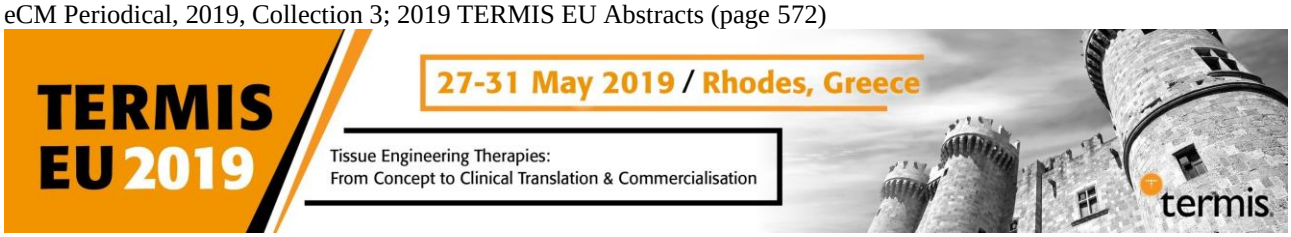
RESULTS & DISCUSSION: pECM-HG showed low amount of DNA residuals and preservation of cell interacting ECM proteins. Rheological measurements showed the formation of 3D hydrogels, dependent on respective pECM-HG concentration. Cell binding and viability assays revealed significantly enhanced cell adherence when seeded on surfaces coated with pECM-HG ($p<0.001$) compared to controls. Furthermore, cell attachment was increased on vascular grafts coated with Pecm HG compared to uncoated and even fibronectin coated controls.

CONCLUSIONS: We have developed a hydrogel from human vascular tissue with high cytocompatibility. Improved recellularization efficiency on vascular grafts by pECM-HG surface coating will support future experiments in the field of vascular tissue remodeling and regeneration using a pulsatile flow bioreactor system. In future applications, pECM-HG will be further tested for its potential to be used in 3D cell printing applications and for its regenerative capacities.

ACKNOWLEDGEMENTS: This research is part of the M3dRES project (858060) supported by the Austrian Research Promoting Agency (FFG).

REFERENCES

[1] Schneider KH et al., Acta Biomater. 2016 Jan;29:125-34.
[2] Schneider KH et al., Biomaterials. 2018 Sep;177:14-26.



Decellularised bovine pericardium scaffold for the application of a hMSC cell sheet

K. Joyce[1, 2], N. Dzhoyashvili[1], Y. Rochev[3,4], S. Rahmani[2]
Presenting Author: Karl Joyce, k.joyce2@nuigalway.ie
[1]Centre for Research in Medical Devices (CÚRAM), Biomedical Sciences, National University of Ireland Galway (NUIG), Newcastle Road, Galway, Ireland [2]Boston Scientific, Galway, Ireland [3]NUIG, Newcastle Road, Galway, Ireland [4]Institute for Regenerative Medicine, Sechenov University, 8-2 Trubetskaya st., Moscow 119991, Russia

INTRODUCTION: Bovine pericardium (BP) is an extensively used biomaterial particularly in the cardiovascular application of bioprosthetic heart valves [1, 2]. However, cytotoxic limitations of the glutaraldehyde treated BP mean no regenerative properties of the tissue in vivo, resulting in a limited lifespan. This study uses a tissue engineering approach to investigate the potential of a decellularized BP (Decell BP) extracellular matrix as a scaffold for the application of a human mesenchymal stem cells (hMSCs) cell sheet [3].

METHODS: Cell sheets of hMSCs formed using a thermoresponsive polymer poly(N-isopropylacrylamide) (PNIPAm) were compared to a cell suspension (1×10^{15} cells/cm^2) seeded onto Decell BP. Characterisation of the ECM structure, attachment and viability of the cell sheet was performed. Histological observations through hematoxylin and eosin (H&E) staining and scanning electron microscopy (SEM) imaging provided qualitative analysis, while the viability and metabolic activity of live/dead and Alamar Blue assays respectively were assessed.

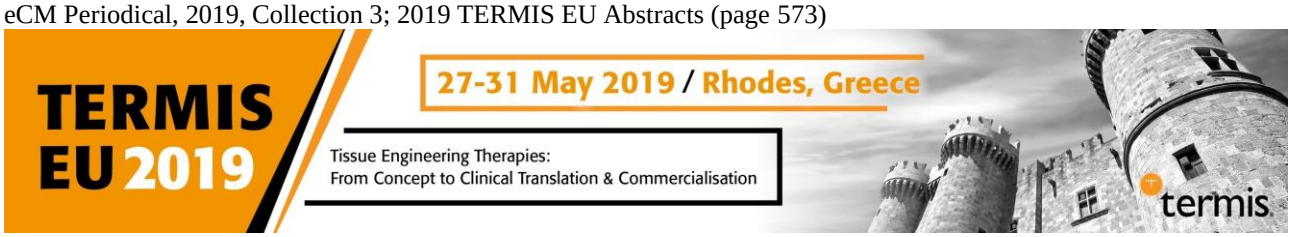
RESULTS & DISCUSSION: Qualitative analysis of H&E staining displayed no gaps between the cell sheet and the decellularized tissue after 7 d. Cytotoxic evaluation of the BP with Live/Dead assay demonstrated strong viability on the Decell BP. There was no significant difference ($p<0.05$) in the viability of a single cell suspension and cell sheets grown on Decell BP.

CONCLUSIONS: Overall this research displayed Decell BP as a viable scaffold material for the implantation of a cell sheet and demonstrated positive results warranting further research into its application for bioprosthetic heart valves.

ACKNOWLEDGEMENTS: Special thanks to all members of the Cúram and the Microscopy Lab of NUIG. This research was supported by Boston Scientific, Galway and the Russian Science Foundation grant 15-15-00132587 (histological analysis).

REFERENCES

[1] Hu, P.P. Clin Med Insights Cardiol, 2012. 6: p. 125-39.
[2] Siddiqui, R.F., J.R. Abraham, and J. Butany, B. Histopathology, 2009. 55(2): p. 135-44.
[3] Owaki, T., et al. Biotechnology Journal, 2014. 9(7): p. 904-914.



Modular design principle for personalized artificial vascular grafts with tunable mechanical properties and degradation speeds

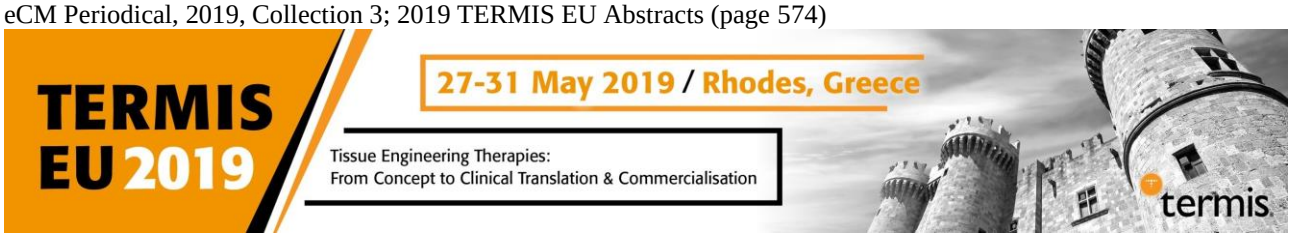
K. Ehrmann[1], C. Grasl[2], H. Bergmeister[3,4], H. Schima[2], B. Podesser[3,4], R. Liska[1,4], S. Baudis[1,4]
Presenting Author: Katharina Ehrmann, katharina.ehrmann@tuwien.ac.at
[1]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria [2]Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria [3]Center for Biomedical Research, Medical University of Vienna, Vienna, Austria [4]Austrian Cluster for Tissue Regeneration, Austria

INTRODUCTION: Cardiovascular bypass surgery currently applies autologous grafts which brings along certain downsides: Blood vessel harvesting can lead to increased donor site morbidity and the approach might suffer from limited graft availability. Using small-diameter artificial grafts would eliminate these short-term side-effects, however, such substitutes tend to clog more easily in the long term. We argue that this disadvantage can be overcome: Natural tissue regeneration can be promoted by rendering the graft to be biodegradable on a similar time-scale as tissue regeneration takes place. By additionally utilizing a modular material-design principle, we are able to tailor the mechanical and degradation behavior of a graft substitute to specific needs.

METHODS: The polymer-class of thermoplastic polyurethanes (TPUs) was chosen as graft material due to their suitable properties: Linear polymer sections (soft blocks) make the material elastic. These are connected via small, hydrogen-bonding components (chain extenders). The aggregated areas of chain extenders (hard blocks) make the material resilient to mechanical stress. This modular architecture of TPUs enables fine tuning of mechanical and degradation properties through the specific exchange of polymer components. Electrospinning allows for the manufacturing of highly porous grafts, aiding biodegradability. In this process, grafts are fabricated by injecting a polymer solution into a high-voltage electric field and collecting the emerging nano-fibers on a rotating rod.

RESULTS & DISCUSSION: We created a pool of low-toxic, degradable molecular compounds for their application as chain extenders in the polymer. A variety of polymers with altering components and component ratios was synthesized. The materials were fabricated into grafts via electrospinning and tested with respect to their mechanical, degradation, and in vivo and in vitro biocompatibility properties. Obtained results show significant changes in material properties even upon minor component changes. This approach gave new TPU materials with improved mechanical properties, less inflammatory response, and faster degradation speeds. The modular design principle enabled the difficult optimization of the balance between mechanical properties and degradability.

CONCLUSIONS: The new materials derived from the modular design principle give rise to the possibility of customized artificial grafts for bypass surgery.



Evidence of gut-liver crosstalk and gut homeostasis in paracetamol toxicity: An in vitro study using impedance based cellular assay

K. Morgan[1], K. Samuel[2], W. Gamal[1], S. Morley[1], P. Hayes[1], P. Bagnaninchi[3], J. Plevris[1]
Presenting Author: Katie Morgan, katie.morgan@ed.ac.uk
[1]The University of Edinburgh Hepatology Laboratory, 49 Little France Crescent, Edinburth EH16 4SB, Scotland [2]Scottish National Blood Transfusion Service, Advanced Therapeutics, The Jack Copland Centre, 52 Research Ave. North, Edinburgh, Scotland [3]MRC Centre for Regenerative Medicine 5 Little France Drive, Edinburgh EH16 4UU, Scotland

INTRODUCTION: Drug induced liver injury accounts for approximately one half of all acute liver failure cases, with Paracetamol (APAP) being one of the most reported hepatotoxins [1]. We used an in vitro approach to model APAP transport from gut to liver where metabolic products (NAPQI) can cause hepatocellular damage. Investigating the mechanisms of a gut-liver axis in vitro may contribute to a better understanding of APAP toxicity.

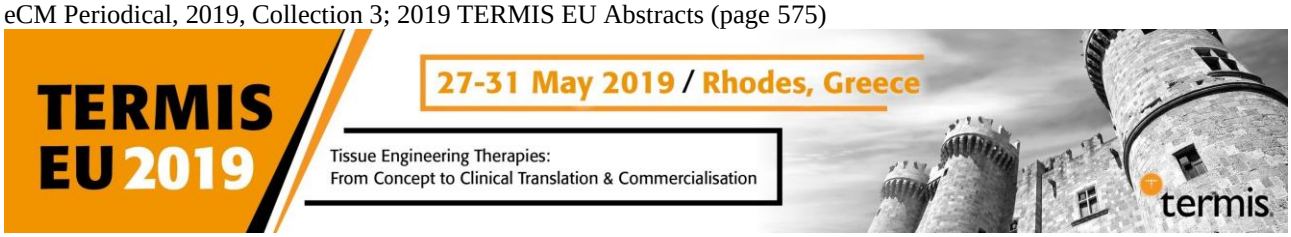
METHODS: The cellular impedance ECIS Z Θ platform was used to study the gut-liver axis and effects of APAP. Caco-2 and HepaRGs were seeded on separate 8W10+E ibidi arrays. The Caco-2 array was collagen coated. Step 1: Previously established concentrations of APAP [2] were applied to confluent Caco-2s (day 10), and impedance monitored for 24 hrs. Step 2: This 'preconditioned' culture medium was then transferred to HepaRGs (day 11) for 24 hrs. Step 3: preconditioned culture medium was then transferred from HepaRGs to fresh Caco-2s (day 12) for 24 hrs.

RESULTS & DISCUSSION: Step 1: using an impedance based cellular assay, we show a tightening of barrier function in Caco-2s treated with 20mM APAP. Step 2: only the highest concentration (20mM APAP) shows substantial loss of impedance on hepatocytes. Step 3: we demonstrate that barrier function has become sensitized on Caco-2s with an increase in impedance at 10mM

CONCLUSIONS: This study demonstrates a potential cross-talk between hepatocytes and enterocytes and reveals a homeostatic effect of gut barrier function in presence of APAP. Exposing hepatocytes to toxic levels of APAP taken from Caco-2's attenuates toxicity at 5 and 10mM concentrations compared to previous study [2] where a dose-dependent loss of tight junctions was observed (5-20mM APAP). Further exposure of preconditioned media on fresh Caco-2s (step 3) shows that tightening of barrier function is now achieved with lower APAP concentration (10mM) to reduce absorption of APAP. It is possible a liver-gut axis regulates APAP absorption through paracrine signals, though more work is needed. This may differ between population phenotypes and potentially be responsible for variability of toxic response in patients with APAP overdose.

REFERENCES

[1]Yoon et al. J Clin Transl Hepatol 4(2): 131–142
[2] Gamal A et al. SREP, 7, 37541.



Electroconductive materials enhance maturation of hiPSC-derived cardiomyocytes in engineered cardiac tissues

K. Roshanbinfar[1], L. Vogt[2], B. Greber[3], S. Diecke[4], A.R. Boccaccini[2], Z. Mohammadi[5], A.S. Mesgar[5], M.M. Dehghan[5], O.P. Oommen[6], T. Scheibel[7], J. Hilborn[8], F.B. Engel[1]
Presenting Author: Kaveh Roshanbinfar, kaveh.roshanbinfar@uk-erlangen.de
[1]Nephropathology, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany [2]Biomaterials Erlangen, FAU, Erlangen Germany [3]Rheincell Therapeutics, Langenfeld Germany [4]MDC Berlin, Germany [5]University of Tehran, Tehran Iran [6]Tampere University of Technology, Tampere Finland [7]University of Bayreuth, Bayreuth Germany [8]Uppsala University, Uppsala Sweden

INTRODUCTION: Stem cell-based cardiac tissue engineering is a promising strategy to treat cardiac diseases. Yet, the major challenge is to generate mature cardiac tissues. While different external stimulations have been used to induce maturation, here, we report that 3D hydrogels containing conductive polymers or carbon nanotubes intrinsically enhance maturation of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes without any external stimulation.

METHODS: hiPSCs were differentiated into cardiomyocytes as reported previously [3] and seeded at 3×10^4 cells/cm^2 on solubilized pericardial matrix-hydrazide-functionalized multiwall carbon nanotubes (PMCNT-gel) or resuspended at 2.5×10^7 cells/ml in a collagen-alginate-PEDOT:PSS hydrogel (eCA-gel) and evaluated for two weeks. Beating movies were acquired and analyzed by MUSCLEMOTION plugin for NIH-ImageJ. Tissues were immunostained for sarcomeric α-actinin, cardiac troponin I and connexin 43 as well as DNA (by DAPI).

RESULTS & DISCUSSION: Analysis of beating movies revealed a significant increase in contraction amplitude of hiPSC-derived cardiomyocytes in conductive engineered tissues compared to those without conductive materials. This was accompanied by formation of patches of aligned cells with an improved intercellular coupling evidenced by a significant increase in connexin 43 expression. Importantly, hiPSC-derived cardiomyocytes reached the sarcomeric length of 1.9 µm in eCA-gels (Figure 1) that is close to that of the adult human cardiomyocyte (2.2 µm).

CONCLUSIONS: Collectively, our data show the beneficial outcomes in using conductive materials in order to enhance maturation of engineered cardiac tissues.

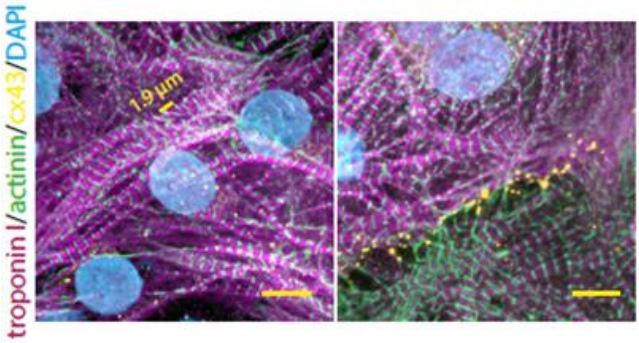
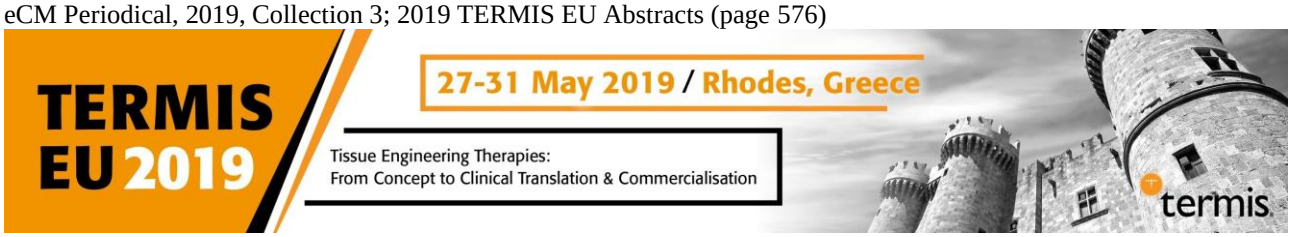


Figure 1: hiPSC-derived cardiomyocytes in the engineered cardiac tissues. Cardiac troponin I (violate), sarcomeric α-actinin (green), connexin 43 (red) and DNA (by DAPI blue). Scale bar: 5 µm.

ACKNOWLEDGEMENTS: This work was funded by the DFG and TRR 225 (Projektnummer 326998133).



Scaffold architecture regulates migration and chondrogenesis

K Sivasubramaniyan[1], CE Tanase[2], A. Lolli[1], M. van Royen[3], SM Best[2], RE. Cameron[2], GJVM van Osch[1,4]

Presenting Author: Kavitha Sivasubramaniyan, k.sivasubramaniyan@erasmusmc.nl

[1]Department of Orthopaedics, Erasmus MC, Rotterdam, The Netherlands [2]Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK [3]Optical Imaging Center, Erasmus MC, Rotterdam, The Netherlands [4]Department of Otorhinolaryngology, Erasmus MC, Rotterdam, The Netherlands

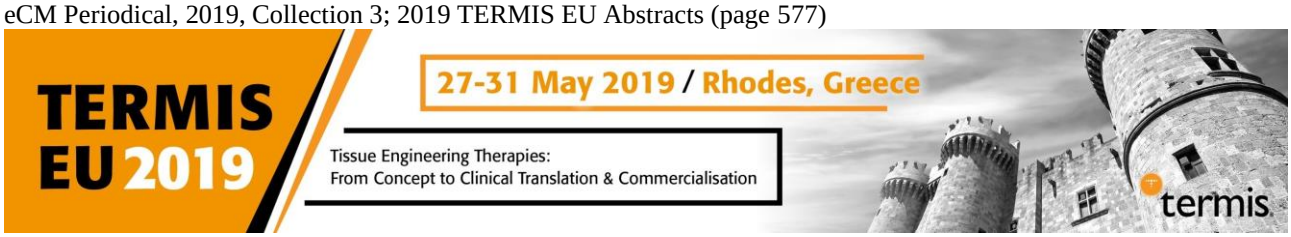
INTRODUCTION: It has been widely postulated that scaffold pore size and crosslinking significantly influence stem cell migration and chondrogenesis. Here we compared 3D ice templated collagen type I scaffolds of different crosslinkings (10%, 30% and 100% carbidimide crosslinking) and pore sizes (80 µm and 170 µm) and established an in vitro and in vivo assay to compare and select biomaterials with migration and chondro-permissive properties

METHODS: For evaluation of in vitro migration, human bone marrow mesenchymal stem/stromal cells (BM-MSCs) were fluorescently labelled with CFDA-SE and seeded on scaffolds (3mm x 3mm). Then the constructs were cultured in static culture for10-16 days, followed by fixation in 4% formaldehyde and optically clearing in gradient solution of benzyl alcohol/benzyl benzoate (BABB) and the samples were imaged with a confocal microscope. Chondrogenesis of BM- and synovial MSCs in the scaffolds was analyzed by histology (glycosaminoglycan deposition). Endogenous cell migration and chondrogenesis was assessed in a bovine osteochondral defect model to simulate a joint-like environment and by implanting the biopsy subcutaneously in immunocompromised nude mice for 4 weeks.

RESULTS & DISCUSSION: Collagen scaffolds with 170 µm pore size supported migration of MSCs significantly better than 80 µm pore size scaffolds. Comparing the crosslinking, 10% crosslinked scaffolds supported migration significantly better than 100% crosslinked scaffolds. In the next step, we compared chondrogenesis in 170 µm pore sized scaffolds with 10% and 100% crosslinking. Our results showed that only 10% crosslinked scaffolds were chondro-permissive while 100% crosslinked scaffolds were less suitable for chondrogenesis with both human BM- and synovial MSCs. The potential of these scaffolds to support endogenous stem cell migration and chondrogenesis was investigated by filling the cartilage defect in an osteochondral biopsy with either10% or 100% crosslinked collagen scaffolds and implanting the biopsy subcutaneously in nude mice. The in vivo results confirmed our in vitro findings and clearly demonstrated that 10% crosslinked 170 µm collagen scaffolds supported migration and chondrogenesis significantly better than 100% crosslinked scaffolds.

CONCLUSIONS: This in vitro 3D system is a promising method for screening of biomaterials to select materials that support migration and chondrogenesis. Using this method, we identified that lower crosslinked, larger pore sized collagen type I scaffolds support migration and chondrogenesis significantly better than smaller pore sized, higher crosslinked scaffolds.

ACKNOWLEDGEMENTS: This work was supported by the European Research Council [ERC Advanced Grant 320598 3D-E] and by a grant from the Medical Research Council, Arthritis Research UK, Reumafonds and the UKRMP.



Four axis additive manufacturing filament deposition system for vascular regeneration

K.A. van Kampen[1], C. Mota[1], L. Moroni[1]
Presenting Author: Kenny van Kampen, k.vankampen@maastrichtuniversity.nl
[1]Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

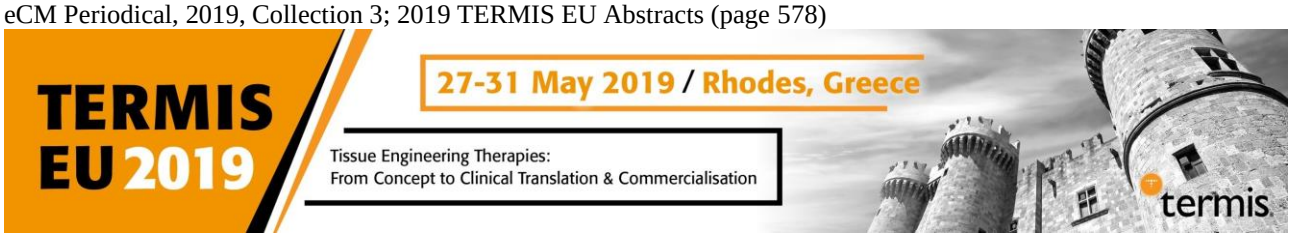
INTRODUCTION: A current issue in vascular graft engineering is the development of a scaffold that is able to substitute the blood vessel and allow its complete regeneration without forming blod clotting. To have functional vessels, a tight lumen has to be formed which facilitates blood flow. In addition, the mechanical properties of the scaffold should match those of the vessel. Techniques such as bioplotting and electrospinning offer a solution in creating improved vascular grafts. However, current bioplotting systems can only create a limited amount of vascular designs due to the need for a supporting structure during the fabrication process. In addition, the filament size in bioplotting might be beneficial for mechanical support, but impairs cell growth to form a lumen. In contrast to bioplotting, electrospinning is able to form scaffolds that support a vascular lumen, but they are difficult to handle and often collapse on their own weight. Here, we developed a 4th axis system which is able to control the diameter of both the supporting and barrier fibers, which can be used for vascular tissue engineering.

METHODS: A 2 mm diameter mandrel was attached to a DC-motor capable of reaching 20.000 rpm. Polycaprolactone (PCL) (Mw 45.000) was molten in a syringe and dispensed on the attached mandrel while the mandrel rotated at 3000 RPM. After the deposition of the thin fibres, the mandrel with fibres was transferred to a custom CNC-machine with a fourth rotary axis, where a script was generated to create the pathway for the supporting fibres. Mechanical assessment such as radial compression, longitudinal tension and three point bending was performed. The supporting fibres were deposited on top of thin fibres and disinfected with 70% ethanol before endothelial cells (HUVEC) were seeded in the lumen of the scaffold.

RESULTS & DISCUSSION: Results showed control over the thin fiber spacing and fiber diameter (15.4 µm ± 0.9 µm). Full control over the deposition of the structural supporting fibers (265 µm ± 19.9 µm) was also achieved. Mechanical testing revealed different mechanical behavior by changing the structural support pattern. Preliminary cell studies showed that seeded HUVECs in the lumen of the scaffold migrated in the course of days towards the outside of the thin fiber membrane.

CONCLUSIONS: The results show a new method to make scaffolds for vascular engineering. The scaffolds show different mechanical behaviors by changing the printing pattern.

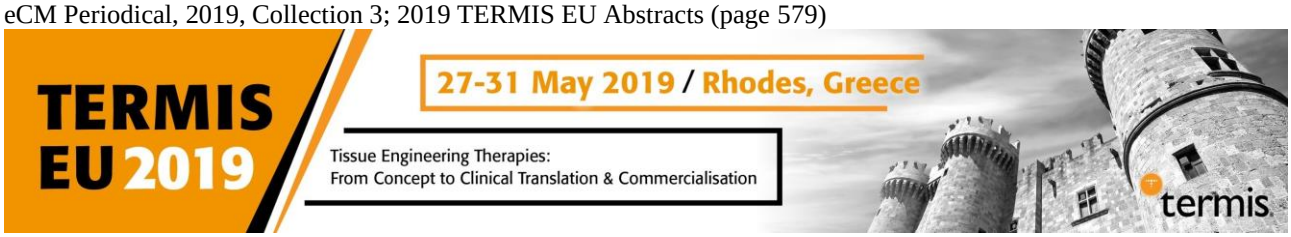
ACKNOWLEDGEMENTS: The authors would like to thank the province of Limburg, H2020 FAST (NMP-7, GA n. 685825), and the ERC Cell Hybridge (GA n. 637308' for providing financial support to this project.



Comparison and blended protocol to induce quiescence in vitro in primary human myoblasts

K. Gudagudi[1], N. Passerin d'Entreves[1], K.H. Myburgh[1]
Presenting Author: Kirankumar Gudagudi, gudagudik@gmail.com
[1]Dept of Physiological Sciences, Stellenbosch University, Stellenbosch, South Africa

Primary human skeletal muscle cells/myoblasts (PHMs) proliferate in vitro and customarily require cell to cell contact for signaling and cell to surface contact for anchoring. Depriving PHMs of these two factors using suspension culture (SuCu) for 48 hours tends to promote reversible cell cycle exit of the PHMs. A different protocol to SuCu used in a recent study from our laboratory has shown that replacing foetal bovine serum (FBS) with Knockout Serum (KOSR, Gibco™) in PHM culture for 10 days without supplementation of any growth factors also tends to drive PHMs into reversible cell cycle exit. The aim here was to compare and then combine these two distinct yet competent in vitro quiescence protocols to form an improved assorted protocol to aid some of the requirements of simulating skeletal muscle regeneration in culture. SuCu with FBS is relatively quick (48 hours) and tends to nudge PHMs into the G0/G1 phase of the cell cycle. However, with SuCu the PHMs while suspended cannot be visualised under a microscope to assess the morphology, unfavorable for interventions and soon after harvesting PHMs lose their quiescent phenotype. In KOSR culture method on the other hand, as PHMs are adhered to the culture flask, morphology can be monitored throughout and is straightforward for interventions. However, this protocol requires 10 days to attain effective quiescence. Since SuCu uses FBS in the preparation of its media, we hypothesised that replacing FBS with KOSR could improve quiescence. We also hypothesised that harvesting PHMs after SuCu and then plating in KOSR media for a brief period could provide stable quiescent PHMs with all the benefits of the KOSR method without requiring 10 days. PHMs were isolated from skeletal muscle biopsies of young and healthy male subjects. Two different suspension culture medias were prepared with either FBS or KOSR. After 48 hours of SuCu, PHMs were harvested using 1x phosphate buffered saline and total RNA was isolated using the phenol-chloroform method. qPCR for Ki67, Myf-5, MyoD and p21was performed using Sybr Green dye (Applied Biosystems instrument). Gene expression levels in SuCu KOSR were divergent compared to SuCu FBS. Specifically, Ki67, Myf5 and MyoD were significantly down regulated with KOSR, while p21 was upregulated with both protocols. Since Ki67 and Myf5 transcription factors are involved in satellite cell proliferation, the significantly reduced levels indicate the SuCu KOSR protocol is more effective compared to SuCu FBS for inducing in PHMs a state of quiescence. Current experiments are testing the mixed-method protocol of SuCu followed by KOSR culture for a brief period to determine if the timeline can be reduced from 10 days, but still provide stable quiescent PHMs. Achieving effective in vitro quiescence in a relatively brief time frame will enhance the study of skeletal muscle regeneration, including initiation of activation from quiescence and the feedback mechanism utilised to induce a return to quiescence to protect the progenitor cell pool for future episodes of regeneration of the tissue.



Continuous culture of hESCs on thermoresponsive polymer surface

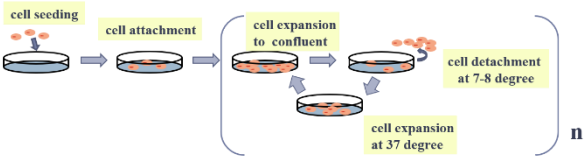
K.J Lin[1], J.S Yang[1], A. Higuchi[1,2]
Presenting Author: Kuan-Ju Lin, st6love5vu@gmail.com
[1]Department of Chemical and Materials Engineering, National Central University (NCU), Zhongli, Taiwan [2]Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama, Japan

INTRODUCTION: The conventional cell cultivation of human embryonic stem cells (hESCs) is batch-type culture, which is expansive and laborious. Hence, we developed a continuous culture system, which culturing hESCs on thermoresponsive polymer-coated dish surface having low critical solution temperature (LCST) (Fig. 1). hESCs were detached by decreasing the temperature of culture medium, which prevents hESCs suffering from enzymatic digestion.

METHODS: In this study, we designed to proliferate hESCs on thermoresponsive dish surfaces. The thermoresponsive dishes were prepared by coating thermoresponsive polymer, poly(N-isopropylacrylamide-co-butylacrylate) (polyNIPAM-BA). The optimal concentration of polyNIPAM-BA for hESCs detachment by decreasing temperature of the culture medium was investigated. The surface water contact angle measurement and X-ray photoelectron spectroscopy were used to analyze the hydrophilicity and chemical element composition of dishes surface, respectively.

RESULTS & DISCUSSION: hESCs were successfully cultured on the polyNIPAM-BA coated dishes surface, and partially detached from the surface by incubating at low temperature (7-8°C) for 30 minutes. The optimal concentration of polyNIPAM-BA for hESCs detachment by decreasing temperature of the culture medium was 8 mg/ml. Subsequently, hESCs were continuously cultured on the dishes coated with optimal concentration of polyNIPAM-BA for 5-7 cycles. Moreover, hESCs maintained their pluripotency and differentiation ability after 5-10 cycles of continuous cultivation on polyNIPAM-BA coated dishes.

CONCLUSIONS: We successfully cultured hESCs on polyNIPAM-BA coated dishes surface and thermally modulated the cell adhesion and detachment. It would be a great benefit for biomedical engineering after applying the continuous harvest system on 3D culture of hESCs applying thermoresponsive surface.



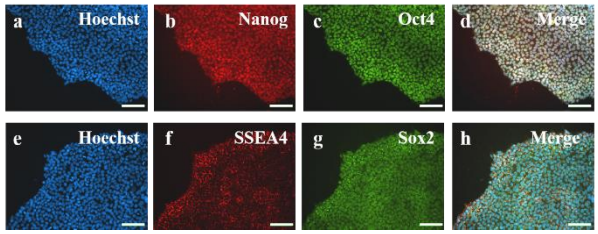
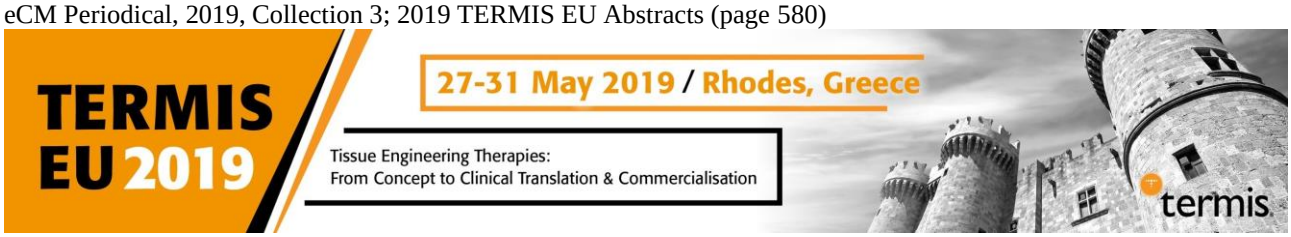


Figure 1: (left) Concept of continuous stem cell harvest. **Figure 2:** (right) Immunostaining characterization of hESC (WA09) pluripotency following continuous culture on dishes coated with 8 mg/ml polyNIPAM-BA. The continuous culture protocol consisted of 5 cycles. Expression of pluripotent proteins: Nanog (b), Oct4 (c), SSEA4 (f) and Sox2 (g). Hoechest staining (a and e). Merged images (d from a-c and h from e-g, respectively). The bar represents 100 μm.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Science and Technology (MOST 107-2119-M-008-002).



Engineered bioactive coatings for 2D/3D titanium implants to enhance osteogenesis and reduce biofilm formation

L. Damiati[1,2], V. Llopis-Hernández[1,2], R. O. C. Oreffo[4],
P. Li[5], P. M. Tsimbouri[1,2], B. Su[3], M. Salmeron-Sanchez[1,5], M. J. Dalby[1,2]
Presenting author: L. Damiati, L.damiati.1@research.gla.ac.uk
[1]Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, UK [2]Biomaterials Engineering Group, University of Bristol, UK [3]Bone and Joint Research Group, University of Southampton, UK [5]School of Engineering, University of Glasgow, UK

INTRODUCTION: Titanium is a common material for the orthopaedic and dental implants. However, an optimal implant should be bioactive towards bone formation and also be bactericidal.[1] Nevertheless, these characteristics are hard to balance without detriment to each other. Thus, here, we employ and antimicrobial high-aspect ratio nanotopogrphy on the Ti scaffolds. Also, we use a simple polymer coating system to help provide osteogenesis on the antimicrobial features. Polyethylacrylate (PEA) can be applied, via plasma polymerization, as a very thin coating, to 2D/3D structures and it causes spontaneous unravelling of fibronectin (FN) upon contact. In the open conformation, FN can be decorated with ultra-low doses of growth factors, such as BMP2. In addition, 3D implant architecture is becoming desirable so that bone modulus can be matched using Ti and cells can be grown inside the scaffold. The selective laser melting technique (SLM) has many advantages to be used to produce scaffolds with different sizes and shapes, with good dimensional accuracy, and good resolution. Here we are focusing on developing antimicrobial/osteogenic 3D surfaces to help with implant lifetime but also to tackle the modulus mismatch between titanium and bone as this leads to implant micromotion and failure.

METHODS: Alkaline hydrothermal treatment was applied to produce an antimicrobial high-aspect ratio nanotopgrphy on the Ti scaffolds. PEA was applied using plasma polymerization at 100 Watts for 90 secs. Scaffolds physical and chemical characteristics were studied using SEM, AFM, WCA, and XPS. MSCs bone mineralisation was examined using Raman spectroscopy, calcein blue, Alizarin red, and Giemsa staining. P. aeruginosa were cultured on the substrates and the number of viable microbial cells was determined by quantitation of the ATP.

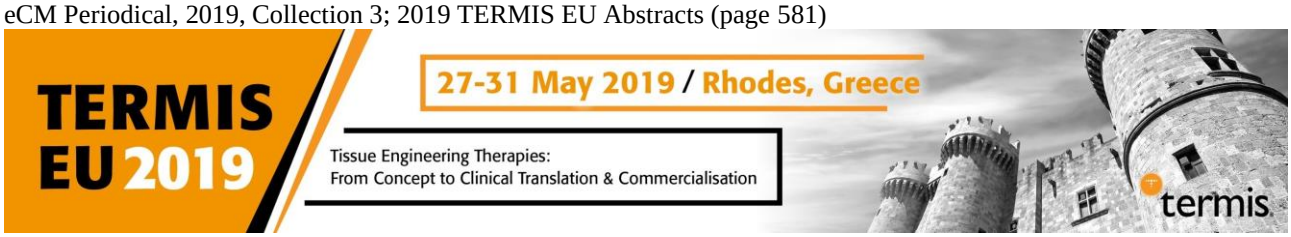
RESULTS & DISCUSSION: Ti surfaces with PEA/FN/BMP2 coating showed improved cell growth, adhesion and bone mineralisation compared with uncoated substrates. Moreover, Ti nanowire surfaces showed a decrease of P. aeruginosa adherence based on the ATP reading. The 3D Ti lattices with 900 µm diameter struts and elasticity ~2 GPa had better MSC adhesion and growth as the bone elasticity is around 7-30 GPa, while the 3D/2h TiO_2 showed a potential bactericidal effect on P. aeruginosa.

CONCLUSIONS: We demonstrate the potential to fabricate 3D Ti implants with topographies that reduce microbial viability and polymer coatings that enhance osteogenesis of MSCs in-vitro

ACKNOWLEDGEMENTS: This project is supported by a studentship to LD from University of Jeddah, Jeddah- Saudi Arabia and EPSRC grant EP/K034898/1. We are grateful for the technical support of Carol-Anne Smith.

REFERENCES

[1] Damiati L. et al. JTE. 2018; 9: 1–16



A drug combination administered via an implantable, polymeric delivery system improves locomotor recovery in a rat model of spinal cord injury

A. Bighinati[1], N. Gostynska[2], M.L. Focarete[2,3], C. Gualandi[3], M. Kurakula[2,3], M. Pannella[2], V.A. Baldassarro[2,4], A. Giuliani[1], M.L. Bolognesi[2,4], N. Passerini[2,4], R.C. Perone[2,4] S. Beggiato[5], L.N. Ferraro[5,6]; E. Zironi[1,2], T. Gazzotti[1,2], M.L. Tondo[1,2], G. Pagiuca[1,2], M. Cescatti[2,6], M. Fernandez[1], L. Lorenzini[1], L. Giardino[1,2,6], L. Calzà[2,4,6]

Presenting Author: Laura Calzà, laura.calza@unibo.it

[1]Department of Veterinary Medicine (DIMEVET), Italy [2]Interdipartimental Center for Industrial Research – Health Science and Technologies, Italy [3]Department of Chemistry "Ciamician", Italy [4]Department of Biotechnology and Pharmacy, University of Bologna, Bologna, Italy [5]Department of Life Sciences and Biotechnology, Section of Medicinal and Health Products, University of Ferrara, Ferrara, Italy; IRET Foundation, Ozzano Emilia, Italy

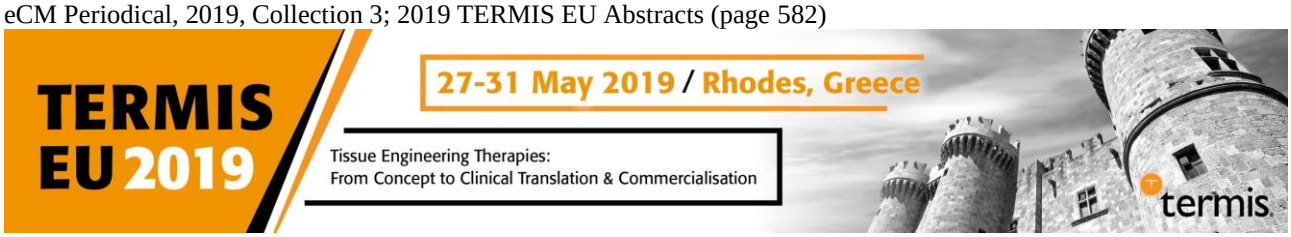
INTRODUCTION: SCI is a cureless condition. Combinatory therapies are regarded as a valuable approach to target the acute, post-lesion phase, in view of the complex cascade of pathophysiology events that leads to the final clinical outcome. We designed a treatment solution based on an implantable biomaterial (electrospun PLLA) loaded with ibuprofen and triiodothyronine (T3) to target two main pathogenic events in acute SCI, i.e. inflammation and demyelination.

METHODS: PLLA scaffolds were prepared by electrospinning technology. The drug-loaded PLLA has been characterized in term of drug release over 14 days (HPLC for ibuprofen; UPLC–MS/MS for T3). The anti-inflammatory effects has been tested in vitro by measuring the TNFα and IL-10 synthesis (mRNA expression level by RT-PCR) in the macrophage RAW 264.7 cell line exposed to LPS (ISO10993); the promyelinating properties by measuring the maturation of primary oligodendrocyte precursor cells (OPC). In vivo efficacy has been tested by implanting the drug-leaded-PLLA in the rat model of contuse spinal cord injury (N=15, mild lesion, performed by Impact One impactor at T8). As control, drug-unloaded PLLA was implated in lesioned rats (N=15). The BBB score for locomotion and gait analysis (performed by the computerized videotracking system CatWalk, Noldus) were used as efficacy primary end-points.

RESULTS & DISCUSSION: We observed the expected time effect (two-ways ANOVA: $F(7,216)=12.53$, $P<0.0001$), starting the recovery at day 7. In PLLA-implanted rats, the observed recovery stabilizes from 21PLD, so that non further improvement is observed. On the contrary, in PLLA+Ibu+T3 rats a significant treatment-effect is observed ($F(7,216)=7.349$, $P=0.0073$), also according to the analyzed time points (interaction: $F(7,216)=2.189$, $P=0.00363$). At the last observational time (49 PLD) only 20% of PLLA-implanted rats reached the BBB score 10, while 60% of PLLA-Ibu-T3 rat reached score 10 and a further 27% reached score 15. This positive effect was also confirmed by the step sequence regulatory index in the gait analysis at post-lesion day 49 (Mann Whitney test, $P=0.0097$)

CONCLUSIONS: The implant of PLLA electrospun scaffold loaded with ibuprofen and T3 significantly improves the functional locomotion outcome of rat with contusive spinal cord injury.

ACKNOWLEDGEMENTS: supported by Regione Emilia Romagna, POR-FESR2014-2020, Step-by-Step project, and by MIUR, Cluster Tecnologici Nazionali, IRMI project.



Silk fibroin scaffolds loaded with hydrogen sulfide (H_2S)-donor for bone tissue regeneration

L. Gambari [1], R. Raggio [1,2], W. Bonani [2]., B. Grigolo [1], A. Motta [2]., F. Grassi [1]
Presenting Author: Laura Gambari, laura.gambari@ior.it
[1]Laboratorio RAMSES, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy [2]Dipartimento di Ingegneria Industriale, Università di Trento, Italy

INTRODUCTION: Although silk fibroin (SF) holds great potential in bone tissue engineering, its capacity to induce osteogenic differentiation is limited; we recently described that the gasotransmitter hydrogen sulfide (H_2S) stimulates osteoblast differentiation and bone formation in vivo. In this work silk fibroin scaffolds were functionalized with a common H_2S-donor (GYY) and the resulting osteogenic potential was assesses both in 3D cultures with human mesenchymal stromal cells (MSCs) within perfusion bioreactor and in vivo in a murine model of ectopic bone formation.

METHODS: Porous SF scaffolds were produced by solvent casting and particulate leaching method and loaded with GYY at concentrations of 1 and 5% w/v. The newly generated scaffolds (SF-GYY) preserved chemical and physical properties, as assessed by SEM, FT-IR, NMR and different thermal analyses techniques [1]. Moreover, scaffolds dose-dependently released H_2S as revealed by amperometri measurements. Human MSCs were seeded into SF scaffold a cultured up to three week in perfusion bioreactor (Cellec Biotech, Basel) and analysed both with historical staining and for osteogenic gene expression by RT-PCR. SF snd SF-GYY scaffolds were also implanted subcutaneously in BL6 mice and assessed for osteogenic differentiation of cells.

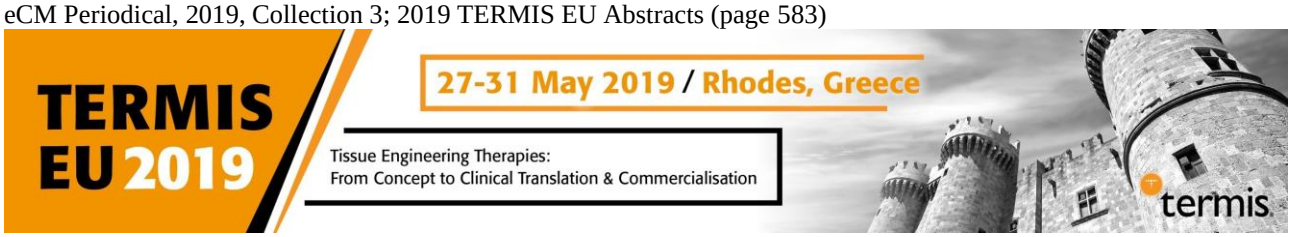
RESULTS & DISCUSSION: The presence of GYY dose-dependently increased the formation of ALP+, mineralized nodules compared to control SF scaffolds. Moreover, analysis of gene expression for 84 osteogenic genes revealed that, in the presence of GYY, 89% and 68% of genes were upregulated, respectively, at day 7 and day 21 in culture compared to control scaffolds. In particular, our data show that H_2S stimulation substantially induced the expression of genes belonging to BMP signaling pathway; Furthermore, SF-GYY showed markedly up-regulated VEGFA expression both at the gene and protein level. Noteworthy, the expression of BGLAP, considered as a prototypical late marker of osteoblast differentiation and bone formation, was also markedly increased in SF-GYY and peaked at D21 reaching a significant 6-fold increase compared to D0 and over 2-fold increase compared to control SF at D21.

CONCLUSIONS: This study demonstrates that loading SF scaffold with H2S donor is a suitable strategy to promote osteoblast differentiation at sites of bone regeneration. We conclude that H2S can significantly improve the efficacy of existing approaches of bone tissue engineering based of SF biomaterials.

ACKNOWLEDGEMENTS: This study was supported by a Grant from the Italian Ministry of Health: Grant Ricerca Finalizzata # PE-2011-02348395

REFERENCES

[1] Raggio R, et al. ACS Biomaterial Science and Engineering, 2018 , 4(8).



Tissue adhesive hydrogels for corneal epithelium and stromal regeneration

L. Koivusalo[1], M. Kauppila[1], T. Ilmarinen[1], S. Miettinen[2], O. P. Oommen[3], H. Skottman[1]
Presenting Author: Laura Koivusalo laura.koivusalo@uta.fi
[1]Eye Regeneration Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland, [2]Adult Stem Cell Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland [3]Bioengineering and Nanomedicine Lab, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

INTRODUCTION: Human pluripotent stem cell- derived limbal epithelial stem cells (hPSC-LESCs) are an exhaustless source of cells for regenerative therapy of the corneal epithelium [1], while human adipose stem cells (hASCs) display considerable potential to regenerate the corneal stroma [2]. However, limited strategies exist for the delivery of these therapeutic stem cells with tissue-like cellular organization to the anterior cornea. With the added risks related to suturing of corneal implants, there is a pressing need to develop new tissue adhesive biomaterials for corneal regeneration.

METHODS: Hyaluronic acid (HA) was modified with aldehyde and carbodihydrazide groups to create hydrazone-crosslinking hydrogels, as previously described [3], with further modification of the carbodihydrazide-component by grafting tissue adhesive dopamine (DOPA) moieties. Tissue-like cellular compartmentalization in the implants was established by encapsulating hASCs inside the hydrogels, with subsequent conjugation of thiolated collagen IV or laminin peptides and hPSC-LESC seeding on the hydrogel surface. We performed rheological characterization, measured swelling and transparency of the HA-DOPA gels, and evaluated their in vitro performance with both cell types. The functionality of the organized cell-laden hydrogel implant was studied in an ex vivo porcine cornea organ culture model.

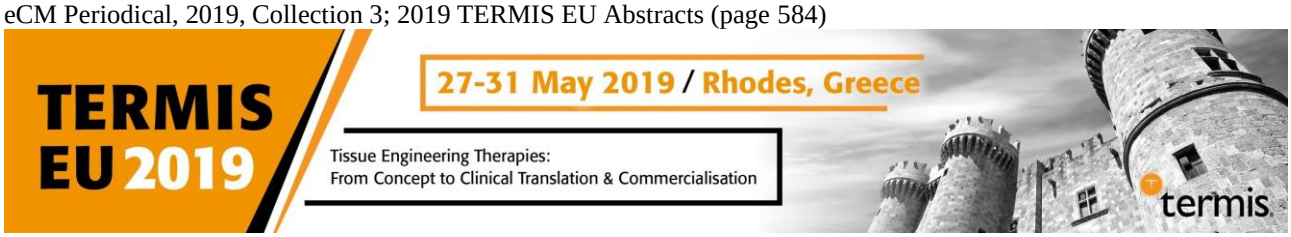
RESULTS & DISCUSSION: The HA-DOPA gels showed remarkable swelling stability and suitable transparency for corneal applications. The encapsulated hASCs in HA-DOPA gels exhibited good proliferation and cell elongation, while the hPSC-LESCs expressed typical limbal epithelial progenitor markers. Importantly, the compartmentalized HA-DOPA implants displayed excellent tissue adhesion when implanted into the porcine corneal organ culture model.

CONCLUSIONS: We have developed a tissue adhesive dopamine-hyaluronic acid hydrogel and shown its proof-of-concept to deliver human stem cells for regeneration of both the corneal epithelium and the underlying stroma. Collectively, these results encourage sutureless implantation of functional stem cells as the next generation of corneal regeneration.

ACKNOWLEDGEMENTS: Financial support for this research was received from Graduate School of Faculty of Medicine and Life Sciences, University of Tampere, Instrumentarium Science Foundation, Academy of Finland and Business Finland.

REFERENCES

[1] Hongisto H et al, Stem Cell Res. Ther. 2017; 8(1):291
[2] Arnalich-Montiel F et al, Stem Cells 2008; 26(2): 570-579
[3] Oommen OP et al, Adv. Funct. Mater. 2013; 23(10): 1273-1280



Raman spectroscopy-based control development for tissue engineered cartilage

L. Power[1], C. Fasolato[2], I. Martin[1,3], D. Wendt[1,3], A. Barbero[3]
Presenting Author: Laura Power, laura.power@usb.ch
[1]Department of Biomedical Engineering, University Hospital Basel, Switzerland [2]Department of Physics and Geology Perugia University, Italy [3]Department of Biomedicine, University Hospital Basel, Switzerland

INTRODUCTION: We are developing tissue engineered cartilage grafts to treat articular cartilage defects using autologous nasal chondrocytes[1] in an ongoing phase II clinical trial. Current methods to assess the quality of engineered tissues are destructive, eliminating the possibility of monitoring grafts continuously and only allows for the assessment of a small part of the graft, which may not be representative of the overall quality. Raman spectroscopy, a method that measures the chemical composition of materials, has the potential to comprehensively and nondestructively characterize engineered cartilage.

METHODS: Native nasal cartilage biopsies were measured with Raman spectroscopy. Nasal chondrocytes were isolated from the biopsies, expanded, seeded onto 3D collagen scaffolds, cultured for up to two weeks in chondrogenic conditions, and measured with a Raman spectrometer. Histological safranin O staining and biochemical quantification of proteoglycans, an important component of cartilage, were performed on the samples and the results correlated with the Raman spectra using statistical learning methods.

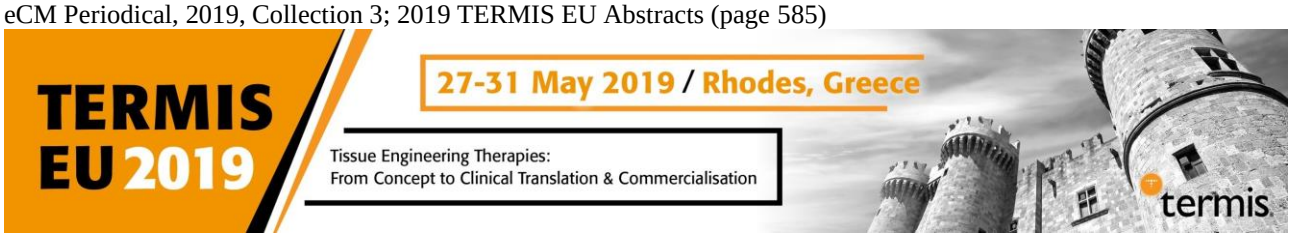
RESULTS & DISCUSSION: The high and low quality native biopsies could be distinguished with a sensitivity of 82% and specificity of 87%. During the two-week process of engineering cartilage, the Raman spectra of the grafts became more similar to the native cartilage spectra. Semi-quantitative histological scores based on the staining intensity and cell morphology of the engineered cartilages (the highest quality corresponds to a maximum score of six) could be predicted from the Raman spectra with a model using LASSO variable selection and multiple linear regression.

CONCLUSIONS: We show that Raman spectroscopy could be used for nondestructive quality controls to assess the starting materials and final graft quality of tissue engineered cartilage – ensuring product quality and facilitating regulatory compliance.

ACKNOWLEDGEMENTS: This work has received funding from the European Union's grant agreement No. 681103 (BIO-CHIP, www.biochip-h2020.eu).

REFERENCES

[1] M. Mumme, A. Barbero, S. Miot, A. Wixmerten et al. (2016) Lancet, 388: 1985.



Shear stress effect on cardiac pathological models

L. Turco[1], N. Karedla[2,] E. Bodenschatz[1], M. Tarantola[1]
Presenting Author: Laura Turco, laura.turco@ds.mpg.de
[1]Max Planck Institute for Dynamics and Self-Organization, Goettingen, Germany [2]III. Institute of Physics-Biophysics, Georg-August-University, Goettingen, Germany

INTRODUCTION: Engineered constructs combined with microtechnologies can be used as reliable in vitro models to study the development of cardiac diseases and the effect of drugs on cellular functions. During left ventricular remodeling (LVR) after cardiac infarction, cells are exposed to increased mechanical loads, in particular shear stress [1]. Due to the lack of experimental tools to quantify the effect that such a stimulation has on cardiac cells, shear stress is often neglected in in vitro and in silico computational models. Thus we investigated the short- and long-term effect of pathological shear stresses on cellular morphology, contractility, and connectivity.

METHODS: Monocultures of 1-2-day-old neonatal ventricular cardiomyocytes and co-cultures with different percentage of cardiac fibroblasts were seeded in channels equipped with electrode arrays (Ibidi, Martinsried) and allowed to reach confluence for 24h. Subsequently, cells were exposed to a continuous laminar flow with different high shear stresses up to 108h. Meanwhile cell passive biological properties, functional and morphological changes were measured and quantified via non-invasive electric cell-substrate impedance spectroscopy. The effect of shear stress on cell size, shape and actin fibers orientation was determined and confocal images were analyzed with a custom-written program.

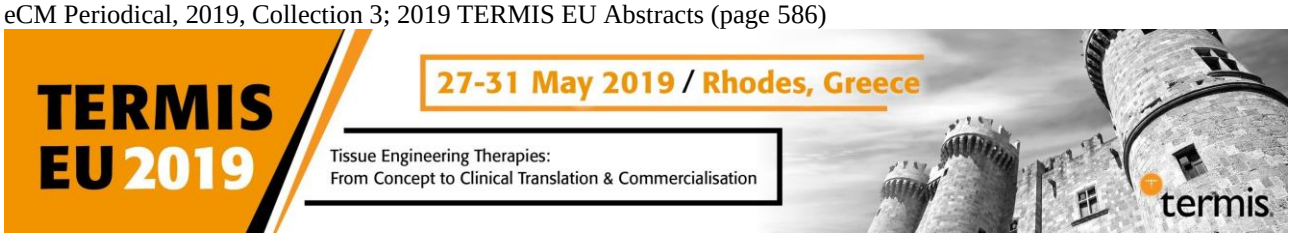
RESULTS & DISCUSSION: By combining impedance spectroscopy and optical microscopy we showed that pathological values of shear stress stimulation lead to an immediate decrease of cell-substrate distance at the flow-onset, further cell spreading up to 48h and a gradual reorientation of actin fibers along the direction of the flow. Beating frequency and cell-cell connectivity of cardiomyocytes increased under shear stress and presented synchronized contraction. Surprisingly, we observe for the first time contractility in in vitro co-cultures with 9:1 ratio of fibroblasts after shear stimulation, whereas the non-sheared co-cultures did not show any activity.

CONCLUSIONS: We could prove that stimulating cardiac cells with high shear stress is a reliable in vitro model to reproduce pathological conditions in vivo. This underlines the importance of taking into account the presence of high shear stress in experimental and modeling studies to understand the pathophysiology during LVR and its influence on mechanoelectrical coupling.

ACKNOWLEDGEMENTS: Financial support was received from SFB937; MWK International Max Planck Research School Excellence Stipend.

REFERENCES

[1] Paul S Crit. Care Nurs. Clin N Am. 2003;15:407-411



Controlled release of biological factors for progenitor cell-mediated endogenous repair of intervertebral discs

L. Frapin[1], J. Clouet[1], N. Henry[1], C. Chedeville[1], M. Fusellier[1], J. Guicheux[1] C. Le Visage[1]
Presenting Author: Leslie Frapin, leslie.frapin@etu.univ-nantes.fr
[1]Inserm, UMR1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS, Nantes, F-44042, France

INTRODUCTION: The recent description of progenitor cells in degenerated intervertebral discs raised the possibility of harnessing their regenerative capacity for endogenous repair[1]. Here, we developed an intradiscal polysaccharide microbead-based delivery system for the sequential release of chemokines and nucleopulpogenic factors. This delivery system would contribute to 1) the recruitment of resident progenitors (CXCL12 or CCL5), followed by 2) the differentiation of the mobilized progenitors (TGF-β1 and GDF5), and 3) the subsequent regeneration of Nucleus pulposus (NP).

METHODS: Pullulan microbeads (PMBs) (100µm) were prepared by a simultaneous crosslinking protocol coupled to a water-in-oil emulsification process. Freeze-dried PMBs were loaded with biological factors then release assays were performed at 37°C for 21 days. To determine the bioactivity of released chemokines and growth actors on in vitro cell recruitment and synthesis of NP-like extracellular matrix (ECM), respectively, human mesenchymal stem cells (hMSCs) were cultured in Transwells during 14 days and histological analysis was performed on loaded PMBs and hMSCs aggregates.

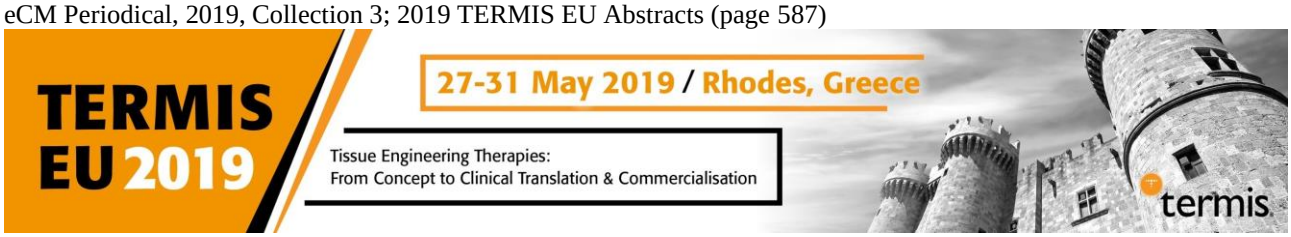
RESULTS & DISCUSSION: All factors were successfully adsorbed on PMBs and a burst release within the 1st day was observed. At day 14, 17% and 68% of CXCL12 and CCL5 were released, respectively and at day 21, 20% and 100% of TGF-β1 and GDF5 were released, respectively. MSCs exhibited an increased tendency to migrate and synthesize an NP-like ECM when cultured in presence of PMBs loaded with chemokines or growth factors.

CONCLUSIONS: PMBs are suitable micro-carriers for the controlled loading and release of biological factors that could boost intervertebral disc endogenous repair. Ex vivo demonstration in ovine IVD model is ongoing, then in vivo proof of concept will be performed in an ovine model of spontaneous IVD degeneration.

ACKNOWLEDGEMENTS: This study was supported by region Pays de la Loire (Paris scientifique), the ANR JCJC and national phD scholarship

REFERENCES

[1] Henriksson HB. et al., Spine. 34: 2278-2287, 2009



Development of a dual miRNA/pDNA delivery system for cartilage tissue engineering

L. Blokpoel Fererras[1,2,3], A. Gonzalez-Vazquez[1,2,3], C. Curtin[1,2,3], J. E. Dixon[4], K. M. Shakesheff[4], F.J. O'Brien[1,2,3]

Presenting Author: Lia Blokpoel Ferreras, liablokpoelferreras@rcsi.ie

[1]Tissue Engineering Research Group, Department of Anatomy, RCSI, Ireland [2]Trinity Centre for Bioengineering, Ireland [3]Advanced Materials and BioEngineering Research Centre, Ireland [4]School of Pharmacy, Wolfson Centre for Stem Cells, Tissue Engineering, and Modelling, Centre of Biomolecular Sciences, University of Nottingham, United Kingdom

INTRODUCTION: The use of gene therapy to induce the chondrogenic differentiation of autologous cells has shown potential as an alternative to current cartilage repair approaches. However, successful differentiation into healthy, stable cartilage has yet to be achieved, in part due to the lack of an efficient and safe gene-delivery system, but also due to a lack of suitable genetic targets.[1] The aim of this project is to develop a non-viral nucleic acid platform system capable of efficiently co-delivering pDNA, to trigger differentiation of human Mesenchymal Stem Cells (hMSCs) towards cartilage, and microRNA (miRNA), to minimize hypertrophy. This co-delivery system will then be incorporated into previously optimized chondrogenic 3D scaffolds.[2]

METHODS: Dual delivery nanoparticles (NPs) were formulated by mixing miRNA and/or pDNA with GET (Glycosaminoglycan Enhanced Transduction) peptide.[3] Nucleic acid encapsulation and NP size were characterized. NP-mediated miRNA transfection efficiency was evaluated using a miR-mimic designed to target the mRNA of the housekeeping glyceraldehyde phosphate dehydrogenase (GAPDH). GAPDH mRNA expression levels in hMSCs were measured by PCR. Transfection efficiency was compared to Lipofectamine RNAiMAX (gold standard).

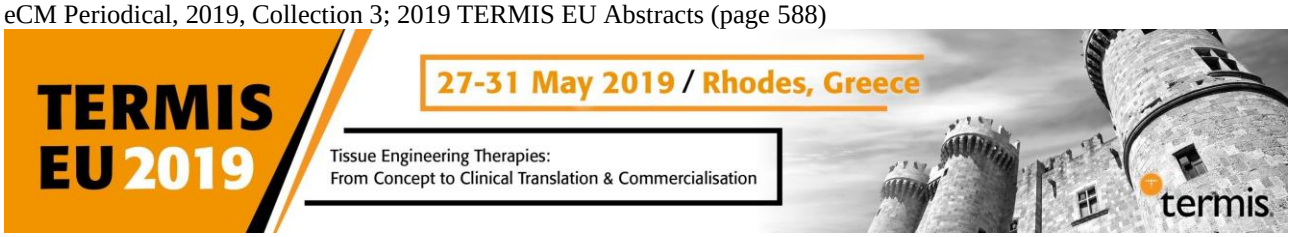
RESULTS & DISCUSSION: Gel retardation assays confirmed the complexation of miRNA by GET. GET formed homogeneous NPs with miRNA and pDNA/miRNA of 31 and 52 nm diameter respectively. Simultaneous delivery of the two genetic cargoes in hMSCs did not have an effect on cell viability or NP uptake. GAPDH mRNA expression was significantly reduced (0.1 fold change) when hMSCs were transfected with the pDNA-miRNA-GET formulation, comparable to Lipofectamine RNAiMAX (Lipo).

CONCLUSIONS: We have successfully developed a NP formulation for the co-delivery of pDNA and miRNA. This represents the first stepping stone in developing an efficient gene therapy that promotes successful, long-term cartilage repair by inducing stable chondrogenesis and inhibiting hypertrophy.

ACKNOWLEDGEMENTS: AMBER: SFI, Research Centres programme, grant SFI/12/RC/2278. ReCAP: ERC Advanced Grant number 788753.

REFERENCES

[1] Graceffa, V et al. Biotechnol. Adv. 2018; Jul.
[2] Raftery, R. M.et al. Adv. Mater. 2016, 28(27), 5447-5469.
[3] Dixon, J.et al. PNAS, 2016;113(3), E291-E299.



Formulation of a functionalized biomaterial to support pancreatic islet viability in transplantation

L. McDonough[1,2], L. Gallagher[1, 2], B. Cavanagh[3], G. P. Duffy[4], H. Kelly[1,2]
Presenting Author: Liam McDonough, liammcdonough@rcsi.ie
[1]School of Pharmacy, Royal college of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Tissue Engineering Research Group, Dept. of Anatomy, RCSI, Dublin, Ireland [3]Cellular and Molecular Imaging Core, RCSI, Dublin, Ireland [4]School of Medicine, National University of Ireland Galway, Galway, Ireland

INTRODUCTION: Pancreatic islet transplant therapy is a promising treatment for type 1 diabetes mellitus. The Diabetes Reversing Implants for enhanced Viability and long-term Efficacy (DRIVE) project aims to develop a functionalized biomaterial to encapsulate pancreatic islets with the aim to improve survival and engraftment of the transplanted pancreatic islets. Herein, we discuss the formulation of a hyaluronic acid (HA) based biomaterial functionalized with an oxygen carrier.

METHODS: The oxygen carrier was prepared as an emulsion, characterized by dynamic light scattering, and incorporated into the HA to give oxygen carrier emulgels (β-gel). The β-gel formulations were characterized rheologically and oxygen properties were assessed using an oxygen sensor. INS-1E [1] cells were used to form pseudoislets (PIs) for in vitro testing. The INS-1E cells and PIs were encapsulated in the lead β-gel formulation and transferred to a shell, cells encapsulated in a HA gel in a shell were used as a control. A serial glucose stimulated insulin secretion assay of low-high-low concentration glucose was performed on encapsulated cells at 24h, 72h, and 168h following encapsulation and the results were read using a rat insulin ELISA.

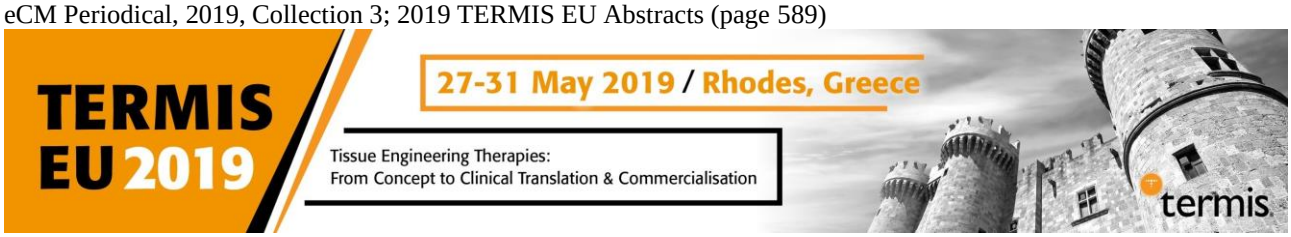
RESULTS & DISCUSSION: The oxygen carrier was successfully prepared as an emulsion and showed stability at 4°C for up to 2 months. The β-gels shear thin substantially with increasing shear stress and show recovery of initial properties following the removal of an applied stress. Preliminary results from encapsulated INS-1E and PIs showed higher concentrations of insulin release in the control HA gel than in the β-gel in normoxia at all time-points.

CONCLUSIONS: A HA based biomaterial was successfully functionalized with an oxygen carrier emulsion. However preliminary in vitro work shows higher insulin release in non-functionalized HA gel than the β-gel in normoxia. Future work will focus on repeating in vitro experiments and testing in hypoxia to better mimic an implant site.

ACKNOWLEDGEMENTS: We would like to thank Professor Maechler from the University of Geneva Medical Center for providing the INS-1E cell line. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement number 645991.

REFERENCES

[1] Merglen et al. (2004) Endocrinology 145:667-678



Chondrogenic differentiation of human mesenchymal stem cells in double-network hydrogel scaffolds for cartilage repair

L. Ifergan[1], S. Cohen[1], H. Simaan-Yameen[1], O. Kossover[1], C. Loebel[2], J. Burdik[2], D. Seliktar[1]
Presenting Author: Liaura Ifergan, liaura.ifergan@gmail.com
[1]Department of Biomedical Engineering, Technion – IIT, Haifa, Israel [2]Department of Bioengineering, University of Pennsylvania, Philadelphia, USA

INTRODUCTION: Autologous chondrocyte implantation (ACI) therapy has most recently been used to treat articular cartilage defects. A next generation of ACI therapy is now being explored using autologous mesenchymal stem cells (MSCs) derived from the patient's bone marrow and incorporated into hydrogel biomaterials. We investigated an innovative hydrogel combination of covalently crosslinked PEGylated fibrinogen (PF) [1] and hyaluronic acid (HA) with guest-host (GH) interactions [2].

METHODS: Bone marrow-derived human MSCs (hMSCs) were seeded into different formulations of double-network hydrogel and maintained in 3D culture for 21 days under chondrogenic conditions. Control gels was cultured under regular hMSCs conditions. Each formulation contained 2% GH component in 8 mg/mL PF. To create a variety of viscoelastic hydrogels with different mechanical properties, we added different amounts of polyethylene glycol diacrylate (PEG-DA): 0.2%, 0.5%, 1% and 2%. We examined cell adaptability and viability by performing Live/Dead assay, using calcein/ethi-dium staining of hMSCs. Moreover, dimethylme-thylene blue (DMMB) assay quantified sulfated-glycosaminoglycan (GAGs) content to evaluate chondrogenic differentiation.

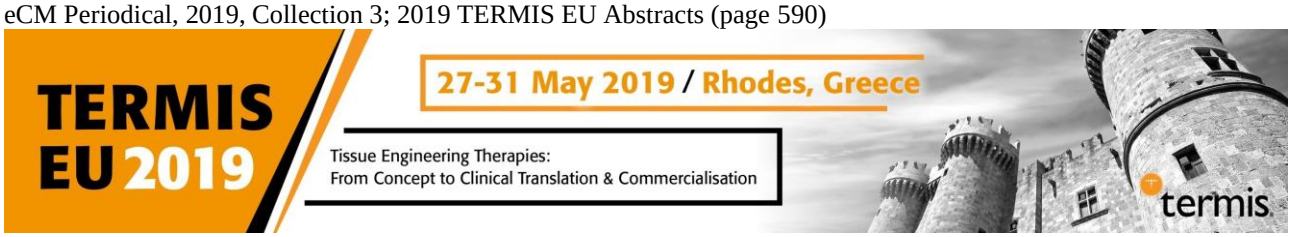
RESULTS & DISCUSSION: Live/dead staining with calcein (green) and ethidium (red) shows that cells were able to create a very well-defined interconnected network, with a relatively high number of viable cells within the hydrogels. Cell differentiation process, partly indicated by GAG production, was normalized by the number of live cells. Two groups, 0.5% and 2% PEG-DA, showed an increase in the mass of GAGs as function of time. Further immunostaining experiments will be performed to verify this relationship.

CONCLUSIONS: The current results illustrate the cell adaptability to this state-of-the-art viscoelastic hydrogel. Along with encouraging viability results, GAG quantification indicates the starting of a successful chondrogenic differentiation pathway.

ACKNOWLEDGEMENTS: USA National Science Foundation (NSF) and United States-Israel Binational Science Foundation (BSF).

REFERENCES

[1] Almany, L., & Seliktar, D. (2005). Biomaterials, 26(15), 2467–2477.
[2] Loebel C et al. Journal of Materials Chemistry B, In Press.



3D printing of thiol-ene photoclick gelatin-based scaffolds for adipose tissue engineering

L. Tytgat[1,2], L. Van Damme[2,3], J. Van Hoorick[1,2], H. Declercq[4], P. Blondeel[3], H. Ottevaere[1], H. Thienpont[1], P. Dubruel[2], S. Van Vlierberghe[1,2]
Presenting Author: Liesbeth Tytgat, ltytgat@b-phot.org
[1]Brussels Photonics, Vrije Universiteit Brussel, Pleinlaan 2 1050 Brussels, Belgium [2]Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Krijgslaan 281 9000 Ghent, Belgium [3]Plastic and Reconstructive Surgery, University Hospital Ghent, De Pintelaan 185 9000 Ghent, Belgium [4]Tissue Engineering and Biomaterials, Ghent University, De Pintelaan 185 6B3 9000 Ghent, Belgium

INTRODUCTION: The development of 3D-printed scaffolds for adipose tissue regeneration has recently gained increasing attention as a result of the exponential growth of lipofilling procedures performed in the clinic[1,2]. However, there remains an urgent medical need to develop materials outperforming the commercially available soft tissue fillers since they lack adipose tissue regeneration potential. In the present work, gelatin has been functionalized with either norbornene or thiol functionalities to enable thiol-ene photoclick chemistry. Subsequently, the hydrogels have been processed via extrusion-based 3D printing thereby resulting in patient-specific scaffolds.

METHODS: Gelatin B was modified with 2.5 equivalents 5-norbornene-2-carboxylic acid or with 5 equivalents N-acetyl-homocysteine thiolactone to obtain GelNB with a degree of substitution (DS) of 53 and GelSH DS72 respectively. Gelatin B modified with methacrylamide functionalities (Gel-MOD DS100) was used as a reference. 3D printing was performed using the Bioplotter pneumatic extrusion printer (Envisiontec, Germany). The 3D-printed scaffolds were physico-chemical characterized in depth. Furthermore, the scaffolds were seeded with adipose tissue-derived stem cells (ASCs) to evaluate the differentiation potential of the ASCs via Bodipy staining.

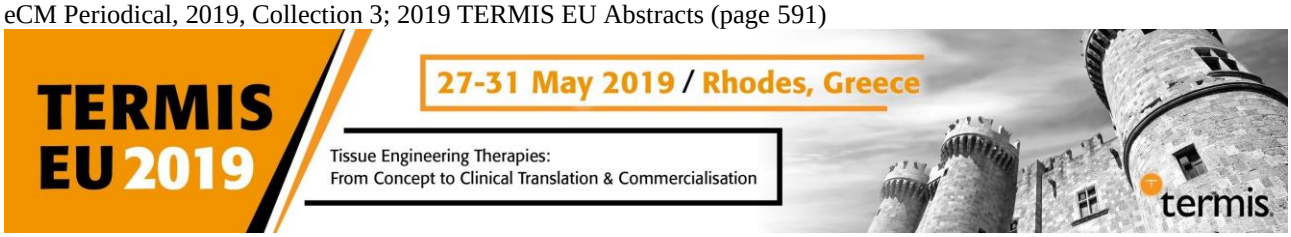
RESULTS & DISCUSSION Cubical shaped gelatin-based scaffolds were printed. The results of the compression tests showed that the Young's modulus of the GelNB - GelSH scaffolds was 1 kPa compared to 1.5 kPa for Gel-MOD. These values are in the same range as the young's modulus of native adipose breast tissue (2 kPa). Furthermore, the mass swelling ratio of the GelNB – GelSH scaffolds was 2 times higher compared to the Gel-MOD constructs (i.e. 52 versus 25). The differentiation potential of both scaffolds was assessed using Bodipy staining. On day 7, small lipid droplets could be seen in the cytoplasmic rim of the Gel-MOD and GelNB – GelSH scaffolds cultured in adipose medium, whereas no differentiation was observed on the scaffolds in the control medium.

CONCLUSIONS: The physico-chemical properties of the scaffolds are comparable with those of native adipose tissue. Furthermore, differentiation into the adipogenic lineage was observed in both Gel-MOD and GelNB – GelSH scaffolds. It can thus be concluded that the developed GelNB – GelSH scaffolds can be a promising candidate for adipose tissue engineering applications.

ACKNOWLEDGEMENTS: Liesbeth Tytgat would like to thank FWO for providing her a PhD fellowship.

REFERENCES

[1] C. Lequeux et al, J. Plast. Reconstr. Aesthetic Surg. **2015**, 68, 1491.
[2] E. Auclair et al, Plast. Reconstr. Surg. **2013**, 132, 558.



microRNA 21 promotes orthodontic tooth movement via TNF-α/ RANKL pathway in T cells

L. Guo[1], L. Wu[2] F. Lin[2], S. Zhu[2], Y. Liu[2]
Presenting Author: Lijia Guo, orthoest@163.com
[1]Department of Orthodontics, School of Stomatology, Capital Medical University, China [2]Laboratory of Tissue regeneration and Immunology and Department of Periodontics, Beijing Key Laboratory of Tooth regeneration and Function Reconstruction, School of Stomatology, Capital Medical University, China

INTRODUCTION: Orthodontic tooth movement (OTM) occurs when under the mechanical forces. The premise of this process is alveolar bone remodeling and OPG/RANKL/RANK axis plays an important role [1-3]. The purpose of this study is to investigate whether MicroRNA-21 can affect OPG/RANKL/RANK axis then affect tooth movement in mice.

METHODS：We established the orthodontic tooth movement model in C57BL/6 mice and miRNA21 knockout mice. Micro CT, H&E staining and TRAP staining was taken to investigate how miRNA21 affect the tooth movement. We also tested RANKL level in serum by ELISA. CD4+T cells were isolated from spleen of the wild type mice then treated the miRNA21 knockout mice.

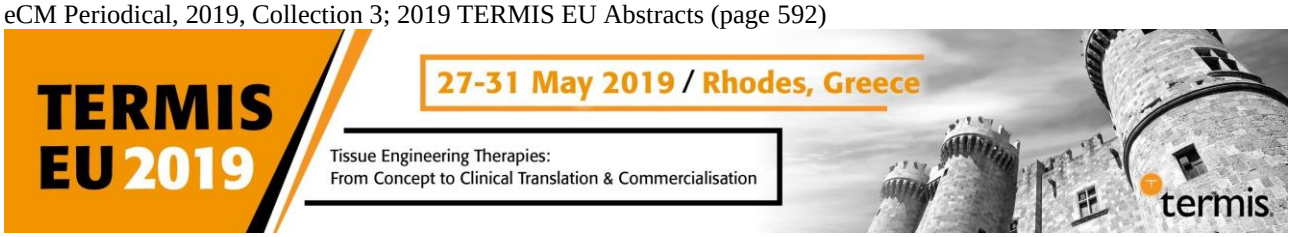
RESULTS：We found that the speed of orthodontic tooth movement was slowed down in miR-21-/- mice. TRAP staining showed that osteoclasts number was decreased, RANKL in serum was also much at a lower level in miR-21-/- mice. Further more, we found CD4+ T cells could partially rescue the OTM in miR-21-/- mice. Further studies showed TNF-α/RANKL pathway involved in miR-21-/- reduced OTM. It suggested that miR-21 promote RANKL secretion by active T cells then influent the maturity of osteoclasts and orthodontic tooth movement.

CONCLUSIONS: microRNA 21 promotes orthodontic tooth movement via TNF- α/ RANKL pathway in T cells. These findings provided an important evidence to understand the systemic factors in orthodontic tooth movement.

ACKNOWLEDGEMENTS: This work was supported by grants from the National Nature Science Foundation of China (81470751 to Y.L, 81600891 to L.G), the Beijing Natural Science Foundation (7172087 to Y.L), The Capital Health Research and Development of Special (2016-2-2141 to Y.L), the Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201703 to Y.B), the Beijing Baiqianwan Talents Project (2017A17 to Y.L), Beijing Municipal Administration of Hospitals' Ascent Plan (DFL20181501 to Y.L), Beijing Municipal Science & Technology Commission（Z16100000516203 to L.G）, Beijing Municipal Administration of Hospitals' Youth Programme (QML20181501), Beijing Dongcheng Excellent Talent (to L.G)

REFFERENCES

[1] Davidovitch, Z., et al., Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. Dent Clin North Am, 1988. 32(3): p. 411-35.
[2] Isola, G., et al., Mechanobiology of the tooth movement during the orthodontic treatment: a literature review. Minerva Stomatol, 2016. 65(5): p. 299-327.
[3] Walsh, M.C. and Y. Choi, Biology of the RANKL-RANK-OPG System in Immunity, Bone, and Beyond. Front Immunol, 2014. 5: p. 511.



A magnesium ion sustained-releasing micro-sized PLGA capsule for the treatment of osteoarthritis

L. Zheng[1,2], Y. Zhang[1,2], Q. Jiang[1,2]
Presenting Author: Liming Zheng, limzheng@126.com
[1]State Key Laboratory of Pharmaceutical Biotechnology, Department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, 321 Zhongshan Road, Nanjing 210008, Jiangsu, PR China [2]Laboratory for Bone and Joint Disease, Model Animal Research Center (MARC), School of Medicine, Nanjing University, Nanjing 210093, Jiangsu, China

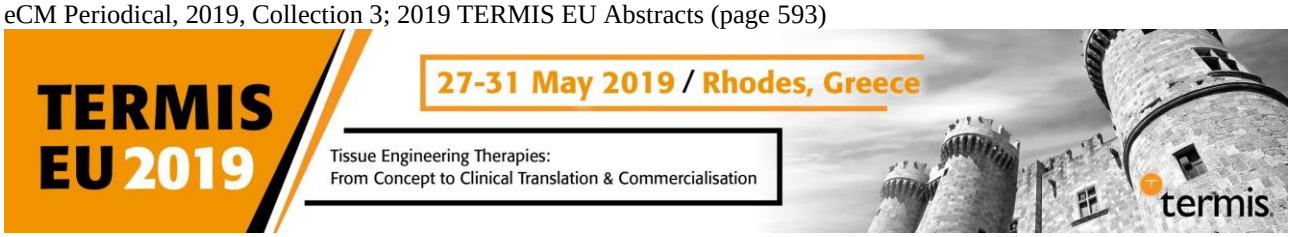
INTRODUCTION: Osteoarthritis has become one of the most popular degenerative disease in the world. Based on the efficiency of magnesium ions (Mg^{2+}) on the treatment of osteoarthritis, we developed a new drug delivery system which can be easily injected into articular cavity to sustainably release magnesium ions for treatment of OA.

METHODS: PLGA was used to encapsule magnesium oxide (MgO) nanoparticles which were hydrophobicly modified and homo-dispersed by stearic acid. The most biocompatible ranges of Mg ions concentration were achieved in PLGA-SA-MgO complex leaching solution in vitro. The fluidic complex was injected into rats' knee joint cavity with osteoarthritis established by meniscus excision. Two and four weeks after surgery, rats knee joint were harvested and histologic sections were then staining by Safranin O/Fast Green and scored by OARSI standard. In vitro, we used IL-1β-incubated SW1353 cells (chondrosarcoma cells) to simulate the osteoarthritis environment. CCK8, Cell flow cytometry, Western Blot and q-PCR were performed for the exploration of possible mechanism.

RESULTS & DISCUSSION: The most biocompatible Mg^{2+} concentrations range from 2mM to 10mM. MgO nanoparticles was modified by stearic acid and then encapsuled in PLGA microspheres. 4 weeks after injecting the complex into rats' knee joint cavity, the tissue attrition of joint cartilage was significantly relieved. In vitro experiments, Mg^{2+} increased the expression of SOX9 and then activate phosphorylation of Akt through synthesis of p110α(one subunit of PI3K) in SW1353 cells. By activating the PI3K-Akt signal pathway, the apoptosis-specific protein Caspases was inhibited and the chondrocyte was protected from apoptosis.

CONCLUSIONS: In conclusion, 10mM Mg2+ are benefit to the treatment of osteoarthritis by anti-apoptosis of chondrocytes. Our preparation of a novel injectable and degradable PLGA-SA-MgO complex with Mg ions sustained releasing can be applied in clinical application for OA treatment.

ACKNOWLEDGEMENTS: Financial support was received from Natural Science Foundation of China (81772402, 81730067), Jiangsu Science and Technology Project (BK20160633) and Postgraduate Research &Practice Innovation Program of Jiangsu Province (KYCX18_0048).



Enhance stem cell therapeutic potency with heparan sulfate glycosaminoglycans

L. Ling[1], X. Ren[2], V. Nurcombe[1], J. H. Hui[1,2], S. M. Cool[1,2]
Presenting Author: Ling Ling, ling.ling@imb.a-star.edu.sg
[1]Institute of Medical Biology, Agency for Science Technology and Research (A*STAR), Singapore 138648, Singapore [2]Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119074, Singapore

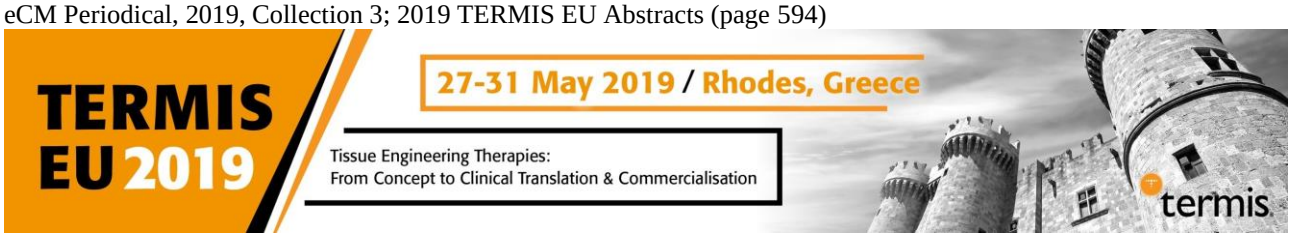
INTRODUCTION: Mesenchymal stem cell (MSC) therapy offers great hope to articular cartilage repair. Due to the low number of MSCs in adult tissues, further culture expansion is necessary prior to their clinical use. However, the ex vivo expansion process often leads to reduced stem cell potency and poor therapeutic outcome. In this study, we developed a novel strategy to enhance the stem cell potency through extracellular heparan sulfate (HS) glycosaminoglycans, a co-receptor for key growth factors for maintaining MSC stemness (e.g. fibroblast growth factor 2).

METHODS: Bone marrow-derived MSCs were isolated from the iliac crest of 11 young male donors. The stemness of MSCs were assessed by assays for growth capacity, telomere length, colony-forming efficiency, MSC surface antigen expression and tri-lineage potential. The therapeutic potency of MSCs were tested in a critical-sized osteochondral defect created in the femoral trochlear groove of thirty-six NIH nude rats and forty-five immune-compromised micropigs (n=6). Fibrin gel was used to deliver the cells. Masson trichrome and toluidine blue staining as well as immunohistochemical staining for collagen type I and II were performed on the tissue sections, and assessed with ICRS II and O'Driscoll scores. Magnetic Resonance Imaging (MRI) and biomechanical test were further conducted in the pig models.

RESULTS & DISCUSSION: An HS variant (termed HS8) with high affinity to fibroblast growth factor 2 significantly increased the number of population doublings of human MSCs without a reduction in their telomere length, colony-forming efficiency, MSC marker expression or tri-lineage potential. Therapeutically, HS8-expanded MSCs (MSC^{HS8}) applied to the rat osteochondral defects improved healing compared with the control MSCs. Histology analysis showed more hyaline cartilage was regenerated in MSC^{HS8} treated defects, and >70% of defects achieved high ICRS II and O'Driscoll scores. In comparison, control MSC treatment had less (~50%) defects and no treatment or fibrin treatment had none (0%) achieving high scores. Similar improvement in osteochondral repair with MSC^{HS8} was also observed in micropig models. MSC^{HS8} significantly increased ICRS II and O'Driscoll scores, reduced osteochondral lesions assessed by Magnetic Resonance Imaging (MRI) and enhanced biomechanical properties compared with control treatments.

CONCLUSIONS: This study highlights the important role of heparan sulfate glycosaminoglycan in growth factor-mediated stem cell activity and great potential for stem cell therapy.

ACKNOWLEDGEMENTS: Financial support was received from Singapore Ministry of Health's National Medical Research Council (NMRC/BnB/0003b/2013) and the Biomedical Research Council of Agency for Science, Technology and Research.



Prolonged intra-articular retention of mesenchymal stem cells by advanced microencapsulation for regenerative joint therapies

L.P. Karbaat[1], N.M. Korthagen[2], M. Baake[1], K. Warmink[3], N. Dupuis[3], S.K. Both[1], K. Coeleveld[3], B. Zoetebier[1], J. Leijten[1], H. Weinans[3], H.B.J. Kaperien[1]
Presenting Author: Lisanne Karbaat, l.p.karbaat@utwente.nl
[1]Department of Developmental BioEngineering, University of Twente, Enschede, The Netherlands [2]University of Utrecht, The Netherlands [3]University Medical Centre Utrecht, The Netherlands.

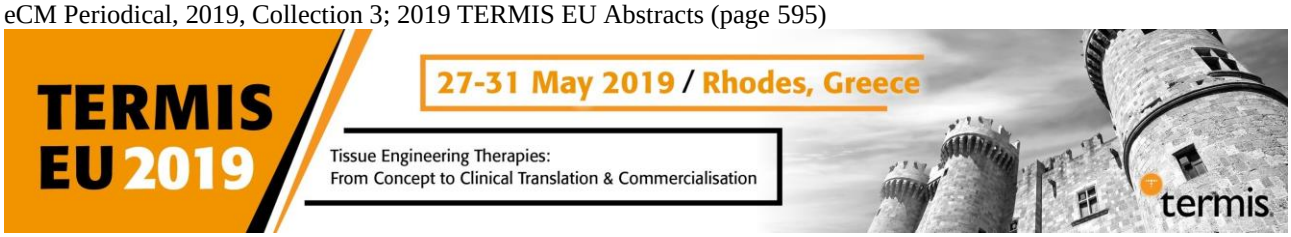
INTRODUCTION: Intra-articular injection of mesenchymal stem cells (MSCs) show therapeutic regenerative potential for patients with osteoarthritis (OA) by restoring damaged cartilage, reducing pain, and increasing motion range in clinical studies. However, clinical efficacy is still limited, which is likely caused by the rapid clearance of MSCs from the synovial cavity. We hypothesize that prolonging the intra-articular retention of MSCs increases their therapeutic potential. Therefore, we developed an advanced micro-encapsulation technique, specifically aimed at retaining MSCs in the joint, while supporting their viability and activity. The goal herein is to assess if micro-encapsulation of MSCs prolongs their intra-articular retention and increases their therapeutic potential.

METHODS: MSCs were harvested from 12 week old Wistar rats, and labelled with a near infrared (NIR) label. An enzymatically crosslinkable polymer and a microfluidic droplet generator were used to encapsulate MSCs in microgels (eMSCs). Viability and metabolic activity were assessed. Microgels with near-infrared labelled MSCs were intra-articularly injected in healthy 12 week old Wistar rats (n=6). For four months, quantitation of the NIR signal was performed using whole animal NIR-imaging. After 8 and 16 weeks, microgels were retrieved and the presence of NIR signal and viability of the eMSCs was confirmed. Additionally, 12 week old Wistar rats (n=12), were fed a high fat diet and underwent a groove surgery to induce a mild OA phenotype. eMSCs were intra-articulary injected one week after the groove operation. Functional performance of OA rats was investigated using gait analysis. At the end point from both studies, histological analysis was performed to provide greater insight in the process and mechanism of action.

RESULTS & DISCUSSION: Microfluidic encapsulation allowed for the formation of homogenous, monodisperse microgels (diameter 100 μm, cv <5%), which contained ~13 cells/gel. In vitro, the MSCs maintained ~60% of their initial metabolic activity and survived for at least four weeks. In vivo, MSC microencapsulation increased the intra-articular retention from four weeks (signal eMSCs vs naked MSCs: 63% vs 13%) to four months (signal eMSCs vs naked MSCs: 11% vs N.A.). Microgels retrieved from the knee joint contained viable, NIR-positive MSCs, confirming that the NIR signal came from the injected cells. Gait analysis shows an improved function of osteoarthritic rats receiving eMSCs over rats receiving naked MSCs or saline controls. Histological analysis corroborated the data.

CONCLUSIONS: Our study shows that encapsulation of MSCs in microgels increases their intra-articular retention to at least four months, while protecting the MSCs against the harsh environment within the joint. Additionally, eMSCs alleviated osteoarthritic symptoms on a functional level. This approach allows for a single intra-articular injection with a significantly extended retention of therapeutic cells within the intra-articular joint cavity.

ACKNOWLEDGEMENTS: Financial support was received from the Europees Fonds voor Regionale Ontwikkeling (00392).



Exploring the potential of dental pulp stem cells for mandibular condyle tissue engineering

L. Utomo[1,2], A.J.W.P. Rosenberg[1], D. Gawlitta[1,2]
Presenting Author: Lizette Utomo, l.utomo-2@umcutrecht.nl
[1]Dept. of Oral and Maxillofacial Surgery and Special Dental Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands [2]Regenerative Medicine Center Utrecht, Utrecht, The Netherlands

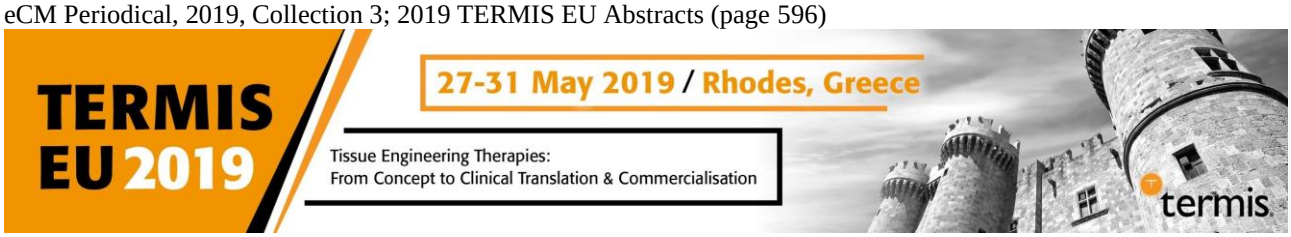
INTRODUCTION: Damage to the temporomandibular joint (TMJ) can be a result of osteoarthritis, congenital diseases, trauma, or tumor excision. The surface of the mandibular condyle is composed of a fibrous zone, covering the hypertrophic zone. A multipotent stem cell population that may be used for tissue engineering (TE) purposes of the TMJ, are dental pulp stem cells (DPSCs). They have great proliferative potential as well as cartilage and bone forming capabilities. The aim of this study was to explore the use of DPSCs for engineering the cartilage of the mandibular condyle. This was done by 1) evaluating which isoform of TGF-β induces fibrous cartilage formation, and 2) by engineering layers of cartilage to mimic the native tissue.

METHODS: Human DPSCs were isolated from the third molars of 3 patients, expanded to passage 4 and differentiated for 35 days in pellet cultures in chondrogenic medium containing TGF-β1, 2, or 3. Pellets cultured without TGF-β were used as controls. The fibrous chondrogenic capacity was evaluated by gene expression analysis of collagen type 1 (COL1A1), COL2A1, COL10A1, aggrecan (ACAN), and cartilage oligomeric matrix protein (COMP), and histology.
Mimicking the surfacing fibrous layer and hypertrophic zone of the mandibular condyle was done by creating a cell-laden bilayered gelatin methacryloyl (GelMA) hydrogel (ø=5mm). The bottom layer contained bone marrow derived mesenchymal stem cells (BMSCs) and the upper layer contained DPSCs ($20*10^6$/mL; h=1 mm/layer). The constructs were chondrogenically differentiated with TGF-β1 for 35 days and evaluated by histological analysis.

RESULTS & DISCUSSION: The differentiation capacity of DPSCs into fibrous tissue was evaluated by comparing the in vitro effects of three TGF-β isoforms. Gene expression levels of COL1A1, COL10A1, ACAN, and COMP were similar between all three isoforms and significantly higher than when the pellets were cultured without TGF-β. COL2A1 was not expressed in any culture condition. Staining with picrosirius red/alcian blue, revealed a collagenous matrix with limited glycosaminoglycan content, in which TGF-β1 showed most favorable regarding matrix deposition and was therefore used in subsequent experiments. By embedding DPSCs and BMSCs in GelMA, we obtained a bilayered cartilage construct. Notably, the deposited fibrous cartilage had similar matrix morphology as that of a native mandibular condyle.

CONCLUSIONS: Based on the expression profiles and morphology of the pellets cultured in various TGF-β isoforms, TGF-β1 appears to be the most favorable growth factor for fibrous chondrogenic differentiation of DPSCs. The generation of a cell-laden bilayered hydrogel construct indicates that DPSCs can be used for TE strategies for mandibular condyle regeneration.

ACKNOWLEDGEMENTS: The authors would like to thank Reilly de Groot for his help with harvesting the dental pulp. This study is financially supported by the AO Foundation (AOCMF-17-17G).



Synergistic enhancement of mesenchymal stem cells differentiation by using a multifunctional peptidic biointerface

L. Oliver-Cervelló[1,2], H. Martin-Gómez[1,2], M.P. Ginebra[1,2,3], C. Mas-Moruno[1,2]
Presenting Author: Lluís Oliver Cervelló, lluis.oliver@upc.edu
[1]Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Department of Materials Science and Metallurgical Engineering, Universitat Politècnica de Catalunya (UPC), Barcelona, Spain [2]Barcelona Research Center in Multiscale Science and Engineering, UPC, Barcelona, Spain [3]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.

INTRODUCTION: Improving the capacity of synthetic biomaterials to induce and guide bone regeneration is a major goal. This may be achieved by combining integrin-binding ligands with growth factors (GFs), such as the osteoinductive bone morphogenetic protein 2 (BMP-2). This approach optimally mimics the healing microenvironment of bone and has been shown to provide synergistic osteogenic effects. Thus, the objective of the present work was to design a novel biomolecular peptidic platform integrating the RGD sequence and a BMP-2 mimetic peptide to synergistically promote the adhesion and osteodifferentiation of MSCs for bone tissue engineering.

METHODS: The multifunctional peptide platform was prepared by solid-phase peptide synthesis and anchored to model glass substrates via chemisorption with catechol groups. Scrambled sequences were used as controls. The surfaces were characterized by means of contact angle, XPS and fluorescence microscopy. The synergistic effect between the two peptides was assessed via cell adhesion, proliferation and differentiation studies with human MSCs.

RESULTS & DISCUSSION: The physicochemical characterization of the biointerfaces demonstrated a successful binding, distribution and stability of the peptidic molecules. The crosstalk between the integrin-binding peptide and the BMP mimetic synergistically enhanced the number of MSCs adhering to the substrates compared to controls. In contrast, cell proliferation was significantly reduced on the biointerfaces, which may be an indication of concomitant differentiation. Indeed, both mineralization (Figure 1) and ALP activity were synergistically increased on RGD/BMP-2 samples in comparison with the presentation of the individual peptide motifs.

CONCLUSIONS: These results demonstrate that simultaneously presenting the RGD sequence and a BMP-2 mimetic peptide, within a molecular-based scaffold, synergistically improves MSC adhesion and differentiation into the osteoblastic lineage, thus opening the way to new biomolecular strategies for engineering bone tissue.

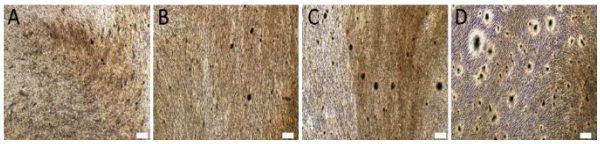
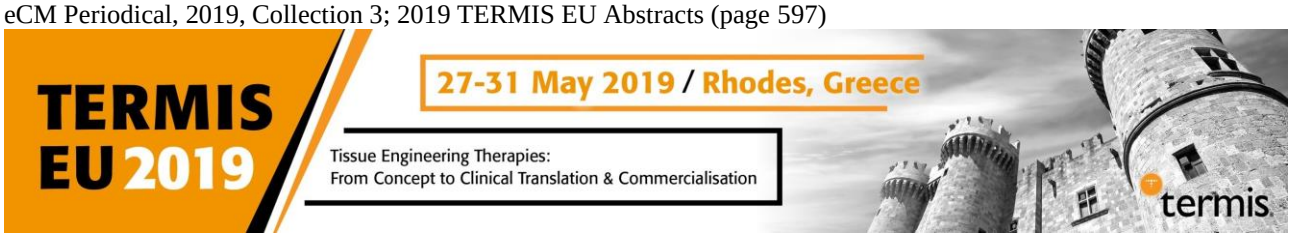


Figure 1: Alizarin Red S staining of calcium deposits after 21 days of incubation. A: Glass control. B: BMP-2 mimetic scrambled platform. C: RGD scrambled platform. D. RGD/BMP-2 mimetic platform. Scale bar 200 μm.

ACKNOWLEDGEMENTS: We thank the MINECO/FEDER (RYC-2015-18566, MAT2015-67183-R and MAT2017-83905-R), and the Government of Catalonia (2017 SGR1165, FI-2018 predoctoral fellowship) for funding.



Gellan gum/poly (ethylene glycol) di-acrylate hydrogels with tunable mechanical properties for articular cartilage engineering

H. Al-Haddad[1], L. Vannozzi[1], D. Trucco[2], G. Lisignoli[2], L. Ricotti[1]
Presenting Author: Lorenzo Vannozzi, lorenzo.vannozzi@santannapisa.it
[1]The BioRobotics Institute, Scuola Superiore Sant'Anna, 56025 Pisa, Italy [2]IRCCS Istituto Ortopedico Rizzoli, SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, 40136 Bologna, Italy

INTRODUCTION: Articular cartilage (AC) is a complex multi-layered structure organized into four zones, namely superficial, middle, deep and calcified layers [1]. Its mechanical properties are varying with depth, and the compression modulus of the superficial and deep zone shows values corresponding to 0.28 ± 0.16 MPa and 0.73 ± 0.26 MPa, respectively [2]. This paper reports preliminary results on mechanically tunable gellan gum/poly (ethylene glycol) di-acrylate hydrogels for mimicking the mechanical properties of such AC zones.

METHODS: Gellan gum (GG, Sigma-Aldrich) was dissolved 1.5 % wt. in deionized water while stirring for 30 min at 80 °C. Then, poly (ethylene glycol) di-acrylate (PEGDA, Mn: 575, Sigma-Aldrich) was added to the GG solution (10 and 15 % wt.), while keeping the temperature at 50 °C. Afterwards, the photoinitiator (Irgacure 2959, 0.5 % wt., Sigma-Aldrich) was added, and the solutions were stirred for 1 h, then degassed. For mechanical testing, each solution was poured in cylindrical wells (diameter: 6 mm, height: 5 mm) and photocrosslinked with UV light (5 and 10 min). Then, samples were immersed in magnesium chloride ($MgCl_2$-$6H_2O$, 1 % wt. in deionized water, Sigma-Aldrich) for 10 min and/or culture medium (Dulbecco's modified eagle medium, DMEM) for 24 h. Each sample underwent compression through an Instron 2444 (load cell: ± 10 N). The Young's modulus was extracted from the first linear region (up to 10%) of the stress-strain curve. Data were analyzed by means of ANOVA and Tukey's tests.

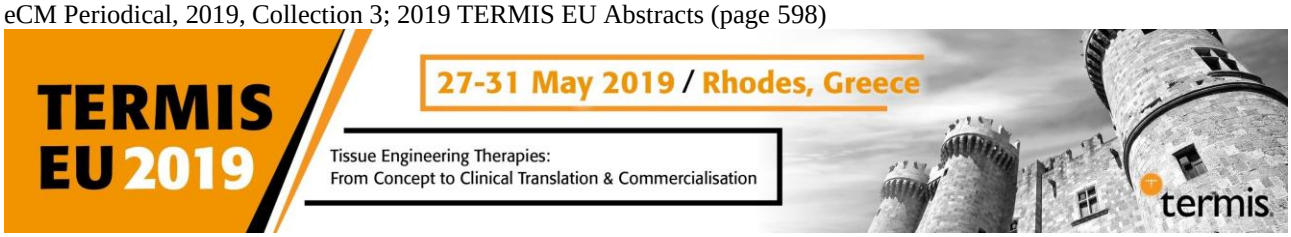
RESULTS & DISCUSSION: Compression data revealed values reflecting the ones of the AC superficial zone in case of PEGDA10 (~ 200 kPa), and AC middle zone in case of PEGDA15 (~ 600 kPa), with significant differences between the use of PEGDA10 and PEGDA15 for all formulations ($p<0.05$). At constant PEGDA concentration, UV exposure time and ionical crosslinking produced a slight change of the modulus.

CONCLUSIONS: The mechanical properties of GG/PEGDA hydrogels can be tuned for matching the stiffness range of the superficial and deep AC zones. These results are promising in view of the fabrication of 3D printed multi-layered structures AC engineering.

ACKNOWLEDGEMENTS: Research supported by the European Commission, through the project ADMAIORA (ADvanced nanocomposite MAterIals fOr in situ treatment and ultRAsound-mediated management of osteoarthritis), funded in Horizon 2020 framework. Grant number: 814413.

REFERENCES

[1] Bhosale A M et al., Br. Med. Bull. 2008; 87(1): 77–95
[2] O'Connell G et al. ACS Biomaterials Science and Engineering. 2017; 3(11): 2657–2668



Design of an injectable porous hydrogel as support for muscle regeneration

L. Griveau [1], C. Emilie [2], R. Debret [1], V. Gache [2], J. Sohier [1]
Presenting Author: Louise Griveau, louise.griveau@ibcp.fr
[1]MATEIS, CNRS UMR5510, Lyon, France [2]INMG, CNRS UMRS5310, INSERMU1217, Lyon, France

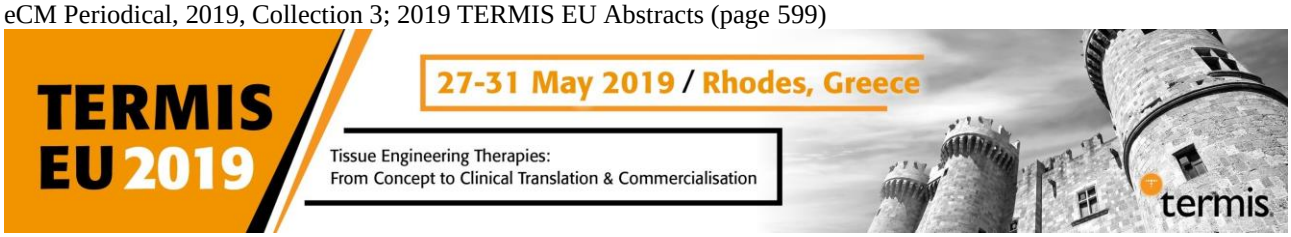
INTRODUCTION: Volumetric muscle loss (VML) resulting from traumatic incidents drastically decreases muscle regeneration capacity and lacks treatments. Hydrogels are promising therapeutic candidates by providing muscle cells support through tailored mechanical properties and potential injection in the wound for a perfect fill. However, as the regeneration potential requires the implanted hydrogels to be porous to allow cell infiltration, it is crucial to obtain a porosity during or after injection. Recently, we have developed a biocompatible and biodegradable hydrogel composed of poly-lysine dendrimers (DGL) and an elastic derived peptide (EDP), cross-linked by polyethylene glycol (PEG-NHS), which requires a preformed porosity to be colonized by cells and is thus non injectable per se. Therefore, the aims of this study were to investigate the potential of this hydrogel as a candidate for skeletal muscle repair, together with the creation of a spontaneous porosity through a novel effervescent approach, compatible with injection.

METHODS: To determine the most suitable environment for myoblasts, dense hydrogels were prepared by mixing different ratio of DGL and PEG-NHS in PBS with or without EDP, and their mechanical properties measured by DMA. Mouse myoblasts (C2C12) were cultured on the hydrogels in proliferative conditions during 24h. Time-lapsed cell proliferation, spreading and mobility were quantified by image analysis. To obtain a porosity compatible with injection, DLG and PEG-NHS were mixed with a carboxylic acid and a carbonated base at different ratios in the presence of a non-ionic surfactant at various concentrations. Cytotoxity was studied by immersing the porous hydrogels in culture medium for 24h and applying the supernatant onto normal human dermal fibroblasts, followed by a live/dead viability cell assay after 24 and 48 hours.

RESULTS & DISCUSSION: As the hydrogels stiffness could be modulated from 10 to 150kPa, the C2C12 behavior in terms of cell proliferation, spreading and mobility on the support was strongly influenced with highest values observed for 150kPa. Interestingly, the presence of the EDP induced similar behavior but for lower rigidities (70 kPa). These two hydrogels of interest were therefore used to develop an effervescent porous formulation. Herein, a selection of acid/base ratios and specific mixing order to the hydrogel components allowed a strong effervescence, concomitant to the hydrogel cross-linking. As a striking result, an interconnected porosity was created, remnant of the effervescently-generated CO2 bubbles. Interestingly, the molarities of acid and base and the addition of different types of surfactants allow to control the resulting pore size, distribution and interconnectivity throughout the hydrogels. However, cell viability was negatively correlated with increasing concentrations of surfactants and acid/bases couples.

CONCLUSIONS: Through a versatile hydrogel, environments of interest for muscle cells in regard of stiffness and composition were highlighted, while a spontaneous, interconnected and tailorable porosity could be induced through an effervescent approach. Cells viability further comforts their potential for in situ injection. On-going experiments focus on the optimal porosity for muscle cells infiltration along with potential to enhance functional tissue repair.

ACKNOWLEDGEMENTS: The French ANR is acknowledged for financial support.



Recolonization of the limbus by epithelial stem cells following the grafting of a tissue-engineered human corneal epithelium

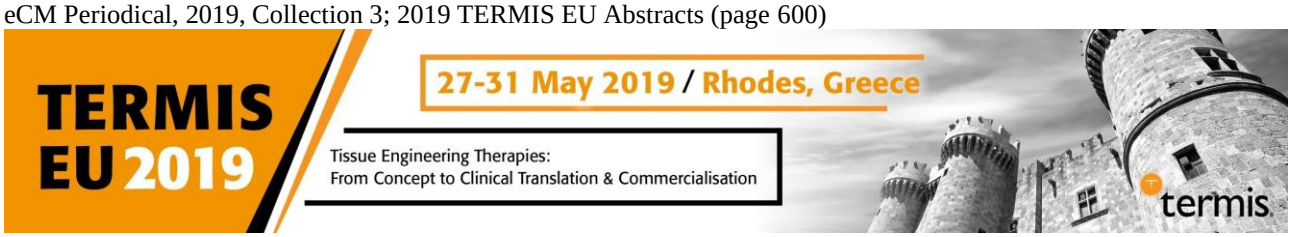
L.-P. Guérin[1], G. Le Bel[1,2], P. Carrier[1,2], R. Bazin[1], L. Germain[1,2]
Presenting author: Louis-Philippe Guérin, louis-philippe.guerin.1@ulaval.ca
[1]Département d'ophtalmologie, Université Laval, CUO-recherche/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada [2]Département de chirurgie, Université Laval, Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada

INTRODUCTION: Limbal stem cell deficiency (LSCD) prevents self-renewing of the corneal epithelium and leads to conjunctivalization and opacification of the corneal surface. Replacement of the epithelium on the eye of a LSCD patient by an in vitro-produced autologous cultured corneal epithelium containing stem cells represents an interesting option to prevent the loss of vision consecutive to LSCD. We tested the hypothesis that limbal stem cells are present in the basal layer of the tissue-engineered epithelium and can recolonize an empty limbus following grafting to a patient with LSCD through the transplantation of autologous cultured corneal epithelial cells.

METHODS: Human corneal epithelial cells (hCECs) were grown as a monolayer in the presence of irradiated fibroblasts of either mouse (i3T3) or human (iHFL) origin and then seeded on tissue-engineered human corneal stromas to allow regeneration of the corneal epithelium. The reconstructed corneas were then exposed to bromodeoxyuridin (BrdU) during 7 days, followed by a chase period of 1, 7 and 21 days. Localization of stem cells was then assessed by indirect immunofluorescence targeting BrdU and keratin 19 (K19). In addition, hCECs were isolated from a limbal biopsy of the contralateral eye of a patient with unilateral LSCD, resulting from a post-shingles neurotrophic keratitis, and cultured in monolayer in the presence of iHFL. The corneal graft was used to treat the affected eye.

RESULTS & DISCUSSION: Twenty-one days after the BrdU pulse, highly positive BrdU cells expressing K19 were observed in the basal epithelial layer of the tissue-engineered epithelium indicating the presence of human corneal epithelial stem cells. The corneal epithelium rebuilt from hCECs grown with iHFL has a more important stem cell population relative to that produced using hCECs co-cultured with i3T3. Moreover, long term restoration of the corneal epithelium was achieved following transplantation of the autologous corneal epithelial graft and relieved the LSCD suggesting that hCECs recolonized the stem cell niche (limbus). A central penetrating keratoplasty was performed in order to replace the damaged stroma. This allowed to recover corneal transparency.

CONCLUSIONS: The tissue-engineered human corneal epithelium possesses a subpopulation of stem cells located in its basal layer that can, once grafted to an eye with LSCD, recolonize the limbus, thus providing permanent restoration of a self-renewing corneal epithelium and reestablishing a clearer vision to the patient.



Segmented microfibers as high-throughput platform to screen 3D cell-materials interactions

L. Gasperini[1,2], A. F. Carvalho[1,2], R. S. Ribeiro[1,2,3], A. P. Marques[1,2,3], R. L. Reis[1,2,3]
Presenting Author: Luca Gasperini, luca.gasperini@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: We propose a high throughput microfluidic-based platform to screen cell-material interactions in which cells are localized in specific segments along hydrogel microfibers. The segments are representative of a wide range of physiologically relevant 3D tissue engineering microenvironments [1].

METHODS: The segmented microfibers are produced with a flow-focusing chip with three or four inlets by the pulsatile flow of polymeric solutions. The individual segments are made from the solution of the inner channels in which human adipose stem cells are encapsulated. Each cellular segment is then separated by an acellular part. When a microfluidic mixer is used, the composition of the cellular segments can vary along the length of the fiber to create linear gradients of materials and cells. Similarly, without the mixer Janus segments can be formed, with two separated compartments whose size ratio varies along the fiber.

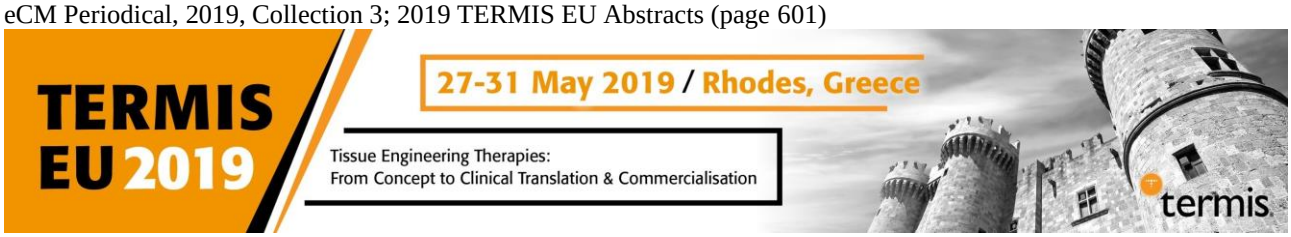
RESULTS & DISCUSSION: The length of the cellular and acellular parts can be independently controlled by changing only the parameters of the process (pressure or flow rate) while the width of the fiber was optimized to ensure optimal nutrient diffusion (300-400 um). The microfiber, from few centimeters to some meters in length, placed on a custom-made transparent spool permits an automated characterization of the cell response and the respective correlation to the original composition of the segment, based on its position along the fiber. Therefore, the identification of the best material/composition for the differentiation of stem cells has been achieved.

CONCLUSIONS: This approach allows the fabrication of multiple separated 3D cell-laden hydrogel-based environments for the screening of cell-materials interactions and selection of the best conditions for the development of improved tissue engineering approaches.

ACKNOWLEDGEMENTS: Advanced ERC-2012-AdG-20120216-321266 project ComplexiTE., FCT SFRH/BPD/109595/2015 (AFC). The Discoveries Centre for Regenerative and Precision Med.

REFERENCES

[1] Gasperini L et al. WO2018078562A1 (Patent)



Patient-specific extracellular matrix-based 3-dimensional cultures are superior to 2-dimensional cultures conditions to model colorectal cancer and liver metastasis

E. D'Angelo[1,2], D. Natarajan[3,4], F. Sensi[1,5], M. Fassan[6], E. Mamman[7], P. Pilati[7], P. Pavan[1], S. Bresolin[1], G. Spolverato[2], M. Piccoli[1,8], M. Agostini[1,2], L. Urbani[3,4]
Presenting Author: Luca Urbani, luca.urbani@researchinliver.org.uk
[1]Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padua, Italy [2]Department of Surgery, Oncology and Gastroenterology, University of Padova, Italy [3]Institute of Hepatology, Foundation for Liver Research, London, UK [4]Faculty of Life Sciences & Medicine, King's College London, UK [5]Department of Women's and Children's Health, University of Padova, Italy [6]Department of Medicine, University of Padova, Italy [7]Unit of Surgical Oncology of the Esophagus and Digestive Tract, Veneto Institute of Oncology IOV-IRCCS, Padua, Italy [8]Department of Biomedical Sciences, University of Padova, Italy.

INTRODUCTION: Colorectal cancer (CRC) is the third most common cancer worldwide and liver is the most common site of CRC metastasis. Relevant tissue culture models are needed to study hepatic metastasis derived from CRC as none of the current models mimic the complex metastatic microenvironment [1]. Here we developed a 3D model of CRC-liver metastasis (CRLM) and matched CRC using patient-derived decellularized matrices to study cell-matrix interaction and drug cytotoxicity.

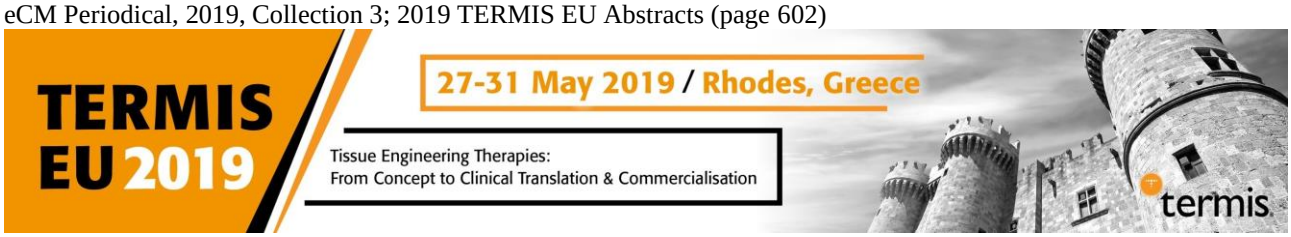
METHODS: Decellularization of patient-derived samples of healthy colon (HC), CRC, healthy liver (HL) and CRLM was performed with a detergent-enzymatic process. Decellularized scaffolds were seeded with CRC cell line HT-29 transfected to express luciferase and use bioluminescence for longitudinal non-disruptive cell tracking.

RESULTS & DISCUSSION: Decellularization preserved tissue-specific biological and ultrastructural properties of CRC and CRLM ECM. Cell proliferation and migration were quantified with bioluminescence for up to 10 days of culture, together with the expression of Ki67, Caspase3 for apoptosis, E-cadherin and Vimentin for epithelial-mesenchymal transition (EMT). The 3D model supported significantly higher cell proliferation and migration in seeded tumour-derived scaffolds compared to matched healthy tissues. Cells cultured in the 3D environment displayed significantly different gene expression profile in respect to 2D cultures. The most represented biological processes in CRLM-seeded scaffolds involved cellular response to stress metabolic processes, to oxygen level and to starvation, demethylation and deacethylation. These data, together with the loss of E-cadherin and increased expression of Vimentin, suggested clear activation of EMT in CRLM seeded scaffolds. Finally, HT-29 cells grown in CRLM scaffolds and exposed to standard 2D IC_{50} of 5-FU were resistant to the treatment compared to cells cultured in 2D conditions.

CONCLUSIONS: The 3D culture model developed with seeded patient-derived decellularized ECM better recapitulates tissue-specific microenvironmental features compared to 2D culture conditions and represents a relevant model for the study of CRLM.

REFERENCES

[1] Katt ME et al. Front Bioeng Biotechnol. 2016;4:12.



Combined 3D bioprinting of skin and adipose tissue as a promising approach for nipple areola complex and breast volume reconstruction

L. Vidal[1], S. Heraud[1], M. Albouy[1], C. Durand[1], A. Thepot[1], M. Dos Santos[1], C. Marquette[2]
Presenting Author: Luciano Vidal, luciano.vidal@labskincreations.fr
[1]Edouard Herriot Hospital, LabSkin Creations, 5, Place d'Arsonval, Bat. 5, 2eme etage, 69003 Lyon, France [2]3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, Bat. Lederer, 1 rue Victor Grignard, 69100 Villeurbanne, France

INTRODUCTION: Current prevailing options for breast volume and nipple areola complex (NAC) reconstruction post-mastectomy are based on different surgical procedures such as autologous skin and adipose tissue flaps, lipofilling or implant based. However, these conventional reconstructive techniques have inconsistent long-term outcomes regarding maintenance of the shape and projection over time and are hindered by multiple post-operative complications, leading to polarizing patient satisfaction rates. To overcome these limitations, our labs developed an innovative approach based on three-dimensional bioprinting approach to accurately deposit cells and biomaterials into precise geometries with the goal of creating anatomically correct biological vascularized adipose tissue and NAC constructs for breast reconstruction.

METHODS: To conduct such research, human fibroblasts, keratinocytes, and adipose derived stem cells combined with human microvascular endothelial cells were mixed in a unique bioink composed of 3 biopolymers and printed with optimal printing conditions and printer functions.

RESULTS & DISCUSSION: Histological characterisation of the 3D bioprinted adipose tissue showed adipocytes cells forming droplets and capillary-like structures homogeneously distributed within the tissue. Nil red staining highlighted the formation of large lipid droplets-containing cells. Immunostaining analysis confirmed the expression of specific mature adipose tissue markers such as FABP4 and perilipin-1 on the surface of lipid droplets. The bioprinted adipose tissue was also able to release adipose tissue-specific adipocytokines and more specifically leptin and adiponectin, demonstrating the functionality of the bioprinted tissue. Additionally, the 3D bioprinted skin was morphologically and biologically representative of the normal human skin. The printed dermis presented a microvascular network expressing CD31 and supported epidermal formation and proliferation to produce a full thickness, well-organized, and terminally differentiated epidermis.

CONCLUSIONS: This work paths the way to the promising development of autologous vascularized adipose tissue and skin for personalized breast volume and NAC reconstruction.

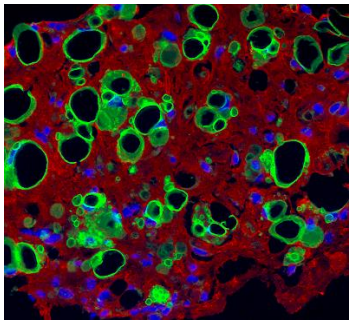
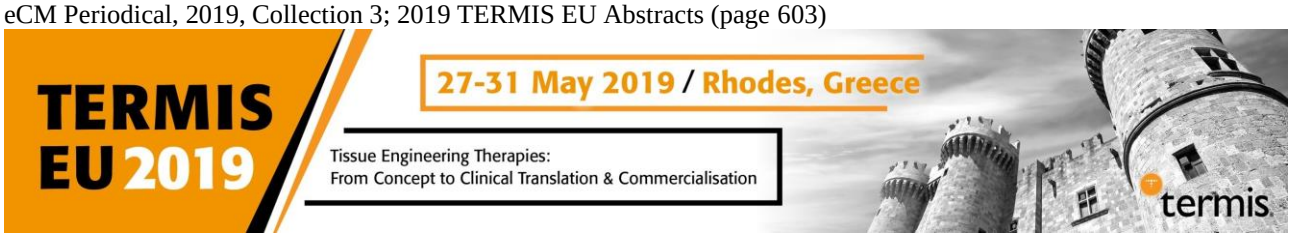


Figure 1: Immunohistochemical characterization of bioprinted adipose tissue: perilipin 1 (green), collagen I (red), nucleus (blue).



Poly(arginine) grafted to biphosphonated dendrons: A novel strategy to design antimicrobial coatings for biomaterial applications

L. Hugoni[1,2], D. V. Nguyen[3], N. Zydziak[4], F. Boulmedais[4], D. Felder-Flesch[3], P. Lavalle[1,2]
Presenting Author: Ludivine Hugoni, hugoni@unistra.fr
[1]INSERM UMR 1121, Strasbourg, France [2]Université de Strasbourg, Faculté de Chirurgie Dentaire, Strasbourg, France [3]CNRS UMR 7504, Strasbourg, France [4]CNRS UPR 22 Strasbourg, France

INTRODUCTION: After the introduction of biomaterials into the body, the surface of implants acts as an optimal substrate for bacterial growth, which increases the risk of developing infections. To modulate bacterial-surface interactions and prevent subsequent infections leading to the implant failure, one strategy consists in designing bioactive coatings on the surfaces. Herein, biphosphonated dendrons (BP-D) were deposited covalently on oxide surfaces. The BP-D presented specific -COOH end groups, which enable to easily functionalize the surfaces with antimicrobial biomolecules such as poly(arginine) (PAR) [1, 2]. Hence, promising coatings for antimicrobial applications were designed here.

METHODS: Prior to PAR functionalization, BP-D (1 mg/mL) were coated on the surface of SiO_2 sensors and the deposition was analyzed by Quartz Crystal Microbalance (QCM). PAR_{10} (1 mg/mL) was then grafted through EDC/NHS chemistry. To characterize the grafting on the BP-D coated surfaces, PAR_{10}-FITC was used and the mean intensity of fluorescence was analyzed by fluorescence microscopy (FM), after 24 h incubation in RPMI 1640 medium at 37 °C. Staphylococcus aureus suspension was deposited on the coated surfaces and incubated for 24 h at 37°C. Bacteria adhered to the surfaces were stained using the BacLight RedoxSensor CTC Vitality Kit, fixed with 4 % PFA to be further observed by FM.

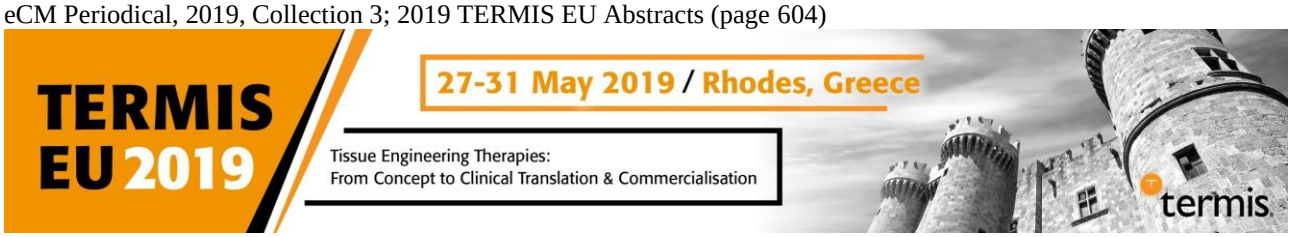
RESULTS & DISCUSSION: Through QCM analysis, optimal conditions for BP-D deposition on surfaces were determined (data not shown). The grafting of PAR_{10}-FITC on BP-D was then checked by FM: higher intensity of fluorescence was observed for the peptide covalently grafted than adsorbed. After the incubation of S. aureus on the coated surfaces, results indicated that only PAR_{10} grafted on BP-D presented a huge decrease of bacterial adhesion.

CONCLUSIONS: Taken together, these results showed the efficacy of PAR10 covalently grafted to BP D for antimicrobial coating. This constitutes a suitable strategy to functionalize surfaces and contribute to the success of implantable devices.

ACKNOWLEDGEMENTS: This study is co-funded through the project Nanotransmed by the European Regional Development Fund in the framework of the INTERREG V Upper Rhine program (Transcending borders with every project) & by the Swiss Confederation, the swiss cantons of Aargau, Basel-Landschaft and Basel-Stadt.

REFERENCES

[1] Özçelik et al. P. Adv. Healthc. Mater. 2015 4 2026-2036 [2] Mutschler et al. Chem. Matter. 2017 29 3195–3201



Growth-arrested adipose-tissue stromal cells and "physiological" hypoxia enhance ex vivo expansion of hematopoietic cells

L.B. Buravkova[1,2], I.V. Andrianova[1,2], E.A. Golikova[1], E.R. Andreeva[1]
Presenting Author: Ludmila Buravkova, buravkova@imbp.ru
[1]Cell Physiology Laboratory, Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia [2]Faculty of Basic Medicine, Moscow State University, Moscow, Russia

INTRODUCTION: The efficacy of long-term ex vivo expansion of stem and progenitor cells (HSPCs) is strongly depends on the feeder cell activity to mimic hematopoietic microenvironment [1, 2]. Here we demonstrated, that combination of mitomycin C (mmC)-induced growth arrest and tissue-related O_2 ("physiological" hypoxia) improved stromal capacity of adipose tissue derived stromal cells (ASCs).

METHODS: ASCs were permanently expanded at "physiological" hypoxia (5%) O_2, growth-arrested with mmC and cocultured with cord blood mononuclears as described earlier [3]. Immunophenotyping, multilineage commitment and RT-PCR analysis of mmC-ASCs were performed. Colony forming units (CFUs), cobblestone area forming cell (CAFCs) were used to detect mmC-ASC stromal activity.

RESULTS & DISCUSSION: MmC growth arrest did not affect viability, stromal phenotype and multilineage potential of ASCs permanently expanded at tissue-related O_2. PCR analysis revealed an up-regulation of genes, encoded molecules of cell-cell (ICAM1, HCAM/CD44) and cell-matrix adhesion (ITGs), extracellular matrix production (COLs) and remodeling (MMPs, HAS1) in growth-arrested ASCs at "physiological" hypoxia in comparison with ambient O_2 (20%). The number of ICAM-1 positive ASCs was increased under low O_2 as well. The share of $CD34^+$HSPCs, CFU number and CAFC colonies was higher at "physiological" hypoxia (Fig. 1). CAFCs considered as long-term culture-initiating cells (LTC-IC) known to support long-term hematopoiesis restoration in vivo.

CONCLUSIONS: Application of mmC-ASCs and "physiological" hypoxia as a feature of HSPC tissue niche provides pronounced stromal effects with predominance of very primitive HSPCs. That may be on demand to create the effective and controlled methodological approaches that ensure long-term production of a large number of HSPCs for regenerative medicine.

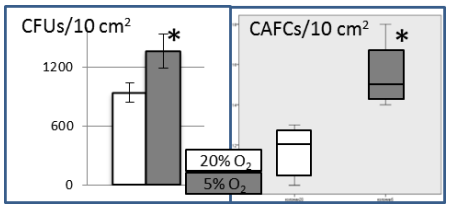
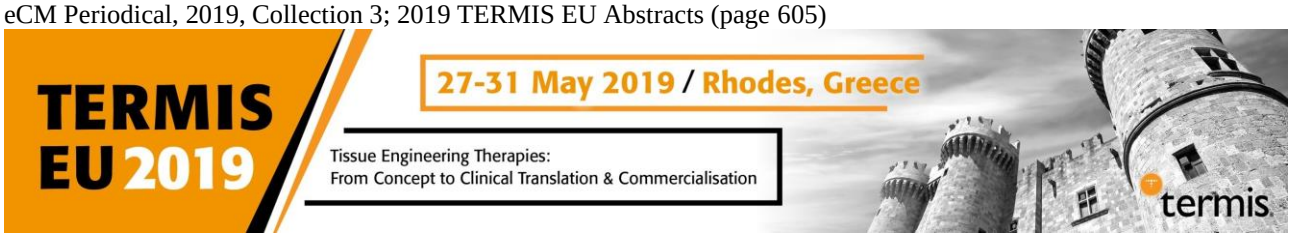


Figure. 1: Committed (CFUs) and primitive (CAFCs) HSPC expansion on mmC-ASCs

ACKNOWLEDGEMENTS: This work was supported in part by "Biomedical technologies: innovative research" Program N18 of Presidium of RAS.

REFERENCES

[1] Kumar S and Geiger H. Trends Mol. Med. 23, 2017;799–819
[2] Sotnezova et al. Acta Naturae. 2016;8:6-16.
[3] Andreeva et al. Plos One. 2015; 10(4):e0124939



Biofabrication of complex tissue scaffolds with controlled porosity and composition gradients

L. Diaz-Gomez[1,2,3], P.D. Kontoyiannis[1,2,3], A.J. Melchiorri[1,2,3], A.G. Mikos[1,2,3]
*Presenting Author: Luis Diaz-Gomez, luisdiaz@rice.edu
[1]Department of Bioengineering, Rice University, 6500 Main Street, Houston, TX 77030, USA [2]Biomaterials Lab, Rice University, 6500 Main Street, Houston, TX 77030, USA [3]NIH/NIBIB Center for Engineering Complex Tissues, USA

INTRODUCTION: the introduction of 3D printing in tissue engineering has allowed the development of intricated structures with high definition and reproducibility due to a precise control over the deposition of the printed materials. However, printing limitations have not allowed the development of radial porosity and composition gradients to mimic the structure of graded tissues, such as bone. The aim of this work was to develop a printing method based on the integration of additive manufacturing and computer-aided design to prepare porous scaffolds containing distinct compositions and porosities that resemble bone tissue structure.

METHODS: Composite poly(ε-caprolactone) (PCL)-based scaffolds of cylindrical form with radial gradients of hydroxyapatite (HA) (higher in the peripheral) and porosity (higher in the core) were printed using a segmented printing method based on the welding of segments containing discrete compositions and strand widths. Morphological and structural analyses were carried out by microCT and SEM. Mechanical tests were performed using a compressive bench. Scaffold mineralization was evaluated after incubation in simulated body fluid (SBF).

RESULTS & DISCUSSION: Tensile test revealed the integrity of the welded fibers and no fracture observed at 200% strain. Structure analysis showed no differences between the pore and strand size and porosity of each distinct region of the gradient scaffolds compared to homogenous controls. Core (67%) and outer (16%) regions showed porosities similar to human trabecular and cortical bone, respectively. HA distribution was homogeneous within each region and no particle aggregation was observed (Fig. 1). Compressive modulus was in the range of human trabecular bone for all compositions and dependent on pore size and HA content. Mineral deposition was significantly higher in the regions of the scaffolds with higher HA content.

CONCLUSIONS: The developed printing methodology allowed the preparation of discrete regions with composition and structural gradients similar to human bone tissue.

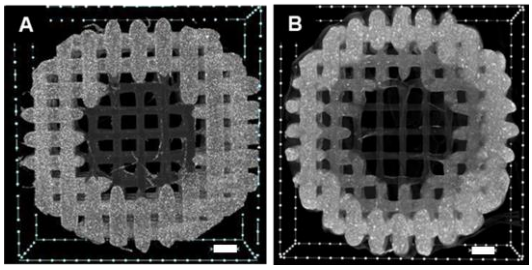
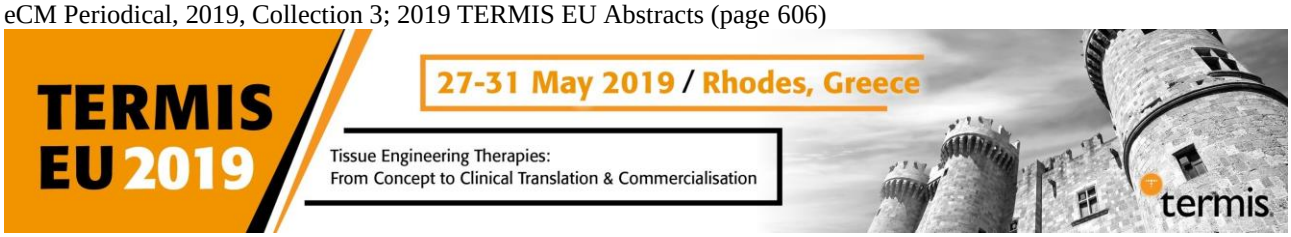


Figure 1: Cross-sectional images generated from microCT reconstructions of multimaterial scaffolds with two (PCL-HA30 and PCL; A) or three (PCL-HA30, PCL-HA15 and PCL; B) distinct regions with specific compositions and pore sizes. Scale bar=500 μm.

ACKNOWLEDGEMENTS: Work supported by the National Institutes of Health (P41 EB023833). L.D-G. acknowledges Xunta de Galicia for a Postdoctoral Fellowship (ED481B 2017/063).



Bioinstructive microparticles for tri-culture 3D tumor spheroids assembly

L. Ferreira[1], V. Gaspar[1], J.F. Mano[1]
Presenting Author: Luís Ferreira, lpferreira@ua.pt
[1]Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

INTRODUCTION: In vitro models capable of closely replicating the matrixial and cellular complexity of the tumor microenvironment (TME) in three-dimensional (3D) platforms are in high demand. In this context, human derived bone-marrow mesenchymal stem cells (hBM-MSCs) have been gathering importance, given their ambiguous influence in tumor progression. Herein, we engineered an hybrid 3D multicellular spheroid (3D-MCTS) based model, combining A549:Fibroblasts:hBM-MSCs with bioinstructive hyaluronan coated microparticles that serve as vehicles for inclusion of tumor-ECM analogous components, and as cell-anchoring hotspots.

METHODS: Distinct ratios of A549, fibroblasts and hBM-MSCs combined with layer-by-layer treated microparticles were seeded in ultra-low adhesion 96-wellplates. Optical microscopy, SEM, histology, viability, necrotic core formation, and CD44 expression analysis were conducted at 7 and 14 days. Optimized conditions were then subject to hBM-MSCs cell-tracking assays and Doxorubicin cytotoxicity screening to access resistance.

RESULTS & DISCUSSION: 3D microspheres provided proper support for cells to self-assemble into compact 3D microtissues and promoted an increase in CD44 expression, as seen in flow-cytometry analysis ($p > 0.05$). 3D-MCTS size and sphere-like morphology was highly reproducible and tri-culture models presented the characteristic solid tumors necrotic core. hBM-MSCs cells-tracking demonstrated a dynamic behavior dependent on present populations and ratios. Moreover, doxorubicin administration revealed hBM-MSCs positive effect on cytotoxic responses in 3D tri-culture models and in dual cultures of hBM-MSCs:A549 at 10:1 ratio.

CONCLUSIONS: Overall, these findings evidence the relevance of hBM-MSCs and cancer-stromal population inclusion in 3D in vitro tumor models, showcasing the importance of testing different cell-to-cell ratios to mimic tumor heterogeneity. Moreover, triculture 3D-MCTS with Hyaluronan-coated microparticles effectively allowed for the integration of pre-existing TME-ECM similar components

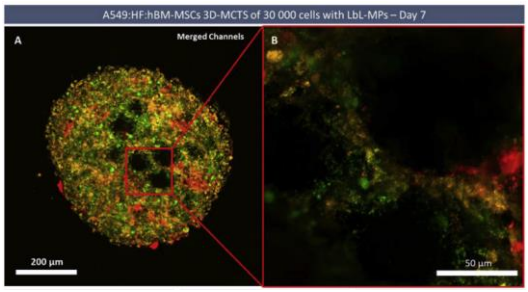
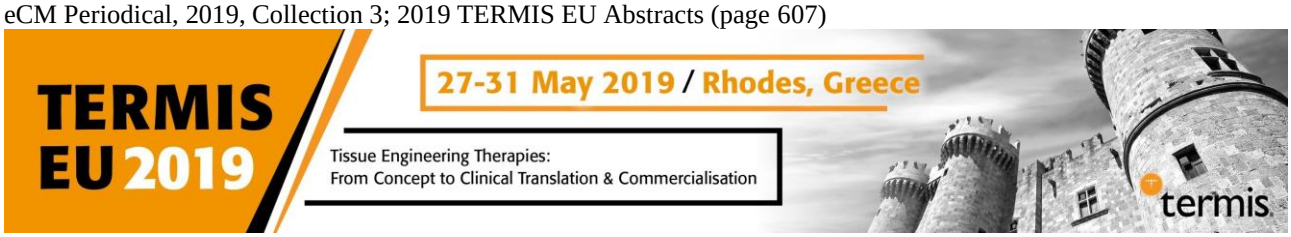


Figure 1: CLSM confocal imaging of cellular organization in tri-coculture LbL-MPs spheroids.

ACKNOWLEDGEMENTS: Financial support was received from the European Research Council grant agreement ERC-2014-ADG669858 for project ATLAS, and by the Portuguese Foundation for Science and Technology (FCT) via Post-PhD grant (SFRH/BPD/119983/2016, Vítor Gaspar), and via PhD grant (SFRH/BD/141718/2018, Luís Ferreira).



A co-culture system based on ASCs and HUVECs in GRGDS-modified gellan gum to promote neurite outgrowth and vascularization

L.A. Rocha[1,2,3], E.D. Gomes[1,2], R.A. Sousa[3], D.A. Learmonth[3], A.J. Salgado[1,2]
Presenting Author: Luís A. Rocha, id6526@alunos.uminho.pt
[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal [2]ICVS/3B's – PT Government Associate Laboratory, Braga, Portugal [3]Stemmatters, Biotecnologia e Medicina Regenerativa SA, Barco, Portugal

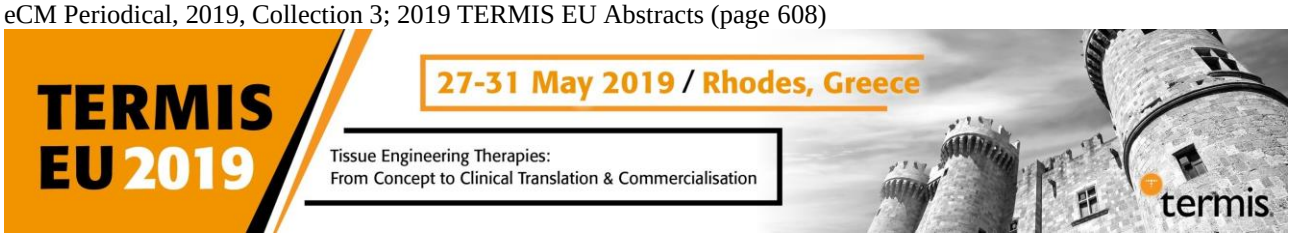
INTRODUCTION: Disruption of the blood spinal cord barrier is fundamental for the poor prognosis following spinal cord injury. Herein, we developed a hydrogel-based approach consisting on the co-culture of adipose-derived stem cells (ASCs) and human umbilical vein endothelial cells (HUVECs) to promote the vascularization of the injury site.

METHODS: Gellan gum modified with GRGDS (GG-GRGDS) was assessed for its angiogenic capacity in comparison to unmodified gellan gum (GG) and collagen (C) using the chick chorioallantoic membrane assay (CAM) for 4 days. Afterwards, we developed a co-culture system using ASCs and HUVECs encapsulated in GG-RGDS to assess its capacity in the neurite outgrowth of DRG explants. Cells were encapsulated in a 1:1 ratio (15000 of each/50 µL of hydrogel) 24 hours before DRG isolation and controls included each cell type cultured alone (using the same densities) and the hydrogel without cells. After DRG seeding, the culture system was maintained during 7 days. Immunocytochemistry allowed to evaluate the growth of neurites and vascular organization of HUVECs.

RESULTS & DISCUSSION: The number of vessels converging towards the hydrogels showed no significant differences between each experimental condition, with GG being followed by C and GG-RGDS. Growth of neurites using GG-RGDS hydrogels demonstrated that neurite occupied area was highest for the co-culture condition, being statistically significant from the condition without cells ($p<0.01$), similarly to the observed for ASCs. Longest neurite followed the same trend, but without statically significant differences ($p<0.05$). Interestingly, HUVECs clearly tended to organize into vascular-like structures in the presence of ASCs within the GG-RGDS, demonstrating the beneficial effects of the presence of these mesenchymal stem cells for SCI vascularization.

CONCLUSIONS: Under the experimental conditions reported, GG-RGDS did not promote increased vascularization using the CAM model. Still, GG-RGDS provided a suitable environment for ASCs and HUVECs encapsulation and neurite outgrowth of DRG explants, in which the presence of ASCs appeared to be pivotal for the arrangement of HUVECs in vascular-like structures. This co-culture system using GG-RGDS demonstrated potential to be further evaluated in vivo in SCI animal models for its capacity of supporting neurite outgrowth and vascular organization at the injury site.

ACKNOWLEDGEMENTS: Financial support by Prémios Santa Casa Neurociências – Prize Melo e Castro for Spinal Cord Injury Research; Portuguese Foundation for Science and Technology. This work has been supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership. ASCs were kindly provided by LaCell, USA.



A novel drug-eluting biomaterial for tracheal tissue engineering

L. Soriano[1,2,3,4], C. O'Leary, C, A. Fagan-Murphy[1,2,3,4],G. Hendy[6], F.J. O'Brien[2,3,4,5], S.A. Cryan[1,2,3,4,5]

Presenting Author: Luis Soriano, luissoriano@rcsi.com

[1]School of Pharmacy and [2]Tissue Engineering Research Group, Royal College of Surgeons in Ireland (RCSI), Ireland [3]Centre for Research in Biomedical Devices (CÚRAM), NUI Galway, Ireland [4]Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Ireland [5]Advanced Materials & Bioengineering Research (AMBER) Centre, RCSI & TCD, Ireland [6]Spraybase®, Avectas, Maynooth, Ireland.

INTRODUCTION: Extensive tracheal damage remains an unmet clinical need as implantable treatment options do not adequately restore a fully functional respiratory tract [1]. The major objective of this study was to develop nanofibrous chitosan-coated polycaprolactone (PCL-CS) scaffolds loaded with all-trans retinoic acid (atRA) and to assess their suitability for tracheal tissue engineering including (i) their biocompatibility and (ii) the effect of atRA on the differentiation of respiratory epithelial cells seeded on the scaffolds.

METHODS: 10% PCL and 5% CS were electrospun (Spraybase®) and atRA was incorporated at three different concentrations (10, 1 and 0.1µg/mg of scaffold). Biocompatibility of atRA-loaded PCL-CS scaffolds was assessed using Calu-3 bronchial epithelial cells under air-liquid interface (ALI) for 14 days. Genetic expression of biomarkers for mucus production (MUC5AC) and cilia formation (FOXJ1), immunocytochemistry and transmission electron microscopy (TEM) were all performed to evaluate and determine epithelial differentiation.

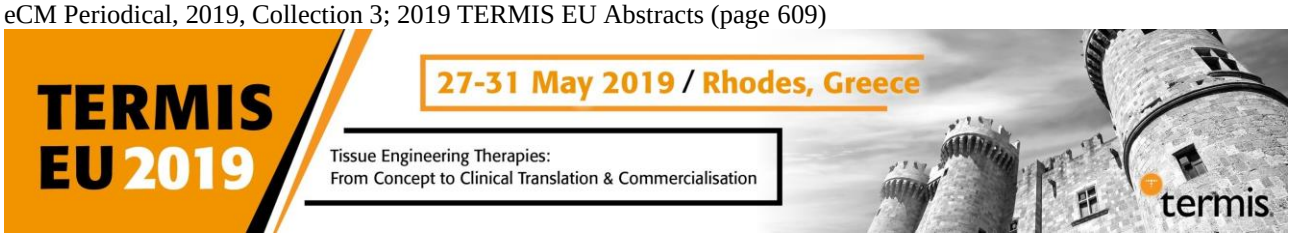
RESULTS & DISCUSSION: atRA-loaded PCL-CS fibres were successfully produced by electrospinning with diameters similar to reported native trachea tissue fibres. All atRA concentrations in the scaffolds supported cell growth and demonstrated comparable biocompatibility to drug-free scaffolds. The incorporation of atRA in the PCL-CS fibres enhanced mucin expression over atRA-free scaffolds and ciliation was observed in all the scaffolds groups.

CONCLUSIONS: atRA-loaded PCL-CS fibrous scaffolds were successfully electrospun as a biocompatible material capable of supporting improved airway epithelial cell differentiation and therefore with potential for application in tracheal tissue engineering.

ACKNOWLEDGEMENTS: Srpaybase®, Avectas. Financial support was received from Science Foundation Ireland (SFI) (Grant Number 13/RC/2073).

REFERENCES

[1] O'Leary C et al. Tissue Engineering Part B: Reviews. 2015 Feb 27;21(4):323-44



Absence of differences in activation of innate immune response by low, middle, and high molecular weight hyaluronan in vitro

L. Kubala[1,2], K. Nešporová[3], B. Šafránková[3], M. Hermannová[3], V. Velebný[3]
Presenting Author: Lukáš Kubala, lukas.kubala@fnusa.cz
[1]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic
[2]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[3]Contipro a.s. Dolni Dobrouc, Czech Republic

INTRODUCTION: Hyaluronan (HA), a linear polysaccharide ubiquitously present in the human body, is commonly employed in tissue engineering due to good biocompatibility [1]. HA potential to activate immune cells and overall to induce adverse immune response are suggested to be dependent on HA molecular weight (MW). Low MW HA should activate immune cells in contrast to high MW HA. Thus, degradation of high MW HA based hydrogel matrices to low MW HA fragments theoretically could promote inflammatory reactions in the body and have negative impact on regeneration potential of HA based materials. However, some current studies do not support this conception and emphasize the importance of the form of preparation of HA, particularly with respect to its purity and origin.

METHODS: We compared the activation of mouse immune cells by HA samples (100, 500, and 997 kDa) prepared from HA originating from rooster comb, and HA samples (71, 500, and 1000 kDa) prepared from pharmacological grade HA originating from Streptococcus equi.

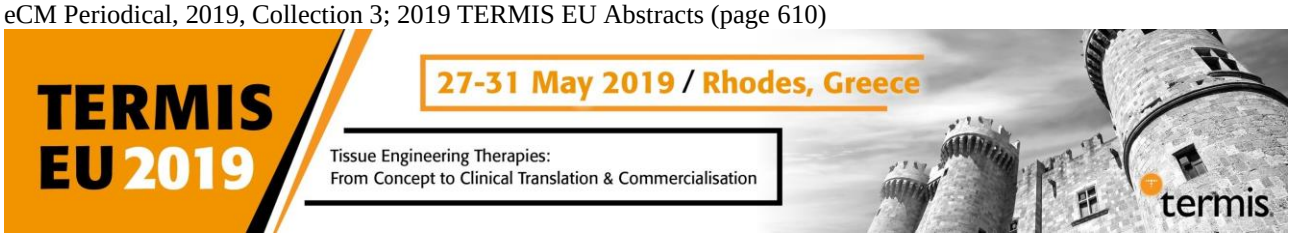
RESULTS & DISCUSSION: In contrast to established theory, only middle and high MW HA originating from rooster comb induced the production of tumor necrosis factor-α by macrophages and in whole blood. Further, all tested preparations of HA failed to induce the expression of inducible nitric oxide synthase, the production of nitric oxide, or the expression of cyclooxygenase 2 in macrophages and splenocytes [2,3]. Importantly, all HA samples originating from rooster comb were found to be contaminated by endotoxin (up to 1.23 EU/ml).

CONCLUSIONS: Hence, low MW HA did not reveal itself to have significantly higher immunostimulatory activity to mouse immune cells compared to middle and high MW HA [2,3]. Similarly, our other study employing human blood innate immune cells showed only very limited stimulatory potential of low MW HA compared to middle and high MW HA [4]. Thus, this suggest that the theory of significant pro-inflammatory potential of low MW HA should be revised. Overall, low MW HA should not have a significant potential to induce adverse immune inflammatory response.

ACKNOWLEDGEMENTS: This work was supported by MEYS CR (project OP VVV INBIO CZ.02.1.01/0.0/0.0/16_026/0008451).

REFERENCES

[1] Huerta-Ángeles G, et al. Front Bioeng Biotechnol. 2018 17(6), 62.
[2] Šafránková B et al. Int J Biol Macromol. 2018;107(Pt A),1-8.
[3] Krejcova D et al. Neuro Endocrinol Lett. 2009; 30 (Suppl 1), 106-11.
[4] Safrankova B et al. Mediators Inflamm. 2010; 2010:380948.



Magnetic nanoparticles loaded human adipose derived stem cells as multimodal tools for regenerative medicine

L. Labusca [1,3], D. D. Herea[1], C Danceanu[1,2], H. Chiriac[1], N. Lupu[1]
Presenting Author: Luminita Labusca, llabusca@phys-iasi.ro
[1]National Institute of Research and Development for Technical Physics (NIRDTP) Iasi, Romania
[2]Alexandru Ioan Cuza University of Iasi [3]Emergency County Hospital Saint Spiridon Iasi, Romania

INTRODUCTION: Iron oxide based magnetic nanoparticles (MNPs) are versatile tools in biology and medicine. MNPs-mediated drug delivery is tested for regenerative medicine (RM) purposes as well as for tumor treatment and diagnostics. Stem cell mediated delivery represents a modality to target remote, metastatic tumors or regenerative sites for controlled drug delivery. The magnetic behavior of MNPs allows their use as contrast agents for MRI. Moreover, remote controlled actuation of MNPs-loaded cells can deliver micro-mechanical stimulation for enhancing adipose derived stem cells differentiation potential.

METHODS: Human adipose derived stem cells (ADSCs) were loaded with proprietary palmitic acid coated magnetite (MNP-PA) ADSCs-MNP-PA complexes were tested for cell viability, proliferative capabilities and magnetic properties. In vitro osteogenic and adipogenic potential of ADSCs-MNP exposed to magnetic field (MF) was tested. ADSCs loaded with MNP-PA and anti-tumor drug mitoxantrone (MIT) were used for in vitro targeting osteosarcoma tissue-like structures.

RESULTS & DISCUSSION: MNP-PA and MNP-MIT were shown to be up-taken by ADSCs. ADSCs-MNP-PA and ADSCs-MNP-PA-MIT complexes retained cell viability and proliferative capabilities compared to non-loaded ADSCs and displayed high magnetization. ADSCs–MNP-PA-MIT were able to target tumor osteosarcoma tissue-like structures in vitro. Distinctly, increased osteogenesis was displayed by ADSC-MNP in MF compared to non-loaded as well as non-MF exposed cells.

CONCLUSIONS: ADSCs loaded with MNP-PA-MIT were able to target tumor osteosarcoma tissue-like structures in vitro. Present results support the design of stem cell mediated delivery of MNP-PA with or without MIT for targeted drug delivery. Also, MF exposure increased osteogenesis in ADSCs-MNPs, fact that needs to be confirmed in vivo.

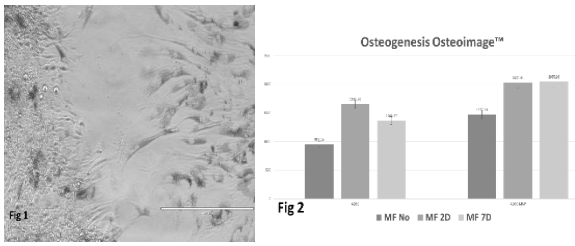
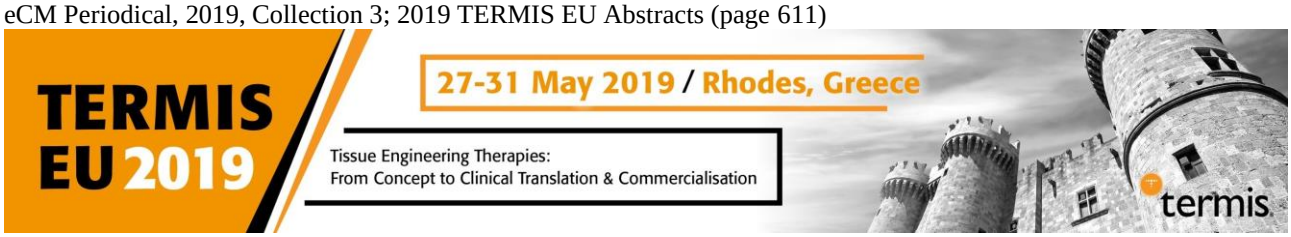


Figure 1: ADSCs-MNP-PA-MIT complexes target osteosarcoma (OS) three-dimensional structures in vitro. Figure 2 shows an increased osteogenesis in ADSCs-MNPs exposed to remote actuation using MF after 2 days exposure (MF2D) and 7 days exposure (MF7D) compared to non-exposed ADSCs-MNP (MF No).

ACKNOWLEDGEMENTS: Financial support was received from Nucleu Proiect PN 19 28 01 01 financed by ANCS Romania



Designing microparticles for tissue engineering: Varying surface topography of polymeric microparticles influences mesenchymal stem cell fate

M. Amer[1], M. Alvarez-Paino[1], K. Shakesheff[1], D. Needham[1,2], M. R. Alexander[1], C. Alexander[1], F. R. Rose[1]
Presenting Author: Mahetab Amer, mahetab.amer@nottingham.ac.uk
[1]School of Pharmacy, University of Nottingham, Nottingham, UK [2]Department of Mechanical Engineering and Material Science, Duke University, North Carolina, USA

INTRODUCTION: Material properties are capable of influencing cell behaviour[1]. The aim of this study was to examine the influence of tailored microparticle design by varying surface topography on stem cell response, which is critical for their use as cell delivery systems for regenerative medicine applications.

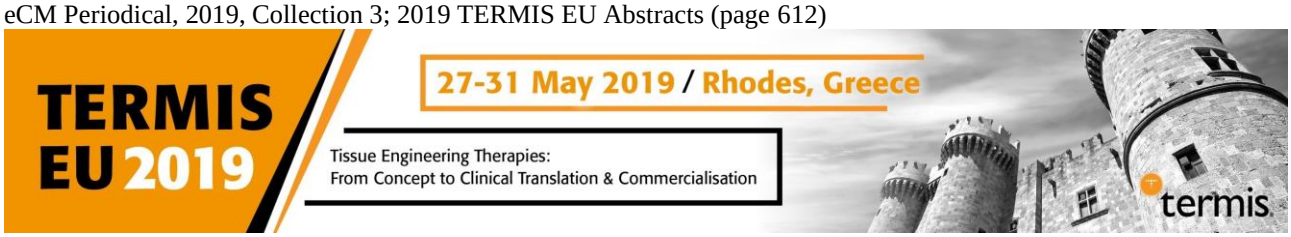
METHODS: Textured PLA microparticles were produced by exploiting phase separation of fusidic acid from PLA during loss of solvent from an oil-in-water emulsion. Fusidic acid then dissolves leaving textured surfaces (Fig 1A). By varying emulsion settings, microparticles of two key morphologies were produced: dimpled 'golf ball' and angular morphologies. To investigate the influence of microparticle topography on cell response, a planar presentation of the particles was developed by heat sintering them into discs before cell culture. The influence of topographical features on attachment and proliferation of primary human mesenchymal stem cell (hMSCs) was investigated. Markers of osteogenesis and comparative gene expression analysis were also assessed in the absence of osteo-inductive supplements.

RESULTS & DISCUSSION: Cell morphology was influenced by the different topographies, with cells spreading on smooth surfaces and adopting more rounded morphologies on dimpled microparticles. In the absence of osteo-inductive supplements, cells cultured on microparticles with dimples exhibited notably increased expression of osteocalcin and mineralisation levels relative to smooth microparticles. Differential gene expression was observed for hMSCs on microparticles compared to 2D-cultured controls. A number of genes were also differentially expressed between smooth and dimpled microparticles.

CONCLUSIONS: Topographically-textured microparticles of varying microscale features were used to investigate stem cell adhesion and subsequent differentiation. This study shows the sensitivity of hMSC attachment and differentiation to microparticle surface topography. It highlights the importance of tailoring topographical design of microparticles and offers the opportunity to control stem cell fate by inducing osteogenesis without use of exogenous osteo-inductive factors.

REFERENCES

[1] Unadkat HV et al. PNAS. 2011;108(40): 16565-70



Deciphering tendons: A systematic review of tendon-specific genes and uncovering the roles of tenomodulin in tendon healing

M. Delgado Cáceres[1], D. Lin[2], D. Docheva[1]
Presenting Author: Manuel Delgado Cáceres, manuel.delgado-caceres@ukr.de
[1]Experimental Trauma Surgery, Department of Surgery, University Regensburg Medical Centre (UK Regensburg), Regensburg, Germany [2]Department of Orthopaedic Surgery, the affiliated Southeast Hospital of Xiamen University, Zhangzhou, China

INTRODUCTION: The establishment of a functional musculoskeletal system depends on a well synchronized development of muscle, tendon and cartilage/bone. The transcription factors Scleraxis and Mohawk as well as Tenomodulin (TNMD, Tnmd), a glycoprotein with cleavable C-terminus, have been identified as key players in tenogenesis. The aims of this study were first, to perform a systematic review of publications based on transgenic mouse models to uncover tendon-specific genes, and second, by surgically induced injury of the Achilles tendons of Tnmd knockout (KO) strain to investigate the role of this gene in the tendon repair process.

METHODS: The search for literature was performed via Pubmed using the terms “tendon”, “tendon phenotype”, “mouse knockout” and “transgenic mouse”. For the injury model of Achilles tendon [2], skeletally mature Tnmd wild-type (WT) and Tnmd-KO mice were used and repair outcomes were assessed at day 8, 21 and 100 (n = 8/genotype/time point) by H&E for overall tissue morphology and BrdU staining for cell proliferation. At day 8, tissue sections were stained with perilipin for fat deposition and collagen IV for blood vessels, and with CD68, CD80, CD163 and F4/80 for macrophage detection.

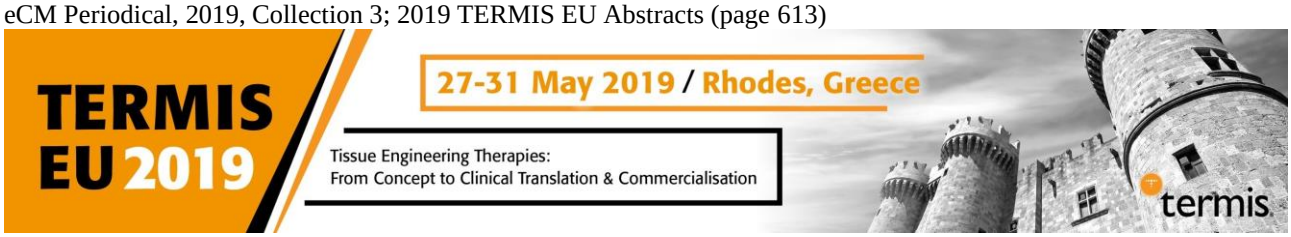
RESULTS & DISCUSSION: Our literature screen revealed 24 tendon-specific genes and in 22 of the respective knockout mouse models, phenotypic changes were detected. Only Scleraxis and TGFbeta-deficient mice presented severe tendon developmental changes, while others, including Tnmd-KO mice, exhibited mild phenotypes [1]. Regarding the Tnmd injury model, histomorphometrical analysis at day 8 revealed inferior scar organization, reduced cell density and large areas filled with fat and blood vessels. Furthermore, Tnmd-KO Achilles tendon scars exhibited an atypical macrophage profile dominated by M1 macrophages. At day 21 and 100, macroscopic observation suggested a persistent delayed repair of the Achilles joint as well as a fattening atrophy of the gastrocnemius muscle. Detailed histomorphometrical analysis of the Achilles tendons at the above time points are currently on the way.

CONCLUSIONS: Our literature search shows that the tendon developmental program is greatly backed up and compensatory effects are common among proteoglycans. Our experimental study demonstrates that the mild phenotype of Tnmd-KO can be greatly challenged by surgical intervention, thus revealing the Tnmd is important for tendon healing as it prevents accumulation of vessels and fat, and regulates the activity of cells during scar formation and remodeling.

ACKNOWLEDGEMENTS: Financial support was received from German Research Foundation, (D.D. Grant Nr. DO1414/3-1).

REFERENCES

[1] M. Delgado Caceres et al. Stem Cells Dev 2018; 27(17):1161-1174
[2] D. Lin et al. Cell Death Dis. 2017; 8(10):e3116



Tropoelastin coated tendon biomimetic scaffolds promote tenogenic commitment of stem cells and deposition of elastin-rich matrix

H. Almeida[1,2], M. Gomez-Florit[1,2,3], R. M. A. Domingues[1,2,3], S. M. Mithieux[4,5], R. A. Pires[1,2,3], R. L. Reis[1,2,3], A. S. Weiss[4,5,6], M. E. Gomes[1,2,3]
Presenting Author: Manuel Gomez-Florit, mgflorit@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal [4]Life and Environmental Sciences, University of Sydney, Australia [5]Charles Perkins Centre, University of Sydney, Australia [6]Bosch Institute, University of Sydney, NSW 2006, Australia.

INTRODUCTION: Tendon is mainly composed of densely packed collagen type-I fibers and elastic fibers parallelly oriented among them, which contribute for its elastic function, structural integrity and resilience. Tendon tissue engineering strategies have often recreated instructive substrates that mimic the anisotropically aligned nano-to-macro structure of the native tissue. Here, we hypothesize that the incorporation of tropoelastin, the soluble precursor of elastin, as a surface coating of tendon mimetic scaffolds will positively modulate cell performance, influencing stem cell differentiation towards the tenogenic lineage and matrix deposition.

METHODS: Continuous and aligned electrospun nanofiber yarns [1] were functionalized with recombinant mature wild-type human tropoelastin (Weiss Lab, Australia) through polydopamine (PDA) tethering. Uncoated and PDA-coated yarns were used as control groups. The coatings were characterized using different techniques. Cell response was evaluated using human adipose-derived stem cells (hASCs).

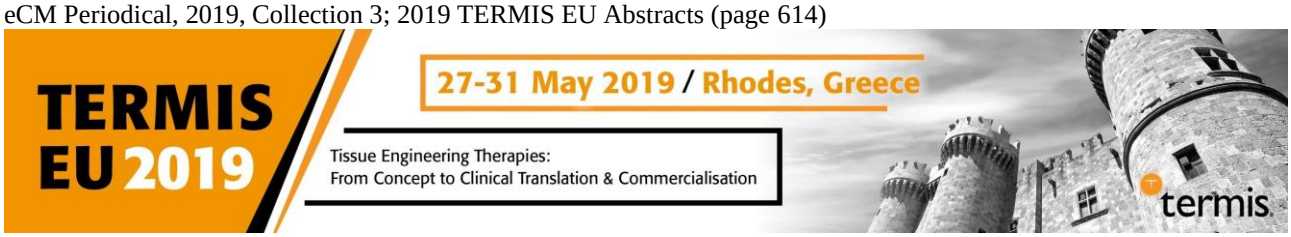
RESULTS & DISCUSSION: After 5 days of culture, hASCs were more elongated and spread on tropoelastin-coated samples, in which the cell aspect ratio is significantly superior to the controls ($p < 0.001$). Immunocytochemistry analysis also suggests that tropoelastin enhances the tenogenic commitment with higher scleraxis expression from day 5 up to day 21, when compared with the other conditions. Additionally, the presence of tropoelastin on the yarns stimulated elastic fiber assembly, with evidence of elastogenesis at day 14 and with visible elastic fibers at day 21, along with collagen type-I deposition. Last, preliminary results suggest that the deposited extracellular matrix presents a higher elastic behavior ($p < 0.0001$) for the tropoelastin-coated samples, compared with the other samples.

CONCLUSIONS: Overall, data suggests that the presence of tropoelastin enhances tenogenic commitment by hASCs, while induces elastic fiber assembly, relevant for tendons physiology

ACKNOWLEDGEMENTS: EU's H2020 programme agreements 706996, 739572, 692333, 668983 and BEAM Program; FCT for SFRH/BPD/ 112459/2015 and NORTE-01-0145-FEDER-000021; Hospital da Prelada (Portugal).

REFERENCES

[1] Laranjeira M et al. Small. 2017; 13:1-13



Imprecise lineage definition associates with functional dissimilarity observed between iPSC-derived MSCs and primary MSCs

M. Xu[1], G. Shaw[1], M. Murphy[1], F Barry[1]
Presenting Author: Maojia Xu, m.xu1@nuigalway.ie
[1]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUIG), Galway, Ireland

INTRODUCTION: Induced pluripotent stem cells (iPSCs) have been considered as a potential alternative source for generating mesenchymal stem cells (MSCs) to meet increasing demands for MSCs in both research and therapeutic applications. Based on the minimal criteria for defining MSCs published by the International Society for Cellular Therapy (ISCT standard) [1], several different protocols have claimed success in the generation of MSCs from iPSCs (iMSCs). However, questions have arisen relating to the phenotypic fidelity of iMSCs because, irrespective of the derivation methods used, the cells generally have less capacity for adipo- and chondro-genesis when compared with primary MSCs. The objective of this study was to understand the cellular mechanism behind these discrepancies.

METHODS: iMSCs were derived using the embryoid body-based outgrowth method. We compared the differentiation ability, immunophenotype and gene expression profiles (GEPs) between multiple iMSC and BM-MSC lines. In addition, the impact of culture expansion on cell state was evaluated.

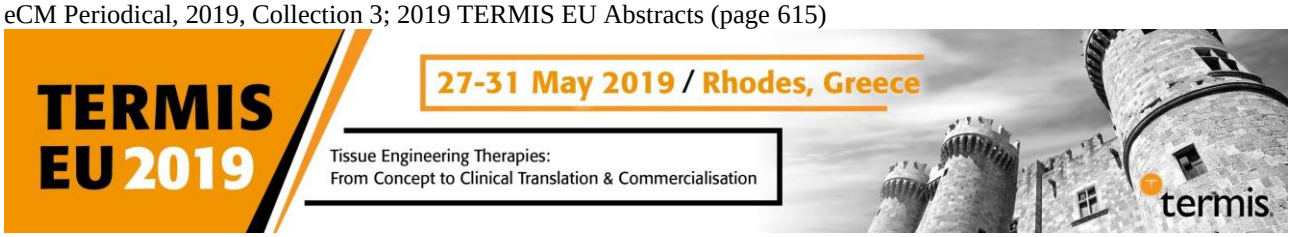
RESULTS & DISCUSSION: Preliminary characterization confirmed that iMSCs met the ISCT standard. However, in consistent with previous findings, the cells displayed significantly negligible adipogenic and chondrogenic capacity compared to BM-MSCs. GEPs analysis revealed that iMSCs expressed very high levels of vascular progenitor cell (VPC) genes (KDR and MSX2), whereas BM-MSCs had significantly greater levels of PDGFRα, a marker of paraxial mesoderm. These distinct GEPs were maintained during culture expansion, indicating that, unlike BM-MSCs, iMSCs were more closely related to VPCs.

CONCLUSIONS: The current understanding of VPCs provides a rationale to explain why iMSCs could meet the ISCT standard but displayed altered differentiation propensity compared to BM-MSCs. Firstly, VPCs and MSCs have partially overlapping immunophenotype, which makes them indistinguishably when examined by typical MSC markers, including CD73, CD90 and CD105. Secondly, although VPCs can differentiate along the osteogenic, chondrogenic and adipogenic pathways, such lineage commitment may not be accomplished in MSCs preferred differentiation condition, particularly for the latter two.
In summary, our results suggest a lineage misidentification of iMSCs, indicate the inadequacy of using the ISCT standard to distinguish different mesodermal progenitors and emphasize a necessity to validate the iMSCs identity with various lineage markers.

ACKNOWLEDGEMENTS: This work was supported by Science Foundation Ireland (SFI 09/SRC.B1794) and the Irish Research Council (GOIPG/2014/96). This study has also received funding from the European Union's Seventh Framework Programme for Research, Technological Development and Demonstration under grant agreement no.223298.

REFERENCES

[1] Dominici M et al. Cytotherapy. 2006; 8:315-31



Bioactive mesoporous silica nanoparticles for stem cell differentiation in bone regeneration

M.T. Tavares,[1,2], M.B. Oliveira[2], V.M. Gaspar[2], J.F. Mano,[2] J.P. S. Farinha,[1] C. Baleizão[1]
Presenting Author: Márcia T Tavares, marcia.tavares@tecnico.ulisboa.pt
[1]CQFM, Centro de Química-Física Molecular, IN—Institute of Nanosciences and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal [2]Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

INTRODUCTION: Stem cells differentiation is usually performed in vitro by exposing stem cells to specific pro-osteogenic pharmaceutics. As an alternative to the inefficient current strategies, one can use carriers containing such factors, which can be internalized by the cells [1]. These factors, once released inside adult stem cells, induce bone cell proliferation and differentiation, and stimulate the expression bone-related proteins [2][3].

METHODS: Bioactive silica mesoporous nanoparticles containing calcium and phosphate ions on their surface and Dexamethasone in their pores (MSN-CaP-Dex) were synthesized by a modified sol-gel method. 100 µg. mL^{-1} of MSN-CaP-Dex were administered to human bone marrow mesenchymal stem cells (hBMSCs), Nanoparticles uptake, cytotoxicity and osteogenic differentiation potential were evaluated in hBMSCs up to 21 days.

RESULTS & DISCUSSION: The MSN-CaP-Dex NPs were delivered to hBMSCs and successfully internalized after 24 h (Fig.1A). Osteocalcin immunoassays revealed the presence of this early marker (Fig.1B1-3) and mineralization studies evidenced the existence of calcium deposition (Fig.C1-3). Stem cells incubated with MSN-CaP-Dex NPs showed higher levels of alkaline phosphatase at day 7 than the cells with osteogenic medium. All results show a significant difference to basal medium.

CONCLUSIONS: The MSN-CaP-Dex NPs showed the ability to induce osteogenic differentiation to levels similar or even higher than osteogenic supplementation. Therefore, MSN-CaP-Dex bio instructed stem cells towards osteogenic lineage in basal conditions and with a single dose.

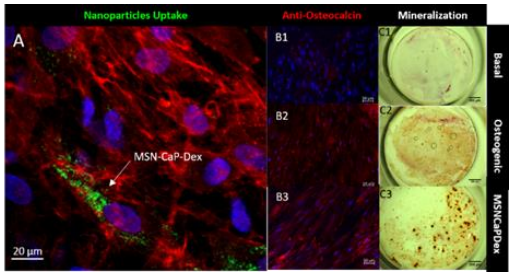
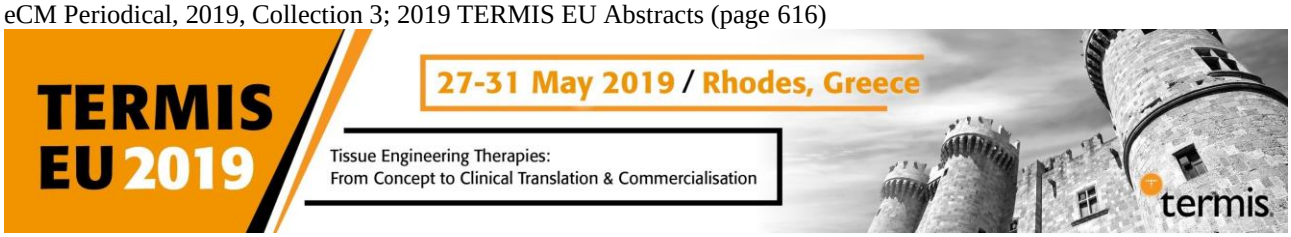


Figure 1: A – MSN-CaP-Dex NPs uptake after 24h, (B1-3) osteocalcin immunolabelling after 14 days of culture, (C1-3) Alizarin red mineralization assay after 21 days of culture.

ACKNOWLEDGEMENTS: Fundação de Ciência e Tecnologia (FCT), Portugal, for the PhD grant PD/BD/114019/2015.

REFERENCES

[1] L. L. Hench Science 2002; 80(295):1014–1017.
[2] Á. J. Leite, et al J. Non. Cryst. Solids 2016; (432):158–166.
[3] Z. Hong et al J. Biomed. Mater. Res. - Part A 200; 88(2):304–313.



Silk fibroin vascular graft: From design to in vitro and in vivo tests

M. Biagiotti[1], A. Chiarini[2], A. Alessandrino[1], I. Dal Prà[2], G.A. Bassani[1], V. Vincoli[1], G. Freddi[1], U. Armato[2]
Presenting Author: Marco Biagiotti, marco@silkbiomaterials.com
[1]Silk Biomaterials Srl, Lomazzo (Co), Italy [2]Human Histology & Embryology Unit, University of Verona Medical School, Verona, Italy

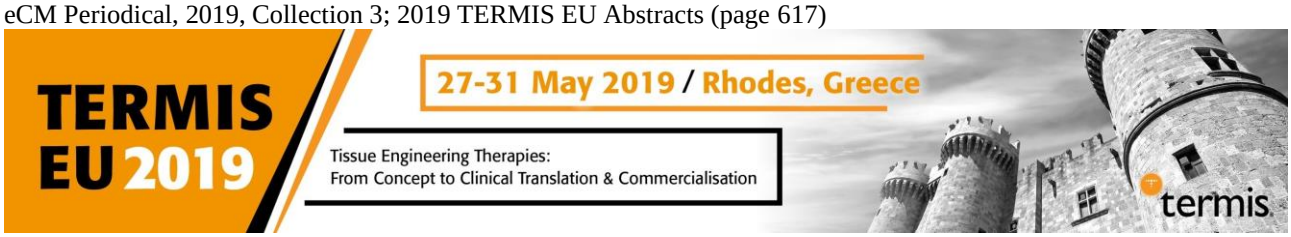
INTRODUCTION: Cardiovascular diseases are the leading cause of death worldwide. The availability of grafts for the treatment of vascular diseases is a real and urgent need. Simulating the three-layered structure of native blood vessel in a biomaterial scaffold is seen as an effective way to mimic the native architecture, the mechanical behavior, and functional features of native arteries. In this study, production and characterization of a novel multi-layered Silk Fibroin Vascular Graft (SF-VG) is reported. In vitro cell interaction studies were performed to investigate the biological response to the device. The results of in vivo preliminary pilot trials on large animals are presented.

METHODS: The SF-VG was manufactured according to a patented technology (WO 2016/067189 A1). Adult Human Coronary Artery Endothelial Cells (HCAECs), Human Aortic Smooth Muscle Cells (HASMCs), and Human Aortic Adventitial Fibroblasts (HAAFs) were used for the in vitro tests. D-Glucose, L-Glutamine, Lactate, the release of type I collagen, and secretion of various cytokines/chemokines were assessed. SF-VGs were implanted along the carotid arteries of female minipigs and sheep and 4 weeks later were explanted and subjected to histopathological analysis.

RESULTS & DISCUSSION: The SF-VG device was manufactured by combining electrospinning and knitting/braiding technologies. Electrospun and textile layers resulted perfectly integrated and did not show slipping or separation when exposed to mechanical stresses. The results of in vitro cell interaction studies demonstrated the biocompatibility of SG-VG. The cumulative curves of consumed glucose and glutamine and released lactate matched the metabolic activities and the expansion of the respective cell populations. HAAF cells significantly curtailed their de novo production of type I Collagen, with a remarkable antifibrogenic upshot, significantly relevant in clinical settings. Interleukin-6 stands out, being the most intensely secreted cytokine. The high basal secretion of Monocyte Chemoattractant Protein-1 and Monocyte Chemoattractant Protein-2 was indicative of a cell proliferation capacity. Interferon gamma-induced protein 10 (known for its antifibrotic and angiostatic properties) and Regulated on Activation, Normal T Cell Expressed and Secreted (known for its ability to regulate leucocyte diapedesis, angiogenesis, and some scarring processes) were expressed at significant levels. Finally, the pilot animal studies showed the feasibility of using SF-VG as vessel grafts in vivo. No lumen stenosis and a persistently good blood flow was observed.

CONCLUSIONS: SF-VG is a small caliber vascular graft entirely made of pure silk fibroin. The fabrication technology allowed manufacturing a multi-layered scaffold characterized by easy handling during surgery and able to avoid the emergence of any biomechanical mismatch at the implantation site. In vitro cell interaction studies showed that the device favor cell adhesion, survival and growth. Long-term in vivo studies to assess the patency and wall restructuration of grafted SF-VGs are already under course in sheep.

ACKNOWLEDGEMENTS: The authors would like to address heartfelt thanks to Dr. Hu Peng (from the First Affiliated Hospital of Zunyi Medical University, China and currently PhD Student in Verona's Laboratory, Italy) for his skillful handling of human cell lines.



Continuous gradient hydroxyapatite-based 3D additive manufactured scaffolds for bone interface tissue engineering

M. Cámara-Torres[1], R. Sinha[1], A. Sánchez[2], A. Patelli[3], C. Mota[1], L. Moroni[1]
Presenting Author: María Cámara-Torres, m.camaratorres@maastrichtuniversity.nl
[1]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands [2]Fundación Tecnalia Research and Innovation, Advanced Materials Department, Sustainable Construction Division, Parque Científico y Tecnológico de Gipuzkoa, Donostia-San Sebastián, Spain [3]Department of Physics and Astronomy, Padova University, Padova, Italy

INTRODUCTION: Hard-to-soft tissue interfaces, such as bone-to-cartilage, exhibit continuous spatial gradients in extracellular matrix composition, which provide unique structural and mechanical properties in the tissue interface. [1] For instance, the concentration of hydroxyapatite (HA), as one of the main bone components, gradually decreases towards the soft tissue. [2] Here, we aim at creating scaffolds with continuous gradients in HA composition to mimic native hard to soft tissue interfaces. The effect of different HA concentrations on the osteogenic differentiation of human mesenchymal stromal cells (hMSCs) is evaluated.

METHODS: Composites of the copolymer PEOT/PBT with different HA concentrations (45 and 20 wt%) were prepared by solvent blending. Using our newly developed AM technology, continuous gradient scaffolds (pores xy = 500 um, pores z= 250 um) with varied HA concentration from 0 to 45 wt% along the axial direction were fabricated, in which the neat PEOT/PBT (i.e. no HA) and the 45 wt% HA composites were continuously mixed during the printing process. Initially, mono-concentration scaffolds (45, 20 and 0 wt% HA) were seeded with hMSCs and cultured for 35 days in basic (FBS + ASAP) or mineralization media (basic media + bGP + dexamethasone) and evaluated as control scaffolds. DNA content and ALP activity of hMSCs were quantified at different time points. Alizarin red staining (ARS) was assessed at the end of the culture period.

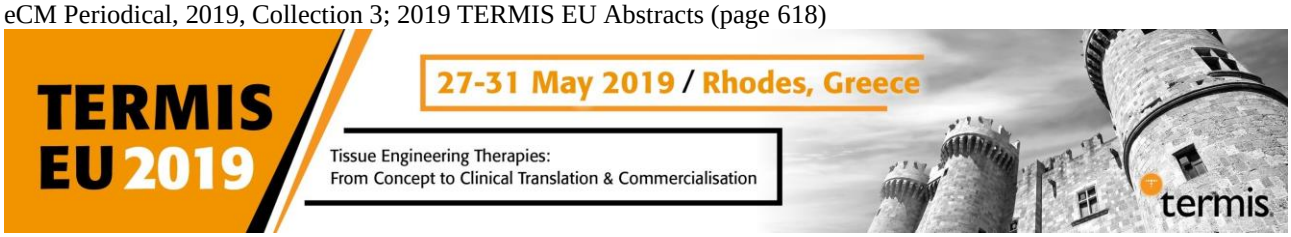
RESULTS & DISCUSSION: DNA quantification demonstrated no significant differences ($p<0.05$) in cell attachment among scaffolds, in spite of the HA concentration. Importantly, ALP activity measurements (day 14, day 21 and day 35) illustrated a gradual decrease in ALP production over the course of the culture in scaffolds with all HA concentrations, suggesting the bone matrix maturation over time. Moreover, ARS semi-quantification at day 35 showed significant increase in mineralization with increasing HA concentration in scaffolds cultured in both mineralization and basic media. Interestingly, scaffolds with 45% HA cultured in basic media, exhibited as much matrix mineralization as neat PEOT/PBT scaffolds cultured in mineralization media, suggesting the ability of the composite material to promote osteogenesis without the addition of any osteogenic factors.

CONCLUSIONS: These data elucidate the possibility of using continuous HA gradient constructs as novel type of scaffolds for bone tissue interface engineering.

ACKNOWLEDGEMENTS: Financial support H2020-NMP-PILOTS-2015 (GA n. 685825).

REFERENCES

[1] Yang, P.J. et al, Tissue Eng Part B Rev, 2009. **15**(2): p. 127-141
[2] Campbell, S.E., et al, Acta Biomat., 2012. **8**(12): p. 4389-4396.



Tenogenic commitment of hBM-MSCs induced by controlled delivery of hGDF-5 and cyclic strain within a 3D multiphase microenvironment

M.C. Ciardulli[1], L. Marino[1], C. Selleri[1], N. Maffulli[1], G. Della Porta[1*]
Presenting author: Maria Camilla Ciardulli, mciardulli@unisa.it
[1]Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi (SA), Italy.

INTRODUCTION: Current therapeutic strategies to manage Tendon/Ligaments injuries are poorly effective. Herein, we focused on novel tissue engineering techniques, including the use of human bone marrow mesenchymal stem cells (hBM-MSCs) and sustained release of growth factors, loaded within a 3D scaffold, as possible methods for tendon repair.

METHODS: The 3D multiphase device, named 3D-HYALOFIB, is formed by a braided hyaluronate elastic band merged with poly-lactic-co-glycolic (PLGA) nanocarriers (200±50 nm) for the controlled release of human Growth Differentiation Factor-5 (hGDF-5) within a fibrin hydrogel seeded with hBM-MSCs. PLGA nanocarriers were fabricated by a proprietary technology and loaded with 130ng/g of hGDF-5. The elastic properties of the braided band (elastic modulus: 6.84±0.02 MPa) allowed cyclic strain delivery to the hBM-MSCs. At different time points, cells tenogenic commitment was evaluated by IHC and qPCR essays.

RESULTS & DISCUSSION: The 3D system was loaded with 100 mg of nanocarriers to deliver 1.5ng/mL/die of hGDF-5 within the 3D structure; a cyclic strain of 10% (4 hours a day) was also applied to transfer a mean load of 9 N with a frequency of 1 Hrz to the tissue-like system along the 11 days of cultivation. The static conditions were also tested. The hBM-MSCs tenogenic commitment was confirmed after 11 days by gene expression of Collagen I (COL1A1: 2.5 fold after 11 days), Scleraxis (SCX-A: 800 fold after 5 days) and Decorin (DCN: 3 fold after 2 days) (Figure 1a) and by immunofluorescence assay with the production of COL1A1 fibers in the extracellular environment (Figure 1b). The dynamic cultivation gave better results and more uniform collagen deposition within the available extracellular volume, nearby the cells. Cytokines gene expression indicated a good cells reaction toward the biomaterials used for 3D-HYALOFIB assembly.

CONCLUSIONS: 3D-HYALOFIB opens concrete perspectives for developing 3D bioengineered models to better understand specific molecular and cellular composition of damaged systems, as well as to develop a functionalized and totally bio-resorbable tendon tissue substitutes.

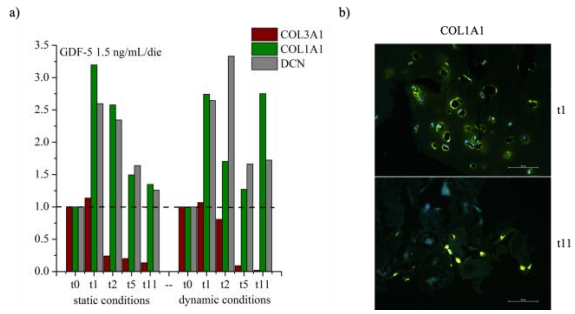
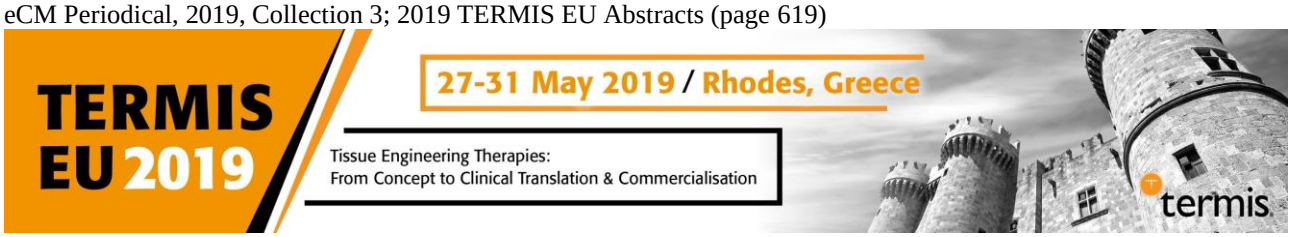


Figure 1: (a) Gene expression profiles for tenogenic markers and (b) COL1A1 pattern in dynamic conditions after 11 days of 3D culture.

ACKNOWLEDGEMENTS: Prof. E. Reverchon (DiIN-UNISA) for nanocarriers fabrication and Prof. E. Giordano (DIE-UNIBO) for the kindly loan of the cyclic strain bioreactor.



A novel xeno-free system for production of clinical grade MSC-secretome

M.E.F. Palamà[1], S. Carluccio[1], D. Reverberi[2], D. Pisignano[3], G. Shaw[4], F. Barry[4,5], M. Murphy[4], C. Gentili[1]
Presenting Author: Maria Elisabetta Federica Palamà, elisabettapalama@gmail.com
[1]Department of Experimental Medicine (DIMES), University of Genoa, Italy [2]IRCCS AOU San Martino-IST, Genoa, Italy [3]Nanoscience Institute CNR-NANO (NEST), Pisa, Italy [4]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland [5]Orbsen Therapeutics Ltd, Galway, Ireland

INTRODUCTION: Osteoarthritis (OA) is a pathological condition that brings to degradation of the articular cartilage. In recent years, human mesenchymal stem cells (hMSCs) are emerging as promising cell therapy candidate for the treatment of this clinical condition. Many studies demonstrate that MSCs attend to tissue repair through secretion of trophic factors.

METHODS: We developed a "donor-to-patient" closed, scalable and automated system for aseptic therapeutic cell manufacturing using a xeno-free (XF) medium. We characterized cells, secretome and extracellular vesicles (EVs) of hMSCs, grown in FBS and in a chemically defined XF medium. We validated also the potential therapeutic benefits of secreted factors, conditioned medium (CM) and EVs isolated from hMSCs cultured in this innovative culture system, for cartilage repair. We treated primary cultures of human Articular Chondrocytes (hACs) with IL-1α to induce an inflammatory process, and with three different concentrations of hMSC-CM for 16 and 48 h. A parallel experiment was performed pre-treating hACs with MSC-EVs for 3h before to induce the inflammation.

RESULTS & DISCUSSION: We demonstrated that our XF system allow to select a highly proliferating-hMSC population with a strong osteogenic potential, both in vitro and in vivo. These cells have a secretion pattern characterized by biological factors involved in homeostasis, wound healing and angiogenesis, beside showing a very strong secretion of EVs. We observed also that under inflammatory condition hACs can internalize and recruit more MSC-derived EVs, especially those derived from cells cultured in our XF system. We investigated the effects of CM and EVs in the activation of different regeneration pathways. We demonstrated that CM derived from MSCs grown in our medium promote a transient inflammation, enhancing levels of IL-6, IL-8, COX-2 and NF-kB, which are then down-regulated, leading to resolution. Our data also showed that our EVs inhibited IL-1α-induced expression of COX-2, indicating their significant anti-inflammatory potential and their protective role in cartilage.

CONCLUSIONS: Our culture system showed to be suitable to isolated hMSCs and cellular product ready-to-use for clinical therapy.

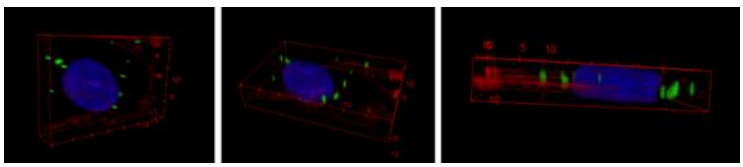
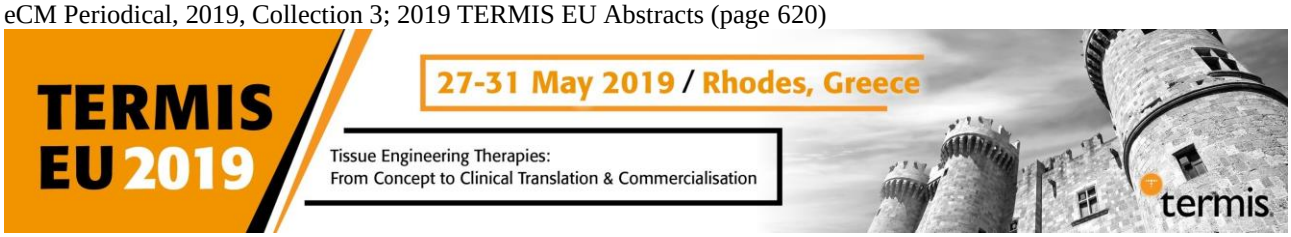


Figure 1: Three-dimensional reconstruction by confocal microscope of hACs internalizing PKH67-labelled EVs. DAPI (blue), phalloidin (red), PKH67 (green).

ACKNOWLEDGEMENTS: Financial support was received from H2020, Autostem (667932).



Nanofibrous structures by thermally induced self-agglomeration of polycaprolactone/chitosan blends for musculoskeletal tissue engineering

A.L. Pereira[1], S. Guieu[1,2,4], A. Semitela[3], P.A.A.P. Marques[3], M.H.V. Fernandes[1,4]
Presenting Author: Maria Helena Fernandes, helena.fernandes@ua.pt
[1]CICECO – Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193 Aveiro, Portugal [2]QOPNA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal [3]TEMA, Department of Mechanical Engineering, University of Aveiro, 3810-193 Aveiro, Portugal [4]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Fibrous structures are widely used in tissue engineering to restore or enhance the normal functions of human tissue. One method used to fabricate micro- and nano-fibers is electrospinning. This technique produces 2D membranes in a simple and rapid way and with several different polymers. Recently, an innovative methodology, thermally induced self-agglomeration (TISA), has emerged to transform electrospun fibers into 3D structures in order to better mimic the natural architecture of the extracellular matrix (ECM) and in turn allow suitable attachment, growth, and proliferation of cells.

METHODS: In this study, nanofibers mats of polycaprolactone (PCL)/chitosan blends using acetic acid/formic acid were fabricated through electrospinning. They were then converted into 3D scaffolds using the methodology TISA, followed by freeze drying (Figure 1).

RESULTS & DISCUSSION: The obtained products were nanofibrous 3D scaffolds with increasing amounts of chitosan (10, 15 and 20%), highly porous (>90%) and with interconnected pores of different sizes. Swelling tests and compression modulus indicated the suitability of the scaffolds for tissue engineering, particularly for cartilage repair. Cell viability studies of the scaffolds was evaluated.

CONCLUSIONS: The similarity of the scaffolds with the ECM in terms of morphology, structure and properties, indicates that the produced architectures are highly promising for their application in musculoskeletal tissue engineering.

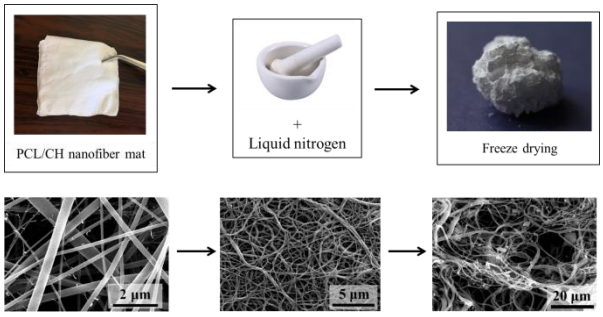
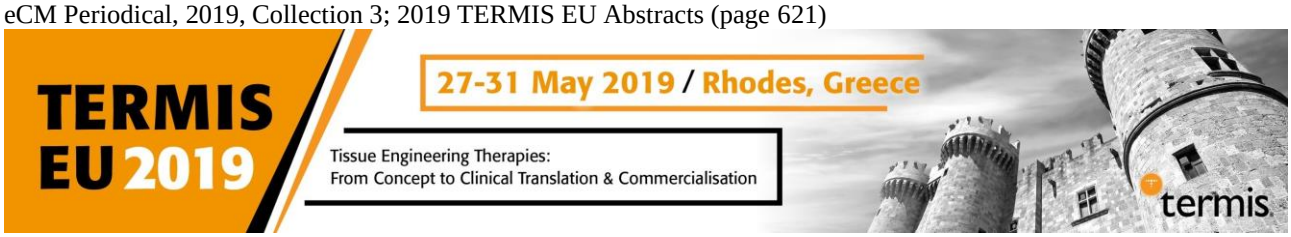


Figure 1: A scheme of TISA technique with SEM images of the materials obtained after each step.

ACKNOWLEDGEMENTS: Thanks are due to University of Aveiro, FCT/MEC for the financial support to the QOPNA (FCT UID/QUI/00062/
2019) and CICECO (POCI-01-0145-FEDER-007679; FCTUID/CTM/50011/2019), through national funds and FEDER, within the PT2020 Partnership Agreement. S. Guieu thanks the pAge project: Life-long protein aggregation (Centro-01-0145-FEDER-000003).



Deciphering the role of non-coding RNAs in osteogenesis and osteoclastogenesis: The impact on bone regeneration

M.I. Almeida[1,2], S. Moura[1,2], A.M. Silva[1,2], S.G. Santos[1,2], M.A. Barbosa[1,2,3]
Presenting author: Maria Ines Almeida, ines.almeida@ineb.up.pt
[1]i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal [2]INEB - Instituto de Engenharia Biomédica, University of Porto, Portugal [3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal

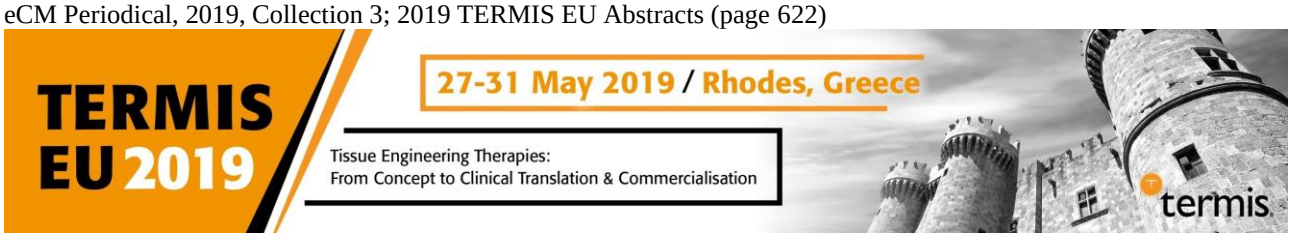
INTRODUCTION Controlled regulation of bone formation and resorption is essential to maintain bone homeostasis. However, these processes are disrupted in several diseases, such as osteoporosis and bone disease associated to multiple myeloma, causing fractures that most often occur in the spine, and lead to pain, long-term disability and death. Current treatments consist predominantly in anti-resorptive drugs that inhibit/prevent bone resorption but are often associated with several side effects. Therefore, the need for new approaches to promote bone homeostasis and regeneration/repair of bone fractures is increasing. Over the past years non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long ncRNA (lncRNAs), have emerged as important post-transcriptional regulators. In this context, we aim to investigate the impact of miRNAs and lncRNAs in osteogenic differentiation and osteoclastogenesis.

METHODS: To achieve this goal, ncRNA expression profile was evaluated during osteogenic differentiation in MC3T3 cell line and in primary human Mesenchymal Stem/Stromal Cells (MSC) and during osteoclast differentiation in RAW264.7 cell line and in human monocytes, by RT-qPCR. Next, the biological effect of the most differently expressed ncRNAs was assessed using ncRNA mimics and inhibitors. To evaluate osteoblast-osteoclast crosstalk, osteoblast-derived culture media at day 7 of differentiation was cultured with osteoclasts and it effect was determined. Finally, ncRNA levels were analysed in bone marrow (BM) of a rats surgical induced with a critical size bone defect.

RESULTS & DISCUSSION: The results showed that miRNAs (miR-195, miR-29a/b/c) are differently expressed during osteogenic differentiation of both MC3T3 cell and human MSC. Also, lncRNA uc.64+, which is an intronic ultraconserved region, is upregulated during osteogenic differentiation in these cell types. Moreover, miR-29 family is altered during osteoclastogenesis of RAW 264.7 and human monocytes isolated from buffy coats. Modulation of miRNA levels, specifically miR-195 inhibition and miR-29, overexpression, in MC3T3 pre-osteoblast cell line lead to an increase of osteogenic differentiation markers and ALP and Alizarin Red O staining (mineralization). Moreover, supernatant from MC3T3-miRNA transfected cells at day 7 of osteogenic differentiation impaired osteoclastogenesis differentiation of RAW 264.7, by altering the number of multinucleated cells and the expression of the osteoclast markers dendritic cell-specific transmembrane protein (DC-STAMP), C-C Motif Chemokine Ligand 2 (CCL2), and Cathepsin K (CTSK). Finally, results show miRNA levels are altered in BM of rats with surgical bone defect.

CONCLUSIONS: Taken together, our data indicate that ncRNAs have the ability to regulate osteogenic differentiation and osteoclastogenesis and are potential candidates for bone regenerative therapies.

ACKNOWLEDGEMENTS: FCT project POCI-01-0145-FEDER-031402 (PORTUGAL 2020 / Programa Operacional da Competitividade e Internacionalização). Associação Portuguesa Contra a Leucemia and Sociedade Portuguesa de Hematologia, in a partnership with Amgen, for their support.



Osteogenic potential of MC3T3-E1 cells on chitosan-graft-poly(ε-caprolactone) copolymers

M. Kaliva[1,2], A. Georgopoulou[2], M. Chatzinikolaidou[1,2], M. Vamvakaki[1,2]
Presenting Author: Maria Kaliva, kalivm@iesl.forth.gr
[1]Institute of Electronic Structure & Laser, Foundation for Research & Technology Hellas, Greece
[2]Department of Materials Science & Technology, University of Crete, Greece

INTRODUCTION: Biodegradable scaffolds are key elements in regenerative medicine. Chitosan (CS) is a natural biocompatible polysaccharide with poor mechanical properties [1]. A way to improve the mechanical properties of CS is by grafting synthetic polymers that exhibit complementary properties along the CS backbone. Polycaprolactone (PCL) is a synthetic biodegradable and biocompatible polyester with good mechanical properties [2]. Herein, we present the synthesis of CS-g-PCL copolymers and the evaluation of their potential for use in bone tissue engineering by examining the osteogenic capability of MC3T3-E1 cells to differentiate to osteoblasts when cultured onto the CS-g-PCL surfaces.

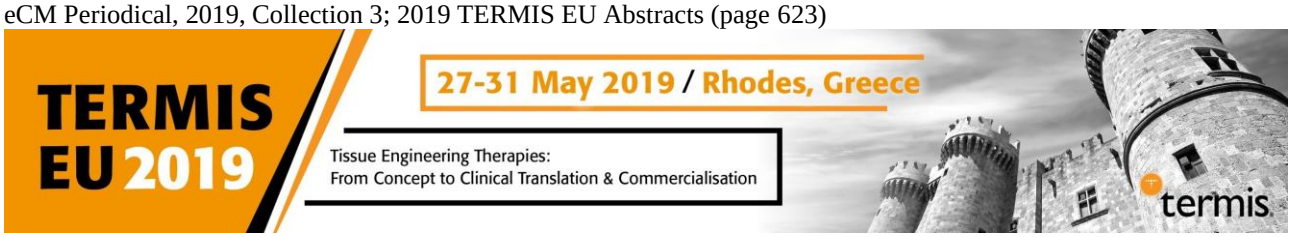
METHODS: CS-g-PCL was synthesized by grafting carboxylic acid end-functionalized PCL chains onto the amine moieties of CS [3]. The chemical structure and the composition of the copolymers were examined by ^{1}HNMR and FT-IR spectroscopies. CS-g-PCL copolymer films were fabricated by spin coating and the biological response of MC3T3-E1 cells on the CS-g-PCL surfaces was examined. The morphology of MC3T3-E1 cells on the material surfaces was examined by scanning electron microscopy, while cell viability on the CS-g-PCL surface was assessed by the PrestoBlue® assay. Finally, the osteogenic potential of MC3T3-E1 cells cultured on the CS-g-PCL surface was evaluated by determining specific early and late markers and comparing them to the TCPS control.

RESULTS & DISCUSSION: CS-g-PCL copolymers were successfully synthesized and their composition was determined by ^{1}HNMR analysis. Cell viability experiments showed that a CS-g-PCL copolymer, of 78 wt% CS content, promotes cell adhesion and proliferation of MC3T3-E1 cells and that the cells retain their characteristic elongated morphology (fig.1a). Furthermore, the study of early markers of osteogenesis demonstrated that the alkaline phosphatase activity levels were significantly higher at both times point compared to TCPS (fig.1b), while collagen secretion was enhanced on day 7. Finally, investigation of the late markers of osteogenesis revealed that calcium biomineralization deposit and osteopontin levels were significantly higher for CS-g-PCL, after 14 days in culture, compared to TCPS [3].

CONCLUSIONS: CS-g-PCL copolymers were successfully synthesized by the 'grafting to' technique. Cell culture experiments showed strong cell attachment and proliferation increase of MC3T3-E1 cells on a CS-g-PCL copolymer of 78 wt% CS content compared to control. Moreover, the increased levels of specific osteogenic markers of the pre-osteoblastic cells cultured on the material surface indicate the potential of the CS-g-PCL copolymers for scaffold fabrication in bone tissue engineering.

REFERENCES

[1] Madihally, S et al. Biomaterials 1999;20(12):1133-42.
[2] Williamson, M.R et al. Biomaterials 2004;25(3):459-465.
[3] Georgopoulou, A et al. Materials 2018;11(4):490.



Biomimetic hyaluronic-based hydrogel enhances endogenous cell recruitment and healing process of osteochondral lesions

M.L. Vainieri[1,2], A. Lolli[2], K. Sivasubramaniyan[2], D. Eglin[1], A. Yayon[3], M. Alini[1], S. Grad[1], G. Van Osch[2]
Presenting author: Maria Letizia Vainieri, letizia.vainieri@aofoundation.org
[1]AO Research Institute, Davos, Switzerland [2]Department of Orthopedics and Otorhinolaryngology, Erasmus MC, Rotterdam, The Netherlands [3]Procore Laboratories, Nes Ziona, Israel

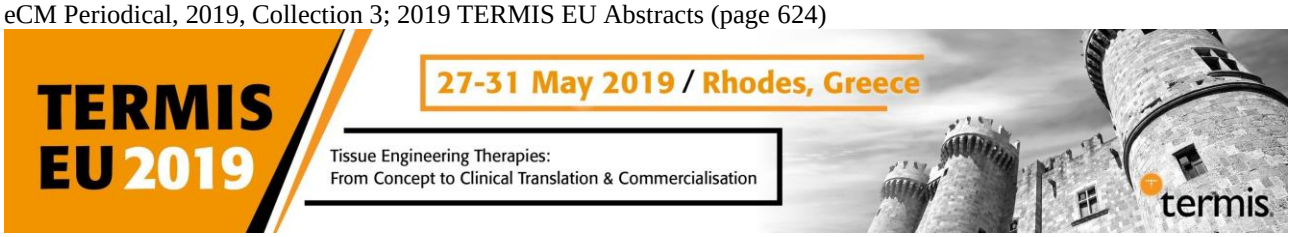
INTRODUCTION: Biomaterials play a pivotal role in tissue engineering, where cells must migrate to the site of the defect through the matrix scaffolding, then proliferate and facilitate the remodeling. We here aimed to test the influence of different hyaluronan (HA)-based hydrogels on cartilage repair by stimulating joint-resident cells. W*e first query cell migration in vitro, testing* a new Fibrin/HA (FB/HA) formulation and HA/Tyramine hydrogels as material cues in the context of migratory barriers using a 3D spheroids-based assay via provision of platelet-derived growth factor-BB. We then implemented this signal in vivo, using an osteochondral explant as a cartilage defect model system.

METHODS: CFDA-SE-labelled bone marrow hMSCs from 3 donors were seeded to form spheroids of 500 cells, embedded in FB/HA or different HA/Tyr cross-linked density hydrogels (150, 300, 600µM H_2O_2) and cultured in aMEM, 1%ITS with/without PDGF-BB. Microscopy analysis was employed to measure the migratory area of the cells from the core. In-vitro chondrogenesis in the hydrogel[1] was determined by qRT-PCR and histology. Osteochondral defects made in bovine osteochondral biopsies[2] were filled with FB/HA and HA/Tyr (150) hydrogels with/without PDGF-BB and subcutaneously implanted in nude mice for 4 weeks.

RESULTS & DISCUSSION: HA hydrogels allowed cell migration from the spheroids in presence of PDGF-BB, inducing progressive increase over three days culture. However, HA/Tyr gels with the lowest crosslinking densities (150µM H_2O_2) were softer and promoted faster migration than stiffer gels, but FB/HA supported the broadest cells migration area (5-fold increase compared to HA/Tyr hydrogels). Both hydrogels supported in vitro hMSCs chondrogenesis, as shown by collagen II and aggrecan expression. By 4 weeks cells were significantly present in FB/HA gels and reached 85-90% of the scaffold area compared to 30% for HA-Tyr untreated groups (**$p<0.01$). Consequently, cell mediated matrix remodeling was significantly enhanced in untreated FB/HA gels in comparison to untreated HA-Tyr gels (GAG production, **$p<0.01$ Fig 2C).

CONCLUSIONS: This study showed a reduction in the size of cartilage lesion and enhanced regeneration of the cartilage using FB/HA hydrogels without exposure of growth factor before implantation. Our system can be employed as advanced platform for pre-clinical screening of biomaterial and for delivering chemoattractants to enhance cartilage repair by endogenous stem cells.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie Grant Agreement No 642414.



Phenotypic and secretory characteristic of mesenchymal stem cells isolated from adipose tissue of ischemic leg patients

M Paprocka[1], A Bielawska-Pohl[1], A Krawczenko[1], L Masłowski[2], A Czarnecka[2], H. Kraśkiewicz[1], D Dus[1]. Presenting Author: maria.paprocka@hirszfeld.pl

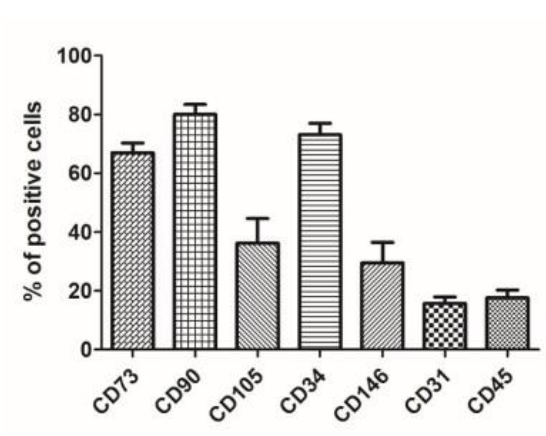
[1]Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland [2]Regional Specialist Hospital, Research and Development Centre, Wroclaw, Poland

INTRODUCTION: Mesenchymal Stem Cells **(**MSC) were isolated from adipose tissue of patients with venous leg ulcers for experimental, autologous transplantation. MSC phenotype and their ability to secrete biologically active factors were evaluated.

METHODS: MSC were isolated from a lipoaspirate (n=5) using closed, fully automated CELLUTION 800 system (Cytori Therapeutics, USA). Directly after isolation the phenotype of cells was evaluated by flow cytometry using the FACSCalibur (Becton Dickinson, CA). Secretory profile was evaluated in supernatants with the use of the RayBio Custom C-series human Cytokine Antibody Array.

RESULTS & DISCUSSION: In evaluated samples the percentage of cells expressing the MSC markers CD73+, CD90+ ranged from 65 to 82%. Similarly high percentage of CD34+ cells was found in all samples. About 20% of CD31+ (endothelial) and CD45+ (hematopoietic) cells were observed in samples (Fig.1).
Cells from all patients produced in vitro IL-8, MMP-3, MCP-1, GRO, TGFβ2, TIMP1 and some of them, additionally, Angiopoietin 2 or VEGF (Fig.2).

CONCLUSIONS: The purity of MSC preparations separated by Cellution 800 was above 60%. Unexpectedly, high percentage of cells expressed CD34 antigen. In supernatants from MSC primary cultures several cytokines with angiogenic activity were found.



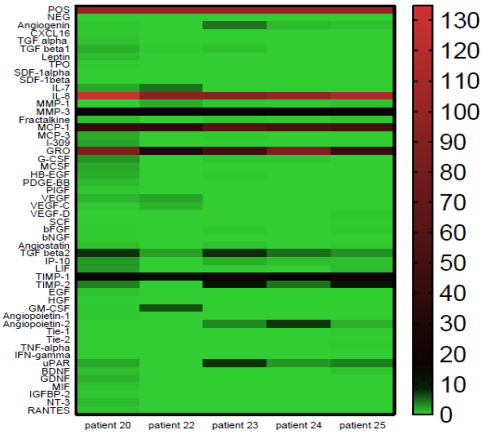
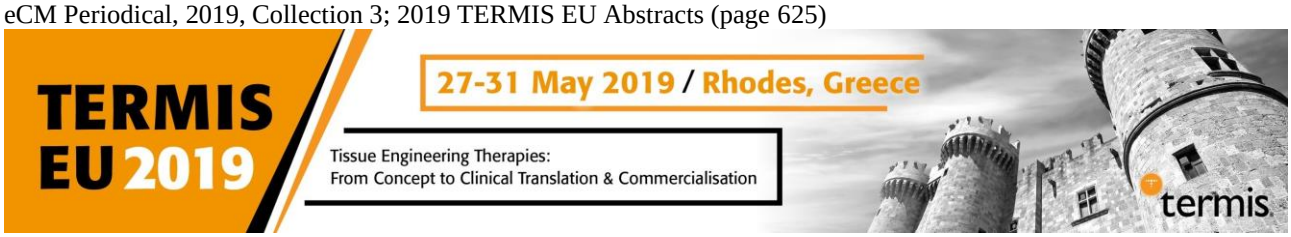


Figure 1: (left) Phenotype and average percentage of cells separated from patient adipose tissue **Figure 2:** (right) Secretion of cytokines by primary cultures of MSC with the use of the RayBio Custom C-series human Cytokine Antibody Array.

ACKNOWLEDGEMENTS: Financial support was received from Polish National Science Center, 2012/07/B/NZ4/01820.



Regulation of pericyte function in angiogenesis and tissue regeneration: The role for T-cadherin

B. Dasen[1], J. Guerrero[1], A. Scherberich[1], S. Verrier[2], I. Martin[1], M. Philippova[1]
Presenting Author: Maria Philippova, maria.filippova@unibas.ch
[1]Tissue Engineering Lab, Department of Biomedicine and Department of Surgery, Basel University Hospital, Basel, Switzerland [2]AO Research Institute Davos, Switzerland

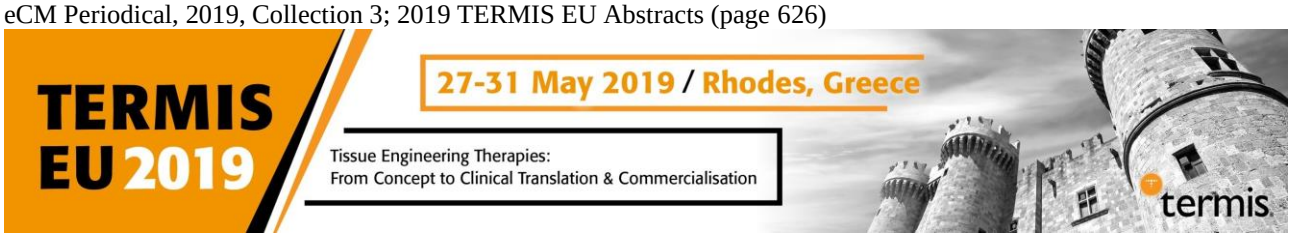
INTRODUCTION: Vascularization and formation of stable functional vascular networks within bioengineered tissue grafts are essential requirements for efficient tissue and organ regeneration. Pericytes are mural cells which play a key role in regulation of vessel sprouting, stabilization and maturation during angiogenesis. Cadherins are adhesion molecules controlling morphogenesis and the maintenance of tissue architecture. Until recently, N-cadherin which forms endothelial-pericyte adherens junctions remained the only cadherin described in pericytes. Here we demonstrate the role for another member of the superfamily, atypical GPI-anchored T-cadherin, in regulation of pericyte function.

METHODS: T-cadherin expression in pericytes from heart, brain and adipose tissue was analyzed by immunohistochemistry. Overexpression or silencing of T-cadherin in human cultured pericytes from placental and adipose tissue was achieved using lentiviral vectors. T-cadherin effects on pericytes were analyzed using CyQuant proliferation assay; migration monitored by time-lapse video-microscopy; invasion in fibrin gel; angiogenesis in Matrigel and in a microfluidic chamber. Pericyte differentiation was studied using flow cytometry analysis of human stromal-vascular fraction (SVF). Gene expression and activity of signaling pathways were measured by qPCR and immunoblotting.

RESULTS & DISCUSSION: T-cadherin is expressed by cultured CD146+/CD34- placental and adipose-tissue pericytes, NG2+ cells from the heart, brain and adipose tissue, and 3G5-antigen+ pericyte-like cells from human aorta. Ectopic T-cadherin expression or silencing in pericytes regulate proliferation, migration, invasion, differentiation and cytoskeleton organization. In cocultures with human endothelial cells from umbilical vein (HUVEC) T-cadherin overexpression promotes and silencing delays pericyte-dependent regulation of endothelial network formation. Changes in T-cadherin expression level modulate expression of pericyte genes relevant for differentiation and angiogenesis, as well as activity of signaling cascades involved in control of pericyte proliferation, migration and phenotype, among them Akt and TGFβ1 pathways. Endogenous T-cadherin expression in pericytes is increased in response to TGFβ1.

CONCLUSIONS: We have identified T-cadherin as a novel regulator of pericyte function. T-cadherin signaling is relevant for control of angiogenesis and vessel maturation and may be a potential target to improve vascularization during tissue engineering and repair.

ACKNOWLEDGEMENTS: The study was supported by Swiss Foundation for Cardio-vascular Diseases and Swiss Heart Foundation.



Dermal equivalents produced with an innovative elasto-mimetic hydrogel

M. Carranca[1], J. Sohier[1]
Presenting Author: Mariana Carranca, mariana.carramca@ibcp.fr
[1]Laboratory of Tissue Biology and Therapeutic Engineering (LBTI), UMR 5305, CNRS, Lyon, France

INTRODUCTION: The dermis is the layer of the skin that provides flexibility and strength to the skin. It is principally composed of fibroblasts, responsible of the production and regulation of the ECM. While collagen is widely used as scaffolds for dermal equivalents, it has weak mechanical properties, batch-to-batch variability and risk of pathogen transfer.[1] This impulse towards the development of new materials for these applications. Interestingly, as well as collagen, the dermal matrix comprises a high amount of elastin, which provide its elasticity.[2] Therefore, we hypothesized that a hydrogel integrating an elastin derived peptide (EDP) with similar physicochemical properties as tropoelastin would be beneficial to recapitulate a dermal equivalent. A hydrogel composed of poly-lysine dendigrafts (DGL) and PEG and encompassing the EDP was therefore developed and evaluated as a support for the formation of an efficient dermal equivalent.

METHODS: Hydrogels were obtained by mixing DGL-G3, PEG-NHS and EDP in PBS and made porous by particulate/leaching technique, using paraffin microspheres as porogens (50-180µm). Dermal equivalents were obtained by seeding human fibroblast for 14, 21 and 42 days in vitamin-c supplemented medium. The quality of the extracellular matrix (ECM) produced through time was evaluated by IHC and zymography. The mechanical properties of the acellular hydrogels were compared with colonized hydrogels through time. To investigate the effect of the EDP, fibroblasts were seeded on 2D hydrogels and observed over 16hrs with time-lapse and IHC.

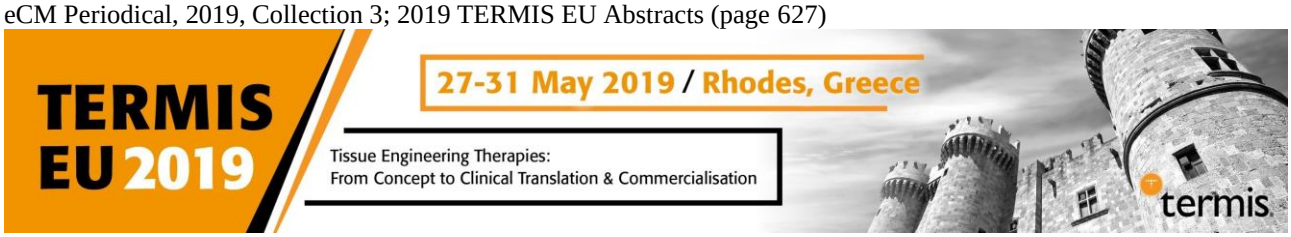
RESULTS & DISCUSSION: Hydrogel's mechanical properties can be modified by changing the ratio of DGL/PEG components. Hydrogels with ratio 2/19 present a complex modulus of 14kPa, close to those reported in the literature for skin [3]. Human fibroblasts can homogeneously colonize the hydrogels after 21 days and synthetize their own ECM. Interestingly, unlike collagen gels, no contraction was observed. Cell density in the hydrogels was increased when the EDP was incorporated, and cells were able to infiltrate deeper and produce a greater quantity of collagen. Zymography showed the presence of pro-MMP, but no activation after 8 and 21days.

CONCLUSIONS: A hydrogel containing an EDP allows to efficiently produce a dermis equivalent without retraction. The presence of EDP in the hydrogels improved cellular colonization and proliferation, resulting in a higher synthesis of ECM. Ongoing experiments are characterizing further the ECM quality and eventually produce a full skin equivalent.

ACKNOWLEDGEMENTS: The authors thank the ARC2 RhoneAlpes and CONACyT for the financial support.

REFERENCES

[1] Franz S et al. Biomat. 2011;32(28):6692-6709.
[2] Kamel R et al. J Am Col Surg 2013;217(3) :533-555
[3] Pailler-Mattei C et al. J Mec Beh Bio Mat 2013;28:474-483



Targeting colorectal cancer using highly specific peptide-modified dendrimer nanoparticles

M. Carvalho[1,2,3], D. Caballero[1,2], S. C. Kundu[1,2], R. L. Reis[1,2,3], J. M. Oliveira[1,2,3]
Presenting Author: Mariana Carvalho, mariana.carvalho@dep.uminho.pt
[1]I3B's Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

INTRODUCTION: Cancer cells from various sources have been reported to express high levels of the 67 kDa laminin receptor, which is responsible for the interactions between cells and laminin of the extracellular matrix [1]. Specifically, the penta-peptide TyrIle-Gly-Ser-Arg (YIGSR), derived from laminin b1 chain, has been identified as the binding site to its cell surface receptor. We have hypothesized that the attachment of YIGSR on the surface of CarboxymethilChitosan (Polyamidoamine) CMCht/PAMAM dendrimer nanoparticles (NPs) would lead to their preferential uptake by metastatic cancer cells over other cells types.

METHODS: CMCht/PAMAM dendrimer NPs were modified using simple EDC chemistry (Fig.1a). After synthesis, NPs were characterized using TEM. NPs size and surface changes were measured using zeta potential (Zeta Sizer) and DLS technologies. The specific internalization of peptide-linked dendrimer NPs by colorectal cancer cells was assessed in standard culture flasks in a co-culture of live stained HCT-116 colorectal cancer cells and L929 fibroblasts.

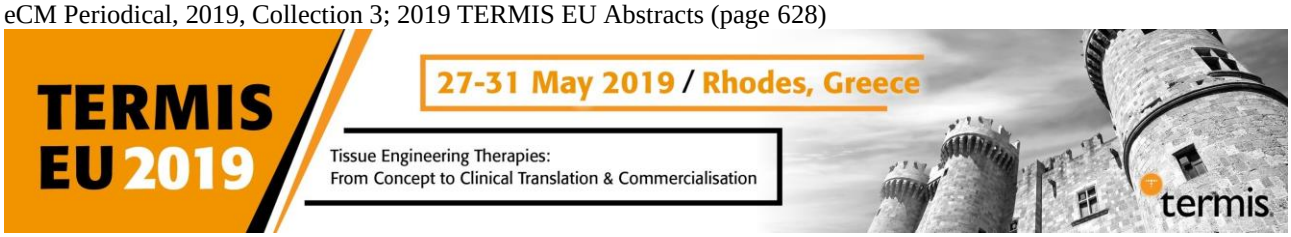
RESULTS & DISCUSSION: We describe a simple method to covalently link the YIGSR peptide to our dendrimer NPs. DLS results show that the diameter of the modified NPs is increased from 54 nm to 130 nm when compared to the non-modified ones. Similarly, the surface charge is less negative compared to the unmodified NPs. This indicates a reduction in the number of carboxylic acid groups on NP surfaces following the peptide binding, The results indicate a targeted internalization of NPs by the HCT-116 cancer cells after 24h of culture, which may probably be due to the overexpression of laminin receptor. Ongoing experiments aim to quantify the internalization efficiency and specificity after seeding the cancer cells both on 2D and 3D environments.

CONCLUSIONS: Our preliminary results confirm the successful modification of CMCht/PAMAM dendrimer NPs with the peptide using simple EDC chemistry. Next, a preferential internalization of the peptide-modified NPs in HCT-116 cancer cells is observed, in contrast to L929 fibroblasts. We intend to improve the specificity and efficiency of drug-targeted therapies on disseminated metastatic cancers, which are the major drawback in current therapeutic approaches.

ACKNOWLEDGEMENTS: Mariana Carvalho acknowledges her PhD scholarship NORTE-08-5369-FSE-000044, funded by Programa Operacional Regional do Norte, Fundo Social Europeu, Norte2020 TERM & SC and European Commission funded project FoReCaST (grant agreement no. 668983).

REFERENCES

[1] Kumazoe M, J Clin Invest, 2013: 62:787-99.



Coating of insulin-producing cells using elastin-inspired biomaterials

M.B. Oliveira[1], C. Martins-Cruz[1], S. Vilabril[1], N. Ehlich[2], A. Ehlich[2], M. González-Pérez[3], C. García-Arévalo[3], J.C. Rodríguez-Cabello[3], J.F. Mano[1]
Presenting Author: Mariana B. Oliveira, mboliveira@ua.pt
[1]Department of Chemistry, CICECO – Aveiro Institute of Materials. Campus Universitário de Santiago. 3810-193 Aveiro. Portugal [2]Ncardia AG, Nattermannallee 1, 50829 Cologne, Germany [3]GIR BIOFORGE, Universidad de Valladolid CIBER BBN, Edificio LUCIA, Paseo de Belén 11, 47011, Valladolid, Spain

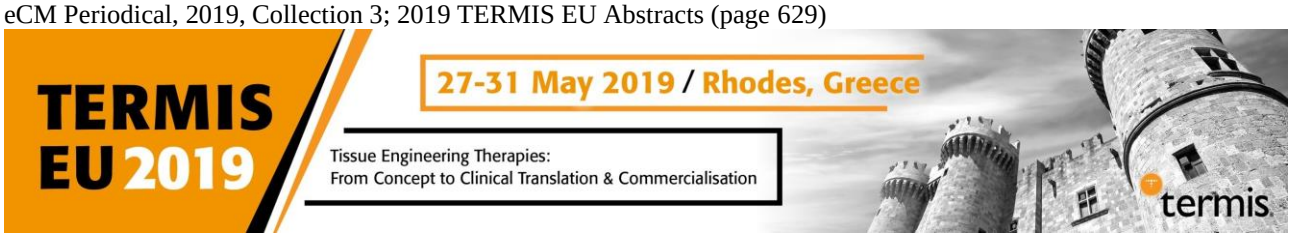
INTRODUCTION: The transplantation of insulin-producing cells is a promising therapeutic approach to treat diabetic patients, with potential to restore their quality of life and reduce costs associated with the disease. Transplanted cells targeting the treatment of diabetes are most often obtained from allogeneic sources, which poses the need for lifelong immunosuppressants intake for patients. Immunosuppressing therapies are associated with several pernicious effects. Encapsulation of functional insulin-producing glucose-responsive cells in biomaterials is a plausible technology to circumvent immunosuppression. Those systems provide transplanted cells with a stealth barrier for the immune system. Despite the short-term success of biomaterial-based encapsulating strategies, transplanted cells (namely, pancreatic islets) have shown a decay of function in time periods of one to five years. Current challenges reported for transplantation strategies targeted at the treatment of diabetes may be circumvented through the development of coatings with high biocompatibility, adequate perm-selectivity to warrant immunogenic protection, and capability to promote angiogenesis to enable host-capsule integration.

METHODS: A method based on the deposition of elastin-inspired biomaterials was developed to coat insulin-producing human cells that were obtained by directed in vitro differentiation of human induced pluripotent stem cells. The cutoff of the coatings was extrapolated from diffusion tests using coated alginate beads to withstand the developed coatings. The assembly of the biomaterials used in the build-up of the coatings was assessed using a quartz crystal microbalance with dissipation mode (QCM-D), and the morphology of model films was assessed by microscopy and atomic force microscopy. The deposition of the coatings on cells was confirmed by confocal microscopy. Cell viability and insulin release were assessed after the coating procedure.

RESULTS & DISCUSSION: The biomaterials used for cell coating were detected on cells' surface by confocal microscopy. For specific cell types an apparently continuous coatings could be detected around the cellular structures. The coating did not impair cellular viability up to 12 days of cell culture. Moreover, insulin passage upon glucose stimulation was also not impaired. Coatings incorporating pro-angiogenic motifs were also successfully developed.

CONCLUSIONS: A method to coat insulin-producing cells was developed without hampering cells' viability or insulin secretion-associated functionality. The immunoprotective effect of the coating as well as its vascularization-inducing property is expected to be proven in vivo using immunecompetent animal models.

ACKNOWLEDGEMENTS: This project has received funding form the European Union´s Horizon 2020 research and innovation program under grant agreement No 646075.



Engineered human chondrocyte shape correlates with nanoscale cytoskeletal stiffness

M. Erb[1], J.C. Lauer[1], M. Selig[1], B. Rolauffs[1]
Presenting Author: Marita Erb, marita.erb@uniklinik-freiburg.de
[1]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Dept. of Orthopedics and Trauma Surgery [2]Dept. of General and Visceral Surgery; Albert-Ludwigs-University of Freiburg, Germany

INTRODUCTION: High throughput image processing and computational analysis are increasingly being used to efficiently quantify cell shape via mathematical shape descriptors for statistically correlating biological form with biological function. Moreover, the standardized engineering of cell shape through micro-patterned adhesion sites, biomaterial stiffness, and biomechanical forces is helpful for better understanding the association of micro-environmental stimuli with cell morphology and associated function. In terms of mechano-transduction, such external stimuli can initiate changes in cytoskeletal properties, which in turn modify focal adhesions and cytoskeletal organization and activate integrin-mediated signal pathways. In this context, we asked how cell shape engineered through micro-contact printed geometrically defined adhesion sites affects the single cell cytoskeletal Young's Modulus, and which aspect of shape measured by mathematical shape descriptors correlates best with cytoskeletal Young's Modulus. Such information would be helpful for controlling cell nano-mechanical properties via engineered cell shape.

METHODS: H-shaped vs. circular fibronectin micro-printed adhesion site geometries (micro-patterns (MPs)) with comparable areas as well as non-patterned control surfaces were used. Chondrocytes (CHs) isolated from human articular cartilage were cultured on the MPs at a density of 3000 cells/cm^2 overnight. In a combination of life cell imaging and nano-indentation, CHs were indented on top of their nucleus, using the Chiaro Nanoindenter from OPTICS11 coupled to an AxioObserver Z1 (Zeiss) with temperature and atmospheric control. The nano-indentation characteristics included a displacement of 10.000 nm, the tip radius was 3 µm and the tip stiffness was 0.055 N/m.

RESULTS & DISCUSSION: The Young's Modulus of H-shaped CHs was 809±139 Pa, of circular CHs was 1179±207 Pa, and of control CHs was 1471±268 Pa. There was a statistically significant correlation between numerically coded CHs shapes (controls coded as 1, h-shape coded as 2, o-shape coded as 3) with the Young's Modulus (correlation coefficient: -0.340, p=0.004, n=72 cells; Fig. 1). The difference in the Young's Modulus between control CHs vs. H-shaped CHs and of control CHs vs. O-shaped CHs was significant ($p<0.05$). Comparable significant correlations were found between numerically coded CHs shapes vs cell area and also cell length but not cell width, roundness, aspect ratio, circularity, or solidity.

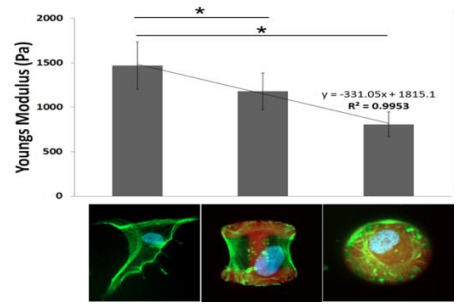
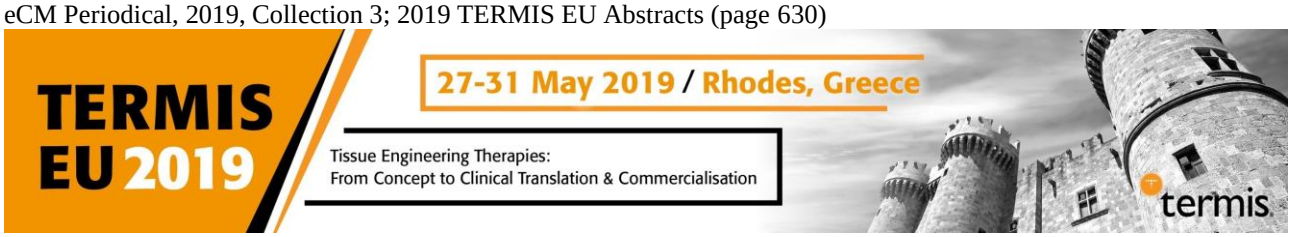


Figure 1: Young's Modulus of shape-engineered chondrocytes vs. control chondrocytes, assessed by life imaging and nano-indentation.



Reinforced 3D printed collagen-based scaffolds for articular joint repair

R.M.F.M. Lemoine[1,2,3], J.M. O'Byrne[1,4], D.J. Kelly[1,2,3], F.J. O'Brien[1,2,3]
Presenting Author: Mark Lemoine, marklemoine@rcsi.com
[1]Tissue Engineering Research Group, Dept. of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Dublin, Ireland [3]Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland [4]Department of Trauma and Orthopaedic Surgery, RCSI, Ireland

INTRODUCTION: Damage to articular cartilage does not heal readily, and often affects subchondral bone resulting in osteoarthritis. The complex layered composition of the cartilage-bone interface suggests multi-layered scaffolds will be required to ensure successful regeneration. To this end, this study used 3D-printed polymer frameworks to reinforce previously developed collagen scaffolds [1,2]. Young's modulus of the reinforcing 3D printed frameworks could be tuned to match cartilage, thus avoiding abrasiveness and producing a porous reinforced collagen scaffold for osteochondral repair.

METHODS: A biodegradable polymer was used to 3D-print (Biobot1, Allevi, USA) five different mesh designs with distinct pore architectures*. The stiffness (Young's modulus) of the meshes was assessed using uniaxial compression testing (Zwick Roell Z005). Collagen -hyaluronic acid and -nanohydroxyapatite (CHyA, CnHA) slurries were incorporated into these meshes and freeze-dried, creating reinforced composite scaffolds. Microarchitecture was assessed by toluidine blue staining and SEM. Mesenchymal stem cells (MSCs) were used to assess the chondrogenic capacity of reinforced CHyA, via sulphated-glycosaminoglycan (sGAG) assay (Blyscan™).

RESULTS & DISCUSSION: The compressive moduli of the five mesh designs ranged from 0.7 to 17 MPa. The softest design (D5) lies in the range of articular cartilage and was used to reinforce the CHyA for chondrogenesis studies, while CnHA was incorporated into the stiffest design (D1) for future studies on osteogenesis. Both CHyA and CnHA scaffolds maintained high porosity in the meshes. GAG deposition in the reinforced CHyA was similar to that observed in non-reinforced control scaffolds.

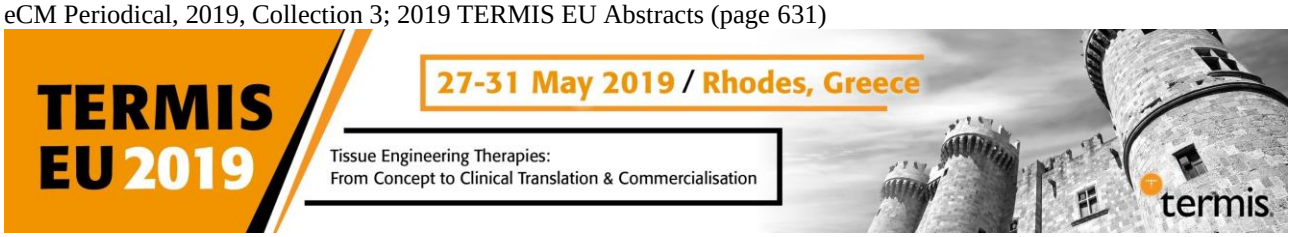
CONCLUSIONS: Multi-layered reinforced scaffolds with mechanical properties suitable for osteochondral repair were created using 3D printing. Reinforcement was successful and did not affect porosity, which is crucial to achieve sufficient cell infiltration. Cartilage regeneration remains a significant challenge in tissue engineering, as biomaterial scaffolds needs to mechanically strong with a composition that can promote chondrogenesis. The composite scaffolds presented here build on success of previously CHyA scaffolds, which combined with 3D printing allows for their application to larger osteochondral defects.

ACKNOWLEDGEMENTS: SFI AMBER Research Centre, grant 12/RC/2278.

REFERENCES

[1] Stack JD (et al) J Tissue Eng Regen Med. 2016 Oct;4(7):524–31; [2] Levingstone T. (et al) Acta Biomater 2016;32:149–60.

* Details not disclosed due to IP restrictions.



A new matrix-based technology platform for the high throughput analysis of 3D cell cultures

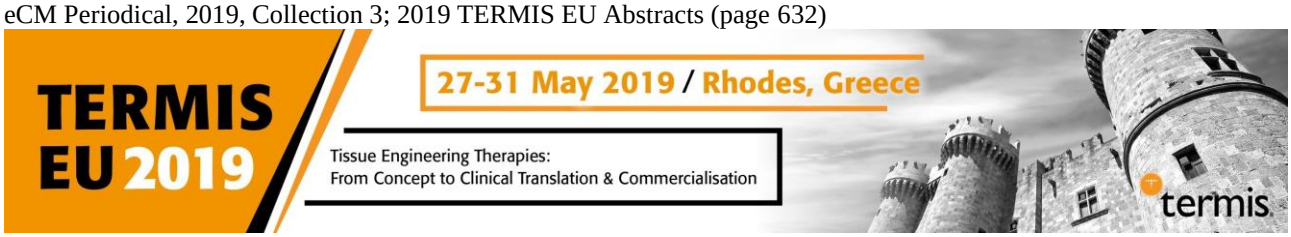
M. Rimann[1], E. Bono[1], S. Stebler[1], A. Picenoni[1], E. Felley-Bosco[2], C. Hund[3], R. Pellaux[3], A. Meyer[3]
Presenting Author: Markus Rimann, rimm@zhaw.ch
[1]Institute of Chemistry and Biotechnology (ICBT), Zurich University of Applied Sciences, Waedenswil, Switzerland [2]Laboratory of Molecular Oncology, Zurich University Hospital, Zurich, Switzerland [3]FGen GmbH, Basel, Switzerland

INTRODUCTION: High throughput (HT) screening is an important process in pharmaceutical drug discovery and R&D. One crucial issue for in vivo-like cell behavior is the mechanical property (stiffness) of the 3D micro-environment. Our aim was to develop a screening platform with human primary cancer cells embedded in alginate-based hydrogel beads with different E-moduli.

MATERIALS & METHODS: Alginates at different concentrations and compositions were used to produce nanoliter-reactors (NLRs), suitable for microtissue (MT) growth and HT analysis. ZL55 cells (mesothelioma), encapsulated in NLRs (Ø=200 µm) formed MTs and remained viable up to 14 days. MTs grown in NLRs with higher E-modulus were smaller compared to those cultured in lower E-modulus NLRs but showed a higher metabolic activity (Ki-67-staining, PrestoBlue). Moreover, NLR stiffness seemed to have an influence on MTs' morphology resulting in round- or oval-shaped MTs in high-stiffness and low-stiffness NLRs, respectively. Podoplanin gene expression increased with increasing alginate bead stiffness, while gene expression of mesothelin and survivin seemed not to correlate with stiffness changes. In order to automate NLR analysis, ZL55 cells were cultured for 14 days in NLRs and then stained with Nile Red to be sorted, according to their biomass, by flow cytometry using a COPAS BioSorter. Sorting procedure did not influence MT viability, a prerequisite for further drug testing. Alginate-free MTs revealed high resistance of ZL55 to cytostatic drugs, such as Taxol, Doxorubicin and Cisplatin. Sorted NLRs will be treated similarly to detect alginate's influence. The final goal will be the generation of clonal patient derived cell lines which then can find application for personalized drugs screening as wells as cancer cell models.

RESULTS & DISCUSSION: As initial proof-of-concept, human primary mesothelioma cells, SDM81, were successfully encapsulated in NLRs made of different alginates. Although primary cells are more sensitive compared to cell line, cells were viable and grew up to 14 days in culture, demonstrating the high potential of the NLR technology for the cultivation and analysis of primary cells.

ACKNOWLEDGEMNTS: Funding: Innosuisse, Switzerland (18364.2 PFLS-LS)



Extracellular vesicles administration using engineered three-dimensional bioactive cardiac scaffolds for myocardial repair

M. Monguió-Tortajada[1,2,3], C. Prat-Vidal[1,4,5], M. Clos-Sansalvador[1], A. Morancho[6], C. Gálvez-Montón[1,4], A. Rosell[6], A. Bayes-Genis[1,4,7,8], S. Roura[1,4], F.E. Borràs[2,3,9]
Presenting Author: Marta Monguió-Tortajada, mmonguio@igtp.cat
[1]ICREC Research Program, Health Science Research Institute Germans Trias i Pujol (IGTP), Can Ruti Campus, Badalona, Spain [2]REMAR-IVECAT Group, IGTP, Spain [3]Dpt of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona (UAB), Spain [4]CIBERCV, ISCIII, Madrid, Spain [5]Center of Regenerative Medicine in Barcelona (CMRB), Hospital Duran i Reynals, Spain [6]Neurovascular Research Laboratory, VHIR, UAB, Spain [7]Cardiology Service, Germans Trias i Pujol University Hospital (HUGTiP) [8]Department of Medicine, UAB, Spain [9]Nephrology Service, HUGTiP, Badalona, Spain

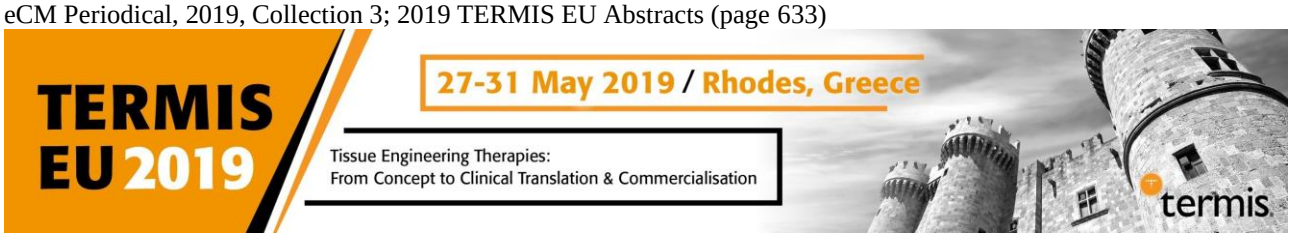
INTRODUCTION: Administration of Extracellular Vesicles (EVs) from mesenchymal stem cells (MSCs) is a promising nanotherapy for cardiac repair after myocardial infarction (MI), based on their immunomodulatory, regenerative, and pro-angiogenic capabilities. However, the optimal EV delivery strategy remains undetermined. We tested the use of biocompatible 3D-engineered cardiac scaffolds as an efficient support for local administration of EVs.

METHODS: EVs from porcine cardiac adipose tissue-derived MSCs (cATMSCs) were purified by size-exclusion chromatography (SEC) and checked for immunomodulation and recruitment capabilities towards allogeneic PBMCs and pro-regenerative cells, respectively. At the same time, EVs were fluorescently labelled for tracking and retention analysis after administration within a peptide hydrogel into cardiac scaffolds, generated by decellularization of healthy cardiac tissue.

RESULTS & DISCUSSION: EVs from porcine cATMSCs reduced allogeneic polyclonal proliferation and pro-inflammatory cytokines production (IFNγ, TNFα, IL12p40), and recruited pro-regenerative cells, including progenitor endothelial cells and allogeneic MSCs. These EVs were successfully retained by two different decellularized scaffolds engineered from cardiac tissues and peptidic hydrogel, with preserved structures and no immunoreactivity.

CONCLUSIONS: These data indicate that 3D-engineered cardiac scaffolds may effectively elicit myocardial local delivery of multifunctional cATMSC-EVs. Administration of EVs using a scaffold may be envisaged as cell-free off-the-shelf product for tissue repair, increasing the local EV dosage and generating a bioactive niche for cell migration and regeneration.

ACKNOWLEDGEMENTS: Financial support was received from Fundació La Marató de TV3 (201516-10, 201502-30), Generalitat de Catalunya (2017-SGR-301, 2017-SGR-483, PERIS SLT002/16/00234), MINECO (SAF2017-84324-C2-1-R), “La Caixa” Banking Foundation, ISCIII (PI17/01487, PIC18/00014, PI16/00981), ISCIII-Feder (REDinREN RD16/0009, TerCel RD16/00111/0006, CIBER Cardiovascular CB16/11/00403).



Sericin, alginate and platelet lysate combined in a biomembrane for the treatment of skin ulcers

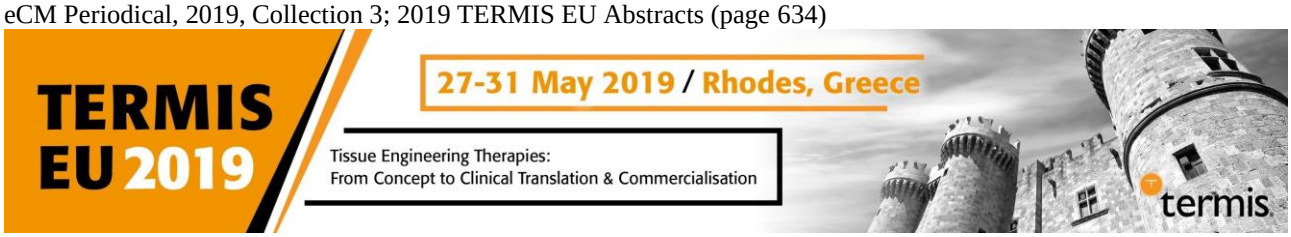
M. Nardini[1,2], S. Perteghella[3], G. Marubbini[3], F. Grillo[4,2], L. Mastracci[4,2], C. Gentili[1,2], R. Cancedda[1], M.L. Torre[3], M. Mastrogiacomo[5,2]
Presenting Author: Marta Nardini, nardinimarta88@gmail.com
[1]Regenerative Medicine Laboratory, Department of Experimental Medicine (DIMES), University of Genova, Genova, Italy [2]IRCCS Ospedale Policlinico San Martino Largo Rosanna Benzi 10, Genova, Italy [3]Cell Delivery System Laboratory, Department of Drug Sciences, University of Pavia, Pavia, Italy [4]Pathological anatomy Laboratory, Department of integrated surgical and diagnostic sciences, University of Genova, Genova, Italy [5]Bioterapy Laboratory, Department of Internal Medicine (DIMI) University of Genova, Genova, Italy

INTRODUCTION: Skin wound healing requires the recruitment and activity of different cell types, such as native immune response cells, endothelial progenitors, keratinocytes, and fibroblasts. Wound treated with Platelet Lysate (PL) gel recovered more quickly in all patients, leading to a reduction of hospital stay and of the related cost. Silk sericin (SS) is a globular and water-soluble protein with a molecular weight up to 200 kDa that, together with fibroin, constitutes the silk cocoon. The treatment of wounds should remove exudate, if present, in order to prevent infections. For this reason, to date, wounds are often treated with dressings based on alginate, which has excellent biocompatibility and ability to adsorb exudate.

METHODS: Sericin (SS), alginate (Alg) and PL were solubilized in distilled water and cast into freeze-dried molds. In vitro tests were performed to quantify the growth factor release from sponge/PL membrane by ELISA kit, the cytotoxicity of membrane and the capability of SS and PL alone or in combination, to induce proliferation and protective effects against oxidative stress. For all this analyses we used bone marrow stromal cells (BMSCs) and human fibroblast (hFB). We also tested the in vivo efficacy of our membranes in an excisional wound healing mouse model. The animals were sacrificed at different times (3, 7, 14 and 21 days and a histological investigation was performed.

RESULTS & DISCUSSION: In vitro results indicated that the transformation of membrane into a gel take place in 48h with a burst of factor release. The presence of SS contributes and support the effect of PL by controlling the release of growing factors. The in vitro experiments showed a key role of the PL as a good inductor of cell proliferation and protector from oxidative stress condition and the SS a good modulator of its release. To be more sure of our hypothesis derived from in vitro experimental data, a wide study in vivo in mouse skin regeneration model was performed.
This in vivo analyses showed how the membrane containing PL led to a faster regeneration of the skin respect to the control one (Alg:SS). We observed an inflammation phase occurred faster in treated lesion due to the presence of PL, that lead to a granulation tissue formation and subsequent collagen deposition and reepithelization and neovascularization. In control lesion we notice a non-complete resolution of the healing process.

CONCLUSIONS: This work propose a handle membrane composed of biomaterial (alginate and sericin) that are biocompatible and have particular mechanical properties, in association with a powerful inductor of cell activation that lead to complete skin regeneration. The SS contribute and support the effect of PL by its controlled release into the lesion.



Endothelial regeneration in coronary arteries from functionalized 3D printed poly-l-lactic acid bioresorbable stent

R. Sabaté [1,2], K. Kolandaivelu[3], R. Schieber[1,2], C. Mas-Moruno[1,2], M. Pegueroles[1,2]
Presenting Author: Marta Pegueroles, marta.pegueroles@upc.edu
[1]Biomaterials, Biomechanics and Tissue Engineering Group, Technical University of Catalonia (UPC), Spain [2]Barcelona Research Center in Multiscale Science and Engineering, UPC, Spain [3]Institute for Medical Engineering and Science, MIT, Cambridge, USA

INTRODUCTION: Bioresorbable stents (BRS) are designed to overcome perceived limitations of drug-eluting stents (DES) by providing temporary support to the vessel wall. Herein, we developed bioactive 3D printed BRS with enhanced mechanical properties by solvent-cast direct-write technique to overcome atherosclerosis whilst regenerating coronary arteries endothelium.

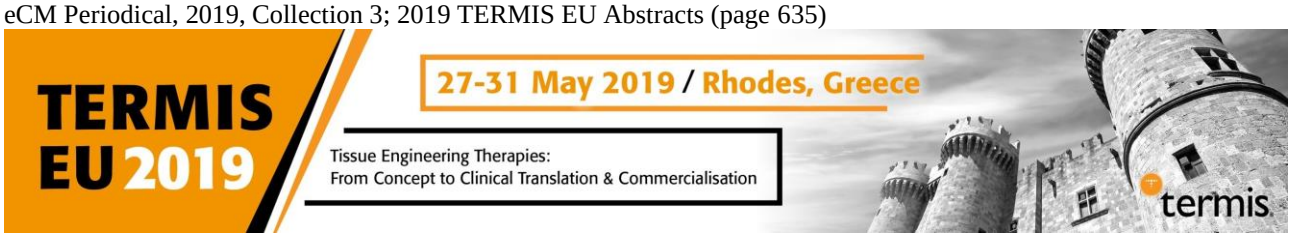
METHODS: 3D printed stents were obtained with a modified fused deposition modelling (FDM) 3D printer (BCN 3D+, BCN 3D technologies). The ink was a solution of poly-l-lactic acid (PLLA, PL65, Purasorb) in chloroform (3.7%v/v) dissolved in a speed mixer (FlackTek). REDV and YIGSR peptides were synthesized by solid-phase and covalently attached to the surface using EDC/NHS. Mechanical assays consisting of crimping the stent and expansion into a silicone tube and average breaking pressure were carried out. HUVEC adhesion and proliferation was characterized by immunofluorescence. HUVEC migration was performed through a wound-healing assay. Thrombogenicity was tested into a prefusion chamber with volunteer donnor's blood. Statistical analysis was realised by non-parametric Mann-Whitney U-test using Minitab software.

RESULTS & DISCUSSION: PLLA biodegradable stents of 3 mm diameter and between 15-20 mm length were obtained by means of a modified FDM 3D printer with different peaks (Figure 1). Actual commercial BRS have struts of 130 μm diameter, while in our study we were able to obtain values down to 80 μm. Thermal treatment eliminated chloroform residues from fabricated stents and increased crystallinity up to 28,7 % compared to non-treated stents.

CONCLUSIONS: The fabrication of a biactive biodegradable stent by 3D printing was successfully achieved by SC-DW with a self-modified FDM. Biological assays confirmed that 3D printed BRS by SC-DW were not cytotoxic and functionalization enhanced endothelial regeneration.

Figure 1: (Left) 3D printed PLLA stents: (a) peak number (n=5); (b) different stents n= 7,10, 15, 20. Average breaking pressure was between 15-17 atm and a good stent deployment was observed into the simulated artery. Biological tests confirmed that 3D printed stents were not cytotoxic independently of the number of peaks. Finally, functionalization enhanced HUVEC migration and did not increase thrombogenicity. **Figure 2:** (Right) Thrombogenicity test on stents with different strut diameter.

ACKNOWLEDGEMENTS: Financial support was received from Spanish Government, MINECO/FEDER, (Grant no: MAT2015-67183-R and Grant no: DTS16/00133).



Innovative 3D brain cell-based model in MINERVA project

M. Tunesi[1], I. Raimondi[1], L. Izzo[1], D. Albani[2], C. Giordano[1]
Presenting Author: Marta Tunesi, marta.tunesi@polimi.it
[1]Dept. Chemistry, Materials and Chemical Engineering "G.Natta", Politecnico di Milano, Milan, Italy
[2]Dept. Neuroscience, Istituto di Ricerche Farmacologiche "Mario Negri" - IRCSS, Milan, Italy

INTRODUCTION: Microbes colonizing the gut (microbiota) can alter brain physiology and be involved in neurodegeneration [1]. The ERC "MINERVA" project aims at developing the first engineered organ-on-a-chip platform to simulate in vitro the key features of the microbiota-gut-brain axis in physiological and pathological scenarios. Herein, we set up an innovative 3D brain cell model potentially suitable to simulate the key hallmarks of Alzheimer's disease (AD) in the brain compartment of the MINERVA device [2].

METHODS: We cultured amyloid precursor protein (APP)-Swedish mutant H4 cells in both collagen/poly(ethylene glycol) (PEG) [3] and collagen/hyaluronic acid (HA) gels. We tested two conditions: a) embedding in hydrogels; b) plating in culture plates and covering by a layer of gel. We varied hydrogel thickness and studied the influence of cell number and volume ratio between medium (or cell suspension) and polymer components on collagen fibrillogenesis by measuring the optical density at 400 nm. We analyzed the samples by transmission electron microscopy (TEM). We varied gel thickness and measured cell metabolic activity with time by MTS assay after embedding $3.125 \cdot 10^5$ cells/cm^2 or plating $2.34 \cdot 10^4$ cells/cm^2. For the best conditions, we examined the trapping of β-amyloid peptide (Aβ) by confocal microscopy.

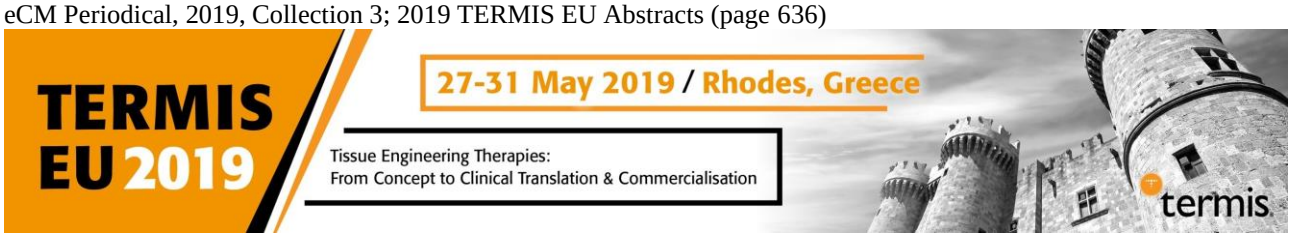
RESULTS & DISCUSSION: For all gels, the reduction in thickness from 5.1 to 1.25 mm did not hinder the gelling process, but influenced the lag time and fibril diameter. TEM confirmed a reduction in fibril diameter when decreasing the thickness. Cell metabolic activity was greater on day 10 for both culturing conditions. For covered cells, regardless of hydrogel composition, the greatest thicknesses led to the lowest metabolic activity. We performed immunofluorescence analyses on samples of 1.25- and 2.5-mm thick. Only for embedded cells, we detected a diffuse signal referable to Aβ.

CONCLUSIONS: Our 3D brain cell model is potentially suitable to recapitulate Aβ accumulation in vitro.

ACKNOWLEDGEMENTS: MINERVA project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement no.724734). We thank C. Maffeis for technical support.

REFERENCES

[1] Mayer E. A. et al. J Clin Invest. 2015;125(3): 926-38
[2] Tunesi M et al. Front. Aging Neurosci. 2016;8:146
[3] Tunesi M et al. Int J Artif Organs. 2013; 36(11):762-74



The role of cell-secreted extracellular matrix proteins in engineered cell niches

U. Blache[1], Y. Devaud[1,2], Q. Vallmajo-Martin[1,2], I. Martin[3], M. Lütolf[2], M. Ehrbar[1]
Presenting Author: Queralt Vallmajo-Martin, martin.ehrbar@usz.ch
[1]Laboratory for Cell and Tissue Engineering, University Hospital Zürich, Switzerland [2]Laboratory of Stem Cell Bioengineering, EPFL Lausanne, Switzerland [3]Department of Biomedicine, University Hospital Basel, Switzerland

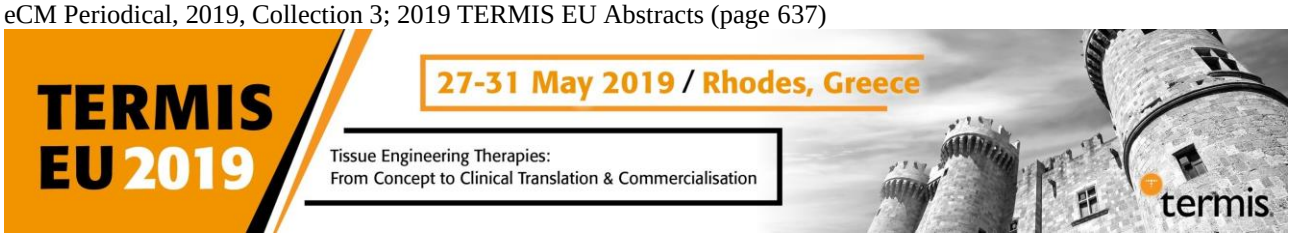
INTRODUCTION: To recreate fully defined three-dimensional (3D) (stem) cell niches, hydrogels with bio-chemically and mechanically tailorable properties were developed. For this a major focus was on the localized presentation or delivery of growth factors and controlling of integrin binding sites. Due to the lack of standardized procedures for the evaluation of extracellular matrix (ECM) components in 3D cultures, limited data on the remodeling of engineered cellular microenvironments and according consequences on cell functions are available. To evaluate ECM proteins and their function in in the establishment of 3D tissue models, we have employed label-free proteomics, RNA-sequencing and co-cultures of endothelial cells with fibronectin-depleted human bone marrow mesenchymal stromal cells (hMSCs).

METHODS: Poly(ethylene glycol) hydrogels (PEG) [1] were optimized for 3D cultures with hMSCs or co-cultures of hMSCs with human umbilical vascular endothelial cells (HUVECs). 3D tissue constructs were cultured in presence or absence of supportive growth factors and evaluated by 1) fluorescence or electron microscopy, 2) harvested by FACS sorting for RNA sequencing, or 3) digested by trypsin for the harvesting of cells and ECM proteins and their processing for label-free proteomics. For fibronectin-depletion hMSCs were treated with siRNA.

RESULTS & DISCUSSION: PEG hydrogels with low stiffness allowed hMSC to spread and deposit various ECM components as shown by immune stains and label-free proteomics. PEG hydrogels allowed temporary spreading of HUVECs but could not support their long-term survival. In contrast co-cultures of HUVECs and hMSCs formed stable 3D vascular networks with morphologies similar to natural capillaries (including vascular lumen). As shown by RNS-sequencing, MSCs derived from co-cultures exhibited a shift in ECM expression when compared to hMSCs from monocultures. When their fibronectin expression was suppressed hMSCs could only partially spread evenin the presence of RGD. Additionally, in absence of fibronection the secretion of all other ECM components was almost completely abrogated. Furthermore, the formation of vascular structures was completely suppressed.

CONCLUSIONS: Our data indicate that in 3D cultures cell-secreted ECM components play a crucial role. This becomes best visible in cultures, where the fibronectin deposition in MSCs is inhibited, resulting in the abrogation of ECM deposition and cell spreading. In co-cultures of hMSCs with HUVECs, hMSCs were shown to play a guiding function in the formation of vascular structures, since vessel formation was critically dependent on MSC-derived matrix components. Taken together, 3D tissue formation in synthetic substrates is significantly modulated by cell-secreted ECM components. Understanding the role of this ECM will thus be crucial for the engineering and manipulation of stem cell niches.

ACKNOWLEDGEMENTS: This work was supported by Swiss National Science Foundation grants 153316, 31003A_141051, 310030_169808 and the European Union's Seventh Framework Programme (iTERM grant agreement No. 607868)



An in vitro multi-culture approach to investigate heterotypic interactions occurring in the early metastatic niche

M. Crippa[1,2], M. Gilardi [3], S. Bersini [3], C. Arrigoni [1], S. Gamba [4], A. Falanga [4], M. Vanoni [5], C. Candrian[1], M. Moretti [1,3,6]
Presenting Author: Martina Crippa, martina.crippa@polimi.it
[1]Ente Ospedaliero Cantonale, Lugano, Switzerland [2]Politecnico di Milano, Milano, Italy [3]IRCCS Istituto Ortopedico Galeazzi, Milano, Italy [4]Papa Giovanni XXIII Hospital, Bergamo, Italy [5]Università Milano Bicocca, Milano, Italy [6]Fondazione Cardiocentro Ticino, Lugano, Switzerland

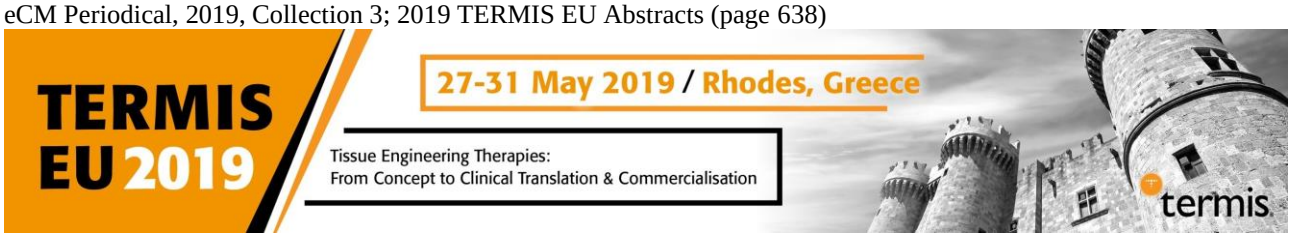
INTRODUCTION: Cancer metastasis is a multistep process during which multiple players establish competitive mechanisms whereby cancer cells experience pro- and anti-metastatic effects. Available in-vitro models are limited for the study of complex interactions, due to implification as compared to physiological tissues. Exploiting our already developed microfluidic model, we aim at replicating the so called early metastatic niche (EMN), characterized by the heterotypic cross-talk between platelets, neutrophils, endothelial cells (HUVECs) and breast Cancer Cells (bCCs), which represents a crucial event for metastatic progression.

METHODS: Microfluidic vascularized models have been generated by co-culturing fibroblasts and HUVECs embedded in a fibrin gel for 4 days. To investigate the role of platelets and neutrophils in breast cancer cells (bCCs) extravasation, we co-cultured bCCs, platelets and neutrophils (isolated from human buffycoat) and after 24 h we injected the cells suspension into the perfusable vessels. We measured bCCs adhesion to HUVECs, extravasation and migration by confocal imaging. We also investigated the effects of a clinically approved anti-platelet drug (αIIbβ3 inhibitor Eptifibatide, 15 µg/ml) on each component of the EMN and the interplay among these components.

RESULTS & DISCUSSION: bCCs extravasation significantly increased in presence of platelets and neutrophils as compared to control conditions (49.99±3.39% vs. 22.66±4.75%, $p<0.001$). Regarding the interactions between EMN components, we saw that neutrophils shifted bCCs towards a rounded shape suggesting a less aggressive phenotype, while the simultaneous presence of neutrophils, platelets and endothelial cells partly reversed the effect. The addition of αIIbβ3 inhibitor significantly reduced the number of extravasated bCCs ($p<0.001$), reducing platelet activation (αIIbβ3) and decreasing SrcY416, FAKY397, (VE)-cadherinY658 phosphorylation, leading to HUVEC junction tightening which could partly explain the reduction in bCCs extravasation.

CONCLUSIONS: Exploiting our approach, we demonstrated how concomitant interactions with several niche components modified the behavior of other cell populations as compared to the effects of the single interactions. As an example, neutrophils acted with a context-dependent behavior, increasing or decreasing bCC aggressiveness when alone or in co-culture with other EMN components. Furthermore, we showed that αIIbβ3 inhibitor decreased bCCs extravasation through concurrent mechanisms acting on different EMN components, highlighting how a clinically approved antiaggregant can contribute to an anti-metastatic effect.

ACKNOWLEDGEMENTS: The Italian Ministry of Health is greatly acknowledged. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Breast Cancer Research Program under Award No. W81XWH-15-1-0092.



Minimally invasive 3D electroconductive biomaterial platforms for cardiac tissue engineering applications

M. Solazzo[1,2], A. Zhussupbekova[3], K. Krukiewicz[4,5], K. Fleischer[3], M. J. P. Biggs[4], M. G. Monaghan[1,2]
Presenting Author: Matteo Solazzo, solazzom@tcd.ie
[1]Trinity Centre for Bioengineering and [2]Dept of Mechanical and Manufacturing Engineering, TCD, Dublin [3]Dept of Physics, TCD, Dublin [4]Centre for Research in Medical Devices, NUI, Galway, Ireland [5]Dept of Chemistry, SUT, Gliwice, Poland

INTRODUCTION: Despite medical advances, myocardial infarction (MI) remains among the main causes of death in western countries. Electroconductive biomaterial scaffolds and electrical stimulation can improve the differentiation of progenitor stem cell sources into cardiomyocytes and the maturation of cardiac engineered organoids. In this study, we aim to process an electroconductive scaffold with optimized features for tissue engineering applications.

METHODS: Poly (3,4-ethylenedioxy thiophene):polystyrenesulfonate (PEDOT:PSS) was functionalized with stabilizing molecules, doped with 4-dodecylbenzenesulfonic acid (DBSA) and processed into films or three-dimensional (3D) scaffolds using spin-coating and freeze-drying respectively. Crosslinking investigations were performed using X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FT-IR) spectroscopy. In parallel, 3D constructs were manufactured and characterised with scanning electron microscopy (SEM), ethanol intrusion measurements uniaxial compression testing, and 2-point probe experiments. Finally, direct and indirect cytotoxicity assays were performed to evaluate biocompatibility.

RESULTS & DISCUSSION: XPS and FTIR demonstrated a unique crosslinking interaction between PEDOT:PSS and the novel molecules. Optimised freeze-drying parameters resulted in highly porous scaffolds with tunable dimensions and aligned or isotropic micro-architecture. Scaffolds can be needle injected into aqueous environments and afterwards gain their original form. Theses scaffolds possess conductivity and mechanical properties in ranges that match that of the myocardium to induce myogenic differentiation.

CONCLUSIONS: We developed a novel functionalized PEDOT:PSS scaffold that exhibits high porosity, tunable morphology, memory shape and low temperature processing,

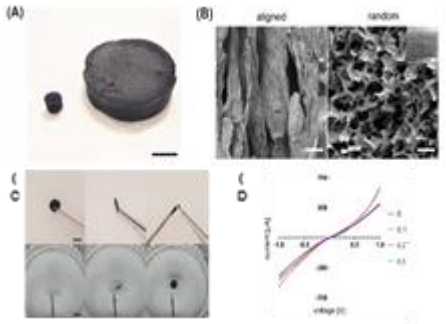
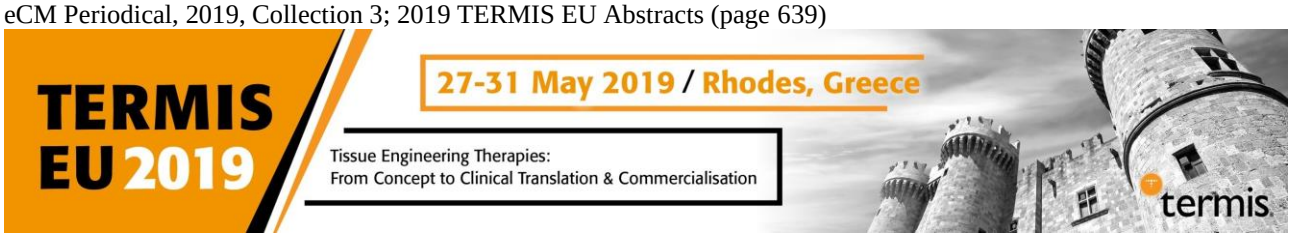


Figure 1: Scaffolds with (A) tunable geometries and (B) aligned or isotropic porous structures. (C) Memory shape effect of scaffolds when injected through a needle into water. (D) I-V curves revealing increase in conductivity with DBSA content. Scale bars: 10mm (A), 200 µm and 1 mm for insets (B), 5 mm (C).



The biologic scaffold immune microenvironment inhibits tumor formation and synergizes with immunotherapy

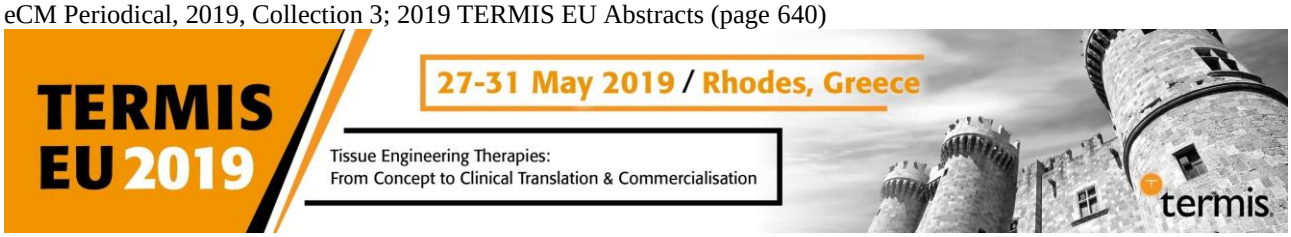
M.T. Wolf[1,2], S. Ganguly[2], D.M. Pardoll[2], J.H. Elisseeff[1,2]
Presenting Author: Matthew Wolf, wolf.matthew.t@gmail.com
[1]Translational Tissue Engineering Center (TTEC), USA [2]The Bloomberg~Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University, Baltimore, MD, USA.

INTRODUCTION: Tissue engineering scaffolds are designed to create an environment that encourages tissue growth. However, many of the same processes and cells involved in tissue healing are associated with tumor progression, including immune cells. Cancer immunotherapy attempts to reverse tumor escape from immune clearance, but is currently unknown whether a pro-regenerative scaffold immune environment affects immunotherapy responses.

METHODS: B16-F10 melanoma cells were co-injected with decellularized urinary bladder matrix (UBM) particles in C57BL/6 mice. Tumor immune infiltrate was characterized by flow cytometry and histology. Cancer immunotherapy response in the UBM microenvironment was interrogated by delivering systemic anti-PD-1 (programmed cell death protein 1) or isotype controls 8 days after cancer injection with UBM. Mice that survived the initial inoculation were rechallenged with B16-F10 and MC38 cells.

RESULTS & DISCUSSION: The UBM biologic scaffold microenvironment inhibited B16-F10 melanoma formation synergistically with anti-PD-treatment. B16-F10 cells were cleared by the combination of UBM and immunotherapy in approximately 20% of mice. These mice were also protected on rechallenge with B16-F10, but were not protected against the unrelated colon cancer cell line MC38 suggesting B16-F10 antigen specific immune recognition. The UBM microenvironment differed substantially from control melanoma tumors. T cell infiltration increased nearly 3-fold with UBM compared to Saline, as did proportions of natural killer (NK) and NKT cells. Histologic staining revealed that the majority of T cells in the UBM microenvironment were CD4+ T helper cells. Tumor growth inhibition with UBM was dependent on CD4+ T helper cells. Tumors readily grew in $Rag1^{-/-}$ mice that lack mature T cells and B cells. However, adoptively transferring CD4+ T cells into $Rag1^{-/-}$ mice rescued tumor inhibition to a similar extent as wild type mice.

CONCLUSIONS: UBM attracts immune cells and creates a unique environment that synergizes with checkpoint blockade immunotherapy to further activate responding T cells. As clinical administration of checkpoint immunotherapy is evolving, pro-healing biologic scaffolds may provide both a vehicle for tissue reconstruction and a mechanism to prime the local immune environment for optimal drug efficacy.



Evaluation of polysaccharide based sealants for soft tissue reconstruction

M. Schnabelrauch[1], K. Wagner[1], J. Becher[1], A. Berg[1], S. Voigt[2], A. Enkelmann[2], G. Schneider[2]
Presenting Author: Matthias Schnabelrauch, ms@innovent-jena.de
[1]INNOVENT e. V., Biomaterials Department, Jena, Germany [2]Jena University Hospital, Department of Otorhinolaryngology, Jena, Germany

INTRODUCTION: The high incidence of soft tissue damage due to trauma or tumor removal asks for new solutions to reconstruct those defects overcoming current limitations. Adhesives able to attach on wet surfaces and bind tissues together seems to be an alternative to suturing and stapling techniques. In this context, we evaluated cross-linkable polysaccharide hydrogel compositions as sealants in soft tissue reconstruction.

METHODS: Crosslinkable units (dialdehyde, carboxymethyl, methacryloyl groups) were introduced into the polysaccharides dextran, levan, chitosan, and hyaluronan using published procedures [1]. The prepared derivatives have been structurally characterized by common analytical techniques. Hydrogel formation was initiated by azomethine-, amide-, or radical polymerization-type crosslinking reactions. The adhesion strength of bonded sealants was determined under defined conditions using a texture analyzer TA-XT2i. In first bonding experiments float glass samples were used followed by substrates from fresh meat (6 hours after slaughter) from different pork tissue samples (mucosa membranes, muscle tissue). In vitro degradation of the sealants was studied gravimetrically in simulated body fluid (SBF) medium at 37°C for defined time intervals. The in vitro cytocompatibility was tested with fibroblast cells using commercial WST-1® and LDH assays.

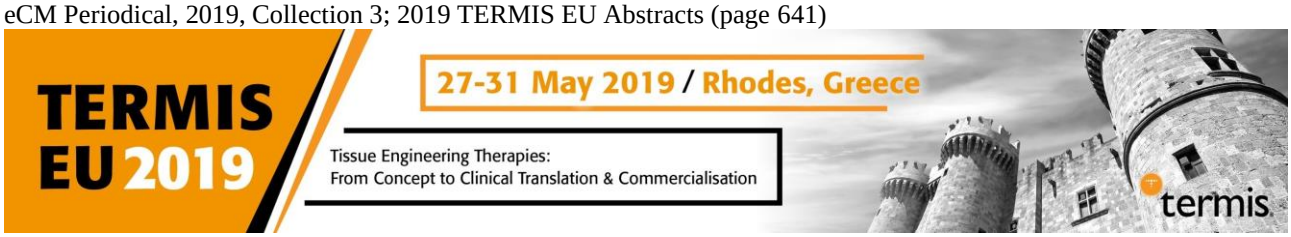
RESULTS & DISCUSSION: Stable hydrogels could be prepared by azomethine-type reaction of chitosan with dextran or hyaluronan dialdehydes and also amide-type reaction of carboxymethyl chitosan after crosslinking with a water-soluble carbodiimide. Hydrogels of levan, dextran and hyaluronan methacrylates can be obtained using conventional photoinitiators. In all cases the curing times of gel formation were below 30 min. The hydrogels showed an excellent in vitro cytocompatibility and a gradual degradability dependent on the type of polysaccharide and the degree of introduced functional group varying from several days to months. Dry bonding of float glass substrates gave highest values for tensile shear strengths in the methacrylate (150-300 kPa) followed by the azomethine (70-80 kPa) and the amide series (20 kPa). Interestingly, under wet bonding conditions, there was only a very slight decrease of tensile shear strengths found for both the azomethine- and the amide-type sealants. In bonding experiments with fresh meat slices an excellent adhesion of the sealant to wet tissue was found and for the azomethine-type sealant tensile shear strengths of up to 1.7 kPa compared to 1.2 kPa for commercial fibrin sealants were received. Whereas bonding of mucosal tissue was rather difficult to achieve, satisfied results could be obtained in bonding tests with pork muscle tissue.

CONCLUSIONS: Chitosan in combination with polysaccharide aldehydes forms rapidly curable, highly cytocompatible, degradable hydrogel sealants with optimal adhesion on wet tissue surfaces and tensile shear strengths for soft tissue in the same range as conventional fibrin glue.

ACKNOWLEDGEMENTS: COST-ENBA CA15216 is gratefully acknowledged.

REFERENCES

[1] A. Berg et al. Int. J. Biol. Macromol. 2018; 107(Pt. B):2312-19



Custom-made bioprinted human cell-laden meniscus prototype

M. Petretta[1,2], C. Cavallo[2], G. Filardo[3], L. Roseti[2], S. Durante[4], U. Albisinni[4], B. Grigolo[2]
Presenting Author: Mauro Petretta, mauro.petretta@ior.it
[1]RegenHU LTD, Villaz-St-Pierre. Switzerland [2]Laboratorio RAMSES, Rizzoli RIT - Research, Innovation & Technology Department, Italy [3]Applied and Translational Research Center, Italy [4]Struttura Complessa Radiologia Diagnostica ed Interventistica, Dipartimento Patologie Ortopediche-Traumatologiche Complesse; IRCCS Istituto Ortopedico Rizzoli Bologna, Italy

INTRODUCTION: Meniscal injuries are often associated with an active lifestyle, and the damage of this tissue renders young patients at higher risk of undergoing meniscal surgery and in time evolving in osteoarthritis (OA). 3D bioprinting has shown great potential in tissue engineering allowing to fabricate customized high-resolution structures starting from imaging data of the patient. The novel technique of simultaneously printing cells embedded into an appropriate biomaterial may be able to produce biological substitutes with enhanced efficiency in tissue regeneration and functional restoration. In this light, an early experience is presented on the production of a custom-made cell-laden collagen-based human meniscus.

METHODS: the 3D model of bioengineered meniscus was based on a Magnetic Resonance Imaging (MRI) scanning, processed using the dedicated software "Mimics" (Materialise, Leuven, BE). LifeInk© 200 purified, highly concentrated Type I Collagen bioink (Advanced Biomatrix, USA) with embedded human Mesenchymal Stem Cells (hMSCs) ($40x10^6$) was printed by means of a 3D Discovery bioprinter (RegenHu, CH). The chosen 3D Discovery printing tool was a microvalve-based inkjet printhead. The printing process was directly performed in culture medium in a sterile Petri dish, kept at 37°C. After printing, cell viability was evaluated by Live & Dead assay.

RESULTS & DISCUSSION: The selected bioink presented good printability and shape-fidelity, allowing the fabricated tissue, obtained by means of a microvalve-based inkjet dispensing technique, to mimic the anatomical model morphology as can be seen in Fig. 1. Moreover, this "cell-friendly" technology allowed hMSCs included into the bioink to be homogeneously distributed within the construct with high viability (95%) for up to 28 days in culture.

CONCLUSIONS: The prototype that we realized, shows the biologic potential of 3D bioprinting technology to provide an anatomically shaped patient-specific construct with viable cells embedded in a biocompatible material. It also sets the starting point for future developments of custom-made tissue-engineered structures toward the optimization of implants to replace damaged tissues.

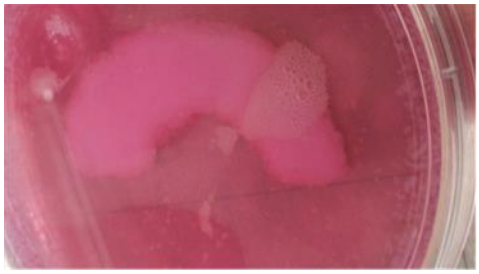
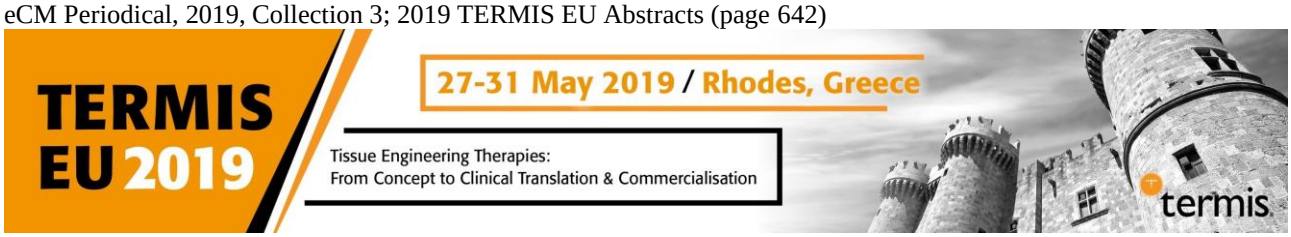


Figure 1: Cell-laden patient specific bioprinted meniscus Prototype.

ACKNOWLEDGEMENTS: Funding was provided by "Progetti Regione Emilia-Romagna POR-FESR 2014-2020: CUSTOM IMPLANTS" and "5x1000 funds, Italian Ministry of Health".



Nasal chondrocytes are potential autologous cell-transplant candidates for treating degenerative disc disease

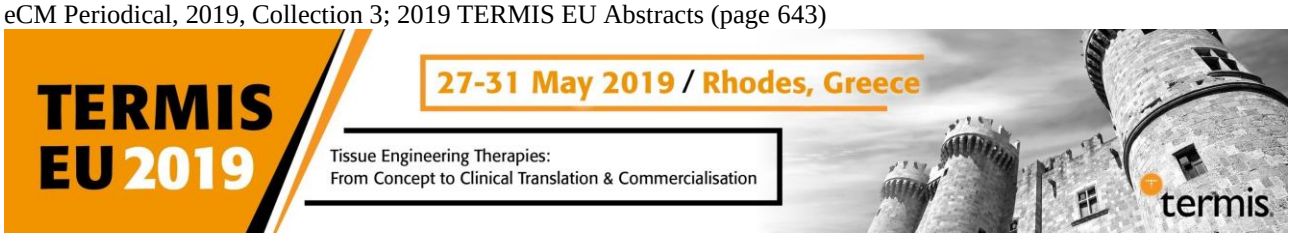
Max H.P. Gay[1,2] & Arne Mehrkens[1], Michael Rittmann[1,2] Andrea Barbero[2], Ivan Martin[2], and Stefan Schären[1]
Presenting Author: Max Gay, max.g@unibas.ch
[1]Department of Spinal Surgery, University Hospital Basel [2]Tissue Engineering, Department of Biomedicine, University Hospital Basel & University of Basel

INTRODUCTION: This project aims to determine whether nasal chondrocytes can be considered as an autologous cell source for cell therapy of disc degeneration by comparing them to MSCs and articular chondrocytes, two cells sources used in phase two clinical trials at the moment.

METHODS: Cells are cultured in in vitro micro-mass culture conditions mimicking facets of a degenerated intervertebral disc, such as hypoxia, low glucose, inflammation, and acidity. The production of the extra cellular matrix is evaluated using immunohistochemistry, quantitative real time PCR, and biochemical analysis.

RESULTS & DISCUSSION: Our data demonstrate MSCs, ACs, and NCs have a similar GAG and Collagen 2 production in response to in vitro conditions simulating singular facets of the IVD environment and the addition of TGFβ1. However, NCs synthesis more of these ECM components than MSCs or ACs when cultured in the same conditions without the addition of the growth factor. Furthermore, growth factor primed NCs maintain an exceeding production of GAG and Collagen 2 compared to both growth factor primed MSCs and ACs in harsher conditions, which combine different characteristics of the degenerated IVD environment. Interestingly, the indifference of ECM production of NCs in response to inflammation factors cannot be linked to the absence of gene expression of the respective receptors. Moreover, NCs as the only cell sources display gene expression of the transcription factor FoxF1, a marker for nucleus pulposus cells.

CONCLUSIONS: In summary, NCs are more similar to nucleus pulposus cells than MSCs and ACs, as they can better produce ECM in an in vitro IVD environment and express the nucleus pulposus marker FoxF1. These findings encourage the assessment that employing NCs in a cell therapy treatment of degenerated disc disease could promote new matrix production in the disc, which could inhibit or delay further disc height loss if not even lead to disc height gain.



Marine polysaccharide-based bioartificial pancreas for type-1 diabetes

M.L. Amin[1], D. Mawad[1], C.C. Sorrell[1]
Presenting Author: Md Lutful Amin, m.amin@student.unsw.edu.au
[1]School of Materials Science and Engineering, UNSW Sydney, NSW 2052, Australia

INTRODUCTION: Destruction of pancreatic islets by autoactivated immune system leads to type-1 diabetes, which affects ~90,000 children every year [1,2]. Implanted islets embedded in a suitable carrier as a bioartificial pancreas are considered to be a potential alternative to native islets. Marine sulfated polysaccharides, fucoidan and carrageenans (λ-, ι-, and κ-), exhibit characteristics similar to those of physiological polysaccharides and they can bind with growth factors and ligands [3]. The aim of the present work was to develop an immunomodulatory construct by copolymerizing these polysaccharides with polyvinyl alcohol (PVA), thus imparting biological functions to the resultant hydrogels.

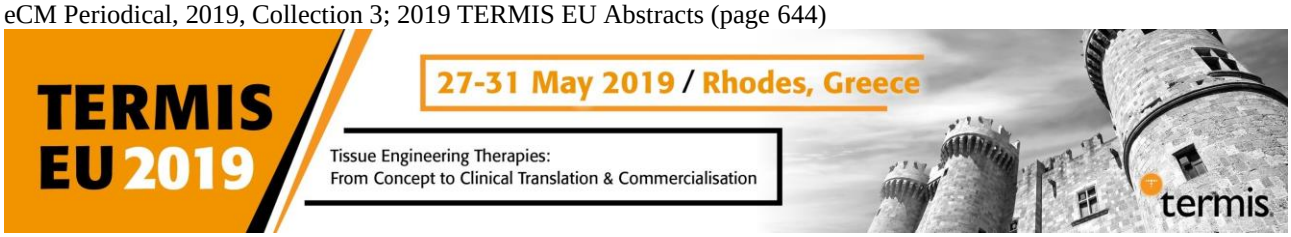
METHODS: PVA, fucoidan, and three commercially available carrageenans were methacrylated (MA) and their cytotoxicities were tested using fibroblast L929 cells. The hydrogel disks (9 or 19 wt% PVA-MA + 1 wt% polysaccharide-MA) were fabricated using 0.025 wt% Irgacure 2959 photoinitiator and they were characterized by mass loss, swelling, and polysaccharide release by dimethylmethylene blue (DMMB) assay (up to 7 days). Further, MIN6 cells were encapsulated in hydrogels and cell viabilities were assessed by live/dead assay. Cellular ATP level and insulin secretion also were evaluated progressively over a period of 32 days. Finally, the cell-containing hydrogels were exposed to a medium containing inflammatory cytokines (IL-1β, TNF-α, and IFN-γ) in order to examine the immunomodulatory characteristics of the hydrogels.

RESULTS & DISCUSSION: The polymers were found to be cytocompatible (>94% cell viability) by the cell growth inhibition assay. The addition of the polysaccharides did not cause considerable changes in mass loss and swelling of the hydrogels. The DMMB assay revealed that >50% of the polysaccharides were covalently bound to the PVA. Significantly, the number of sulfate groups in each polysaccharide repeating units correlated directly with the viability of the encapsulated MIN6 cells. Hence, >95% cell viability (32 days) was observed for PVA-fucoidan and PVA-λ-carrageenan hydrogels, both of which contain the maximum of three sulfate groups. The encapsulated cells formed islet-like spheroids inside the hydrogels and had consistent ATP level. The glucose-stimulated insulin secretion assay revealed that the cells retained their insulin-secretion profile. Further, hydrogels containing fucoidan and λ-carrageenan were observed to protect the cells from inflammatory cytokine-induced apoptosis.

CONCLUSIONS: The present work reports successful design of a bioartificial pancreas, revealing the importance of maximizing the number of polysaccharide sulfate groups. Consequently, fucoidan and λ-carrageenan in PVA-based hydrogels can be leveraged to enhance islet survival and functionality, indicating that these hydrogels can be used for in vivo islet implantation for the treatment of type-1 diabetes.

REFERENCES

[1] Katsarou A et al. Nat Rev. 2017;3:17016 (17 pp.)
[2] Wang Z et al. Clin Rev Allergy Immunol. 2017;52(2):273-86
[3] Silva TH et al. Biomatter. 2012;2(4):278-89



3D printed vascular channels and their maturation by flow stimulated bioreactor

K. Meenakshi[1], D.E Sindhuja[1], A.K Shukla[1], Sandeep[2], S.N Rath[1*].
Presenting Author: Meenakshi Kamaraj, BM18RESCH01004@iith.ac.in
[1]Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Telangana, India
[2]Department of Vascular and Endovascular Surgery, Nizam's Institute of Medical Sciences, Hyderabad, India

INTRODUCTION: Vascular tissue engineering requires the implants which avert the restenosis, thrombosis and immunological reactions and must withstand physiological pressures without any leakage [1]. Flow induced physiologic system plays a pivotal role in cell alignment and vessels maturation [2]. This study explores the influence of mechanical stimulation on the 3D printed co-culture system consisting of Endothelial cells (EC) and Mesenchymal stem cells (MSC) on the vessel maturation and efficacy of the graft.

METHODS: Alginate-Gelatin bioink with EC and MSC's were prepared and-a tubular CAD design is 3D printed. Medium was changed for every two days during the static culture of the 3D printed scaffolds. To study the effect of physiological pulsatile flow stimulation on the 3D printed scaffolds a bioreaction chamber was 3D printed using an FDM 3D printer with extensions to support perfusion. FDA/PI, Alamar Blue, Picogreen, SEM, Mechanical testing and IHC were performed at week 1 and week 3 for both static and dynamic conditions.

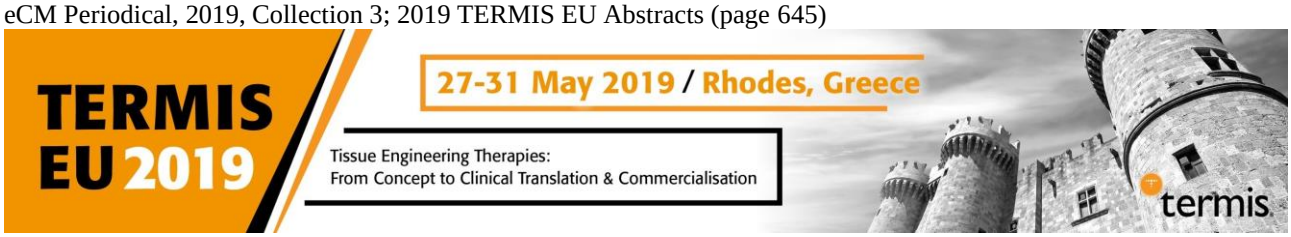
RESULTS & DISCUSSION: The surface morphology and structural integrity of the scaffolds were observed using Scanning Electron Microscopy (SEM) and adherence of cells on the top layer was evidenced. Live dead staining shows better biocompatibility and proliferation in dynamic conditions. The custom made bioreactor to simulate the physiological condition of blood vessel did not show any a toxic effect. Dynamic culture conditions allow the cells to mature and express genes for the vascular tissues such as CD31, VEGFR and vWF. Significant increase in mechanical strength and expression of angiogenic markers were observed in 3D printed vascular grafts cultured under dynamic conditions with flow stimulation when compared with static conditions.

CONCLUSIONS: ECs and MSCs are maturing together in dynamic conditions and are collectively responsible for increase in the mechanical strength of the vascular grafts. The mechanical stimulation provided externally is also contributing to the enhanced maturation of the coculture system. Vascular grafts fabricated and matured using these bioreactors have huge potential in vascular tissue engineering.

ACKNOWLEDGEMENTS: Financial support was received from Department of Biotechnology (DBT) India, Ministry of Human Resource and Development (MHRD) India.

REFERENCES

[1] William G Chang et al, Regenerative Medicine. 2017; 7.
[2] Sivarapatna A et al, Biomaterials. 2015; 53:621-633.



Mechanical properties of a PPF/pericardium biohybrid for small-diameter vascular graft applications

M. Kimicata[1,2], J. Allbritton-King[2,3], P. Swamykumar[2,3], M. Santoro[2,3], J.P. Fisher[2,3]
Presenting Author: Megan Kimicata, mak5467@terpmail.umd.edu
[1]Dept. of Materials Science and Engineering, U. of Maryland, College Park, MD, USA [2]Center for Engineering Complex Tissues, U. of Maryland, College Park, MD, USA [3]Fischell Dept. of Bioengineering, U. of Maryland, College Park MD, USA

INTRODUCTION: Extracellular matrix (ECM) is a fibrous network produced by cells that has been employed in tissue engineering endeavors. The objective of this work is to improve the mechanical properties of decellularized pericardial ECM by reinforcing it with poly(propylene fumarate) (PPF).

METHODS: PPF was synthesized and diluted with diethyl fumarate (DEF) at a PPF:DEF ratio of 2:1.[1,2] Bovine pericardium was freshly isolated and used as is (nECM), decellularized (dECM), or decellularized, dehydrated with serial dilutions of ethanol (20%, 40%, 60%, 80%, and 100%), coated with the 2:1 PPF:DEF solution, and crosslinked in the presence BAPO (dECM+PPF) (Fig 1A). The aforementioned tissue decellularization utilized a previously published protocol.[3] Half of the samples were digested in collagenase for four hours to simulate in vivo degradation. The samples were tensile tested to quantify tensile properties (Fig 1B) and suture retention strength (Fig 1C).

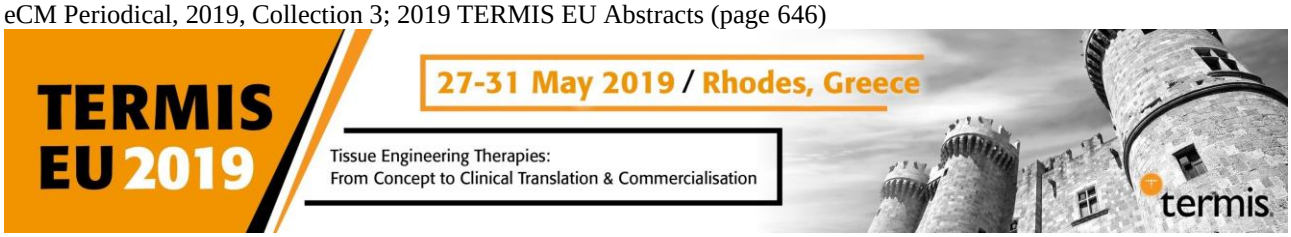
RESULTS & DISCUSSION: The toughness of both native and decellularized pericardium (nECM and dECM), decreases significantly ($p<0.005$) with collagenase digestion, while the pericardium that has been reinforced with PPF (dECM+PPF) does not experience a significant change ($p>0.05$). The coupling of pericardium with PPF prevents a loss of toughness in the pericardium when exposed to collagenase. Similarly, the maximum suture retention force after collagenase degradation of pericardium reinforced with PPF (dECM+PPF) is significantly higher than that of the decellularized pericardium (dECM). The application of PPF reduces the detrimental effects of collagenase on the suture retention strength of the pericardium.

CONCLUSIONS: PPF reinforcement methods improve the tensile behavior and suture retention of pericardial ECM, despite collagenase degradation for applications in small diameter vascular grafts. These trends will be utilized in future research to understand the cell response in the biohybrid vascular grafts.

ACKNOWLEDGEMENTS: This research was supported by the NIBIB/NIH Center for Engineering Complex Tissues (P41 EB023833) and the NSF CBET (5246870).

REFERENCES

[1] Bracaglia L et al. Tissue Eng Part A. 2014; 20(21-22):2807-2816
[2] Kasper F et al. Nat Protoc. 2009; 4(4):518-525
[3] Gui L et al. Tissue Eng Part C Methods. 2010; 16(2):173-184



Innovative preclinical models to evaluate new treatments for osteonecrosis of the knee

M. Maglio[1], E. Della Bella[1*], A. Roffi[1], G. Krishnakumar[2], H.A. van Boxtel[3], S.G.J.M. Kluijtmans[3], J. Knychala[3], G. Giavaresi[1], G. Filardo[1], E. Kon[4], M. Fini[1]
Presenting Author: Melania Maglio, melania.maglio@ior.it
[1]IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy [2]Bannari Amman Institute of Technology, Tamil Nadu, India; [3]Fujifilm Manufacturing Europe B.V., Tilburg, The Netherlands; [4]Humanitas Clinical and Research Center, Milan, Italy
*current author affiliation: AO Research Institute, Davos, Switzerland

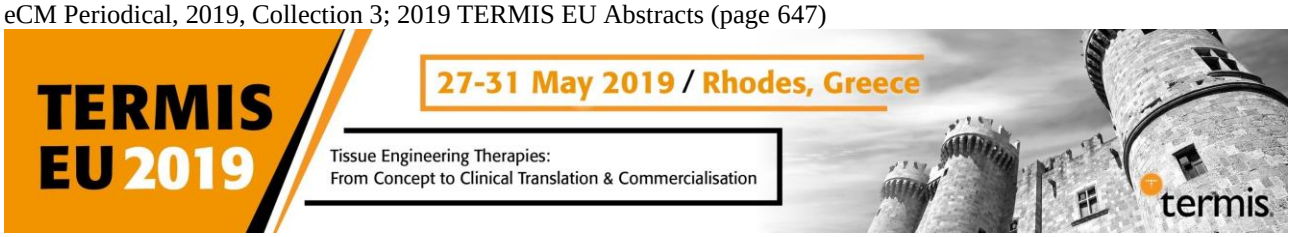
INTRODUCTION: Knee is the second most common site of osteonecrosis after femoral head with very demanding management, often requiring invasive surgical procedures. Novel material formulations, easier to use and combinable with biological adjuvant, might offer new therapeutic possibilities, but the relevant blank in preclinical models makes the evaluation of innovative therapy difficult. Aim of our work was to assess the osteogenic potential of injectable calcium phosphate pastes (biomimetically precipitated nano-size HA in Recombinant Collagen-like Peptide solution), and to study the set-up of a preclinical model of knee osteonecrosis, both in vitro and in vivo.

METHODS: In vitro study: Bone marrow human mesenchymal stem cells were co-cultured with two calcium phosphate formulations for 2 weeks and tested for viability and osteogenic differentiation. An in vitro culture model was set up with rabbit femoral condyles for 4 weeks to compare the effectiveness and feasibility of two methods for necrosis induction: ethanol injection and cycles of freezing/thawing, evaluated with viability assay, RNA yield and histology. In vivo study: a rabbit model of knee osteonecrosis was set up via injection of absolute ethanol. After 4 weeks, rabbits were randomly treated with the calcium phosphate material resulting more suitable after in vitro culture, with and without autologous bone marrow concentrate. After 8 weeks outcomes were evaluated histologically.

RESULTS & DISCUSSION: In vitro study: Bone marrow human mesenchymal stem cells cultures showed that both materials tested stimulate osteogenic differentiation, increasing the expression of RUNX2, COL1A1, VEGF. As for osteonecrosis induction protocols, both treatments induced a sharp drop of viability without recovery over time in comparison to control group, reduction in RNA yield with poorer A260/280 and A260/230 and RNA degradation, and histological signs of necrosis. In vivo study: After 8 weeks, untreated group showed histopathological signs of bone necrosis, with empty lacunae and signs of substitution of normal bone marrow with adipose tissue. Histology in treated groups highlighted high bone metabolic activity, with presence of osteoblasts along trabeculae, lacunae generally filled with osteocytes and reactive bone marrow.

CONCLUSIONS: Results obtained from the study proved that the materials tested can be suitable for application in regenerative medicine protocols. The feasibility of the in vitro model adopted open up the possibility to use such a long term bone culture for preliminary therapy evaluations and its reliability allowed to successfully set up an in vivo model of knee osteonecrosis, never described in literature.

ACKNOWLEDGEMENTS: Financial support was received by “BIO-INSPIRE” project, Training program on new bio-inspired bone regeneration (FP7-PEOPLE-2013-ITN Grant n° 607051).



Human plasma & platelet lysate or horse serum results in neuron-like cells but has no effect on adult human bone marrow MSC skeletal myogenesis

D. Barisic[1], B. Rolauffs[1], M. L. Hart[1]
Presenting Author: Melanie Hart, melanie.lynn.hart@uniklinik-freiburg.de
[1]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis Lab, Dept.of Orthopedics & Trauma Surgery, Medical Center-Albert-Ludwigs University of Freiburg, Germany.

INTRODUCTION: Mesenchymal stromal cells (MSCs) have great potential in cell-based therapies for restoring structure and function of many tissues including neuromuscular tissues. While human adipose- (ADSC) and umbilical cord-derived (ucMSCs) were shown to differ-entiate into a striated muscle phenotype using horse serum (HS) [1-3], to date bone marrow-derived MSCs (bmMSCs) have only been shown to differentiate into striated muscle using 5-azacytidine, a chemotherapeutic DNA-methylation inhibitor [4-5], or a complex mix of cytokines including bFGF, PDGF, forskolin and neuregulin, followed by transfection with notch intracellular domain [6]. However, neither of these approaches is clinically feasible. Hence, this study investigated the effects of a GMP-compliant medium on the differentiation of bmMSCs into a neuro-muscular phenotype vs. the standard HS protocol commonly used to differentiate ADSCs, ucMSCs and C2C12 cells.

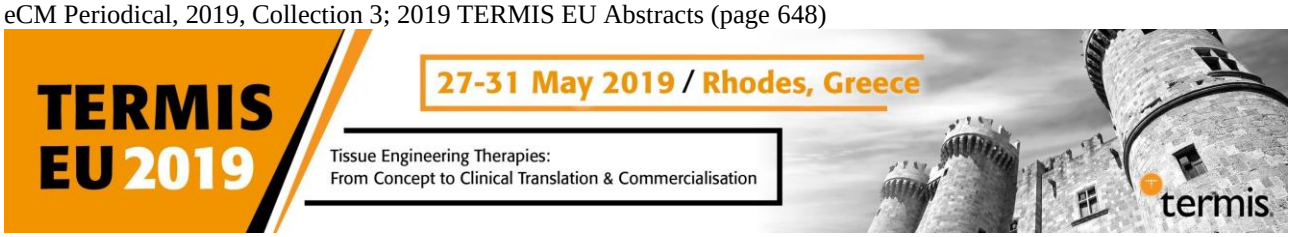
METHODS: bmMSCs were expanded in DMEM, 5% human plasma (P) and platelet lysate (PL). At P2 cells were placed in 8-well chamber slides or 12-well plates (3000 cells/cm^2) and further cultured in P/PL medium or 5% HS, 0.1µM dexamethasone, and 50µM hydrocortisone for 14 days. Expression of myogenic and neurogenic markers were quantified (ddPCR and immunofluorescence).

RESULTS & DISCUSSION: P/PL expansion (day 0) followed by treatment of bmMSCs with P/PL or HS (day 14) led to essentially zero expression of myogenic markers MyoD, MyoG, Myf5, and ACTA1 and low Desmin expression (Table I). P/PL expansion led to a high baseline expression of Coronin1b, vs. SJCR10 cells or mature skeletal muscle, which was not further increased with P/PL or HS. Yet P/PL led to a significant increase in GAP43 expression vs. HS (day 14). Moreover, the protein expression of GAP43 and Desmin was significantly increased by both P/PL and HS (Fig. 1, day 14 vs. day 0).

CONCLUSIONS: Adult bmMSCs expanded in GMP-compliant medium do not undergo myogenesis at day 14 using P/PL or the commonly used HS method to differentiate other MSC types. However, P/PL, and to a lesser extent HS, potentially stimulated a neuron-like phenotype.

REFERENCES

[1] Gang et al. 2004; Stem Cells, 22**,** 617-24
[2] Mizuno et al. 2002; Plast Reconstr Surg
[3] Zuk et al. 2002; Mol Biol Cell, 13**,** 4279-95
[4] Drost et al. 2009; AnnNYAcad Sci, 1176
[5] Stern-Straeter et al. 2014; IntJMolMed, 33
[6] Dezawa et al. 2005; Science, 309**,** 314-7



The effect of aligned electrospun fibres, macromolecular crowding and growth factor supplementation in tenocyte culture

D. Tsiapalis[1,2], M. Doulgkeroglou[1,2], S. Rana[1,2], Y. Bayon[3], D.I. Zeugolis[1,2]
Presenting Author: Meletios-Nikolaos Doulgkeroglou, m.doulgkeroglou1@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), NUI Galway, Galway [2]Ireland, Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland [3]Medtronic, Sofradim Production, Trevoux, France

INTRODUCTION: In vitro expansion of tenocytes leads to phenotypic drift and loss of function [1]. Different microenvironmental cues has been employed to maintain the phenotype of tenocytes [2, 3]. Herein, in this study we venture to assess the synergistic effect of aligned Biosyn® fibres, macromolecular crowding and TGFβ3 supplementation in tenogenic phenotypic maintenance.

METHODS: Human tendons were kindly provided from University Hospital Galway, after obtaining appropriate licenses, ethical approvals and patient consent. Afterwards, tenocytes were extracted using the migration method. To produce electrospun fibres, a 10 % w/v solution of Biosyn® in 1,1,1,3,3,3-Hexafluoro-2-propanol was prepared and supplied by a syringe pump at a flow rate of 20 μl/min. Aligned fibres were produced by electrospinning onto a collector covered with aluminium foil which was rotated at 3,000 rpm. SEM analysis was performed to visualise the fibres and to quantify fibre diameter. At passage 3 tenocytes were seeded onto the fibres and were supplemented with 5 ng/ml TGFβ3 and 50 μg/ml Carrageenan. Cell morphology was assessed using rhodamine/phalloidin staining. Cell proliferation and metabolic activity were assessed using Picogreen® and alamarBlue® assays. ECM synthesis and deposition were assessed using immunocytochemistry analysis. All experiments were performed at least in triplicate. MINITAB (version 16; Minitab, Inc.) was used for statistical analysis.

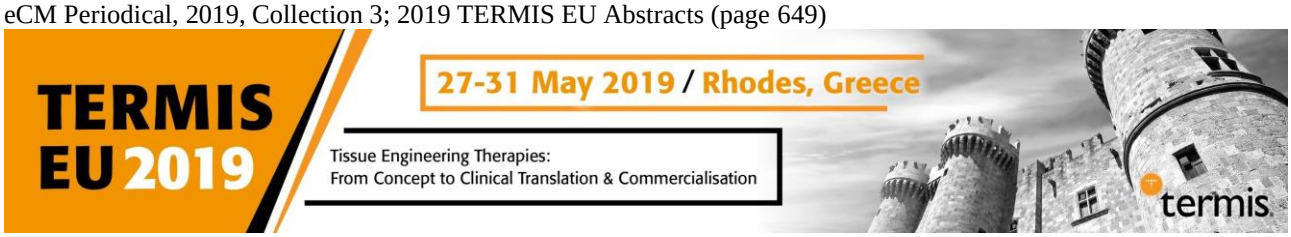
RESULTS & DISCUSSION: SEM analysis revealed fibre alignment with a range of diameter between 800-1600 nm. Cell morphology showed tenocyte arrangement to the direction of the fibres. Cell proliferation was significantly enhanced on cells seeded in fibres and supplemented with TGFβ3 and/or CR when added in serial fashion. Immunocytochemistry analysis demonstrated that human tenocytes seeded on aligned fibres and supplemented with TGFβ3 and CR at serial fashion increased synthesis and deposition of collagen type I, which is the major component of tendon ECM, as well as the tendon-specific ECM proteins collagen type III and V. In addition, collagen fibres were also aligned to the direction of the fibres mimicking collagen orientation in the tendon tissue.

CONCLUSIONS: Collectively, results suggest that the synergistic effect of surface alignment, MMC TGFβ3 can accelerate the formation of ECM-rich substitutes, which may stimulate tenogenic phenotype maintenance. Currently, further analysis for tendon specific genes is being conducted to validate our promising results.

ACKNOWLEDGEMENTS: Tendon Therapy Train (H2020-MSCA-ITN-2015-ETN) (Grant Number: 676338) and Science Foundation Ireland (Grant Number: 13/RC/2073)

REFERENCES

[1] Yao, L., et al., Tissue Eng. 12(7): p. 1843-9, 2006.
[2] Kumar P et al. Adv Drug Deliv Rev. 84: p. 240-56, 2015.
[3] Spanoudes K et al., Trends Biotechnol. 32(9): p. 474-82, 2014



Novel helix aspersa extract loaded chitosan scaffolds for hard tissue regeneration

M. Perpelek[1], S. Tamburaci[2], F. Tihminlioglu[2], A. Karakasli[1], H. Havitcioglu[1]
Presenting Author: Merve Perpelek, merveperpelek@gmail.com
[1]Instıtute of Healt Science, Department of Biomechanics, Dokuz Eylul University, Narlidere, İzmir, Turkey [2]İzmir Institute of Technology, Department of Chemical Engineering, Gulbahce Campus, Urla, İzmir, Turkey

INTRODUCTION: Cartilage damage is a highly observed clinical situation in orthopaedic treatments. Although there are traditional treatments known as autografting and allografting techniques, they have risk factors such as donor site morbidity, infections and immunogenic reactions [1,2]. Recently, studies have focused on tissue engineering applications as alternative methods to replace surgical procedures. In this study, it was aimed to fabricate bioactive Helix aspersa extract loaded chitosan scaffolds with slime and mucus incorporation and to investigate the composite structures in terms of physical, morphological and biological compatibility for bone-cartilage regeneration.

METHODS: Chitosan-slime and chitosan-mucus scaffolds were prepared with snail (Helix aspersa) secretions at different amounts (0.5%, 1% and 3%). Scaffolds were fabricated via lyophilization method. Morphology of scaffolds were determined by Scanning Electron Microscopy (SEM) and chemical composition by Fourier Transform Infrared Spectroscopy (FT-IR). In addition, swelling ratio test, open porosity determination, biodegradation test, biomechanical analysis (compression tests) and antimicrobial tests for five different strains were performed. In vitro bioactivity of chitosan-slime and chitosan-mucus scaffolds has been investigated with Saos-2 and SW 1353 proliferation. Osteogenic effect of extracts is investigated with ALP activity, osteocalcin secretion and biomineralization, whereas, chondrogenic potential of scaffolds is evaluated with GAG assay, hydroxyproline content and COMP assay.

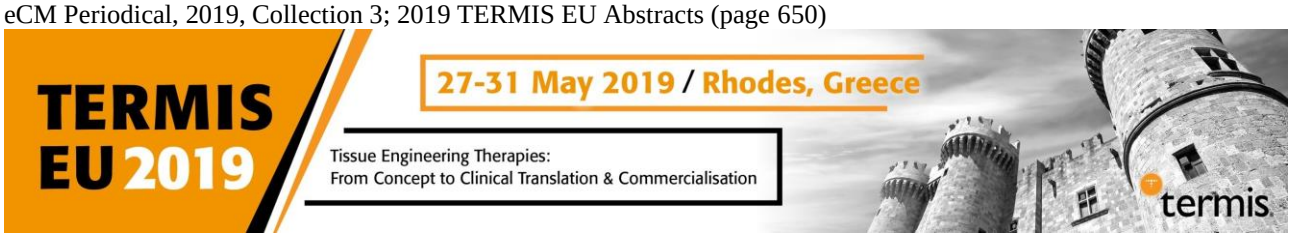
RESULTS & DISCUSSION: As a result, scaffolds containing snail extracts have been produced as intended. As the extract incorporation in polymer matrix increased, the mechanical strength was enhanced, and biodegradation rate increased. However, swelling ratio of chitosan scaffolds decreased with extract incorporation. SEM images showed that the pore size of the scaffolds decreased with higher extract content when compared to control group. Furthermore, porosity of all scaffold groups was obtained above 80%. The antimicrobial tests indicated that snail extracts showed antimicrobial effect on two bacterial strains out of five.

CONCLUSIONS: The scaffolds which have produced for bone and cartilage provided antimicrobial characteristics as well as suitable morphological and mechanical properties.

ACKNOWLEDGEMENTS: The authors are greatful to Izmir Institute of Technology (Iztech) Biotechnology and Bioengineering Research and Application Center (IZTECH BIOMER) for antimicrobial tests and FT-IR analysis, Center for Material Research (IZTECH CMR) for SEM imaging.

REFERENCES

[1] Temenoff JS et al. Biomaterials 2000;21:431-34
[2] O'Driscoll SW J Bone Joint Surg Am. 1998;80:1795-812.



Novel hydrogel for corneal regeneration

M. Suku [1,2], A. Laiva[2], A. Ma[1,2], A. Zeidan[1], F. J O'Brien[2,3], M.B. Keogh[1,2]
Presenting Author: Michael B. Keogh mkeogh@rcsi-mub.com
[1]School of Medicine, Royal College of Surgeons in Ireland, Bahrain [2]Tissue Engineering Research Group, RCSI, Dublin, Ireland [3]Advanced Materials and Bioengineering Research Centre (AMBER), Dublin, Ireland

INTRODUCTION: Human cornea is one of the most transplanted tissue worldwide, but faces intense shortage in donors. Gelatin-based hydrogels are commonly used in corneal tissue engineering, but they lack mechanical and thermal stability. It is hypothesized that gelatin can be reinforced by the addition of the protein crosslinking enzyme transglutaminase (Tgase) and polysaccharide gum acacia (GA), to obtain a novel hydrogel for corneal regeneration. Hydrogels will be assessed for biocompatibility using human adipose derived stem cells (ADSC) and 3D printing will be used to design moulds to form the implant.

METHODS: Hydrogels were prepared using 15% w/v of gelatin, 2.5% w/v Tgase and varying concentrations of GA (3, 2, 1, and 0.5% w/v). The time taken for solidification and gel thermal stability was measured at 37ºC. Thin films of hydrogels were prepared in 24 well plates by drying them in 40ºC overnight. 50,000 ADSCs seeded on rehydrated hydrogels and cultured for 48h. ADSCs cultured on the tissue culture plates acted as controls. Images of the cell were taken to assess biocompatibility. The gels were moulded into the shape of a corneal implant using 3D printed corneal moulds.

RESULTS & DISCUSSION: All the gels except the pure gelatin gel control were found to be thermally stable. 3% GA gels solidified fastest (18 mins) versus 0.5%GA which solidified in 25mins. Cells remained viable and gels were found to be biocompatible with ADSCs. Cells cultured on gels with 0.5%GA showed highest similarity to controls grown on tissue culture plates (Fig.1: a&b). Hydrogels containing 0.5%GA were chosen to be carried forward to be moulded in the shape of cornea. Blender was used to computationally design a 3D printed mould. After converting to STL format designs were printed on a Stratasys 3D ABS printer (Fig. 1 C). Moulds required treatment using acetone vapor exposure for smooth the printed surfaces.) 0.5% GA hydrogels were set using the 3D printed moulds resulting in corneal bioimplants (Fig. 1d).

CONCLUSIONS: This study shows that gelatin hydrogels with transglutaminase and Gum acacia can be used to form hydrogels. These hydrogels are biocompatible to ADSCS. Using 3D modelling it is possible to design and 3D print moulds to set the hydrogels into a corneal implant morphology. Current studies are examining the differentiation of ADSCs into corneal keratocytes within this formulation and potential for 3D bioprinting cornea implants.

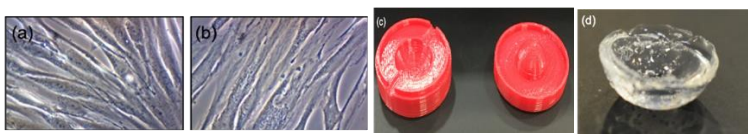
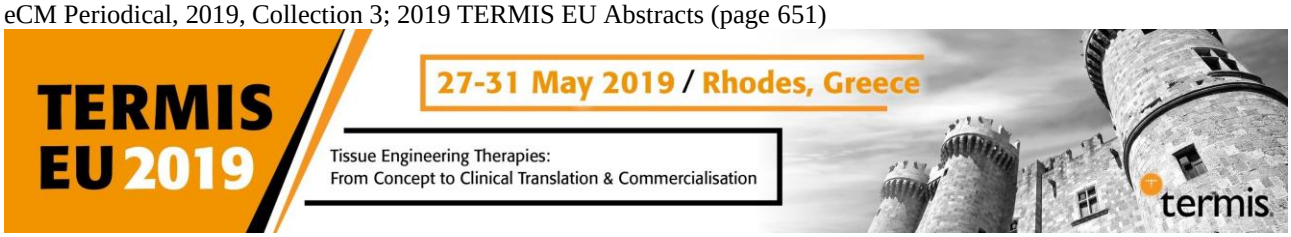


Figure 1: (a) ADSCs tissue culture plate monolayer culture was found to be morphologically similar to (b) ADSCs cultured on gels with 0.5%GA. (c) 3D printed corneal mould and (d) the gel with 0.5%GA shaped into cornea using the mould.

ACKNOWLEDGEMENTS: Financial support was received from RCSI Bahrain.



Production and analysis of bioprinted skeletal muscle tissue for drug development

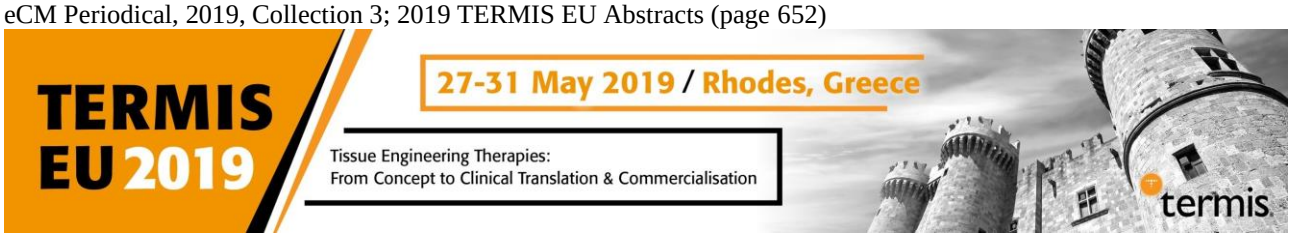
M. Rimann[1], M. Nosswitz[1], A. Alaves Reyes-Furrer[2], J. Goldowsky[3], V. Revol[3], F. del Principe[4], A. Baader[4], D. Marty[5], J. Burri[6], H. Keller[2]
Presenting Author: Michael Nosswitz, noss@zhaw.ch
[1]Center for Cell Biology & Tissue Engineering, Institute of Chemistry and Biotechnology (ICBT), Zurich University of Applied Sciences, Waedenswil, Switzerland [2]Musculoskeletal Diseases, Novartis Institutes for BioMedical Research, Basel, Switzerland [3]Instruments & Tools for Life Sciences, Centre Suisse d'Electronique et de Microtechnique CSEM, Alpnach, Switzerland [4]Life Imaging Services LIS, Basel, Switzerland [5]Weidmann Medical Technology, Rapperswil, Switzerland [6]Multi-Scale Robotics Lab, Department of Mechanical and Process Engineering, Swiss Federal Institute of Technology ETH, Zurich, Switzerland

Novel treatments against muscle-related diseases are becoming important due to the growth of the aging population. Currently, no robust medium throughput in vitro skeletal muscle model exists to assess drug effects on muscle contraction. Muscle functionality strongly depends on the three-dimensional (3D) structure and the high level of tissue organization. Thus, we use bioprinting to produce highly ordered contractile muscle tissues. With increasing tissue model complexity, the cultivation as well as the read-out are becoming more demanding. In this study, we produced functional human skeletal muscle tissues in a specialized 24 well plate. Each well contained two posts around which the muscle tissue formed. With integrated electrodes, the muscle tissue was electrically stimulated and contractions were monitored optically. Human skeletal muscle-derived cells (SkMDCs) mixed in Matrigel are deposited in inkjet-mode around the posts into the wells. After differentiation of the tissues and formation of myofibers the constructs are stimulated with electrical pulses resulting in tissue contractions. Contractile forces of about 400 µN were determined using a force measurement set-up. These forces were not able to bend posts made of polypropylene as previously published [1] because the post were too stiff and unflexible. Thus, in this project, different thermoelastic polymers (TPEs) were injection molded to produce softer posts with different stiffnesses. The posts made of TPE were tested via cellular force microscopy (CFM) to determine post deflection when applying a defined force of 400 µN. The post deflection will be monitored optically and serve as a quantification of muscle contractility/activity. The goal of the project is to develop an automated platform to produce and cultivate human muscle tissues suspended between two flexible posts. In an incubation chamber, the tissues are then subjected to electrical stimulation leading to post deflection, which is monitored with a camera. With this system 24 similar human muscle tissues are produced that can be used to monitor drug effects, while analyzing contraction differences. In the end, this platform may lead to a reduction in animal experimentation because it is human cell-based, more standardized and medium throughput-compatible.

ACKNOWLEDGEMENTS: We thank the innovation promotion agency Innosuisse Switzerland (Project No.: 16313.1 and 27901.1)

REFERENCES

[1] Laternser S et al. SLAS Technol. 2018 Dec;23(6):599-613



A strong, biomimetic and cytocompatible bone tissue adhesive

M. Pujari-Palmer[1], D. Wenner[1], R. Pujols[1], G. Insley[1, 2], P. Procter[1], H. Engqvist[1]
Presenting Author: Michael Pujari-Palmer, michael.palmer@angstrom.uu.se
[1]Applied Materials Sciences, Engineering Sciences, Uppsala University, Uppsala, Sweden [2]GPBio LTD. Rathkeale, Ireland

INTRODUCTION: A bone tissue adhesive must bond to protein and mineral surfaces, in wet and fatty environments [1]. A new, phosphoserine modified calcium phosphate cement (PM-CPC), which acts as a tissue adhesive, is described herein. PM-CPC strongly bonded multiple tissue types (1-4 MPa in shear, cortical bone), cured rapidly in liquids, and contained biocompatible precursors [2]. The cured adhesive retained many of the beneficial properties of the base material (ceramic), including supporting cell proliferation for bone scaffold/tissue engineering applications.

METHODS: Adhesive was prepared by mixing O-phospho-L-serine (Flamma) with alpha tricalcium phosphate (αTCP), and/or calcium silicate (CaSi), and water (0.25 L/P). Shear test Fresh bovine cortical bone tissue was cut into cubes (1 cm^3), 0.2g of PM-CPC was applied to the surface, and cubes were cured in water at 37^{o}C for 24 hours. A shear force was applied at 1mm/ minute on an MTS (Shimadzu AGS-X). The adhesive microstructure was investigated with x-ray diffraction (D8 Advance, Bruker) and scanning electron microscopy (Merlin SEM, Zeiss). Cytocompatibility PM-CPC or brushite was injected by syringe, cured for 3 minute, leached in 200mg/mL DMEM for 24 hours, neutralized and sterile filtered. The same process was repeated on the raw material powders (40mg/mL). Viability was determined in human dermal fibroblasts (HDFn) by alamar blue fluorescence (Invitrogen, 10% solution, 24h exposure) at 560nm/ 590nm (Tekan plate reader, normalized to untreated cells).

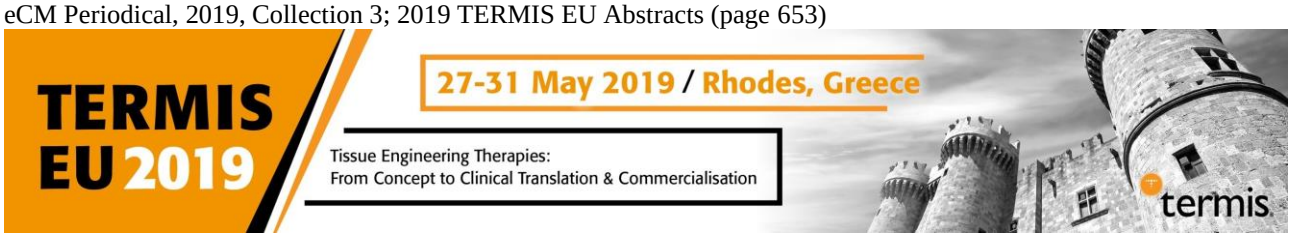
RESULTS & DISCUSSION: Various PM-CPC formulations produced strong bone adhesion, up to 380N (average, ≈2-3MPa). PM-CPCs were extremely tacky and bonded hard and soft tissues, and biomaterials such as collagen, underwater, without needing surface treatments (polish, etch). The set adhesive was disordered (XRD amorphous), and the internal architecture contained a unique organic/inorganic interface. Extracts from brushite produced greater cytotoxicity than PM-CPC formulations. The pH of the adhesive depended upon the ratio of acidic amino acid to basic calcium salt. Though formulations with low pH were cytotoxic, neutral formulations and extracts were cytocompatible (>80% viability).

CONCLUSIONS: PM-CPC was cytocompatible and bonded tissues stronger than other ceramics or naturally derived adhesives. Simple phosphorylated amino acids produce entirely novel properties in calcium phosphate and silicate bioceramics.

ACKNOWLEDGEMENTS: #RMA15-0110, EIT Health head start grant (2018 HS PoC 2018-HS-0046).

REFERENCES

[1]Farrar, D. et al. Int. J. Adhes. 2012, 33, 89–97.
[2] Pujari-Palmer, M. et al. Materials. 2018; 11(12): 2492.



Remote control of cell signalling using magnetic nanoparticles for neuronal cell differentiation

M. Rotherham[1], T. Goodman[2], T. Nahar[1], N. Telling[1], M. Gates[2], Alicia El Haj[1,3]
Presenting Author: Michael Rotherham, m.rotherham@keele.ac.uk
[1]Institute for Science and Technology in Medicine, Keele University, UK [2]School of Life Sciences, Keele University, UK [3]Institute for Translational Medicine, University of Birmingham, UK

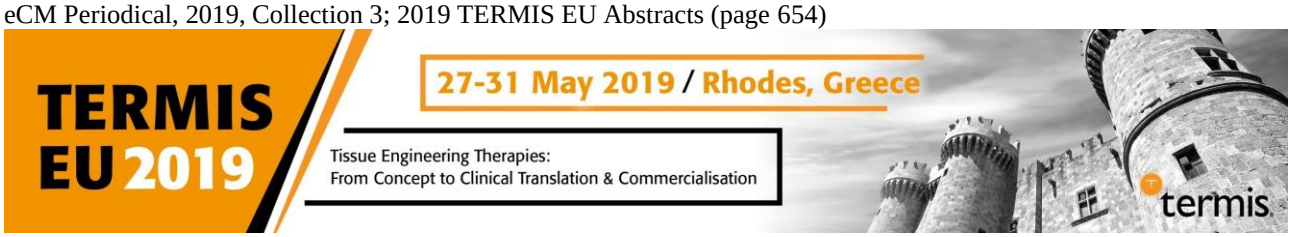
INTRODUCTION: Cell signalling pathways such as the Wnt pathway and Trek ion channel signalling are important regulators controlling cell fate and play roles in orchestrating neuronal development. These pathways are therefore attractive targets for development of modulators that regulate signalling activity and neuronal precursor cell behaviour. Cell receptors that initiate signalling cascades and neural progenitor differentiation can be targeted using ligand functionalised magnetic nanoparticles (MNP). Alternating magnetic fields can then be used to induce MNP torque or twisting. The translational force applied to the target receptor through MNP may induce receptor activation or opening of ion channels thereby triggering differentiation pathways. The aim of this research was to investigate the effects of remote activation of the Wnt receptor Frizzled and Trek1 K^+ channel using MNP and magnetic fields and to assess neuronal differentiation of progenitor cells in response. The translational aspects of this approach was also investigated using rat brain slices, cultured ex vivo, which were injected with MNP labelled neuronal pre-cursor cells and magnetically stimulated.

METHODS: Target receptor expression was assessed in SH-SY5Y and neural progenitor cells using rtPCR. 250nm MNP were coated with peptides or antibodies allowing MNP tagging to Frizzled/Trek receptors. Remote MNP-receptor complex stimulation was performed in 1h-3h sessions using alternating magnetic field gradients provided by a commercial magnetic force bioreactor. Downstream signalling activity was monitored by tracking β-catenin mobilisation and TCF/LEF luciferase reporter activity. Neuronal marker expression was then probed using immunohistochemistry both in vitro and in ex vivo embryonic rat brain slices to assess the effects of remote MNP mediated signalling activation on neuronal differentiation of injected progenitor cells.

RESULTS & DISCUSSION: Expression of Wnt receptors Frizzled1, 2 and the Trek1 K^+ channel were confirmed in SH-SY5Y cells with increases in Frizzled expression observed during neuronal differentiation. β-catenin mobilisation and TCF/LEF luciferase reporter activity both increased in response to MNP and magnetic field stimulation. Expression of dopaminergic markers DAT and TH was augmented in vitro in MNP labelled monolayer cultures stimulated with magnetic fields. In ex vivo rat brain slices marker expression was maintained in the injected MNP labelled neuronal progenitor cells.

CONCLUSIONS: Our results demonstrate that remote activation of cell signalling pathways using targeted magnetic particles and alternating magnetic fields can be used to influence neuronal signalling and precursor cell differentiation. This unique approach opens a potential avenue for novel therapeutic treatments, in the form of injectable cell therapies, with potential applications for treating neuro-degenerative diseases such as Parkinson's disease.

ACKNOWLEDGEMENTS: We gratefully acknowledge MICA Biosystems who provided the magnetic force bioreactor and the European Union's Horizon 2020 research and innovation programme (grant agreement no. 686841) who funded this research.



Stimulation of muscle regeneration by extracellular vesicles

F. Magarotto[1], M. Grassi[1,2], A. Sgrò[2], M. Saggioro[1,2], M. Andretta[2], E Maghin[1,2], M Piccoli[3], M Jurga[4], G Perilongo[2], P Gamba[2], M Muraca[2] M Pozzobon[1,2]
Presenting Author: Michela Pozzobon, michela.pozzobon@unipd.it
[1]Stem Cells and Regenerative Medicine Lab, Institute of Pediatric Research Città della Speranza, Padova, Italy [2]Dept of Women and Children Health, University of Padova, Italy [3]Biomedical Sciences Dept, University of Padova,[4]The Cell Factory BVBA (Esperite NV), Italy

INTRODUCTION: The need of new biomaterials to replenish the loss of muscle mass is currently a challenge. While the implant of extracellular matrix (ECM) from decellularized tissues provide the best biocompatible scaffold, on the other hand ECM alone produce limited muscle recovery. We are now aware that several intercellular signals mediating tissue renewal, vascularization and immune regulation, are convoyed via extracellular vesicles (EVs), biologically active microparticles composed of a lipid bilayer produced by cells. The aim of this work is to analyze the muscle regeneration in a murine model of volume muscle loss after implant of ECM engineered with EVs.

METHODS: ECM samples were obtained using a detergent-enzymatic protocol and were embedded with EVs isolated from Wharton Jelly mesenchymal stromal cells (EV-MSC) and BJ fibroblast cell line (EV-BJ). EVs were obtained through ultrafiltration, characterized by citofluorimetric analysis, and quantified with MTA and qNano instruments. ECM-EVs were transplanted in mice after tibialis anterior damage (ECM-PBS and ECM alone were used as control). 72 hours post implant, EVs local injection was performed. After 7, 15 and 30 days, samples were analysed by histology, immunofluorescence and qPCR.

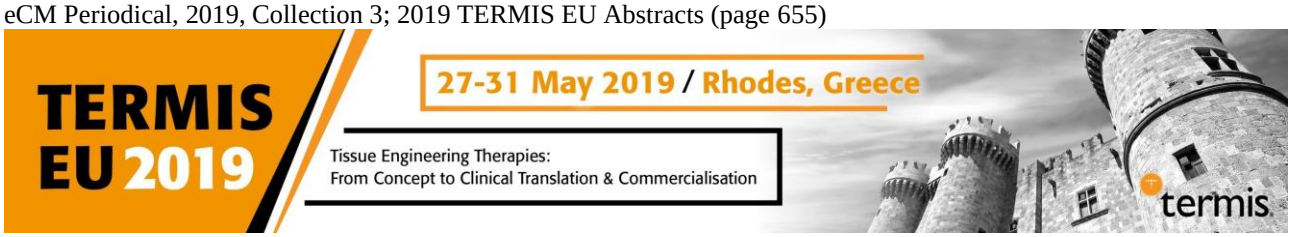
RESULTS & DISCUSSION: The macrophagic response in mice EV-MSC treated was clearly directed toward tissue rebuilding as confirmed by qPCR results on M2 and myogenic markers. 30 days post implant the fibrosis (collagen quantification) was significantly reduced in the same group of mice. vW+ cells -indicating neo-angiogenesis- and new born centrally nucleated fibers (CNFS) where present in a statistically higher percentage (***=$p<0.005$;****=$p<0.0001$.) again in EV-MSC treated mice in respect to the other experimental groups.

CONCLUSIONS: These preliminary results underlined the pro regenerative action of EV-MSC in a chronic model of volume muscle loss.

ACKNOWLEDGEMENTS: Financial support was received from University of Padova grant number BIRD 2016.

REFERENCES

[1] Sicari M et al. Sci Trans Med. 2014; 6, 234ra58
[2] Piccoli M et al et al. Methods Mol Biol. 2018; 1577:87-93
[3] Tkach M et al. Cell. 2016; 164(6):1226-1232



Hybrid scaffold with enhanced patterning for treatment of limb ischemia

M. Carrabba[1], M. Fagnano[1], E. Jover Garcia[1], A. Thomas[1], E. Avolio[1], G. Vozzi[2], A. Perriman[3], P. Madeddu[1]
Presenting author: Michele Carrabba, mc14021@bristol.ac.uk
[1]School Translational Health Science, University of Bristol, Bristol, UK [2]Research Centre 'E. Piaggio', University of Pisa, Italy [3]School of Chemistry, University of Bristol, UK

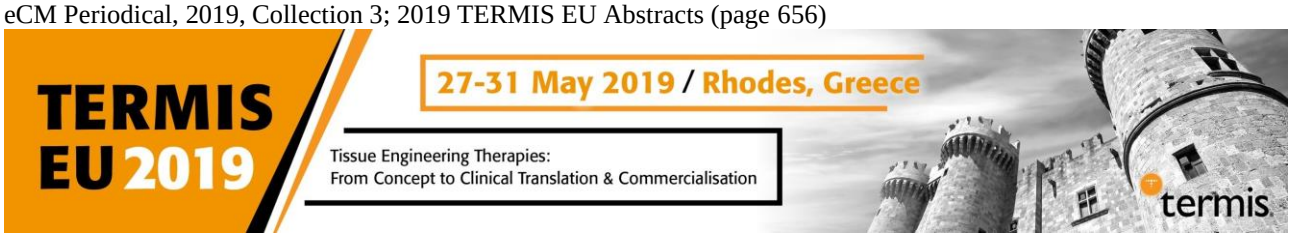
INTRODUCTION: Cell Therapy has been explored as potential of Critical Limb Ischemia (CLI) in alternative to traditional interventional approaches. Despite the initial promising results, cell therapy showed limitations in cell retention and absence of supportive environment. To overcome these limitations, we propose the design and development a new cells delivery system based on a hybrid scaffold able to stimulate reparative angiogenesis embedding vascular cells (pericytes and endothelial cells) in a mouse model of peripheral ischemia.

METHODS: The development of the hybrid scaffold was divided in two main steps: 1) composite scaffold fabrication and 2) cellularization. 1) Using a customized 3D printer, synthetic structures were fabricated with the shape of channels and woodpile. Polycaprolactone (PCL) 10% and Poly-lactic-co-glycolic acid (PLGA) (15%) were used to compare the effect of different mechanical properties. The synthetic structures were then covered with a layer of gelatin (GL) electrospun nanofibers crosslinked with γ-glycidypropyltrimethoxysilane (GPTMS), forming a nanometric network with adhesive features similar to those of the natural ECM. Morphology and mechanical properties of the scaffolds were characterized through SEM and tensile testing. 2) Adventitial Progenitor Cells (APCs), isolated from saphenous vein, were seeded at 10,000 cells/cm^2 onto PCL- and PLGA-based scaffold for 5 days to create a confluent layer. Cell density, proliferation and viability were performed. On top of the confluent layer of APCs, a patterned co-culture of APCs and HUVECs were then bioprinted. A hydrogel solution formed by 6% Sodium Alginate and 13% F127 Pluronic was loaded with a co-culture of APCs and HUVECs in ratio 1:4 and density of 4million/ml, extruded in lines with a Bioprinter and crosslinked with 20mM $CaCl_2$ to guarantee spatial organization. To validate the approach, scaffold which performed better in vitro were implanted with and without cells around the femoral artery of mice with unilateral LI. The end-points of the test were evaluated with microCT.

RESULTS & DISCUSSION: Scaffolds with channel and woodpile shapes were successfully fabricated, achieving line width of 121±5μm and thickness of 75±5μm, Mechanical properties showed significant differences depending on the geometry and on material, with the PCL displaying values closer to the tissue of femoral artery. In vitro assays showed higher performances of the channel-shaped scaffold compared to woodpile ones, while no differences were noticed between PCL and PLGA. Viability of Bioprinted APCs and HUVECs was observed always around 70%, and the aligned pattern maintained in the period of the investigation. Based on these results, PCL- and PLGA-channel shaped scaffold with and without cells were implanted around the occluded femoral artery. Flowmetry assessment showed that the group implanted with cellularized PLGA-AG/PL recovered faster as compared with vehicle (11 vs. 18 days, respectively, p=0.01). Qualitative analysis of vasculature tree was evaluated with microCT at time point of 3 weeks.

CONCLUSIONS: Data show the potential of the novel hybrid scaffold as cell delivery system to improve the efficacy of cell homing and increasing pro-angiogenic effect in ischemic tissues.

ACKNOWLEDGEMENTS: British Heart Foundation PhD studentship (FS/15/7/31307) and MRC



Secretomes derived from fetal and adult MSCs for cartilage regeneration

M. Fenu[1], I. Ribitsch[1], G.L. Oreff [1], H. Baik,[1] S. Gültekin[1], F. Jenner[1]
Presenting Author: Michele Fenu, michele.fenu@vetmeduni.ac.at
[1] Department for Companion Animals and Horses, Veterm, University Equine Hospital, Vetmeduni Vienna, Vienna

INTRODUCTION: Traumatic injuries affecting articular cartilage do not heal spontaneously and often lead to osteoarthritis. As opposed to adult tissue, injured fetal cartilage undergoes scarless healing[1]. This difference might be due to inherent variance of mesenchymal progenitor cells. Even though advantageous effects of MSCs on cartilage repair have been demonstrated, use of cells as therapeutics is limited by several factors[2]. Hence recently, exploiting paracrine signaling of cell secretomes has gained attention as potential therapy [2]. In this work we aimed to evaluate the effect of secretomes derived from fetal and adult MSCs for cartilage regeneration.

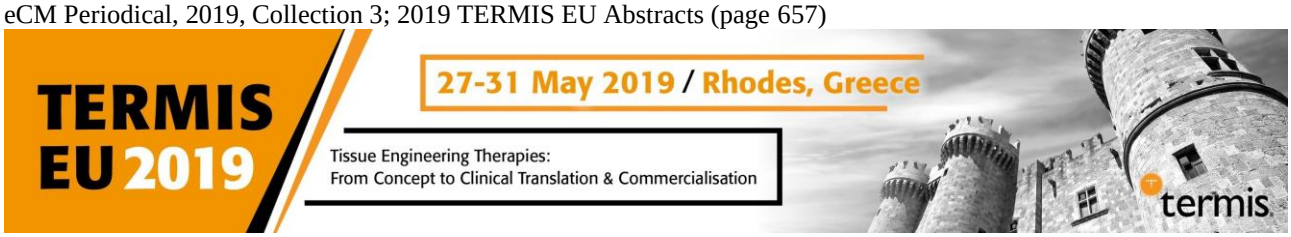
METHODS: Ovine primary cells (MSCs and Chondrocytes) were isolated from healthy donors following standard procedures for both adult and fetal donors. Experiments were designed to simulate injury to adult chondrocytes followed by treatment with secretome derived from either adult or fetal MSCs. MSCs were left to adhere overnight. Following medium change (all experiments were performed in serum free medium - STEM MACS), secretome was produced for 24 hours and subsequently used as treatment. Adult chondrocytes were exposed to inflammatory stimuli (10 ng/µl IL1β and 10 ng/µl TNFα) for 24h before treatment with either fetal or adult secretome. Chondrocyte migration (scratch assay), proliferation (PicoGreen) and metabolic activity (MTT) were evaluated in monolayer, while gene expression was evaluated in pellet culture. Q-PCR was performed for several inflammation markers, as well as extracellular matrix (ECM) degradation and production related genes.

RESULTS & DISCUSSION: Migration, proliferation and metabolic activity assays showed differences in the effects induced by fetal compared to adult MSC secretome. In both cases, MSCs derived secretome increased chondrocyte migration and proliferation compared to untreated chondrocytes. Gene expression revealed anti-inflammatory properties of adult and fetal secretomes although differences between the two cell sources were found. Also expression of genes related to ECM production was differently regulated by secretomes of fetal and adult MSCs.

CONCLUSIONS: Results of this study indicate a positive influence of secretomes derived from fetal and adult MSCs on adult injured chondrocytes. Variance in their effects might be due to key factors driving fetal regeneration as compared to adult repair. While the underlying mechanisms of action still need to be investigated, the secretome derived from fetal MSCs might represent a valid treatment option to achieve cartilage regeneration. Future studies will need to address the qualitative as well as quantitative differences in protein content between adult and fetal MSC secretomes as well as confirm our current results in vivo.

REFERENCES

[1] Ribitsch at al. DMM. 2018; 11(7):1-11
[2] Veronesi et al. J Cell Physiol. 2018; 233:4423-42



The catabolic-to-anabolic shift in the osteoarthritic cartilage after knee joint distraction in dogs occurs after the distraction period

M. Teunissen[1], J. Popov-Celeketic[2], K. Coeleveld[2], B.P. Meij[1], F.P.J.G. Lafeber[2], M.A. Tryfonidou[1], S.C. Mastbergen[2]
Presenting Author: Michelle Teunissen, m.teunissen@uu.nl
[1]Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands [2]Rheumatology & Clinical Immunology, University Medical Center (UMC) Utrecht, Utrecht University, Utrecht, The Netherlands

INTRODUCTION: Knee joint distraction (KJD) is a joint-preserving treatment strategy for severe osteoarthritis (OA) that provides long-term clinical and structural improvement [1]. Data from both human trials and animal models indicate clear cartilage regeneration from 6 months and onwards post-KJD [1, 2]. However, recent work showed that during distraction, the balance between catabolic and anabolic indicators is directed towards catabolism, as indicated by collagen type 2 markers [3], proteoglycan (PG) turnover and a catabolic transcription profile [unpublished data]. As such, this study investigates the cartilage directly and 10 weeks after joint distraction in an animal model in order to elucidate the shift from a catabolic to an anabolic cartilage state.

METHODS: Knee OA was induced bilaterally in 8 dogs according to the groove model [2]. After 10 weeks of OA induction, the right knee received joint distraction, employing the left knee as an OA control. After 8 weeks of distraction, and after 10 weeks post-KJD, 4 dogs were euthanized. Respectively, macroscopic cartilage degeneration, PG content (Alcian Blue), and PG synthesis ($^{35}SO_4^{2-}$-incorporation rate) were assessed [2].

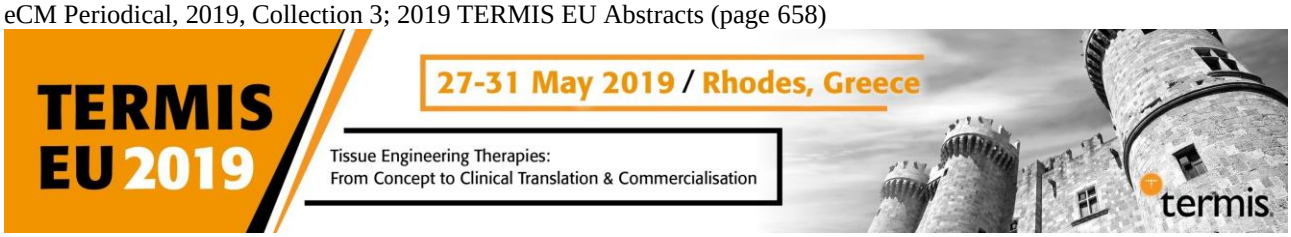
RESULTS & DISCUSSION: Directly after KJD, macroscopic cartilage damage of the right tibial plateau was higher compared to the left OA control (OARSI score: 1.7±0.2 vs 0.6±0.3; $p < 0.001$). 10 weeks post-KJD this difference persisted (OARSI score: 1.4±0.6 vs 0.6±0.1; $p < 0.05$). Biochemical analysis of the tibia cartilage directly after KJD revealed a lower PG content (20.1±10.3 mg/g vs 23.7±11.7 mg/g). At 10 weeks post-KJD this difference in PG content was less (24.8±6.8 mg/g vs 25.4±7.8 mg/g). The PG synthesis rate directly after KJD appeared significantly lower vs. OA (1.4±0.6 nmol/h.g vs 5.9±4.4 nmol/h.g; $p < 0.001$)). However, 10 weeks post-KJD this difference was not detected (3.7±1.2 nmol/h.g vs 2.9±0.8 nmol/h.g), and the synthesis rate in the distracted knee was increased compared to directly after distraction ($p < 0.01$).

CONCLUSIONS: Further in-depth investigation of the material is ongoing; these first results suggest that the shift from a catabolic to an anabolic state occurs within the first weeks after joint distraction, mostly reflected in the biochemical changes. As such, the post-distraction period seems to be essential in identifying key-players that support intrinsic cartilage repair.

ACKNOWLEDGEMENTS: Financial support was received from the TTW Technology Foundation: Perspectief P15-23.

REFERENCES

[1] Jansen MP et al. Osteoarthritis Cartilage. 2018 Dec;26(12):1604-1608
[2] Wiegant K et al. Arthritis Rheumatol. 2015 Feb;67(2):465-74
[3] Jansen MP et al. Cartilage. 2019 accepted



Formation of elastin-like recombinamer membranes in a two-phase system

M. González-Pérez[1], I. González de Torre[2], Matilde Alonso[1], José Carlos Rodríguez-Cabello[1]
Presenting Author: Miguel González-Pérez, mgonzalezperez@bioforge.uva.es
[1]BIOFORGE Lab, University of Valladolid − CIBER-BBN, Paseo de Belén 19, 47011 Valladolid, Spain [2]Technical Proteins Nanobiotechnology (TPNBT) S.L., Valladolid, Spain

INTRODUCTION: Reactions leaded by diffusion forces confined at the interface between two immiscible liquids present singular characteristics difficult to replicate in other environments. Although these systems have proven to be of great interest for preparing self-assembled physical peptide-based hydrogels [1], very little efforts have been exerted employing covalently cross-linked polypeptides, such as elastin-like recombinamers (ELRs) [2]. While other biomaterials are limited in terms of tunability, ELRs offer the possibility to introduce both specific bioactivities and physico-chemical reactivities. Herein has been exploited these two particularities, the employment of free-catalyst click modified ELRs versions in combination with a two-phase solution to produce thin moldable elastin-based membranes.

METHODS: Two ELRs were designed, encoded in double strand DNA plasmid and expressed in E. Coli bacteria. Purified polypeptides were chemically modified to incorporate the click chemistry groups, dissolved at the same concentration in an aqueous and an organic immiscible solutions previous mixing. Four different concentrations were assayed to prepare the membranes inside a tubular container. Isolated membranes both hydrated and lyophilized were observed by ESEM and SEM microscopy. Franz cells were used to study the diffusion rate of five FITC-dextran molecules in a 4-150kDa range during 21 days.

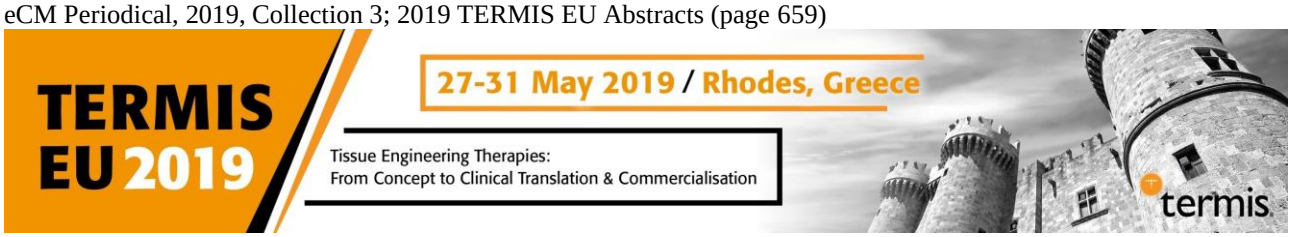
RESULTS & DISCUSSION: A tailored device was designed to contain the two-phase system. The initial concentration of the two solutions put in contact determined the thickness of the membranes obtaining the thinnest thickness with the lowest concentration. Similarly, the smallest fluorescent labelled polysaccharide depicted the fastest diffusion rate finding half-diffusion times starting from 3 up to 9 days. Both hydrated and lyophilized versions of the membranes showed an internal and superficial porous appearance.

CONCLUSIONS: A reproducible methodology has been implemented to construct tunable soft membranes in terms of both thickness and diffusive properties. In addition, the high biocompatibility of the recombinant elastin-based materials employed enables their use as a versatile scaffold for developing new systems for tissue-engineering applications

ACKNOWLEDGEMENTS: This work was funded by the European Commission (H2020 programme) through the ELASTISLET project (NMP-2014-646075) and MINECO (PCIN-2015-010, MAT2016-78903-R, FPU15-00448 to MGP).), Junta de Castilla y León (VA317P18) and Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León.

REFERENCES

[1] Azevedo H S et al. Peptides and Proteins as Biomaterials for Tissue Regeneration and Repair. 2018; 245-281.
[2] Mata A et al. Acta Biomaterialia. 2017; 58, 80-89.



Vascularized skeletal muscle scaffold composed of cell-laden aligned micro/nanofibers fabricated by cell electrospinning

M. Yeo[1], J. Lee[1], W. Kim[1], J. Lee[1], H. Kim[1], S. J. Chae[1], J. Kim[1], D. Yoon[2], G.H. Kim[1]
Presenting Author: Miji Yeo, yeomiji@skku.edu
[1]Department of Biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea
[2]Burn Institute, Hangang Sacred Heart Hospital, College of Medicine, Hallym University, Seoul, South Korea

INTRODUCTION: Tissue engineering is developed to construct a functional tissue to repair or restore the damaged tissues/organs [1]. For instance, vascularization plays an important role, which enables to remove wastes and deliver nutrients. In addition, various tissues like skeletal muscle have uniaxially aligned vasculature to sustain the viability of the anisotropic tissues. Since microcirculation is derived from the longitudinally oriented blood vessels [2], an aligned microstructure should be achieved for vascularized skeletal muscle. Thus, an aligned micro/nanofibrous scaffold fabricated via cell electrospinning will be proposed to provide physical cues to induce cell alignment and elongation.

METHODS: Human umbilical vascular endothelial cells (HUVECs) at 5×10^6 cells/mL were mixed with alginate/polyethylene oxide (PEO) solution as a bioink. Then, the bioink was electrospun onto the polycaprolactone (PCL) with and without aligned topological cues. Then, C2C12 myoblasts at 1×10^5 cells/mL myoblasts were seeded on the specimens.

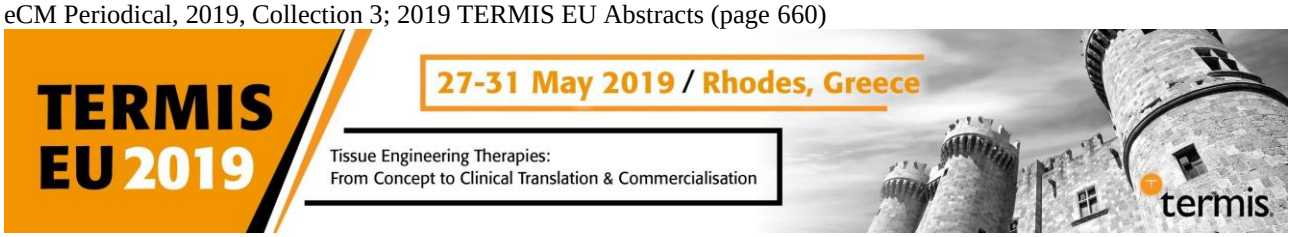
RESULTS & DISCUSSION: To assess feasibility of cell electrospinning using HUVECs, initial cell viability was measured by live/dead staining. Electric field was varied from 0.050 to 0.125 kV/mm, and over 90% cell viability was observed for 0.050-kV/mm and 0.075-kV/mm electric field. However, cell viability significantly decreased for 0.100-kV/mm electric field. Moreover, cell morphology was captured by CD31 at 14 days. The HUVECs on aligned PCL stretched along the micro/nanopattern while the HUVECs on non-aligned PCL were in ellipsoidal shape. This aligned vascular structure accelerated myogenesis and cell-cell interaction among myoblasts and HUVECs.

CONCLUSIONS: In brief, aligned micro-/nano-pattern guides cells in anisotropic structure.

ACKNOWLEDGEMENTS: This research was supported by the National Research Foundation of Korea (NRF) Grant funded by the Ministry of Science and ICT for Bio-inspired Innovation Technology Development Project (NRF-2018M3C1B7021997) and the National Research Foundation of Korea grant funded by the Ministry of Education, Science, and Technology (MEST) (NRF-2018R1A2B2005263).

REFERENCES

[1] Lanza R et al. Science. 1993; 260:920-6
[2] Cittadella Vigodarzere G et al. Front Physiol. 2014;5:362



Mechanisms of antifibrotic effects of mesenchymal stromal cells mediated by extracellular vesicles

M.S. Arbatsky[1], N.A. Basalova[1,2], G.D. Sagaradze[1,2], O.A. Grigorieva[2], N.I. Kalinina[1], A.Y. Efimenko[1,2]
Presenting Author: Mikhail Arbatsky, m.s.arbatsky@belozersky.msu.ru
[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, M.V. Lomonosov Moscow State University, Moscow, Russia [2]Institute for Regenerative Medicine, Medical Research and Education Center, Lomonosov Moscow State University, Moscow, Russia

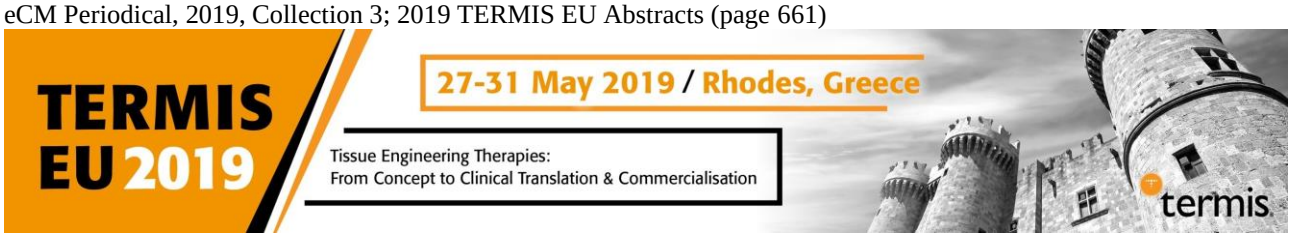
INTRODUCTION: Mesenchymal stem/stromal cells (MSC) are able to regulate tissue repair after injury, including the prevention of excessive fibrosis. Extracellular vesicles (EV-MSC) were shown to mediate most of MSC effects and could be involved in their antifibrotic activity. However, the mechanisms of these effects, particularly the role of microRNA (miR) within EV-MSC, are poorly understood.

METHODS: Human adipose-derived MSC (primary and immortalized line ASC52telo, ATCC) were used to analyze the content of EV-MSC. Ultrafiltration was used to isolate EV from MSC conditioned medium. Exosomal RNA was isolated and libraries for mass parallel sequencing for the analysis of miR were constructed. Using the TargetScan7.2, HMDD, miR2Disease, miRwayDB databases, miR associated with the development of fibrosis and their representation in EV-MSC were analyzed. Clustering was performed using the David database. To assess the contribution of selected miR (miR-21 and miR-29) to the biological activity of EV-MSC, vesicles transfected with anti-miR-21 or anti-miR-29 were added to fibroblasts treated with TGF-β to induce myofibroblast phenotype.

RESULTS & DISCUSSION: From 2652 miR 270 were detected in the EV-MSC. According to the results of cluster analysis it was shown that among the targets of detected miR there were factors associated with the development of fibrosis. 372 unique miR and 20 genes are associated with TGF-β, of which 24 miR are reliably represented in EV-MSC; 368 unique miR and 22 genes are associated with actin, 23 are represented; 21 miR and 1 gene are associated with fibrosis, 21 are represented; 5 miR and 1 gene are associated with collagen, 5 are represented.
Thus, multiple miR might be involved in EV-MSC-mediated regulation of fibrosis. Impact of selected miR were validated by the model of TGF-β-induced fibroblast differentiation into myofibroblasts. The addition of EV-MSC inhibited TGF-β-induced differentiation fibroblasts in myofibroblasts. Addition of EV transfected by scramble or miR-21 inhibitor did not affect EV-MSC-mediated inhibition of differentiation. Transfection of vesicles with miR-29 inhibitor reduced the effect of EV-MSC on TGF-β-induced differentiation of fibroblasts into myofibroblasts.

CONCLUSIONS: Our data indicates the impact of miR in antifibrotic activity of EV-MSC. Further study will enable the development of drugs and biomaterials based on miR within EV-MSC that could be promising for regenerative medicine and antifibrotic therapy.

ACKNOWLEDGEMENTS: The study was conducted using biomaterials collected and preserved in the frame of the project “Scientific basis for national bank-depositary of living systems” using the equipment purchased as a part of Lomonosov Moscow State University Program of Development and supported by Russian Foundation for Basic Research (#18-015-00525).



Restoration of extracellular matrix proteins in vocal fold scars after mesenchymal stem cell implantation: An experimental study

M. Svistushkin[1], S. Kotova[2,3], A. Shekhter[2], V. Svistushkin[1], A. Akovantseva[4], A. Frolova[1,4], A. Fayzullin[2], S. Starostina[1], P. Timashev[2,3,4]
Presenting Author: Mikhail Svistushkin, swistushkin@yandex.ru
[1]ENT Department, Sechenov University, 6 Bolshaya Pirogovskaya St., Bldg. 1, Moscow, 119991, Russia [2]Institute for Regenerative Medicine, Sechenov University, 8 Trubetskaya St., Moscow, 119991, Russia [3]N.N. Semenov Institute of Chemical Physics 4 Kosygin St., Moscow, 119991, Russia [4]Institute of Photonic Technologies, Research center "Crystallography and Photonics", 2 Pionerskaya St., Troitsk, Moscow, 108840, Russia

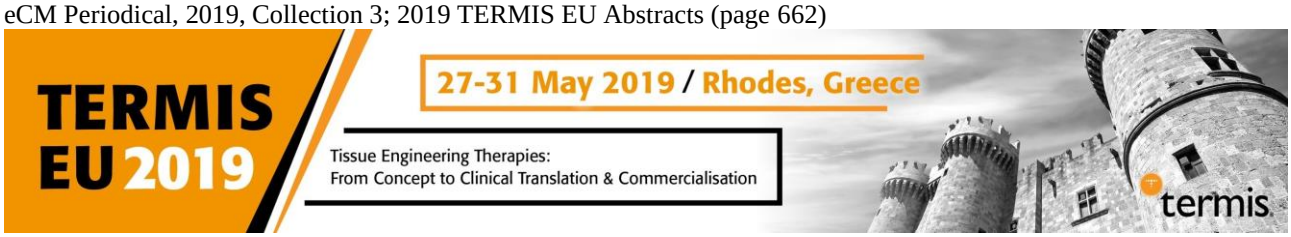
INTRODUCTION: Vocal fold scars are a common multietiological disease appearing with persistent voice impairment. Due to a highly complex microstructure of the extracellular matrix's (ECM) proteins in the vocal fold mucosa, it is one of the most difficult problems in laryngology, in spite of the great variety of existing treatment techniques. In our study, we follow the regenerative effects of mesenchymal stem cells (MSC) on the structure of vocal folds with cicatricial lesions.

METHODS: Twelve laboratory rabbits underwent a surgical modeling of mature scars of the vocal folds. Ninety days after the first surgery, the vocal fold scar was excised with the simultaneous implantation of autologous bone marrow derived MSC ($6 \cdot 10^5$ cells) or a saline for the experimental or control groups, respectively. The restoration of the vocal folds ECM was studied 90 days post-implantation via a histological estimation of 11 pathological signs, scar thickness measurement, immunohistochemistry for collagen type I and type III and atomic force microscopy (AFM) studies.

RESULTS & DISCUSSION: The analysis of the semi-quantitative data detected significant differences in the following morphological signs: lack of fibroblasts, irregular architectonics of collagen fibers, density of collagen fibers with the prevalence of alteration in the control group (p-value 0.086). There was also a visible trend of a scar width reduction from the control to the MSC-treated group. According to the data of immunohistochemical analysis, the collagen type III content was by 45.5% lower than that of collagen type I in the control group ($p<0.0001$). At the same time, no distinguishable difference in the intensity and prevalence of staining for collagens type I and type III was observed in the intact vocal folds and in the MSC-treated group. The AFM study revealed a statistically significant increase in the average Young's moduli (p-value 0.013) in the control group and returning to their initial values (p-value 0.59) in the MSC-treated group. The thicknesses of collagen fibrils in the intact vocal fold ECM was slightly higher compared to the scar tissue in the control group. In MSC- treated group this indicator did not differ from the intact tissue.

CONCLUSIONS: Our results have shown that the ECM structure in the scar tissue of the vocal folds with implanted MSC is more similar to that in the normal mucosa of the vocal folds than to that of the untreated scars. According to our study results, AFM provides clear and understandable data for accurate assessment of the recovery of the vocal folds ECM proteins and may be used in further studies.

ACKNOWLEDGEMENTS: This study has been financially supported by Russian Foundation for Basic Research Grant. No. 18-02-00658.



Multicomponent hydrogel materials as bioreactors for tissues production

M. Tsurkan[1]. M. Binner[1], S. Tsurkan[1], J. Bessert[1,2], D. Carsch[1], R. Selzer[1], C. Werner[1,3]
Presenting Author: Mikhail Tsurkan, tsurkan@ipfdd.de
[1]Leibniz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials Dresden, Germany [2]Institute of Anatomy, Medical Faculty Carl Gustav Carus, Technische Universität Dresden Germany [3]Center for Regenerative Therapies Dresden (CRTD) at Technische Universität Dresden, Germany

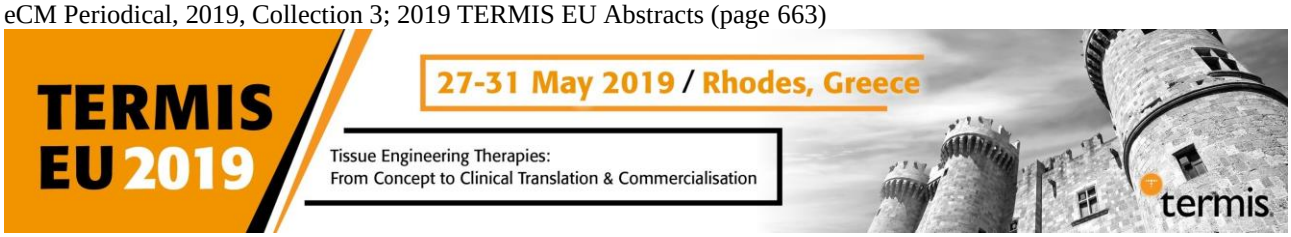
INTRODUCTION: Regenerative therapies that restore the function of damaged tissues and organs via the use of a patient's own cells is a highly desirable cure for many presently incurable pathologies and diseases. Over the last years, biological advances have allowed for the expansion and implementation of primary human and stem cells for the production of nearly any human tissue and organ like cellular masses (organoids) ex vivo, leading the way for their use in regenerative therapies. Nevertheless, state of the art technologies do not allow for the undamaged release of produced tissue or cell assemblies from their culture platform, which is a necessary step before their clinical application. Here, we highlight the recent progress of our lab in the development of orthogonal synthetic strategies for the formation and functionalization of biodegradable biohybrid hydrogels composed of covalently cross-linked four-arm poly(ethylene glycol) and various glycosaminoglycan (GAG) polysaccharides with peptide conjugates. The combination of these biohybrid materials with an orthogonal, enzymatically degradable peptide material concept was utilized to create a new bio-orthogonal approach for ex vivo tissue production (Figure 1) thus utilizing the material as a bioreactor platform. The potential of this technique was shown by formation of a human cornea endothelial lamella tissue suitable for surgical implantation in descemet membrane endothelial keratoplasty (DMEK).

METHODS: Human corneal endothelial cells (HCEC) were seeded on functionalized hydrogel culture carriers and cultured until a functional tissue was formed. The culture carrier was subsequently enzymatically degraded with FXa enzyme, which is both specific and gentle, to release free-floating cell sheets. The formed tissue was characterized in terms of viability (live-dead) and functionality (marker proteins).

RESULTS & DISCUSSION: Release of the cultured HCEC layers show the suitability of enzymatically-degradable hydrogels for HCEC sheet generation of physiologically relevant size (~1 cm) as shown in the Figure 1. The cells cultured on the hydrogels exhibit a regular morphology and appropriate metabolic activity. Cells were positive for expression of function-associated marker proteins ZO-1, Na+/K+-ATPase, and the extracellular matrix proteins fibronectin, laminin and collagen type IV. The detached, floating HCEC sheets were transplanted onto porcine corneas in vitro to show their suitability as a potential medical transplant.

CONCLUSIONS: This technology allows any live cells, tissue, or organoids, to be encapsulated, manipulated, and released from this hydrogel materials without damaging the biological sample. Father application will include human umbilical vein cells (HUVC) for vascular regeneration and human fibroblast for connective tissue regeneration.

ACKNOWLEDGEMENTS: Financial support was received from EXIST 03EFJSN114.



Triple function in one coating: Towards anti-adhesive, bactericidal, and cell instructive medical implants

M. Hoyos-Nogués,[1,2,3] J. Gil[1,2,3], C. Mas-Moruno[1,2]
Presenting Author: Mireia Hoyos-Nogués, mhoyos@uic.es
[1]Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Universitat Politècnica de Catalunya (UPC), Barcelona, Spain [2]Barcelona Research Center in Multiscale Science and Engineering, UPC, Barcelona, Spain [3]Bioengineering Institute of Technology (BIT), Universitat Internacional de Catalunya (UIC), Sant Cugat del Vallès, Spain

INTRODUCTION: Strategies to inhibit bacterial colonization are crucial to prevent infection on biomaterial surfaces, a major threat in modern medicine [1]. However, simultaneously achieving efficient antibacterial potential while positively supporting host eukaryotic cells functions remains a challenge for successful biomaterial-host tissue integration [1]. Here, we describe a method for the development of a trifunctional coating that repels contaminating bacteria, kills those that adhere, and promotes osteoblast adhesion.

METHODS: Titanium model substrates were functionalized with polyethylene glycol (PEG) via electrodeposition. Subsequently, the polymeric layer was further coated with a peptidic platform (PTF) combining cell adhesive (RGD) and antibacterial sequences (LF1-11 peptide) [2] using silanization. Physicochemical characterization was performed with contact angle analysis, interferometry, XPS and FTIR. The response of sarcoma osteogenic cells (SaOS-2) was evaluated in vitro by means of cytotoxicity test (LDH) and immunofluorescence analysis. The antibacterial activity was measured using Streptococcus sanguinis via live/dead and SEM analysis.

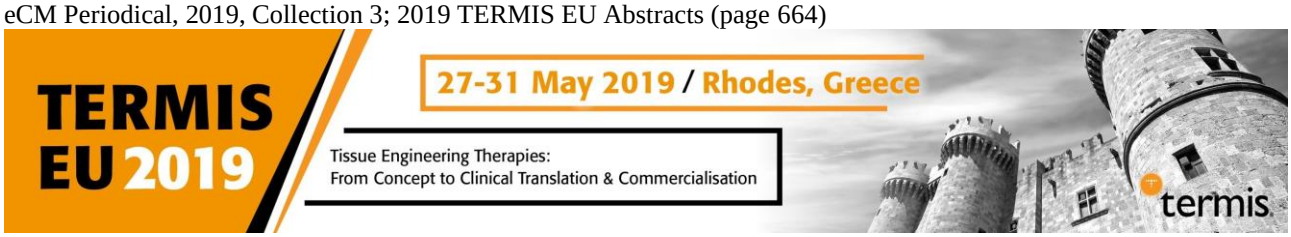
RESULTS & DISCUSSION: PEG coatings significantly inhibited protein adsorption and SaOS-2 attachment; however, the presence of cell adhesive domains (PTF) efficiently rescued osteoblast adhesion, yielding higher values of cell attachment and spreading compared to controls ($p<0.05$). The repellent properties of PEG resulted in a significant inhibition of S. sanguinis attachment ($p<0.05$). This antibacterial potential was further increased by the bactericidal peptide, yielding values of bacterial adhesion below 0.2% ($p<0.05$) [3].

CONCLUSIONS: The antibacterial properties of our coating strategy respond to the antifouling character of PEG, which repels bacteria, and the bactericidal properties of the PTF. Such combined effect drastically inhibited the adhesion of S. sanguinis. Simultaneously, the coating supported very good levels of osteoblast adhesion. Taken together, this new trifunctional coating represents a promising approach to address both bacterial infection and poor material osteointegration.

ACKNOWLEDGEMENTS: Financial support from the MINECO/FEDER (RYC-2015-18566, MAT2015-67183-R and MAT2017-83905-R) and the AGAUR (2017 SGR1165) is appreciated.

REFERENCES

[1] Mas-Moruno C, et al. Adv. Healthcare Mater. 2018; 1801103
[2] Hoyos-Nogués M, et al. ACS Appl. Mater. Interfaces 2017; 9: 21618-30
[3] Hoyos-Nogués M, et al. Colloid. Surf. B: Biointerfaces 2018; 169:30-40.



Scripting a trainable machine learning tool for single cell shape measurement in complex biological situations

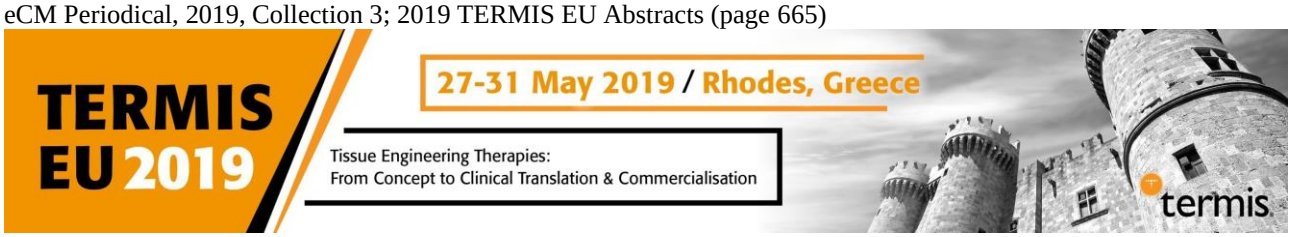
M. Selig[1], M. Hanak[1], B. Walters[2], B. Rolauffs[1]
Presenting Author: Mischa Selig, mischa.selig@uniklinik-freiburg.de
[1]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Dept. of Orthopedics and Trauma Surgery; Albert-Ludwigs-University of Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany
[2]Department of Biomedical Engineering, University of Michigan, Michigan, MI 48109, USA

INTRODUCTION: Automated, user-independent quantitative analysis of biological images for analyzing cell morphology is of great need as high amounts of morphological data could be generated and correlated with biological readouts such as mRNA expression. However, single cell shape analyses are difficult in complex biological situations such as high cell densities. Here, we present a novel trainable machine learning tool for automated and exact single cell measurement in complex biological situations.

METHODS: A cell segmentation tool, based on the WEKA machine learning algorithm was developed for single cell measurement in complex biological situations. Throughout the process, step by step evaluation situations were established and used in a feedback loop for software-improvements. Then, human users vs. scripted WEKA was compared in standardized tests.

RESULTS & DISCUSSION: First, the WEKA script was adapted to establish so-called image classifiers and image regions of interest (ROIs) representing e. g. the nucleus were used to train the "nucleus classifier" and others. These classifiers were applied for segmenting images into nucleus, cytosol, protrusions and background, which enabled the automated, trainable segmentation of cells vs. background. Separation of neighboring cells was achieved using a watershed algorithm. Combining these approaches allowed reliable single cell separation; an segmentation illustration of two adherent hMSCs derived from mixing and seeding hMSCs stained with the CellTracker Blue with hMSCs stained with CellTracker Green. First tests demonstrated that the scripted WEKA was significantly faster than two human users ($p<0.05$). Assessing accuracy, we used the difference in pixels between the segmented images generated by the trained algorithm vs. human segmentation. The data demonstrated significant differences in the amount of pixels between the two human users and between the algorithm and the human users, indicating significant quantitative differences in the final segmentation result. Interestingly, there were no significant pixel differences between repeated algorithm segmentations, indicating a high reproducibility. After adapting the algorithm to quantify a panel of quantitative shape descriptors such as cell area, aspect ratio (AR), roundness, length, width, circularity, and solidity, these data was further used to compare human users vs. scripted WEKA. Statistical tests demonstrated significant differences in shape descriptors between the WEKA and untrained human users ($p<0.05$) but not trained users.

CONCLUSIONS: We established an automated, user-independent, user-friendly, and exact machine learning tool for quantifying the geometrical shape of single cells. Moreover, these tests demonstrated that a trained subjective user can be as good as the trained WEKA script but that an untrained user was not as good as the trained WEKA script.



Towards understanding why nanoclays are osteogenic

M. Mousa [1], O. Kelly[2], J. Doyle[2], N. D. Evans [1], R. O. C. Oreffo [1], J. I. Dawson [1]
Presenting Author: Mohamed Mousa, M.A.Mousa@soton.ac.uk
[1]B & J Research Group, Stem Cells & Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton, SO16 6YD, UK [2]BYK Additives Ltd., Widnes, Ceshire, UK

INTRODUCTION: Laponite clay nanoparticles are emerging as a new class of biomaterials with exciting opportunities for regenerative medicine applications [1]. High profile studies have demonstrated [2] the osteogenic properties of Laponite, however, the mechanism(s) underlying nanoclay bioactivity remain poorly understood. Cell uptake and release of degradation products such as Li^+ are frequently cited mechanisms. In this work, we investigated the effect of Laponite clay nanoparticles on osteogenic differentiation of Human Bone Marrow Stromal Cells (HBMSCs) and the role of these frequently proposed mechanisms for clay bioactivity.

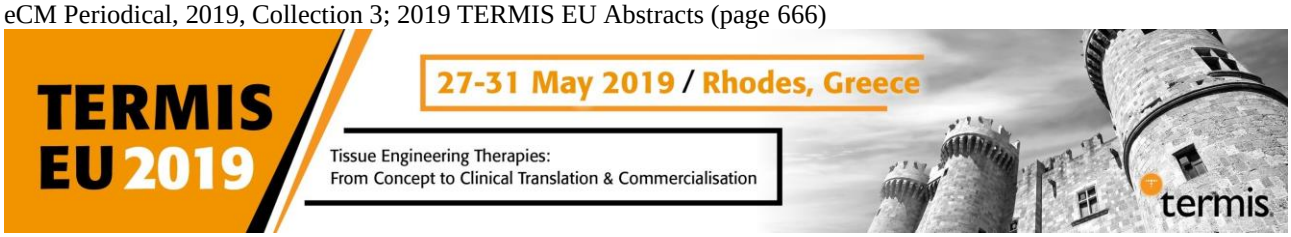
METHODS: i) role of Laponite degradation products: Various Li^+ modified Laponite formulations were generated and their effect on HBMSCs osteogenic differentiation was investigated at both basal and osteogenic medium conditions at clay doses of 0 – 100 µg/mL. ii) Role of direct Laponite/ALP interaction: the adsorption kinetics of ALP enzyme on clay surfaces was investigated at physiological conditions (pH = 7.6 & 37ºC) and the catalytic activity of adsorbed vs. free enzyme was measured. https://www.neb.com/products/p0757-p-nitrophenyl-phosphate-pnpp
iii) Role of Laponite endocytosis: HBMSCs were pre-treated with chlorpromazine hydrochloride (CPZ), a clathrin-mediated endocytosis inhibitor, for 2 hours, then incubated with both CPZ (5 µg/mL) and Laponite (0 – 100 µg/mL) in basal and osteogenic conditions for 72 hours. At day 3, ALP activity of HBMSCs was measured using the p-nitrophenol phosphate (pNPP) colorimetric assay.

RESULTS & DISCUSSION: Laponite nanoparticles induced osteogenic differentiation of HBMSCs at an early stage in a dose-dependent manner. This was confirmed by a significant increase in ALP Activity and upregulation of osteogenic genes (e.g. RUNX2 & Collagen I) at day 3, as well as enhanced Ca-P mineral deposition at day 14. Modified Laponite chemistries achieved variation in their Li^+ content. However no attenuation or increase in clay bioactivity was observed between the modified clays tested. ALP enzyme displayed a strong binding affinity for Laponite, following a Langmuir adsorption isotherm. However Laponite significantly reduced ALP acivity rather than enhanced it (V_{max} of adsorbed enzyme < free enzyme). Finally, the addition of an endocytosis inhibitor did not show significant effect on the dose dependent (0 – 100 µg/mL) osteogenic effect of Laponite on the cells.

CONCLUSIONS: Cellular uptake of clay nanoparticles and their subsequent degradation, has been frequently cited as the main mode of action for clay bioactivity. Our data, however, indicate that clay exert their osteogenic properties extracellularly and independent of their degradation products. Furthermore, our data confirms that such early-observed clay-mediated increase in ALP activity was not due to a direct clay/enzyme interaction, suggesting a role for biophysical models, such as, clay-cell membrane and clay-ion interactions in cell culture medium [1]. Studies seeking to elucidate these mechanism(s) are ongoing.

REFERENCES

[1] M. Mousa et al. Biomaterials, vol. 159. pp. 204–214, 2018.
[2] A. K. Gaharwar et al. Adv. Mater., vol. 25, no. 24, pp. 3329–3336, 2013.



Regulation of genotypic and phenotypic profile of amniotic epithelial stem cells while varying fiber diameter size in aligned PLGA electrospun scaffolds

M. El Khatib[1], V. Russo[1], M. Schnabelrauch[2], R. Wyrwa[2], F. Lazzaro[3], A. Mauro[1], P. Berardinelli[1], M.R. Citeroni[1], O. Di Giacinto[1], M. Turriani[1], M. Ancora[4], B. Barboni[1]
Presenting Author: Mohammad El Khatib, melkhatib@unite.it
[1]Faculty of Bioscience and Agro-Food and Environmental Technology, Italy [2]INNOVENT, Department of Biomaterials, Germany [3]Department of Research & Development, AssutEurope Italy
[4]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale", Italy

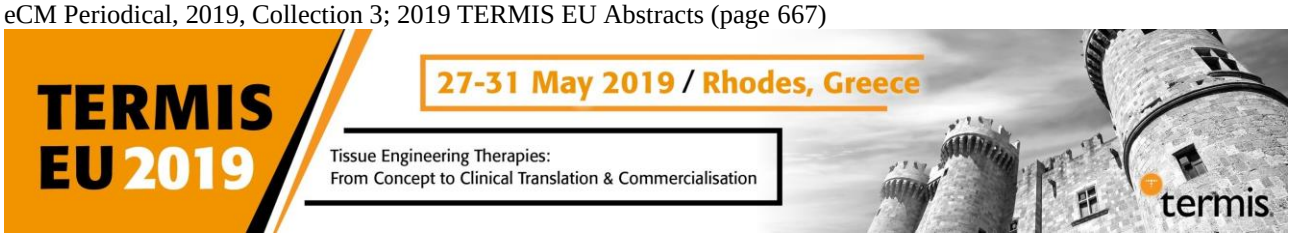
INTRODUCTION: The performance of amniotic epithelial stem cells (AECs) has retained great attention in the field of regenerative medicine given the fact of their ability to differentiate into tenogenic lineage. To increase their potential to completely regenerate tendon injuries, it is essential to develop innovative tissue engineering solutions as tendon bio-mimetic scaffolds possessing mechanical and structural properties similar to the native tissue. In this context, PLGA electrospun scaffolds with highly aligned fibers and different diameter size were fabricated to test and compare their mechanical properties and tenoinductive potential on AECs.

METHODS: PLGA electrospun scaffolds with highly fiber alignment possessing two different fiber diameter size (PLGA1 with 1.25 µm and PLGA2 with 2.5 µm), and PLGA with randomly oriented fibers, used as control, were fabricated. The produced scaffolds were characterized for their structure (SEM), their wettability, their mechanical properties, and their influence on AECs. In particular, seeded ovine AECs onto the PLGA electrospun scaffolds were compared for DNA quantification (4h and 48h), viability (Calcein AM and propidium iodide), spatial distribution and morphology (Phalloidin), and teno-differentiative potential by analyzing collagen type 1 (COL1) protein and gene expression of mature tendon markers (COL1 and TNMD) after 24h, 48h, and 7 days culture.

RESULTS & DISCUSSION: PLGA1 with small fiber diameter size showed a high-water uptake percentage value compared to PLGA2 ($p<0.05$) while both values were significantly lower than that of PLGA-R ($p<0.05$). Additionally, both aligned scaffolds possessed similar ultimate tensile stress value ($p>0.05$) whereas fracture strain value was significantly higher in case of PLGA2 compared to PLGA1 ($p<0.05$). In fact, it was significantly higher on PLGA-R respect to PLGA1 and PLGA2 scaffolds ($p<0.05$), whereas in PLGA2 scaffold it was slightly higher compared to PLGA1 ($p<0.05$). Interestingly, spindle-like tenocyte morphology of AECs seeded onto both PLGA aligned fibers was observed just after 24h of culture, whereas cells on PLGA-R retained their cobblestone typical morphology ($p<0.05$). The number of elongated cells increased significantly by increasing the culture time on both aligned fiber scaffolds with a faster elongation rate onto PLGA1 ($p<0.05$), confirming low cell proliferation. Moreover, AECs seeded onto both aligned fibers expressed COL1, the main protein of a tendon, in their cytoplasm already at 24h of culture. These results were also confirmed by analyzing mature tendon specific markers COL1 and TNMD gene expressions which have shown an increasing upregulation in both PLGA scaffolds with aligned fibers ($p>0.05$) during the analyzed culture periods, compared to those in PLGA-R scaffolds ($p<0.05$).

CONCLUSIONS: Fiber diameter size of electrospun scaffold and not just its topology can be challenging by regulation cell fate and teno-differentiative potential.

ACKNOWLEDGEMENTS: Funded by MIUR -project PON R&I 2014-2020.



Human placenta derived membrane is a favourable natural biomaterial for vascular tissue engineering

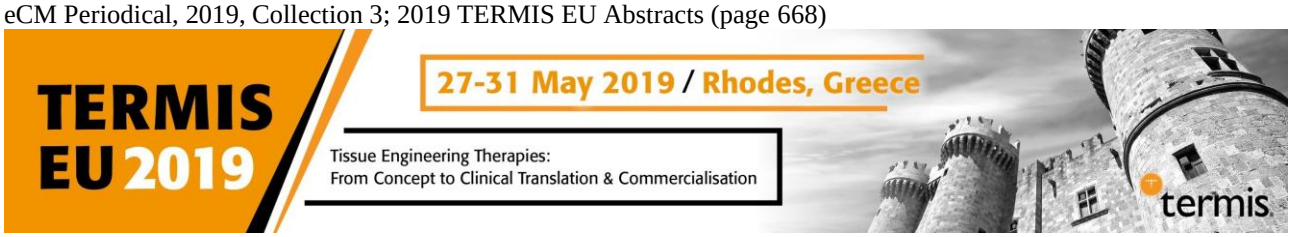
M.H. Farjoo[1], H. Niknejad[1]
Presenting Author: Mohammad Hadi Farjoo, m.farjoo@sbmu.ac.ir
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

INTRODUCTION: Human placenta derived amniotic membrane, as a natural biomaterial, has many characteristics, such as low immunogenicity, antibacterial, anti-inflammation, anti-fibrosis, and rich extracellular matrix ingredients, which make it a promising source for vascular tissue engineering. In addition, a single layer of amniotic epithelial stem cells (similar to a monolayer of endothelial cells in the vessels) which have capability to differentiate into the cells of three germ layers boosts the idea of using amniotic membrane as vascular substitute.

METHODS: This work evaluated the feasibility of constructing a vascular conduit from the human amniotic membrane and implanting it in the external jugular vein of juvenile sheep. Also, endothelial differentiation capability of amniotic epithelial stem cells and angiogenesis inducing activity of epithelial and mesenchymal sides of amnion were assessed.

RESULTS & DISCUSSION: At 6 and 48 weeks after implantation, the grafts were fully patent and the internal surface was smooth, and shiny, without any sign of thrombus formation. No inflammation or fibrosis was evident. Scanning electron microscopy revealed a confluent layer of cells with normal endothelial cell morphology. There was a monolayer of cells positive for von Willebrand factor in histology sections. The combination of BMP-4 and VEGF synergistically increased the expression of endothelial markers in in vitro cultured amniotic epithelial cells. Both epithelial and mesenchymal sides of amnion showed increased angiogenesis after removing epithelial cells. Platelet aggregation and P-selectin production assays, Prothrombin time (PT), activated partial thromboplastin time (aPTT), clotting time (CT) and haemolysis (%) tests showed that both the epithelial and mesenchymal sides of the amnion are hemocompatible.

CONCLUSIONS: The results of the current study demonstrate that the human amniotic membrane is a proper substitute for vascular tissue engineering. However, further studies will be required to investigate human amniotic membrane as an implant in the arterial circulation.



Evaluating the design and fabrication of tissue engineering scaffolds based on triply periodic minimal surfaces

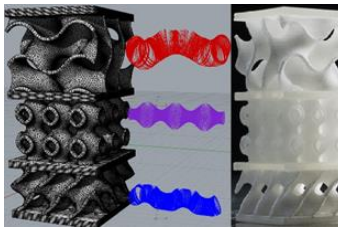
M. Nauman Jalil[1], H. Millward[1], A. Lewis[1]
Presenting Author: M Nauman Jalil, mohammad.jalil@cbmwales.co.uk
[1]Wales Centre for Advanced Batch Manufacture, University of Wales, Swansea, Wales

INTRODUCTION: One of the routes tissue engineering research can take is to provide a scaffold, which acts as a temporary artificial extracellular matrix for cells. An important aspect of scaffold design is the variety of pore diameters required to optimize cell culture development. This work details how triply periodic minimal surfaces (TPMS) [1-2] consist of pore profiles with a variable pore diameter that could accommodate the requirements for an optimized 3D cell culture.

METHODS: Scaffold geometries were designed in Rhinoceros 3DI, and their pore profiles were evaluated using a protocol developed in the visual programming language Grasshopper. CAD models were converted to printable 3D meshes and fabricated using the ProJet 3500 printer in a biocompatible polymer. Micro CT reconstructed models of 3D printed scaffolds were used to compare the fabrication accuracy of these pore profiles. Scaffolds were also seeded with human endothelial cells (HECV) in order to observe their attachment and viability using confocal microscopy.

RESULTS & DISCUSSION: The design work helped to develop a protocol for the 3D modelling and fabrication of bio-mimetic scaffolds geometries. The comparative analysis of Micro CT reconstructed models to CAD models showed how variable pore profiles can be fabricated using 3D printing. This profile results in a gradually changing pore diameter, which can range from 100 µm to 1000µm depending on single unit size, and the specified wall thickness required for stable fabrication. The cell seeding work provided insight into how endothelial cells arrange themselves within a pore.

CONCLUSIONS: The results of this work show that TPMS geometries may be a suitable candidate for scaffolds with bio-mimetic architecture and provide variable pore profiles that could play a role in optimizing 3D cell culture.



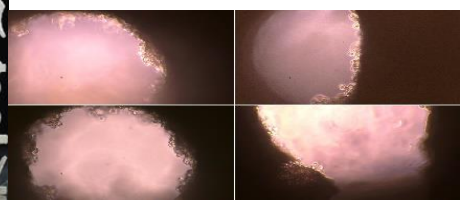
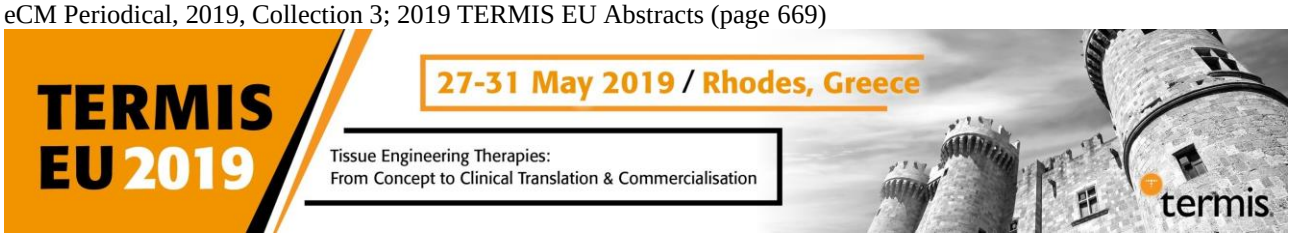


Figure 1: (left) Variable pore profiles of three different TPMS stacked geometries. **Figure 2:** (right) Inverted Microscope Images of endothelial cells lining curved pore wall.

ACKNOWLEDGEMENTS: This PhD was funded by University of Wales scholarship. Micro CT was performed by Professor Richard Johnston from the Materials Research Centre in Swansea University. Cell Seeding work was done with Professor Keith Morris at CSHS in Cardiff Metropolitan University.

REFERENCES

[1] Srinivasan R et al. Medical Image Analysis. 2006; 10(5):693-712
[2] Sanjairaj V et al. ACS Appl. Bio mater. 2018; 1: 259-269



Is there a need for a fellowship advisor for doctors? Analysis of the first 100 surveys from My-Fellowship.com, a Swiss online platform

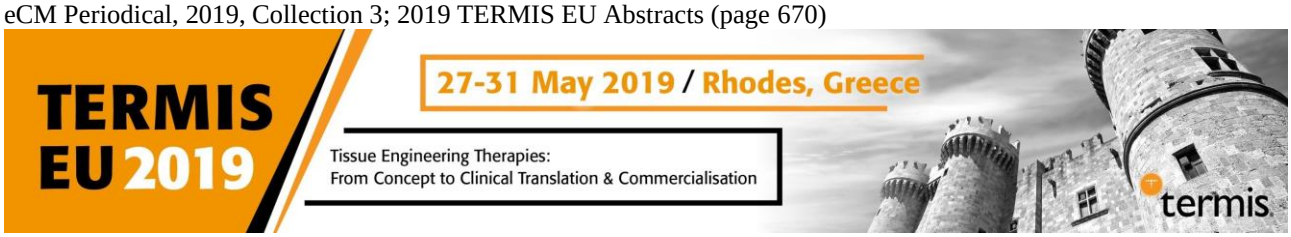
M.E. Taha[1], S.A. Müller[1], L. Audigé[1], M.O. Gauci[2], N.F. Friedrich[1], A. Lädermann[3]
Presenting author: Mohy Taha, mohy.taha@usb.ch
[1]Department of Orthopaedics & Trauma Surgery, University of Basel, Basel, Switzerland [2]Department of Orthopaedic and Sport Surgery, University Institute of Locomotion and Sports, Pasteur Hospital, Nice, France [3]Division of Orthopaedics and Trauma Surgery, La Tour Hospital, Meyrin, Switzerland; Department of Orthopaedics and Trauma Surgery, University of Geneva, Geneva, Switzerland

INTRODUCTION: Fellowships are common in many countries, whereas other countries do not offer special training after residency. Therefore many doctors seek for a fellowship after completing their residency. Fellowships became an essential part of professional medical training. Finding a suitable fellowship is essential. Physicians and institutions may have different expectations regarding fellowships, which can lead to frustration and wasted resources for both. One obstacle for doctors is finding reliable feedback from previous fellows regarding a specific fellowship and being able to contact that person for further advice. In addition, for both doctors and institutions alike, financing a fellowship can also prove a challenge.

METHODS: A website was created as well as a 1 minute survey using REDCap electronic data capture tools. The project was initiated during an international Orthopaedic and Trauma meeting in Switzerland. Moreover, the website was posted on Linkedin, Facebook and a newsletter explaining the project was sent to 1749 doctors worldwide per e-mail.

RESULTS & DISCUSSION: 2 weeks after the project initiation there were 1632 visits (1091 new users) to the website from 50 countries, mainly USA (37.9%), Switzerland (36.8%), Egypt (7.2%) and Germany (3.2%). 100 surveys from participants from 24 countries were completed: Switzerland (56%), Egypt (7%), France (5%) as well as Australia, Brazil, India and the United Kingdom (3%). The main specialities were Orthopaedic & Traumatology (59%), General Surgery (12%) and Internal Medicine (6%). Most of the participants were potential fellows (43%), previous fellows (26%) or individuals and/or institutions who would like to offer a fellowship (12%). The participants were mainly interested in a fellowship database (75%), connecting to other fellows (67%), giving/receiving feedback about a fellowship (61%) or receiving financial support (41%).

CONCLUSIONS: The results of the first surveys suggest that there is an interest in an online fellowship advisor including a database for fellowships worldwide, a platform for fellows to connect to each other with the ability to give and receive feedback about a fellowship. Accordingly an IT company was assigned to build the platform with the needs of doctors and institutes in mind. Financing a fellowship remains a challenge for many participants.



Assembly of functionalized silk together with cells to obtain proliferative 3D cultures in a network of microfibers

U. Johansson[1], M. Widhe[1], N. D. Shalaly[1], I. L. Arregui[2], L. Nilebäck[1], C. P. Tasiopoulos[1], C. Åstrand[1], Per-Olof Berggren[3], C. Gasser[2], M. Hedhammar[1]
Presenting Author: Mona Widhe, widhe@kth.se
[1]Department of Protein Science, [2] Department of Solid Mechanics, KTH Royal Institute of Technology, SE-106 91 Stockholm, Sweden, [3]The Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institute, S-171 76 Stockholm, Sweden

INTRODUCTION: Tissues are built of cells integrated in an extracellular matrix (ECM) which provides a 3D network with specific sites for cell anchorage. By genetic engineering, motifs from the ECM can be fused to recombinant silk proteins. Such a silk protein, FN-silk, which harbors a motif from fibronectin, has the ability to self-assemble into microfibers under physiological-like conditions. Herein we describe a method by which mammalian cells are added to the silk solution before assembly, and thereby get uniformly integrated between the formed microfibers.

METHODS: 12 different cell types were investigated for incorporation during the silk assembly process. The studied cell types, mainly isolated human primary cells, were selected to include a wide range of adherent cells, originating from various tissues of the body. Silk was assembled to microfibers in a random foam network or aligned fiber bundles (Fig. 1). Cell spreading, viability and proliferation was investigated. The differentiation capacity of stem cells after integration into silk was also evaluated.

RESULTS & DISCUSSION: Cells with elongated morphology and distinct focal adhesion points were found already after 3h in the silk network. In contrast to parallel cultures in RGD-coupled alginate, the cells in FN-silk were highly proliferative, quickly spreading out with proliferative cells also in the innermost parts. Cells remain viable in silk for at least 90 days. The method is also scalable to macro-sized 3D cultures. Silk fibers with integrated cells are both strong and extendable, with mechanical properties similar to that of artery walls. The described method enables both differentiation of stem- or precursor cells in 3D and facile co-culture of several different cell types. For example, inclusion of endothelial cells leads to the formation of vessel-like structures throughout the tissue constructs.

CONCLUSIONS: Silk-assembly in presence of cells constitutes a viable option for 3D culture of cells integrated in a microfiber ECM-like network, with potential as base for engineering of functional tissue.

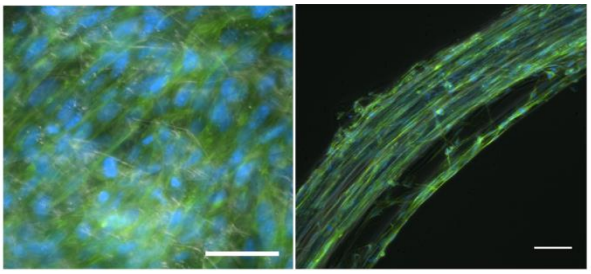
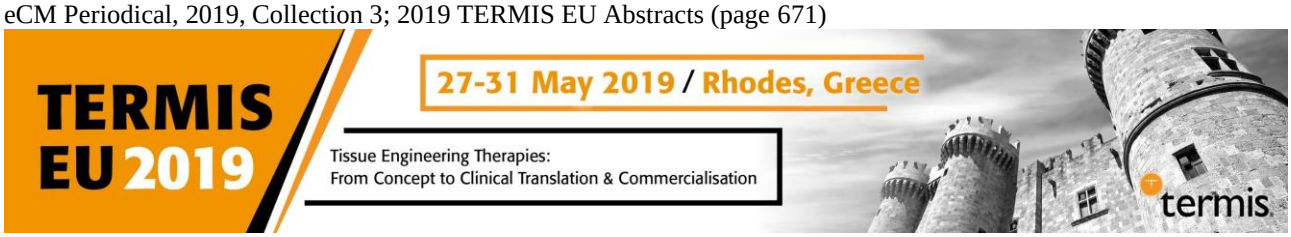


Figure 1: Differential Interference Contrast micrographs of the silk microfibers (white) in fiber (left) and foam (right) with mouse mesenchymal stem cells. Actin filaments are visualized by phalloidin (green) and cell nuclei by Dapi staining (blue). Scale bar = 50 µm.



Thorough investigation on the encapsulation of drugs with different wettability in thermosensitive micellar hydrogels

M. Boffito[1], R. Laurano[1], V. Chiono[1], G. Ciardelli[1]
Presenting Author: Monica Boffito, monica.boffito@polito.it
[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy

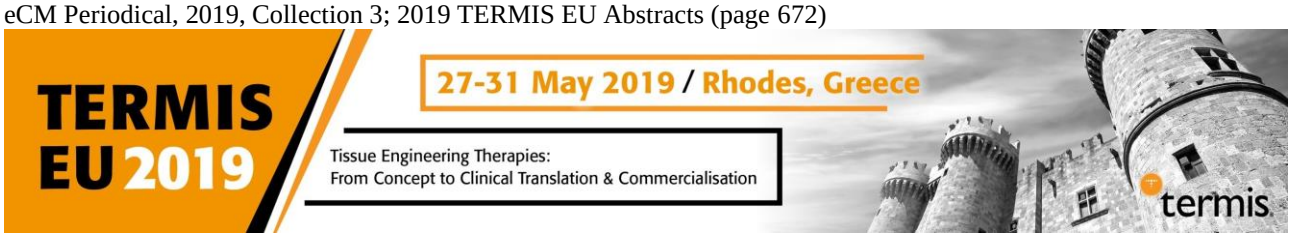
INTRODUCTION: Injectable hydrogels for the targeted release of therapeutics are a promising tool in the treatment of a wide variety of pathologies. In order to optimize gel design, a thorough understanding of gel/therapeutic interactions is required to predict payload release timing and its effects on gel properties. Herein, we designed thermosensitive micellar gels based on an amphiphilic poly(ether urethane) (PU), that were loaded with ibuprofen (IBU, hydrophobic drug) or ibuprofen sodium salt (IBUSS, hydrophilic salified form of IBU). IBU and IBUSS arrangement within the gels and their interactions with PU micelles were studied by rheology, dynamic light scattering (DLS) and colorimetric assay using 1,6-diphenyl-1,3,5-hexatriene (DPH) as marker of micellization.

METHODS: PU was synthesized starting from the triblock copolymer Poloxamer 407, an aliphatic diisocyanate and 1,4-cyclohexane dimethanol. Hydrogels were prepared in saline solution at a 15% w/v according to [1]. IBU and IBUSS were then loaded (1 mg/ml) and the resulting systems were rheologically characterized by strain sweep, frequency sweep and temperature ramp tests. Swelling and stability to dissolution were also evaluated. Drug/PU micelle interactions were studied by DLS and DPH assay on PU solutions (0.5% w/v) loaded with IBU and IBUSS [1].

RESULTS & DISCUSSION: Gel loading with IBU resulted in a decreased onset of the gelation process compared to PU_IBUSS and PU gels (9 °C for PU_IBU vs 14 °C for PU_IBUSS and PU). This result suggested that IBU hydrophobic nature induces its encapsulation within PU micelles during their nucleation, thus leading to the achievement of the critical micellar volume required for gelation onset [1] at lower temperature compared to the other samples. In fact, PU_IBUSS did not change its gelation onset compared to PU gel, suggesting that this drug is mainly located in the interstitial space among the micelles. DLS further proved this hypothesis, showing that micelles in PU_IBU had a significantly higher hydrodynamic diameter compared to PU_IBUSS and PU solutions (58 nm for PU_IBU vs 37 and 40 nm for PU_IBUSS and PU). Additionally, PDH assay showed that the critical micellization temperature of PU_IBU was clearly lower compared to PU and PU_IBUSS solutions (9 °C for PU_IBU vs 22 °C for PU and PU_IBUSS). Interestingly, irrespective of the nature of the encapsulated drug, drug-loading seemed to slightly accelerate the kinetics of gel formation and development compared to drug-free PU hydrogels (gelation points in frequency sweep test at 25°C were 58, 50 and 45 rad/s for PU, PU_IBU and PU_IBUSS), thus suggesting that encapsulating either hydrophilic or hydrophobic drugs do not have detrimental effects on the temperature-driven sol-to-gel transition of micellar gels. Additionally, no significant effects were observed in gel swelling and stability to dissolution in aqueous media upon drug encapsulation.

CONCLUSIONS: We reported for the first time the potential of rheology, DLS and DPH assay in characterizing drug arrangement within gels and their interaction with micelles.

ACKNOWLEDGEMENTS: The H2020 EU funding for Research & Innovation project "MOZART" (MesopOrous matrices for localiZed pH-triggered releAse of theRaPUTic ions and drugs, H2020-NMP6-2015) is acknowledged.



Mechanotransduction at the nuclear pore complex investigated at the molecular level: The role of SUN1

F. Donnaloja[1], E. Jacchetti[1], F. Rigoldi[2], M.T. Raimondi[1], M. Soncini[2]
Presenting Author: Monica Soncini, monica.soncini@polimi.it
[1]Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milan, Italy [2]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy

INTRODUCTION: It was demonstrated that stem cells cultured in 3D environments mimicking the native niche are able to maintain their stemness [1]. The cell fate seems to be correlated to the nuclear roundish morphology, far from the spread one of adherent cells. We hypothesize that the nuclear shape results from the system of forces transmitted to the nucleus through integrins, actins, nesprins and SUN proteins. We investigate the force distribution among different structures supposed to be connected to Sun1 protein, such as lamina and the nuclear pore complex (NPC). Here we focus on the prediction of SUN1 N-terminal domain folding to understand its affinity with aforementioned structures, and therefore better characterize force transmission to the NPCs.

METHODS: According to literature, we define the SUN1 N-terminal domain ranging from 1 to 300 amino acids. I-Tasser software returns possible templates. The best template is used to obtain SUN1 N-terminal predictive model by Swiss Model Software (two further templates are under evaluation). To estimate the model stability, we performed a simulated annealing simulation (Trange:300-500K; Tinc:5K/10ps) in explicit water using NAMD software and CHARMM force field. To validate the method, we run the same protocol to the solved SUN2 C-terminal domain (PDB: 4DXS).

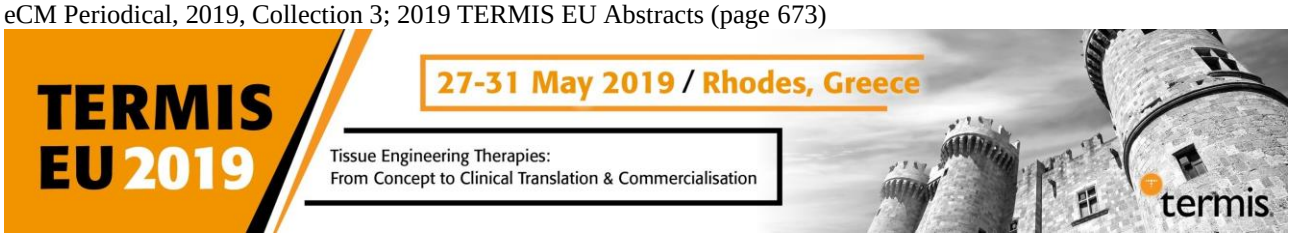
RESULTS & DISCUSSION: After the annealing procedure, the SUN1 N-terminal model results stable as proved by the Root Mean Square Deviation value (9,15±0.52) in equilibration phase. Moreover, the timeline secondary structures proves preserved arrangement during the equilibration phase. By comparison with SUN2 C-terminal domain structure, we can consider the predicted structure as promising model for SUN1 N-terminal domain.

CONCLUSIONS: The implemented protocol for folding prediction returns a promising protein template for further investigations. The proposed model is required for studying the SUN1 N-terminal domain affinity with the NPC structure (in particular with Nup153 protein) and lamina. We are experimentally testing the SUN1-Nup153 binding via Bio-layer interferometry technique. In future work, we will identify the SUN1 N-terminal specific binding sites to investigate the force distribution at nuclear envelope site. We hypothesise that a different grade of affinity modulates the force distribution influencing cell mechanotransduction and fate, both in physiology and in disease.

ACKNOWLEDGEMENTS: ERC grant NICHOID, Grant Agreement n. 646990

REFERENCES

[1] Nava M. et al. J Tissue Eng Regen Med 2017



Opening of chromatin in cancer cells by switching substrate elasticity

M. Rabineau[1,2,4], C. Ehlinger[1,2,4], F. Flick[1,2,4], Y. Haikel[1,2,4], P. Schaaf[1,2,4], B. Senger[1,2,4], P. Lavalle[1,2,4], L. Kocgozlu[1,2,4], J.-N. Freund[3,4], D. Vautier[1,2,4]
Presenting Author: Morgane Rabineau, morgane.rabineau@inserm.fr
[1]Inserm UMR-S1121, Université de Strasbourg (UdS), France [2]Faculté de Chirurgie Dentaire (UdS), France [3]Inserm UMR-S1113, (UdS), France [4]Fédération de Médecine Translationnelle, Strasbourg, France

INTRODUCTION: Cancer cells are characterized by their proliferative potential, ability to metastasize and high degree of plasticity. Increasing evidences support the central role of the mechanical properties of the cellular microenvironment in cell fate. Reducing the rigidity of an adhesion substrate leads to massive death of human SW480 colon cancer cells [1]. Chromatin de-condensation appears as the priming early event in cell reprogramming [2]. Whether chromatin plasticity of cancer cells is influenced by the Young's modulus of the cellular microenvironment has not been addressed so far.

METHODS: The behavior of SW480 cancer cells was assayed on polyelectrolyte multilayer films with an elastic modulus of 20 kPa (short-hand notation E_{20}) as a selective soft substrate model for cell survival. To highlight the survival process developed by cancer cells, cells that were resistant on E_{20} were recovered and amplified on supraphysiologically stiff culture glass slides and replated on E_{20}.

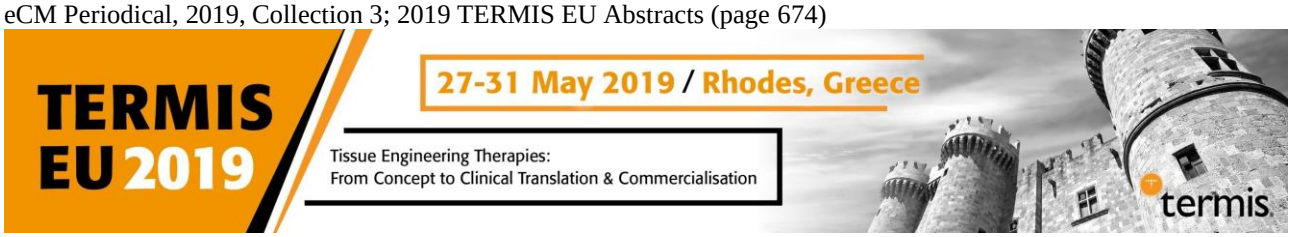
RESULTS & DISCUSSION: A first contact with the soft substrate E_{20} caused massive SW480 cell death by necrosis, whereas about 7% of the cells did survive exhibiting a high level of condensed chromatin (21% heterochromatin). These surviving cells were round with poor motility, and characterized by limited focal adhesions. However, four consecutives hard / soft cycles elicited a strong chromatin de-condensation (6% heterochromatin) in correlation with increase of cellular survival (about 90%), cell spreading, formation of focal adhesions and higher cell motility. Furthermore, cell survival appeared reversible, indicative of an adaptive process rather than irreversible gene mutation(s). This adaptation process was associated with modifications in gene expression patterns. Interestingly, expression of the homeobox gene CdX2 and the nuclear receptor family gene Hnf4α, two major regulators of intestinal homeostasis with tumor suppressor activity was downregulated, as well as stimulation of the Macc 1 gene involved in metastasis.

CONCLUSIONS: At the ultrastructural level, the de-condensed chromatin induced by mechanical means appeared to be very similar to those obtained chemically by trichostatin A or by the molecule CYT296. This work represents a new step towards understanding how cancer cells resist to mechanical stresses. A totally new approach for chromatin de-condensation only based on mechanical properties of the microenvironment without any drug mediation is thus demonstrated.

ACKNOWLEDGEMENTS: Financial support was received from Alsace Contre le Cancer 2012, 2014 and 2019, La Ligue contre le cancer 2014 (GE-BFC) and ANR "FORCELL" (ANR-12-BSVE5-0021).

REFERENCES

[1] Rabineau M et al. Plos One. 2012; 7:e50443
[2] Gaspar-Maia A et al. Nat Rev Mol Cell Biol. 2011;12:36-47



Recombinant human type I collagen compositions for 3D bioprinting of tissues and organs

R. Zarka[1], J. Seror[1], O Grinberg[1], O. Shoseyov[1,2], N. Orr[1]
Presenting Author: Nadav Orr, nadav@collplant.com
[1]CollPlant Ltd, Ness-Ziona, Israel, [2] The Robert H. Smith Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

INTRODUCTION: Collagen, the major component of connective tissues, is frequently considered the first choice for use in 3D printing of tissues and organs. Tissue extracted collagen however suffers from number of problems, including batch-to-batch inconsistency, structural damage to the molecule, allergic response, risk of disease transmission, and exposure to residual components from the source tissue. Recombinant type I human collagen (rhCollagen) was processed from tobacco plants that were genetically engineered to express 5 genes associated with the production of human Type I collagen [1]. The purified, naïve, rhCollagen is superior to tissue extracted material in major characteristics including safety, mechanical properties and biological signaling [2-3].

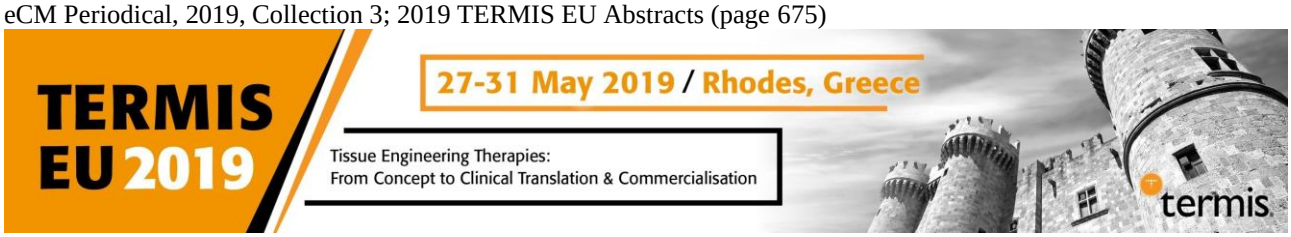
METHODS: Recombinant type I human collagen was purified in large scale under cGMP conditions. The purified material was modified with photo-reactive molecules to serve as photocurable media in 3D Bioprinting. Specific compositions (BioInks) were designed by formulating the modified rhCollagen alone, or with additional components to match different printing technologies and mimic variety of tissues. The compositions were characterized for physical and biological properties.

RESULTS & DISCUSSION: rhCollagen and it's modified versions can be used for 3D printing at room temperature thus avoiding the typical gelation of tissue extracted collagen at these conditions. RhCollagen modified with methacrylic groups (rhCollagen-MA) or thiols demonstrated excellent compatibility with different photoinitiation systems at a broad range of wave lengths including 365, 405 and 500nm. The unique viscosity and shear thinning properties of modified rhCollagen allowed the flexibility to formulate BioInks compatible, with or without cells, with the major printing technologies including extrusion, ink-jet, laser and stereolithography. Scaffolds comprised of BioInk compositions including the modified rhCollagen alone, or in combination with natural or synthetic polymers and cross linkers exhibited physical properties matching natural tissues and demonstrated excellent support for cells proliferation and vitality including a series of primary and differentiated human cells.

CONCLUSIONS: These data demonstrate that photo-curable modified rhCollagen can serve well as a major component in many 3D Bioprinting systems where a need to mimic the natural environment including the physical and biological niches is critical.

REFERENCES

[1] Stein H et al. Biomacromolecules ;5-10:2640 2009
[2] Willard JJ et al. Tissue Eng Part A 2013; 19(13-14):1507-18
[3] Shilo S et al. 2013; Tissue Eng Part A. 19(13-14):1519-26



3D microfluidic chip based mesenchymal stem cell differentiation

N. Dhiman[1,2], P. Kingshott[2], S. N. Rath[1]
Presenting Author: Nandini Dhiman, bm15resch11002@iith.ac.in
[1]Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Kandi, Telangana, India [2]Department of Chemistry and Biotechnology, Faculty of Science and Engineering Technology, Swinburne University of Technology, Hawthorn, Victoria, Australia

INTRODUCTION: 3D microfluidic (μF) devices or organ-on-chips provide a more physiologically relevant microenvironment for stem cells to proliferate or differentiate as compared to traditional cell culture methods [1]. Umbilical cord derived stem cells (UMSC) have advantages over bone-marrow or adipose-derived stem cells, such as, no ethical issues, ease of procurement, and collection of large number of cells [2]. Herein, we attempted to compare the osteo-differentiation potential of UMSC with different chemical factors in a collagen type-I-based 3D μF device.

METHODS: 3D PDMS μF device was fabricated using photolithography and replica molding. Human UMSC were differentiated into osteolineage in 3 different platforms – 3D μF device, 3D collagen beads, and 2D 24-well plate – using osteoinduction medium (OIM) containing 10 mM β-glycerophosphate 100 nM dexamethasone, and 50 μg/ml L-Ascorbic acid. Media were changed every day for μF device and every two days for other controls. FDA/PI, ALP staining, immunofluorescent staining and qPCR were conducted after 1, 7 and 14 days.

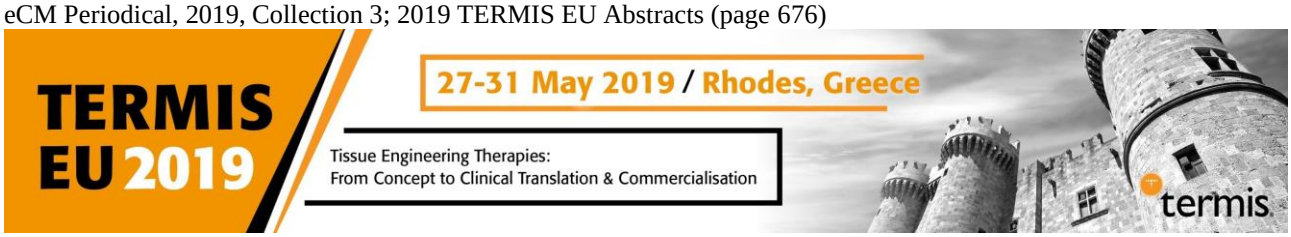
RESULTS & DISCUSSION: The PDMS devices fabricated for 3D stem cell culture contain three channels with central channel containing a hydrogel (with cells) flanked by media for cell growth and differentiation. The hexagonal posts provide surface tension to hold the gel without any leakage. Preliminary results in the 2D and 3D groups showed early osteo-differentiation in OIM-treated samples as compared to the control samples exposed to normal medium.

CONCLUSIONS: As the 3D microfluidic device helps in replicating the stem cell differentiation pathway, as also observed in 3D beads in our study, the application potential of the 3D μF device is multi-fold, especially with respect to bed-side testing of mononuclear stem cell fraction of blood for therapeutic purposes.

ACKNOWLEDGEMENTS: This work was funded by the Nano Mission project, DST (Project Reference No. SR/NM/NT-1095/2016) and the Swinburne University Postgraduate Research Award 2018-19 (SUPRA). This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF).

REFERENCES

[1] Ertl P et al. Trends in Biotech. 2014; 32(5):245-253
[2] Hu L et al. Biomed Res Int. 2013;2013:438243



A customized in vitro platform for cartilage thermo-mechanobiology

N. Nasrollahzadeh[1], L. A. Applegate[2], D. P. Pioletti[1]
Presenting Author: Naser Nasrollahzadeh, naser.nasrollahzadeh@epfl.ch
[1]Laboratory of Biomechanical Orthopedics, Institute of Bioengineering, EPFL Lausanne, Switzerland [2]Regenerative Therapy Unit, University Hospital of Lausanne (CHUV), Lausanne, Switzerland

INTRODUCTION: Careful design of biomaterials properties, cell-scaffold interaction and in vivo like stimulation is required to guide chondrocytes differentiation and facilitate clinical translation of engineered cartilage. Since articular cartilage is a poro-viscoelastic tissue with high dissipative capacity, temperature increase following joint loading could occur [1]. Given this self-heating phenomenon, a customized biomimetic in vitro platform was developed, to study cartilage thermo-mechanobiology.

METHODS: To induce a stable cell-scaffold interaction, we firstly RGD functionalized the developed stiff and fatigue resistance porous hydrogels with recapitulated cartilage mechanical properties. This ligand grafting process was performed in two steps for functional group activation and peptide conjugation while maintaining desired mechanical properties. In addition, a novel and modular home-made bioreactor was designed to apply stress/strain and desired temperature scheme during culture of cell-laden hydrogels while CO_2/O_2 and humidity levels are maintained. The gradual temperature increase from 32°C (corresponding to knee temperature at rest) to 39°C (after exercise) during cyclic compression was based on the measured in vivo data inside intra-articular region of the knee during jogging [2]. The synergetic or decomposed effects of temperature and loading on human epiphyseal chondro-progenitor (hECP) cells response were then evaluated.

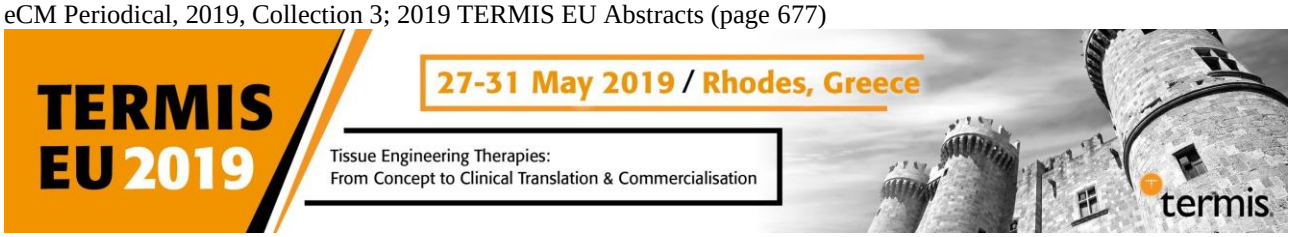
RESULTS & DISCUSSION: Not only cells attachment was enhanced within the RGD functionalized hydrogels in static culture, but also the cells-hydrogel interaction following biophysical stimulus was better preserved. Moreover, a synergetic effect following thermo-mechanical stimulation was observed in upregulation of Sox9, one of the main regulators of chondrogenic differentiation process [3]. In parallel, Twist1 which is known as chondrogenesis inhibitor [3] was significantly downregulated following thermo-mechanical stimulation. The total extracted RNA from samples varied minimally by intermittent thermo-mechanical stimulation contrary to significant decrease of total RNA for standard culture of hECP cells above knee cartilage temperature at rest (37°C vs 32°C).

CONCLUSIONS: Collectively, intermittent thermo-mechanical stimulation as simulation of self-heating phenomenon following joints physical activity can enhance chondrogenesis while maintaining cells metabolism.

ACKNOWLEDGEMENTS: This work was supported by the Swiss National Science Foundation (#310030_149969 / 1).

REFERENCES

[1] Abdel-Sayed P et al, J. Mech. Behav. Biomed. Mater. 2014; 30, 123.
[2] Becher C et al, BMC Muscul. Disord. 2008; 9, 46.
[3] Gu S et al, J. Biol. Chem. 2012, 287, 21082.



What controls endothelial sprouting? Interstitial flow vs. shear stress

N. Salehi-Nik, S.S. Seyedipour, P. Padmanaban, F. Stein, J. Rouwkema
Presenting Author: Nasim Salehi-Nik, n.salehinik@utwente.nl
Department of Biomechanical Engineering, Technical Medical Centre, University of Twente, 7522NB Enschede, The Netherlands

INTRODUCTION: During angiogenesis, endothelial cell (EC) sprouting occurs when select ECs lining a vessel are exposed to stimulatory factors. Growth factor gradient has been proved to stimulate angiogenesis. However, the effect of shear stress on cell sprouting has been controversial [1-2]. Since certain level of media perfusion is required to support cell viability as well as to maintain the stability of the newly formed vascular network, it is necessary to fine-tune shear stress in engineered tissues. In the present study, we investigated the effect of different amounts of shear stress and interstitial flow on EC sprouting with the aim of finding a new approach to control vascular formation in thick engineered tissues.

METHODS: To determine how different ranges of interstitial flow and shear stress modulate sprouting, we developed a microfluidic platform that contains a crinkle line with different angles (Channel 1), a straight line (Channel 2) and a hydrogel channel in between. Both channels 1 and 2 were lined with a monolayer of ECs. Mural cells and ECs were mixed with the hydrogel and filled in the channel between the two EC lined channels. To assess the effect of shear stress on cell sprouting, the pressure drop in different parts of the channel 1 was kept constant. To apply interstitial flow in different directions, positive or negative pressure difference was considered between two channels. Shear stress and interstitial flow profiles were calculated using COMSOL simulation.

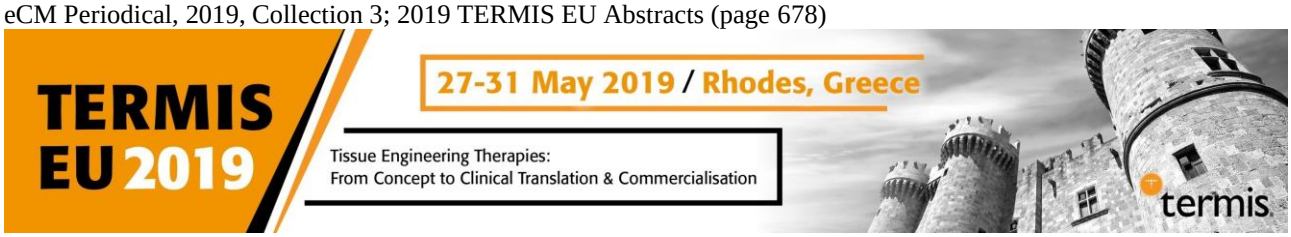
RESULTS & DISCUSSION: Biological self-assembling of ECs resulted in tube formation within the hydrogel after one week. ECs lined the channels 1 and 2 started to sprout and connect to the capillary bed based on the amount of shear stress they experienced. High amounts of shear stress restricted angiogenesis. In contrast, low amount of shear stress was suitable to initiate and support ECs sprouting. Interstitial flow was required to direct ECs connection to the capillary bed within the hydrogel, and making perfusable vascular networks.

CONCLUSIONS: Using a microfluidic platform, we found that biomechanical forces at special ranges direct sprout formation and can be used to control vascularization within engineered tissues.

ACKNOWLEDGEMENTS: The work is supported by an ERC Consolidator Grant under grant agreement No. 724469.

REFERENCES

[1] Song JW et al. PNAS 2011; 108(37):15342-47
[2] Lee VK et al. Cell Mol Bioeng. 2014; 7(3):460-72



Mesenchymal stem cells from alveolar mucosa as a new source of myogenic progenitors

N.V. Kosheleva[1,2,3], A.A. Pulin[1]
Presenting Author: Nastasia V. Kosheleva, n_kosheleva@mail.ru
[1]FSBSI "Institute of general pathology and pathophysiology", Moscow, Russia [2]Lomonosov Moscow State University, Faculty of biology, Moscow, Russia [3]FSBEI FPE "Russian Medical Academy of Continuous Professional Education" of the Ministry of Healthcare of Russia, Moscow, Russia

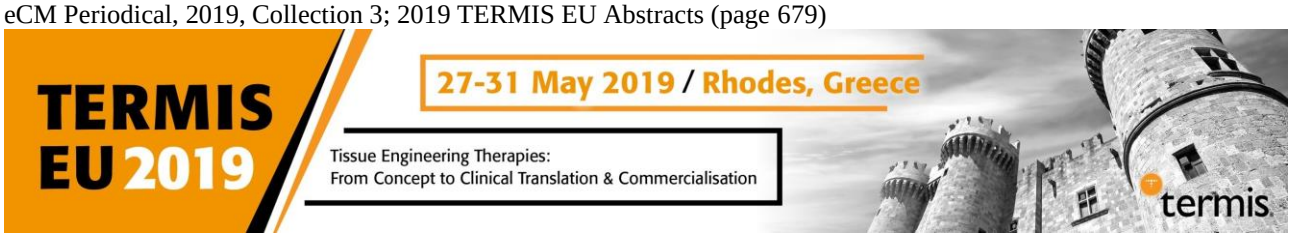
INTRODUCTION: In case of musculoskeletal dysfunction and muscular dystrophy, one of the key problems of replacement cell therapy is availability of autologous cell sources with potential to differentiate in myogenic direction. Mesenchymal stem cells (MSC) are one of the sources of myoblasts during muscle tissue regeneration. Moreover it is known that viability and differentiation potential of MSC are elevated when they are cultured under 3D conditions in the form of cell spheroids. The aim of this work was to study myogenic potential of MSC from alveolar mucosa (MSC-amc) in 2D (monolayer) and 3D (spheroids) culture.

METHODS: To isolate human MSC-amc, we collected the biopsies of alveolar oral mucosa from healthy donors (n=10) after obtaining their informed consent. We used cultures at passages 3-4. Growth culture medium consisted of DMEM/F12 and 20% FBS (HyClone, USA), induction medium contained DMEM low glucose and 2% Horse Serum (BioInd, USA). Spheroids were formed under standard conditions (37°C; 5%CO_2) on agarose plates with micro-wells made using 3D Petri Dishes (Microtissue, USA). Appearance of myotubes in 2D culture and dynamics of spheroids' formation were monitored via live light time-lapse microscopy in Cell-IQ device (CM Technologies, Finland). Cells in monolayer and 7-day spheroids were fixed in 4% paraformaldehyde for immunocytochemical analysis of MyoD, sarcomeric alfa-actin and MEF 2A+2C expression. For in vivo experiments on rabbits allogenic MSC-amc from suspension or in spheroids were injected in injured calf muscles ($3*10^6$cell/200mkl 0,9% NaCl). Muscles were studied on 20, 28 and 35 days after operation.

RESULTS & DISCUSSION: In 2D culture after induction or in 40% spontaneously MSC-amc were able to differentiate in myogenic direction. Cells formed multinucleated myotubes and expressed a marker of myogenesis early stages MyoD. In spheroids obtained from MSC-amc by day 7 both in induction and growth culture medium we observed the hallmarks of myogenic differentiation with the formation of muscle tubes. Differentiated spheroids did not contain early progenitor cells, there was no expression of MyoD, but more differentiated well-formed myofibrils with characteristic peripheral nuclei arrangement, expression of MEF and cross-striation, marked by antibodies against sarcomeric alfa-actin were observed. On the model of calf muscle injury suspension of MSC-ams reduced size of scar, whereas spheroids from MSC-amc promoted full organotypic recovery of muscle tissue.

CONCLUSIONS: 3D culturing of MSC-amc in form of spheroids can stimulate effective spontaneous myogenic differentiation maintain tissue-specific functional characteristics over a long period. Spheroids from MSC-amc can become an alternative accessible and less invasive source of myogenic cells in vitro and for cell replacement therapy in vivo.

ACKNOWLEDGEMENTS: Financial support was received from Russian Science Foundation (grant № 17-75-30066).



Enhanced human bone marrow mesenchymal stromal cell adhesion and survival on scaffolds promotes bone formation

M. Mebarki[1], L. Coquelin[1], P. Layrolle[3], S. Battaglia[3], M. Tossou[1], P. Hernigou[1,2], H. Rouard[1], N. Chevallier[1]
Presenting Author: Nathalie Chevallier, nathalie.chevallier@efs.sante.fr
[1]INSERM U955-E10, IMRB-Paris Est University, Engineering and cellular Therapy unit, Etablissement Français du Sang, Creteil, France [2]Orthopaedic Surgery Department, Henri-Mondor AP-HP Hospital, Creteil, France [3]INSERM U957, Lab. Pathophysiology of bone resorption, Faculty of Medicine, University of Nantes, Nantes, France

INTRODUCTION: In order to induce an efficient bone formation with human bone marrow mesenchymal stromal cells (hBMSCs) associated to a scaffold, it is crucial to determine the key points of the hBMSCs action after in vivo transplantation as well as the appropriate features of a scaffold.

METHODS: For this purpose, we compared the behavior of hBMSCs,in vivo, by species specific RT-qPCR, immunostaining and histology, when grafted onto two biomaterials allowing different bone potential. To this aim, the cancellous devitalized Tutoplast®-processed bone (TPB) and the synthetic hydroxyapatite/β-tricalcium-phosphate (HA/βTCP) which give at 6 weeks 100% and 50% of bone formation respectively, were chosen. Biomaterial structure was analyzed by micro-computed tomography and Fourier transform infrared spectroscopy.

RESULTS & DISCUSSION: We first showed that hBMSCs adhesion is two times favored on TPB in vitro and in vivo compared to HA/βTCP. Biomaterial structure analysis indicated that the better cell adhesion on TPB is associated to its higher and smooth open pore architecture as well as its content in collagen. Our 6 week time course analysis, showed using qPCR that only adherent cells are able to survive in vivo giving thus an advantage in term of cell number on TPB during the first 4 weeks after graft. We then showed that grafted hBMSCs survival is crucial as cells participate directly to bone formation and play a paracrine action via the secretion of hIGF1 and hRANKL which are known to regulate the bone formation and resorption pathways respectively.

CONCLUSIONS: Altogether our results point out the importance of developing a smooth and open pore scaffold to optimize hBMSCs adhesion and ensure cell survival in vivo as it is a prerequisite to potentiate their direct and paracrine functions.

ACKNOWLEDGEMENTS: This work was supported by EFS Ile de France (APR - 2014) and by the 7th Framework Program of the European Commission through the project REBORNE no. 241879. M. Mebarki was supported by Assistance Publique – Hôpitaux de Paris. The authors are grateful to Franck Chiappini for helpful statistical analysis, Guy Louarn from the Institut des Matériaux de Nantes and to the SEM platform of University Paris-Est, with special thanks to Dr Patrick Ausset for his precious help. The authors are grateful to the company Ceraver, which kindly provided the HA/βTCP ceramics.

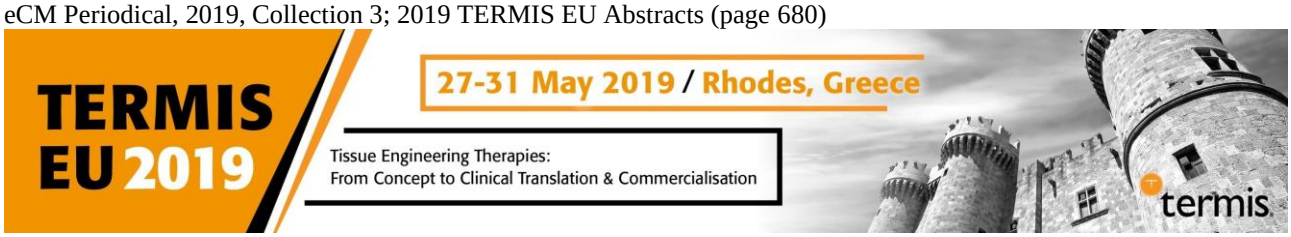


Photo-crosslinkable collagen precursors for vascular tissue engineering

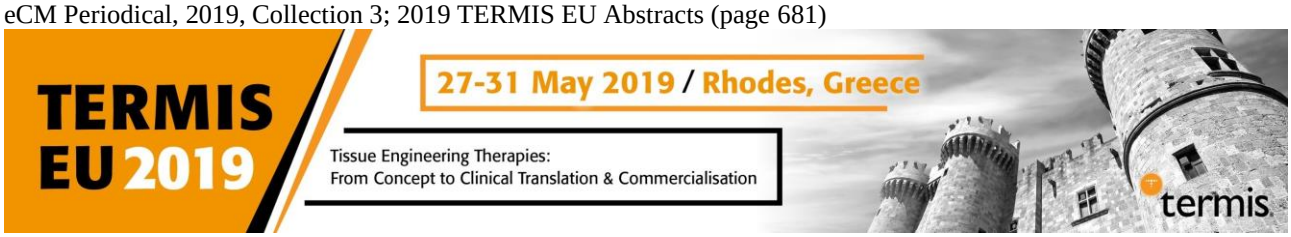
N. Pien[1,2], D. Pezzoli[2], D. Camasao[2], F. Bray[3], C. Rolando[3], M. Albu[4], D. Zeugolis[5], J. Martins[6], D. Mantovani[2], S. Van Vlierberghe[1], P. Dubruel[1]
Presenting Author: Nele Pien, nele.pien@ugent.be
[1]Polymer Chemistry & Biomaterials Research Group, Centre of Macromolecular Chemistry, Ghent University, Belgium [2]Laboratory for Biomaterials and Bioengineering, CRC-I, Laval University, Canada [3]Miniaturisation pour la Synthèse, l'Analyse & la Protéomique (MSAP), Université Lille 1, France [4]Department of Collagen Research, National Research & Development Institute for Textiles and Leather, Romania [5]Regenerative, Modular & Developmental Engineering Laboratory, National University of Ireland Galway, Ireland [6]NMR and Structure Analysis Unit, Ghent University, Belgium

INTRODUCTION: The aim of vascular tissue engineering (VTE) is the design of responsive, living conduits, with properties similar to those of native tissue. Collagen type I, being the main component of native vessels, is a promising scaffold material for vascular TE owing to its favourable biological properties and to the ability of cells to remodel its matrix. The present work targets the development of collagen precursors functionalized with photo-crosslinkable moieties while preserving the biocompatibility, and to develop collagen-based hydrogel films with tunable mechanical properties.

METHODS: In brief, methacrylated collagen was prepared by reaction of the primary amines of collagen with 0.5 or 1 eq methacrylic anhydride (MeAnH). An ortho-phthalic dialdehyde (OPA) assay was applied to evaluate the functionalization of the collagen precursors. Identification of the biopolymer peptide sequence was performed via mass spectrometry analysis (Orbitrap) along with bioinformatic analysis on unmodified and functionalized collagen precursors. The position of the introduced double bonds was also determined via proteomics. Crosslinked collagen films were characterized in depth in terms of crosslinking efficiency, gel fraction, swelling ratio and mechanical properties using rheology. Potential in vitro cytotoxicity of the functionalized collagen was evaluated using human umbilical vein endothelial cells (HUVECs). Cell viability, proliferation and morphology were examined via indirect and direct in vitro assays.

RESULTS & DISCUSSION: The OPA assay showed that the targeted lysine groups were successfully modified, with a degree of substitution of 85 and 98% for the 0.5 and 1 eq MeAnH, respectively. In addition to the amino acid quantification and localization, the position of the introduced double bonds was found to have a homogeneous methacrylamide distribution throughout the collagen backbones. Rheological measurements confirmed that an increasing number of crosslinkable moieties yields a higher storage modulus (i.e. 4.8 kPa versus 9.3 kPa), due to a higher network density. High gel fractions (i.e. 87 and 91 %) were obtained. The crosslinking efficiency determined via HR-MAS NMR spectroscopy indicated the occurrence of an efficient crosslinking (i.e. 86 and 88% double bonds being consumed during crosslinking). In vitro assays to evaluate cell-biomaterial interactions of the crosslinked hydrogel films indicated an excellent cell viability (i.e. 97% and 94%) and cell adhesion.

CONCLUSIONS: Collagen type I has been successfully functionalized with photo-crosslinkable moieties. The developed collagen precursors have tunable mechanical properties while maintaining biocompatibility.



Validation of the NANT 001 bioreactor system for automated adipose-derived mesenchymal stem cell expansion

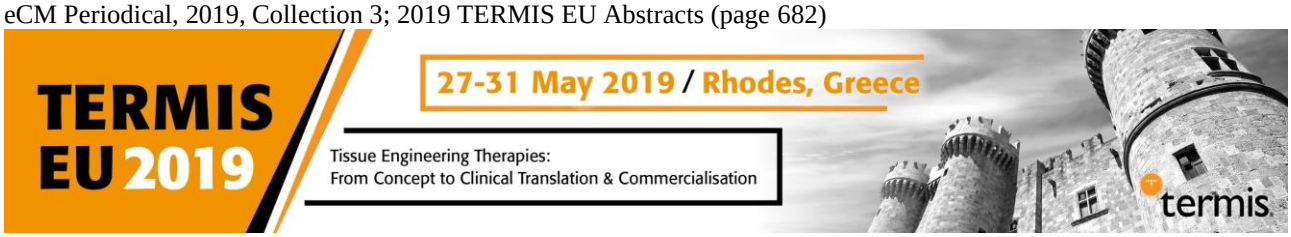
N. Duffy[1], J. Fitzgerald[1], A. Paulitti[2], F. Curcio[3], G. Cattaruzzi[2], F. Barry[1]
Presenting Author: Niamh Duffy; nduffy@nuigalway.ie
[1]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway, Ireland, [2]VivaBioCell S.p.A. Udine, Italy [3]Dipartimento di Area Medica (DAME), University of Udine, Udine, Italy

INTRODUCTION: Adipose-derived mesenchymal stem cells (ASCs) are an attractive choice for regenerative therapies due to their minimally invasive method of isolation. As with any source, the need for cell expansion to produce therapeutically useful quantities of cells bring challenges. Manual production of stem cells is labor-intensive, expensive and poses a high risk for contamination. The NANT 001 is an innovative bioreactor that has evolved from manual cell culture principles to introduce a high level of automation. This work reports on the validation of this system for the production of ASCs compared with conventional manual expansion processes.

METHODS: ASCs at passage 1 from a healthy donor were suspended in α-MEM supplemented with 10% Fetal Bovine Serum, seeded into either the bioreactor system or Corning 636 cm^2 CellSTACK culture chambers at a density of 2000 cells/cm^2 and incubated at 37°C, 5% CO_2. After 24 hours, and when the cells reached 50% and 90% confluency, the monolayer was washed with PBS and the growth media was replaced. Cells were automatically harvested 24 hours after reaching 90% confluence. Three validation runs were performed. Growth kinetics, cell viability, sterility, trilineage differentiation capacity and surface immunophenotype were assessed.

RESULTS & DISCUSSION: Cell growth kinetics and yield from the bioreactor were comparable to those obtained from the manual process. On average, an inoculation of $1.3x10^6$ cells yielded $52x10^6$ cells from the NANT 001 and $55x10^6$ from the manual process in a 6-day expansion. Viability was not affected by the bioreactor process with >95% cell viability recorded from all 3 runs. Supernatants from all cell cultures tested negative for microbial contamination. There were no significant differences in the adipogenic, osteogenic and chondrogenic differentiation propensity of the cells regardless of expansion process. Expression of surface markers in all cases was consistent with IFATS standards for ASC phenotype.

CONCLUSIONS: Collectively, these data illustrate the potential of using the NANT 001 bioreactor for automated expansion of ASCs. The risk of contamination is decreased by reducing the use of open procedures. Ongoing work consists of a GMP validation of the system for the expansion of ASCs from the stromal vascular fraction of adipose tissue. Based on these observations, the NANT 001 bioreactor represents an effective option for closed, automated and aseptic manufacturing of autologous cell products for applications in cell therapy and regenerative medicine.



Medical device coatings to mitigate the foreign body response

N. Welch[1], M. A. Hossain[2], R. Bathgate[2], S. Mukherjee[3], J. Werkmeister[3], J.D. Wade[2], D. Winkler[1,4,5], H. Thissen[1]
Presenting Author: Nicholas Welch, Nicholas.Welch@csiro.au
[1]Commonwealth Scientific and Industrial Research Organisation (CSIRO), Melbourne, Australia [2]The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia [3]Hudson Institute of Medical Research, Melbourne, Australia [4]La Trobe University, Melbourne, Australia [5]Monash Institute of Pharmaceutical Sciences, Melbourne, Australia.

INTRODUCTION: The foreign body response (FBR) to an implanted biomaterial leads to the formation of a fibrotic capsule. This capsule can disrupt tissue-implant interactions such as those necessary for communication between a nerve cell and an implanted electrode. Human gene-2 relaxin (H2 relaxin) has exhibited anti-fibrotic activity in vivo. A synthetic short-chain soluble peptide, B7-33, mimics the anti-fibrotic actions of relaxin in vivo. Here, we present a method for the incorporation of B7-33 into polymer coatings for medical device applications.

METHODS: Poly(lactide-co-glycolide) (PLGA) and B7-33 were co-dissolved in DMF to the desired concentrations and then applied to 96 well-plates before being placed in a vacuum system to remove the solvent.
An in vitro reporter gene assay for cyclic adenosine monophosphate (cAMP) activity in human embryonic kidney cells stably expressing the relaxin family peptide receptor 1 (HEK-RXFP1) was used to assess B7-33 activity. Surfaces were seeded with HEK-RXFP1 cells and incubated for 6 hours. Cells were then lysed and reporter gene stimulated Beta-Galactosidase activity measured using a colorimetric substrate to evaluate the relative cAMP responses.

RESULTS & DISCUSSION: Figure 1 demonstrates that the PLGA+B7-33 surface coating (red) dose-dependently activates RXFP1 to a similar level as the soluble B7-33 peptide (blue) though with a 10-fold lower EC50. The dried B7-33 control (green), in the absence of PLGA, demonstrates activity akin to the soluble B7-33.

CONCLUSIONS: The cAMP assay offers a validation method for the development of B7-33 peptide coatings. Here, we have demonstrated that a polymer/peptide surface coating can successfully agonize RXFP1 with an equivalent potency to the soluble B7-33 peptide. This result may indeed open the door to the next-generation of anti-fibrotic device coatings.

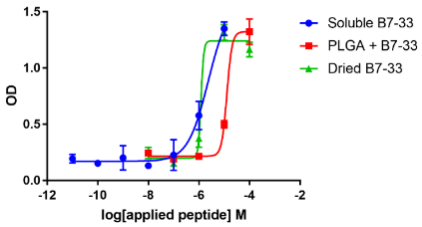
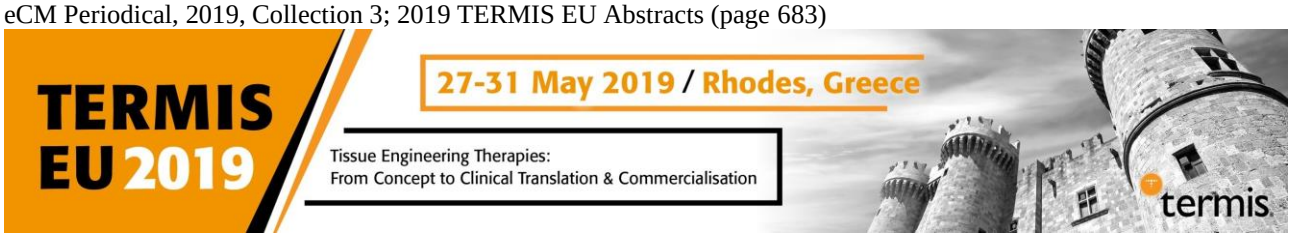


Figure 1: Dose-response cAMP activity of soluble B7-33, PLGA+B7-33 coating, and dried B7-33 control as a function of ligand concentration.

ACKNOWLEDGEMENTS: Financial support was received from a VESKI Victoria Fellowship and the Australian French Association for Science and Technology (AFAS) Associate Award



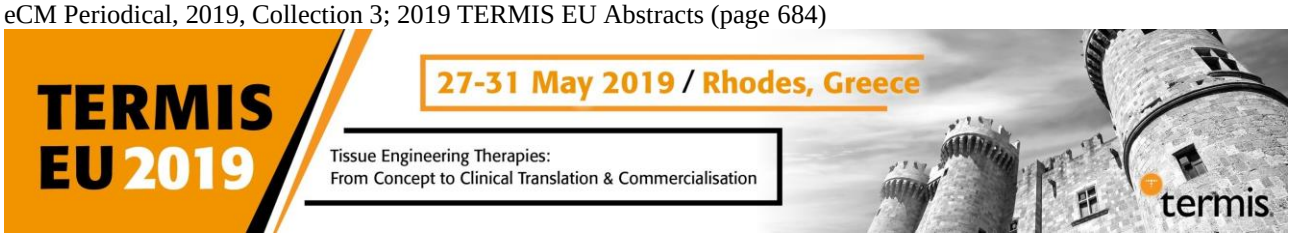
Unpicking the permanent adhesion of barnacle cypris larvae

N. Aldred[1]
Presenting Author: Nick Aldred, nicholas.aldred@ncl.ac.uk
[1]School of Natural and Environmental Sciences, Newcastle University, NE1 7RU, UK.

The permanent adhesion of larvae to surfaces represents a pivotal point in the life-cycle of barnacles, as the free swimming organism commits to a sessile existence. While surface selection and settlement by barnacles is clearly of ecological interest, it is also a focus for more applied research into biological adhesives and antifouling. The permanent adhesive of the cypris larva is one of nature's most impressive but least well understood glues. In the context of marine biofouling, a multi-billion-dollar industry, cyprids and their adhesion are of particular interest as the colonizing stage of barnacles, and arguably more important than the adult form as the target of fouling control strategies. Better knowledge of cyprid adhesion would therefore contribute to the development of coatings that resist barnacle adhesion without the need for biocides. This talk will outline recent progress towards understanding the larval adhesion system including its protein and non-protein components - both lipids and carbohydrates, as well as proteins, are required for successful adhesion. The dynamics of cyprid attachment to surfaces will be discussed, as well as implications for the development of novel fouling-resistant coatings and bio-inspired adhesives.

Figure 1: A barnacle cypris larva, imaged from beneath, exploring a glass surface.

ACKNOWLEDGEMENTS: Support was received from the COST Action CA15216 "European Network of BioAdhesives" ENBA.



3D printed poly-ether-urethane scaffolds for bone tissue engineering

N. Contessi Negrini[1,2], F. Bongiorni[1], L. Trombi[2,3], D. D'Alessandro[3], S. Danti[2,4], S. Farè[1,2]
Presenting Author: Nicola Contessi Negrini, nicola.contessi@polimi.it
[1]Dept. of Chemistry, Materials and Chemical Engineering "G.Natta", Politecnico di Milano, Milan (IT) [2]National Interuniversity Consortium of Materials Science and Technology, Florence, Italy [3]Dept. of Surgical, Medical, Molecular Pathology and Emergency Care, University of Pisa, Pisa, Italy [4]Dept. of Civil and Industrial Engineering, University of Pisa, Pisa, Italy

INTRODUCTION: Regeneration of bone defects by tissue engineering is a challenging field that requires the design of scaffolds with suitable morphological, structural and biocompatible properties. Unlike traditional fabrication technologies, 3D printing enables manufactures with defined pore sizes and geometries, with patient-specific macroscopic shape. We propose 3D printed thermoplastic poly-ether-urethane (TPU) as a scaffold for bone tissue.

METHODS: TPU scaffolds were fabricated by fused deposition modeling (Sharebot 42). Twelve layers were printed in three layer-by-layer patterns at 90°, 45° and 60° relative angle. Morphology, printed filaments dimension and pore size were characterized. Weight variation tests were performed in culture medium at 37 °C. Compressive mechanical properties were tested in wet conditions at 37 °C by Dynamic Mechanical Analyzer. In vitro indirect cytotoxicity tests were performed using L929 cells (ISO 10993). In vitro cytocompatibility and osteogenic differentiation were investigated using bone marrow-derived human mesenchymal stem cells (hMSCs). Metabolic activity of hMSCs was evaluated by AlamarBlue® assay up to 14 days. Histological analysis by Hematoxylin & Eosin (H&E) and von Kossa staining was used to study hMSC morphology and osteogenic differentiation. Data were statistically analyzed by ANOVA.

RESULTS & DISCUSSION: The morphology of the printed scaffolds matched the one of the CAD-designed models, after printing process optimization (Fig. 1). Printed filaments diameter was 600 µm; pore size varied from 0.005 mm^2 (45°) to 0.1 mm^2 (90°), with 60% average porosity. Weight variations values were up to 75%; all scaffolds were stable up to 21 days. The compressive modulus ranged in 2-4 MPa, depending on the printed pattern. In vitro cytotoxicity tests proved the absence of cytotoxic effects. An increase of metabolic activity was detected during the culture and osteo-differentiated hMSCs colonized the pores of the scaffolds with mineral ECM deposition (Fig. 1).

CONCLUSIONS: Scaffold morphological and structural properties are suitable for bone tissue regeneration, proving adhesion, proliferation and differentiation of hMSCs.

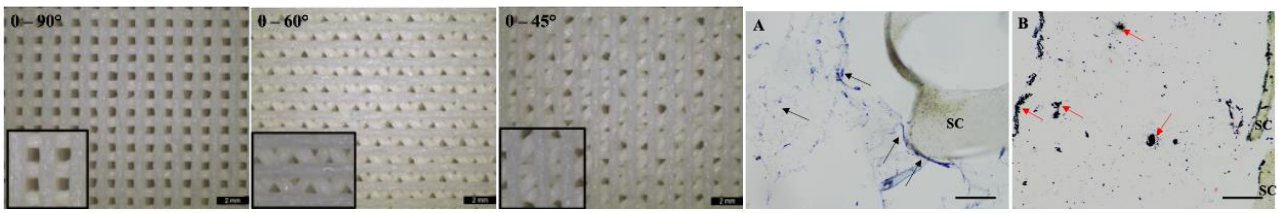
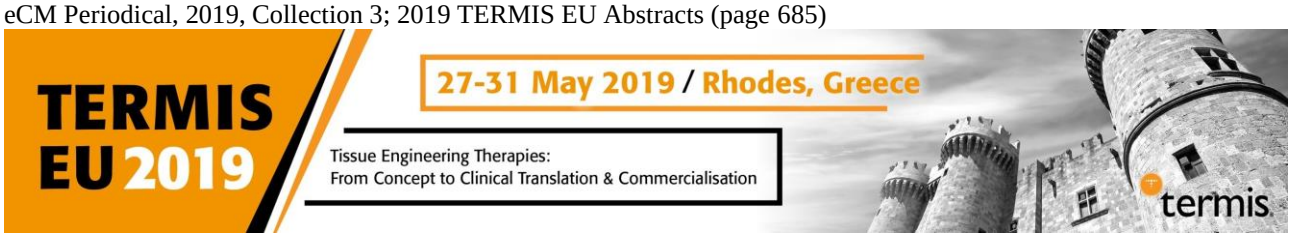


Figure 1: (left) Morphology of 3D printed TPU scaffolds with different patterns (scale bar: 2 mm).
Figure 2: (right) (A) H&E and (B) von Kossa staining of hMSCs on 3D printed TPU (60°). Scale bar: 100 µm (SC = scaffold).

ACKNOWLEDGEMENTS: Authors thank Sharebot (IT) for the 3D printer.



Chondrogenic differentiation of human embryonic stem cells is enhanced with the application of a Wnt platform

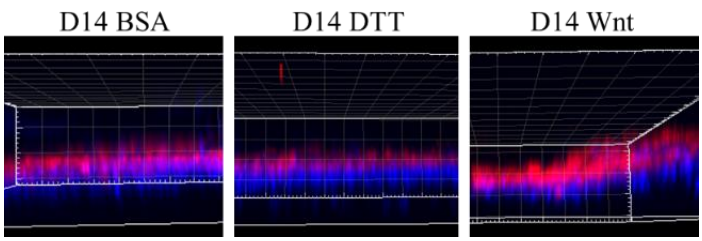
N.C. Foster[1], S.J. Kimber[2], A.J. El Haj[3]
Presenting Author: Nicola Foster, n.c.foster@keele.ac.uk
[1]Institute of Science and Technology in Medicine, Faculty of Health, Keele University, Staffordshire, United Kingdom [2]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom [3]Healthcare Technology Institute, School of Chemical Engineering, University of Birmingham, United Kingdom

INTRODUCTION: Osteoarthritis, resulting from cartilage degradation, poses a great financial burden to healthcare providers. Long-term relief is not possible with current gold-standard treatments; thus there is a need for tissue-engineered alternatives. Immobilised Wnt3a can induce asymmetrical cell division in pluripotent stem cells. Human embryonic stem cells (hESC) can yield large numbers of chondroprogenitors, however, the risk of residual pluripotent cells with the potential to form teratomas, presents a barrier to their clinical application. Use of a Wnt platform as a substrate for differentiation may result in a cartilaginous construct with a more homogenous population of chondroprogenitors.

METHODS: A 14-day directed differentiation protocol (DDP) [3] was initiated on hESC. On d4, cells were split and transferred to Wnt3a-modified polycaprolactone (PCL) discs. Acellular fibrin gels were placed on top of cells on the following day and the DDP was completed. Gene expression analysis and immunocytochemistry (ICC) were carried out on d9 and d14 samples. For control samples, bound Wnt ligands were inactivated with dithiothreitol (DTT) before cell seeding. An additional control group of polymers was incubated with 5% BSA instead of Wnt3a.

RESULTS & DISCUSSION: Constructs on the Wnt-modified PCL show significantly increased ACAN expression on d9 compared to BSA controls and significantly increased COLII expression on d14 compared to DTT controls. A migrating population of differentiating cells is more evident in the Wnt3a-modified group (figure 1).

CONCLUSIONS: Use of the Wnt platform, in combination with the DDP, may yield hydrogels with enhanced chondrogenic potential.



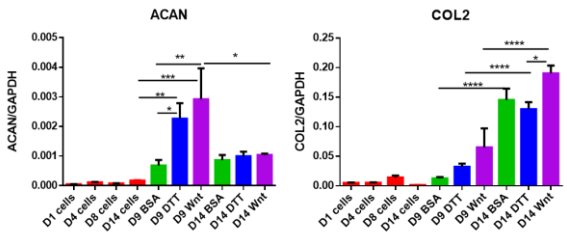
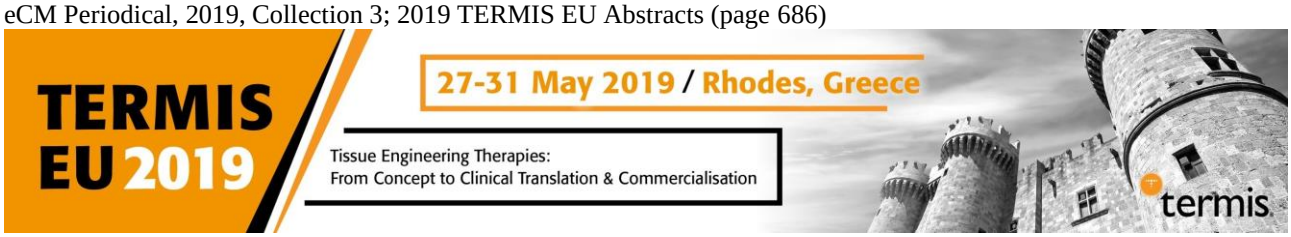


Figure 1: (left) Enhanced migration of SOX5-positive cells in Wnt3a group. SOX5 (red) and DAPI (blue). **Figure 2:** (right) ACAN expression significantly higher in d9 Wnt3a group and COLII expression significantly higher in d14 Wnt3a group (n=4).

ACKNOWLEDGEMENTS: The authors would like to thank the UKRMP Hub for funding



Development of a 3D human intestine mucosal model to aspects of inflammatory bowel diseases

N.J. Darling[1], S.A Przyborski[1,2]
Presenting Author: Nicole Darling, n.j.darling@durham.ac.uk
[1]Department of Biosciences, Durham University, Durham, UK [2]Reprocell Europe, Sedgefield, UK

INTRODUCTION: Intestinal epithelial function is assayed using the in vitro industrial standard Caco-2 monolayer. However, this model has numerous limitations including the lack of communication between different cell types and is not readily adaptable to mimic diseased intestinal tissue. We aim to adapt this intestinal model to better recapitulate the native intestine architecture by culturing Caco-2 cells with multiple types of cells, including immune cells, in a three-dimensional microenvironment and hypothesise that this model can be adapted to mimic aspects of inflammatory bowel disease (IBD).

METHODS: Human dermal fibroblasts (HDFn) were seeded onto Alvetex® Scaffold inserts and cultured for 7 or 14 days. After 7 days, differentiated U937 macrophages can be added to the HDFn and cultured for a further 7 days. After a total of a 14 day of HDFn pre-culture period, Caco-2 cells were seeded and cultured for a further 21 days to allow differentiation to occur.
Functional properties of the model were assayed with Transepithelial Electrical Resistance (TEER) measurements and tissue construct morphology was observed by histology, electron microscopy and immunofluorescence.

RESULTS & DISCUSSION: Fibroblasts deposit a rich collagenous extracellular matrix, resembling the lamina propria, within which immune cells integrate into. Co-culture of the stromal compartment with epithelial cells results in the formation of an intact barrier due to the presence of a highly polarised monolayer of differentiated cells. This is evidenced by the columnar appearance of cells in conjunction with the formation of a microvilli brush border.

CONCLUSIONS: The 3D mucosal construct more closely resembles the architecture of the human intestine than previous in vitro models. Data thus far has indicated that the model has improved functional characteristics over the Caco-2 monolayer and is more like in vivo tissue. Future experiments will endeavor to stimulate the immune cells within the model to recapitulate features of IBD.

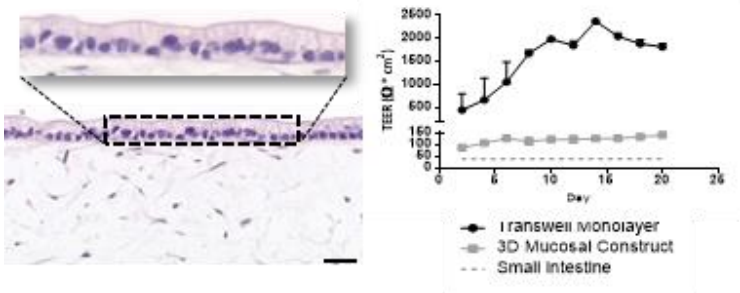
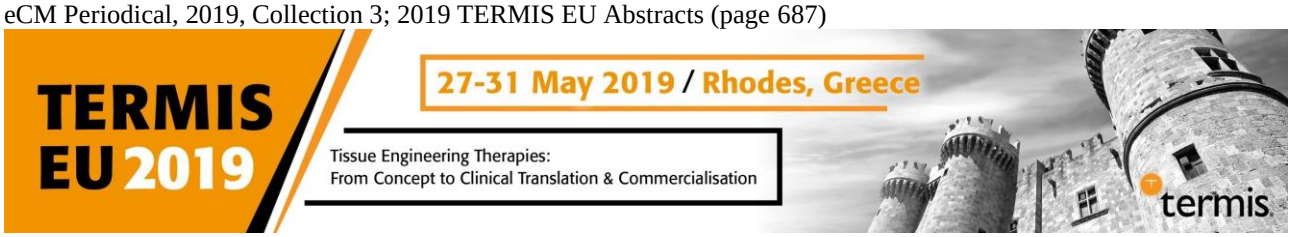


Figure 1: H&E staining of Caco-2 cells cultured for 21 days on top of fibroblasts cultured for 14 days prior to Caco-2 cell seeding. Scale bar: 50 µm. TEER throughout the 21 day epithelial cell culture of the Transwell insert, co-culture model and the small intestine.

ACKNOWLEDGEMENTS: The authors would like to thank the NC3Rs for funding this project and Reprocell Europe for providing human tissue samples.



Scaffolding and bioprinting via advanced laser technologies

N.V. Minaev[1]
Presenting Author: Nikita Minaev, minaevn@gmail.com
[1]Laboratory of Laser Nanoengineering, Institute Photon Technologies (IPT), Federal Scientific Research Centre "Crystallography and Photonics" RAS, Russia

INTRODUCTION: In recent years, laser technologies have found wide applications in various fields of medicine and biomedical researches such as plastic surgery, oncology, ophthalmology and others. With the use of laser radiation, it became possible to form scaffolds and tissue-engineering structures with desired properties, and to manipulate of living micro objects, such as microorganisms and cells. This report presents our results in developing of several laser technologies for biomedicine and tissue engineering.

METHODS: Several laser technologies and methods are used to solve some biomedical tasks: - Technologies of single- and two-photon polymerization and surface-selective laser sintering were used for formation of scaffolds from the original biocompatible and bioresorbable materials. - Laser photopolymerization was used for creating an anisotropic structured material based on collagen sponge and photoreactive polylactide. - The LIFT (laser induced forward transfer) printing method has been used to functionalize scaffolds by living cells and cell spheroids. - The method of the laser engineering microbial systems (LEMS) based on the LIFT method was developed.

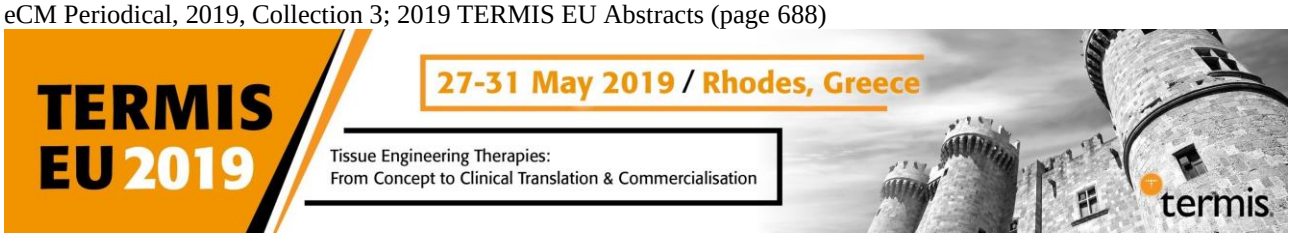
RESULTS & DISCUSSION: Original photosensitive compositions containing natural polysaccharides (chitosan) and polylactides have been developed. Chitosan scaffolds, fabricated by two-photon polymerization appeared non-cytotoxic and suitable for treating the spinal cord injuries and other neuronal degenerative diseases [1]. Three-dimensional scaffolds with improved hydrophilic and osteoinducing properties were produced using the surface-selective laser sintering (SSLS) method from modified polylactide [2]. LIFT method of the laser printing, was used for moving cartilage cells and cellular spheroids to the surface of the scaffolds. This made possible to build tissue-engineering construction for regeneration of complex skeletal tissues. The LEMS technology has been developed for cultivating of difficultly cultivated or uncultivated microorganisms from soil sources. The ability to obtain a wider biological diversity of microorganisms from the trivial soil, compared with standard methods of cultivation was demonstrated [3].

CONCLUSIONS: The combination of the proposed laser methods and technologies is promising for solving a number of important problems in the field of medicine, tissue engineering and microbiology.

ACKNOWLEDGEMENTS: Financial support was received from by the Ministry of Science and Higher Education within the State assignment FSRC «Crystallography and Photonics» RAS in part of «laser engineering of microbial systems», Russian Science Foundation (Project No. 18-32-20184) in part of «development of laser technologies scaffold formation».

REFERENCES

[1] T. S. Demina et al., Polymers (Basel), 9(12), (2017), 302.
[2] E. A. Grebenik et al., Biomedical Materials, 13(5), (2018) 054103.
[3] M. V. Gorlenko et al. Journal of Biological Engineering (2018) 12:27



A close look at peri-implant wound healing inside the living body in real-time

N. Khosravi[1,2,3], A. Maeda. Korntner[3], R. DaCosta[3], J.E. Davies[1,2]
Presenting Author: Niloufar Khosravi, niloufar.khosravi@mail.utoronto.ca
[1]Faculty of Dentistry, University of Toronto, Toronto, ON, Canada [2]Institute for Biomaterials and Biomedical Engineering, University of Toronto, ON, Canada [3]Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada [4]University of British Columbia, Vancouver, BC, Canada

INTRODUCTION: Recent decades have been characterized by advancement of medical implant design to improve the clinical success rate. One of the challenges in the implant field is to develop surfaces capable of controlling and guiding tissue healing. Neovascularization is considered an essential prerequisite to osteogenesis as the mesenchymal progenitors of osteogenic cells have a perivascular origin. Thus, our aim was to examine the effect of nanosurfaces on peri-implant neovascularization and mesenchymal cell recruitment in vivo.

METHODS: We have developed a cranial implant window model to track peri-implant wound healing intravitally over clinically relevant time scales as a function of implant topography [1]. Combined with in vivo cell labeling strategies, it allows direct visualization of neovascularization as well as migration and function of peri-vascular stromal cells in the peri-implant wound site.

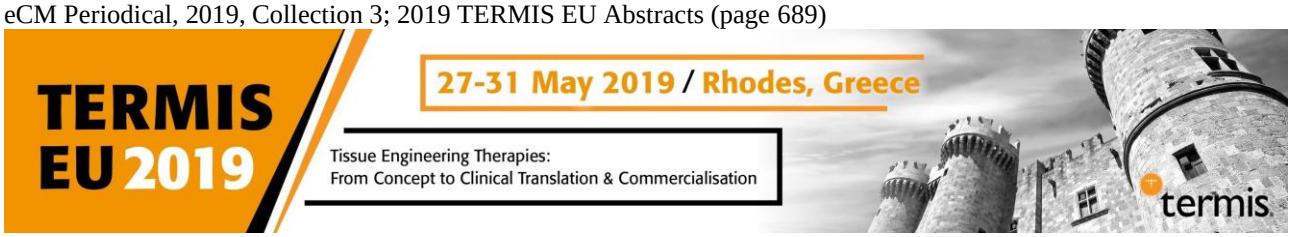
RESULTS & DISCUSSION: Quantitative intravital confocal imaging showed that the microvascular density around a nano implant surface (TiNT) was significantly higher than a machined surface (TiMA) at days 7, 11, and 28 post-implantation. Nano surface contributes to the development of a radially arranged vascular structure with hierarchical branches spatially closer to the surface of the Ti-implant. Use of nanosurfaces accelerated the recruitment of perivascular mesenchymal cells (PMCs) that were localized in the periosteum. Microscopic and histological evidence showed that the PMCs were the progenitors of multiple cell types including osteogenic cells. The early population of the peri-implant wound site by PMCs was correlated with the growth of the neovasculature. Further µCT assessment of the bone formation showed that the changes in the rate and pattern neovascularization and mesenchymal cell recruitment resulted in a change in the mechanism of peri-implant bone healing from distance to contact osteogenesis.

CONCLUSIONS: The knowledge gained from the current study informs the design of endosseous implants capable of controlling tissue healing and overcoming delayed peri-implant wound healing as seen in conditions such as hyperglycemia.

ACKNOWLEDGEMENTS: Zimmer-Biomet Dental Inc. for fabrication of the implants. The Animal Resources Center at University Health Network; the Facility of Advanced Optical Microscopy (AOMF).

REFERENCES

[1] Khosravi N et al. Tissue Eng. Part C Methods. 2018; 24(11):617-27.



Hyaluronan scaffold improves gut barrier function and epithelial integrity in ulcerative colitis

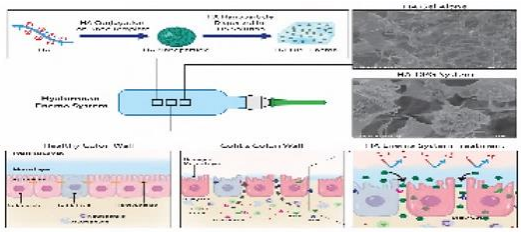
N.G. Kotla [1], R. Singh [2], V.R. Jala [2], Y. Rochev [1], A. Pandit [1]
Presenting Author: Niranjan G. Kotla, n.kotla1@nuigalway.ie
[1]Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway, Ireland
[2]Department of Microbiology and Immunology, James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA

INTRODUCTION: The active stages of intestinal inflammation (in Crohn's and ulcerative colitis diseases) is characterized by intermittent wounding and inflammation in the effected intestinal regions [1,2]. The progress in mucosal/epithelial damage and inflammation causes a decrease in transepithelial resistance and increases the gut permeability [3]. Herein, we report a hyaluronan based hydrogel enema system that protects the damaged gut epithelium by enhancing the tight junction proteins, mucosal repair and helps in maintaining the epithelial gut barrier integrity [Figure 1].

METHODS: A HA-enema scaffolding system was fabricated by embedding high molecular weight ($1.1x\ 10^6$) HA particles in a HA hydrogel system. Anti-inflammatory studies of the system were conducted in mouse bone marrow derived macrophages (BMDMs). Human colon epithelial like cells (Caco-2, T-84) were used to test the trans-epithelial electrical resistance (~TEER), permeability effect by transwell assay and tight junction proteins up regulation by western blotting. Further, the system efficacy and barrier integrity were tested in dextran sodium sulphate (DSS) induced colitis mice model, in vivo.

RESULTS & DISCUSSION: HA particles in the hydrogel-enema were found to be 320±100 nm in size (Figure 2A). Cell culture (BMDMs) experiments showed a significant decrease in induced cytokines TNF- α, IL-6 secretions (Figure 2B). Treatment upon Caco-2 cell transwells, HA-hydrogel enema and Naïve HA increased the percentage ~TEER, and decreased the FITC-dextran flux (permeability) compared to LPS treatment (Figure 2C). Up regulation of the gut epithelial tight junction proteins (Cldn-4, Ocldn) were observed after HA-gel enema (100 µg/ml) treatment on Caco-2 cells (Figure 2D).

CONCLUSIONS: These data illustrate the beneficial effect of HA-hydrogel enema in mucosal protection of the damaged gut epithelial and improvements to the intestinal barrier function.



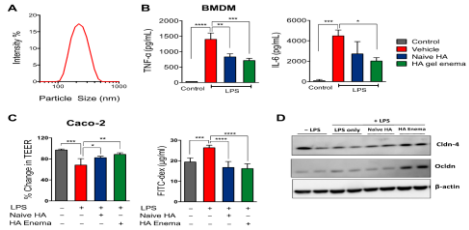
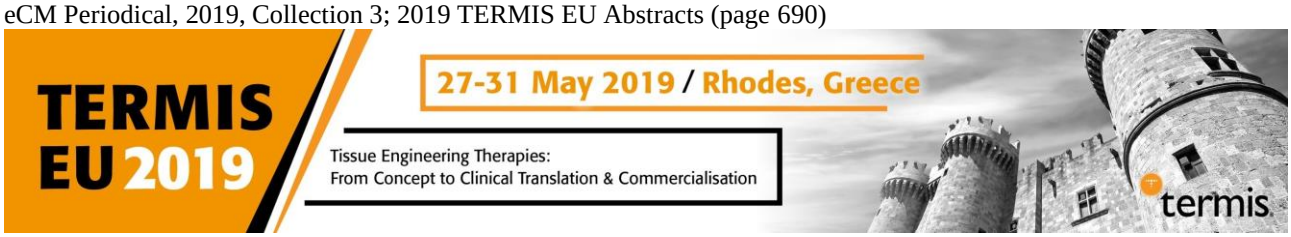


Figure 1: (left) Fabrication of HA-hydrogel enema, respective SEM images and pictorial illustration of hydrogel enema protection, repair on the damaged gut epithelial mucosa in colitis. **Figure 2:** (right) DLS size intensity graph of HA particles (A), Decreased TNF- α, IL-6 activity in BMDMs (B), Effect of percentage ~TEER and permeability on Caco-2 cells (C). Enhancement of tight junction proteins in Caco-2 cells after enema treatment (D).

ACKNOWLEDGEMENT: Financial support was received Science Foundation Ireland (SFI) and is co-funded under the European Regional Development Fund under Grant Number13/RC/2073.



Development of the mini-liver, mini-testis, and diabetic islet by ECM-loading method for drug screening

T. Akama[1], F. Tao[1], Y. Asajima[1], T. Ono[1], K. Toya[1], N. Kojima[1]
Presenting Author: Nobuhiko Kojima, nobuhiko@yokohama-cu.ac.jp
[1]Graduate School of Nanobioscience, Yokohama City University, Yokohama, Japan

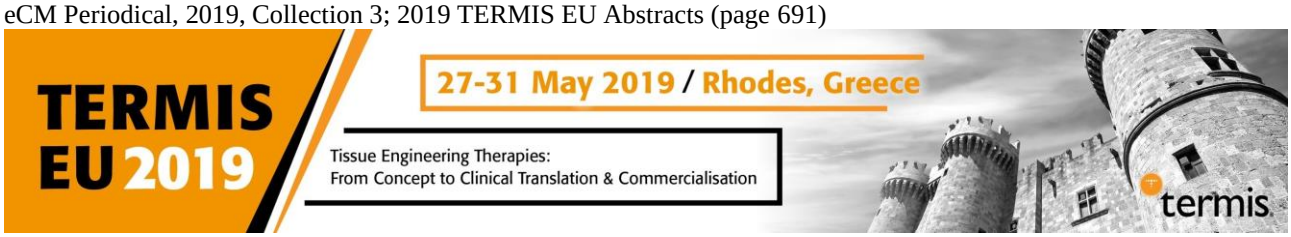
INTRODUCTION: Multicellular spheroids are thought to be useful for drug screening. However, it was hard to fill extracellular matrices (ECMs) into the spheroids, which improve structures and/or functions of them. For example, Matrigel has a potential to support cell polarity and tubulogenesis. Hyaluronic acid (HA) is detectable around of islets in a healthy condition but internal of them in a diabetic one. We developed a method to fill thin-layered ECMs into the spheroids. In this report, we show results that ECM filling enhanced cell-polarity of the mini-liver, formation of seminiferous tubules of the mini-testis, and producing the diabetic islet having reducing glucose-dependent insulin secretion.

METHODS: We used human hepatoma cell lines Hep G2 and Huh-7 for the mini-liver, mouse (ICR) testis cells for the mini-testis, and mouse (C57BL/6NcrSlc) islet cells for the diabetic islets. When we use hepatoma cell lines, they were suspended in culture medium at 2 x 10^6 cells/ml. To prepare mouse testis cells, the testes isolated from day 1.5 neonatal mice were digested by collagenase reagents. The testis cells were suspended in culture medium at 1 x 10^8 cells/ml. In the case of the islet cells, they were prepared at 8 x 10^6 cells/ml. Spheroids were formed by the methylcellulose method. Methylcellulose was dissolved at 3% into the each medium suitable for the cell type. We injected 1 µl of the cell suspension into the methylcellulose medium. Aggregation of cells was occurred in 10 to 30 min, thereafter, they formed spheroid depending on their adhesion and self-organization properties. The spheroids were cultured for 1 day (the mini-liver and islet), or 2 days (the mini-testis) in the methylcellulose medium, and isolated for additional culture in the normal medium without methylcellulose. Thin-layered ECMs (Matrigel for the mini-liver and mini-testis, HA for the diabetic islet) was filled into the spheroids by dissolving ECM to the cell suspensions. Cells and dissolving ECM were gathered and condensed after injection into the methylcellulose medium. Additional culture was done 1 day for the mini-liver and islet, or 12 days (5 days in normal medium and 7 days in medium with Di(2-ethylhexyl)phthalate (DEHP), a chemical agent showing reproductive toxicity) for the mini-testis. Structures, or protein expression and localization were confirmed by observation of paraffin sections with hematoxylin-eosin staining and immunohistochemistry. Glucose dependent insulin secretion was surveyed by ELISA.

RESULTS & DISCUSSION: The mini-liver with thin-layered Matrigel had improved localization of ZO-1and claudin-1 compared to the normal spheroids without ECM. In addition, localization of MRP2 and formation of bile canaliculi are enhanced. The mini-testis with thin-layered Matrigel showed relatively matured seminiferous tubules and those tubules had sensitivity to the DEHP.

CONCLUSIONS: Thin-layered ECM filling into the spheroids is effective to form high-value-added the mini-organs for drug screening.

ACKNOWLEDGEMENTS: Financial support was received from Japan Agency for Medical Research and Development (AMED), Terumo Foundation for Life Sciences and Arts, and Life Innovation Platform Yokohama.



Quality control test for human epithelial cell sheet

N. Kanai[1, 2], K. Ohse[3]
Presenting Author: Nobuo Kanai, nobukana@tmig.or.jp
[1]Healthy Aging Innovation Center, Tokyo Metropolitan Geriatric Medical Center [2]Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University [3]Research Team for Geriatric Medicine, Tokyo Metropolitan Geriatric Medical Center, Japan

INTRODUCTION: There is a common problem in the developing the quality control test of cell therapy products due to the complexity of the mode of action. In this study, we have developed the quality evaluation method concerning about the mode of action of epithelial cell sheet product. The results of traditional evaluation methods (cell number count or cell purity measurement) do not always correlate with the product efficacy. Therefore, we have developed the simple quality control test using qPCR array card to measure the expression levels of critical quality genes. We firstly analyzed the comprehensive gene expression profiles of epithelial cell sheet products which were fabricated in GMP-grade using next generation sequencer (NGS). As a result of NGS analysis, the genes of secretory factors which associate with the mode of action of epithelial cell sheet (enhancement of cell migration, prevention of the inflammatory immune cells infiltration and inhibition of fibrosis) were highly expressed in the epithelial cell sheets in comparison with oral submucosa. We also assessed the in vitro activity of conditioned medium of epithelial cell sheet products for the exerting the therapeutic effect. Next, we validated whether these secretory factors correlated with the mode of action of epithelial cell sheet by in vitro assay and selected the candidate genes on the basis of the result of this validation and the literature research. Finally, we analyzed the gene expression levels of the selected genes in the epithelial cell sheet products by RT-qPCR and determine the expression threshold for the evaluation of epithelial cell sheet quality. In this research, several candidate genes were identified for the evaluation of the quality of epithelial cell sheet product. It is necessary to evaluate the eligibility of these genes for the accurate quality control test by searching the association between the result of the therapeutic effect and the gene expression levels of candidate genes. Our newly developed method enables us to evaluate the product quality more rapidly and accurately than the traditional method. In consequence, our method will contribute the industrialization of regenerative medicine product.

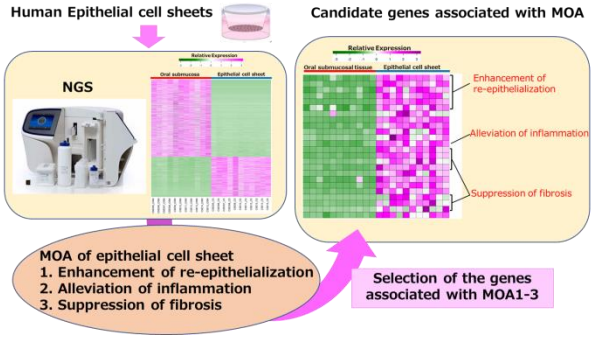
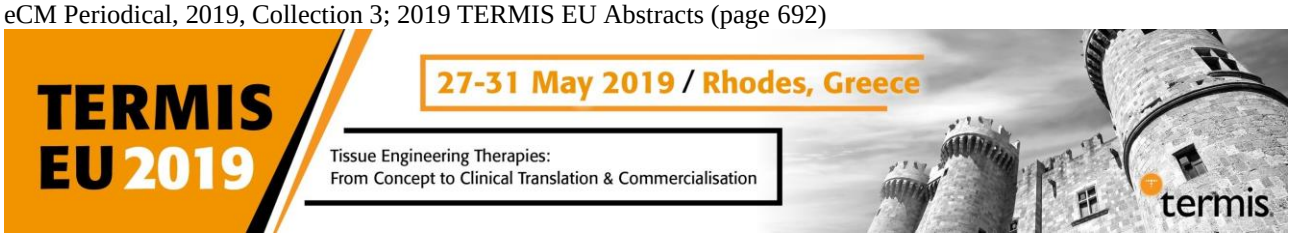


Figure 1: Scheme of the candidate genes selection for quality control test**.** At first, we analyzed the global gene expression profiles of epithelial cell sheet products. Next, secretory factors associated with the mode of action (MOA) of epithelial cell sheet product were selected.

ACKNOWLEDGEMENTS: Financial support was received from Japan Agency for Medical Research and Development in the “Project focused on Developing Key Evaluation Technology: Manufacturing Technology for Industrialization in the Field of Regenerative Medicine.”



Investigation into the effect of BMP-12 and TGF-β$_1$ on stimulation of tenogenic markers in serum-free cultured human adipose-derived stem cells (ADSCs)

N. D. Falcon[1], A. Saeed[2]
Presenting Author: Noelia D Falcon, n.dominguez-falcon@uea.ac.uk
[1,2]School of Pharmacy, Norwich Research Park, University of East Anglia, Norwich, Norfolk, UK

INTRODUCTION: ADSCs are at the forefront of stem cell therapy due to less invasive isolation procedures and its higher yield in cell number after purification. Growth Factors (GFs) such as Transforming Growth Factor β (TGF-β) and Bone Morphogenetic Proteins (BMPs) have been investigated as effective tools for driving tenogenic differentiation of stem cells. For example, recent studies highlighted the promising effect of BMP-12 in the tenogenic differentiation of ADSCs [1]. Other studies have compared the effect of different GFs in the upregulation of key markers involved in tendon commitment both in ADSCs [2] or other stem cell lines [3]. Herein, we focused on the characterisation and the differentiation potential of human ADSCs in the presence of several GFs in the absence of serum.

METHODS: ADSCs were serum starved and treated with different customised media as the differentiation factor. After specific time points, several techniques were performed: 1) Analysis of messenger RNA (mRNA) for common tendon markers was performed by Real-Time Polymerase Chain Reaction (RT-PCR). Likewise, markers for other cell lineages were analysed. 2) Protein expression was confirmed by Western blotting. 3) Cellular localisation of the markers was evaluated by Immunocytochemistry (ICC) techniques.

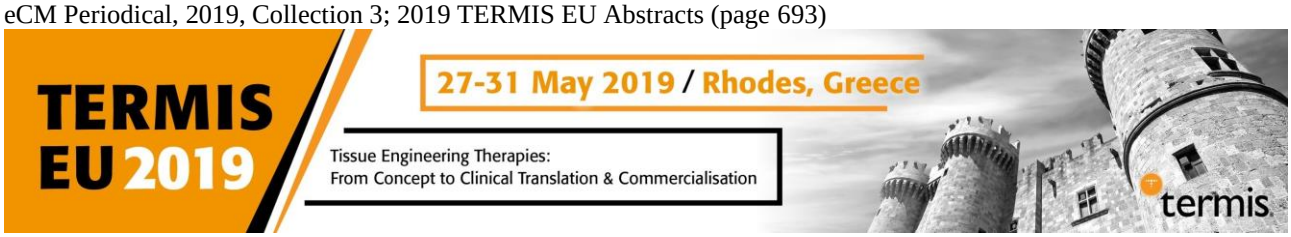
RESULTS & DISCUSSION: In this study, we evaluated the tenogenic effect of different GFs on ADSCs. Scleraxis, a transcription factor involved in tendon development, was upregulated at the mRNA level, but the expression was significantly different depending on the GFs used. Collagen type 1, the most abundant type in tendon, was greatly induced. Interestingly, deposition of Collagen I on the extracellular matrix (ECM) by the ADSCs was altered depending on the media used, which was confirmed by ICC. Additionally, cell morphology and confluency changed depending on the GF used.

CONCLUSIONS: Our data suggest that specific environments may be needed for the tenogenic development and potential application for the treatment of the injuries without overstimulation. Overall, we believe ADSCs represent a promising approach for tendon regeneration and repair.

ACKNOWLEDGEMENTS: Financial support was received from Rosetrees Trust Fund, Arthritis Action UK and University of East Anglia, Faculty of Science.

REFERENCES

[1] W Zarychta-Wiśniewska et al. BMC Cell Biology 2017. 10.1186/s12860-017-0129-9
[2] H Shen et al. PLoS One 2013. 10.1371/journal.pone.0077613
[3] Yin Z et al. Stem Cells Transl Med 2016. 10.5966/sctm.2015-0215



Strategies to improve mesenchymal stromal cell therapeutic effect: Application to pelvic radiotherapy side effects

L. Moussa[1], C. Demarquay[1], A. Sémont[1], G. Réthoré[2], J. Guicheux[2], D. Barritault[3], P. Weiss[2], N. Mathieu[1]
Presenting Author: Noëlle Mathieu, noelle.mathieu@irsn.fr
[1]Laboratoire de Radiobiologie Médicale (LRMED), IRSN, SERAMED, Fontenay-aux-Roses, France. [2]Regenerative Medecine and Squeleton Laboratory (RMeS), INSERM UMRS 791, Université de Nantes, Centre Hospitalier de Nantes, Nantes, France [3]Société OTR3, France

INTRODUCTION: Healthy tissues surrounding abdomino-pelvic tumours may be impaired during radiotherapy and could lead to chronic gastrointestinal complications with substantial mortality. Injection of Adipose- derived Mesenchymal Stromal Cells (Ad-MSC) represents a promising therapeutic strategy. However, many stem cell clinical trials do not confer expected beneficial effect, suggesting a real need to accelerate research towards the successful clinical application. We hypothesized that heparan sulfate (HS)-mimetic injections that restore the extracellular matrix network and enhance the biological activity of growth factors, associated with local injection of MSC protected in a hydrogel that improves cell engraftment and cell survival, could improve the therapeutic benefit of MSC treatment.

METHODS: We used an experimental model of radiation proctitis developed in rats that reproduces severe colonic mucosal damages and fibrosis similar to those observed in patients treated by radiotherapy [1]. We tested injections of HS-m, local injection through endoscopy of Ad-MSC embedded in Si-HPMC hydrogel as well as combinations of these various treatments. The therapeutic benefit was evaluated by endoscopy, histology and functional parameters as epithelial barrier were also tested.

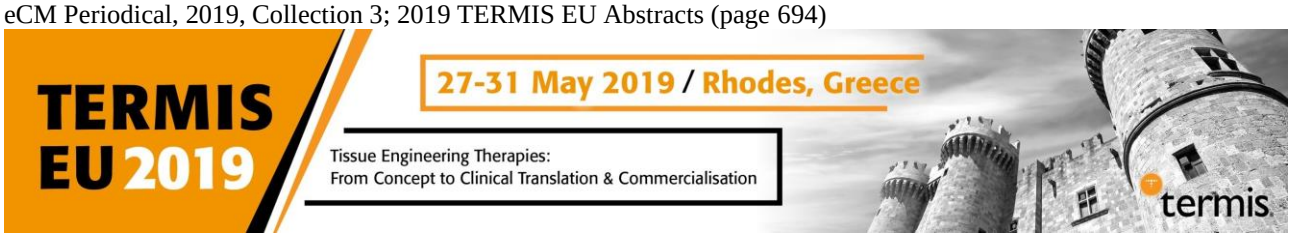
RESULTS & DISCUSSION: We demonstrated that hydrogel loaded-Ad-MSCs were viable, able to secrete trophic factors and responsive to the inflammatory environment. In animal model, Ad-MSC+Si-HPMC improve colonic epithelial structure and hyperpermeability compared with no embedded cells. This therapeutic benefit is associated with greater engraftment of Si-HPMC-embedded Ad-MSCs in the irradiated colonic mucosa [2]. We also demonstrated that combination of HS-m to hydrogel-embedded MSC treatment enhances the therapeutic benefit of MSC therapy alone. Indeed, for the first time, a decrease of the injury score in the ulcerated area was observed. We also demonstrated that the combined treatment favored the epithelial regenerative process. Finally, using an animal model of colonic surgery after irradiation, we demonstrated that the combined treatment improved rat survival, healing of the anastomosis and scar quality assessed by collagen deposit [3].

CONCLUSIONS: In this study, we identified a new way, clinically applicable, to optimize stem-cell therapy and could be proposed to patients suffering from severe colonic defect after radiotherapy.

ACKNOWLEDGEMENTS: Financial support was received from French “Agence Nationale de la Recherche” (ANR-13-RPIB-0008).

REFERENCES

[1] Semont A et al. PlosOne. 2013; PLoS One 8(7)
[2] Moussa L et al. Biomaterials. 2017; 115:40-52
[3] Moussa L et al. Sci Rep. 2019 Jan 17;9(1):164



Effective vascularization and efficient bone formation in osteogenic grafts requires VEGF dose control

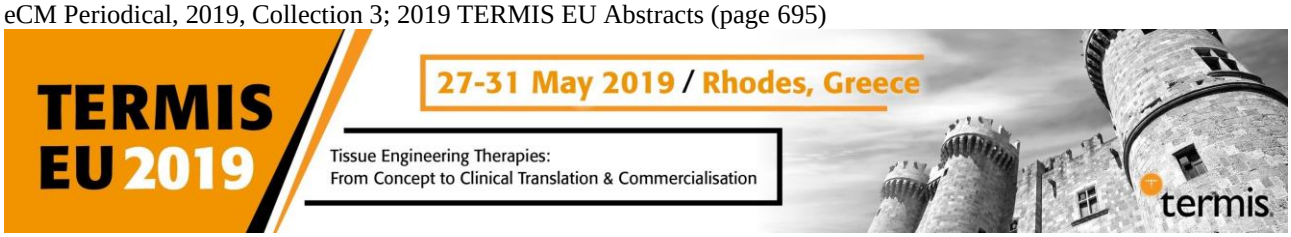
A. Grosso[1], A. Lunger[1,2], M.G. Burger[1,2], F. Mai[1], J.A. Hubbell[3], P.S. Briquez[3], V. Sacchi[1], D.J. Schaefer[1,2], A. Banfi[1], N. Di Maggio[1]
Presenting Author: Nunzia Di Maggio, nunzia.dimaggio@usb.ch
[1]Cell and Gene Therapy, Department of Biomedicine, Basel University Hospital, Basel, Switzerland [2]Department of Plastic, Reconstructive, Aesthetic and Hand Surgery, Basel University Hospital, Switzerland [3]Institute of Molecular Engineering, University of Chicago

INTRODUCTION: Spontaneous vascularization of large-size bone grafts based on bone marrow-derived mesenchymal stem cells (BMSC) is insufficient and requires therapeutic stimulation to ensure progenitor survival and bone formation. However, sustained VEGF over-expression from genetically modified BMSC leads to excessive osteoclast activation and bone resorption. Recently, we found that short-term delivery of recombinant VEGF protein cross-linked in fibrin hydrogels could prevent osteoclast recruitment while ensuring increased vascularization. Here we investigated the role of VEGF dose on the coupling of angiogenesis and osteogenesis, in order to define a VEGF therapeutic window for vascularized bone grafts.

METHODS: Recombinant VEGF was engineered with a transglutaminase substrate octapeptide (TG-VEGF) to allow cross-linking into fibrin hydrogels. Osteogenic constructs were prepared with human BMSC and hydroxyapatite granules in a fibrin hydrogel containing different TG-VEGF concentrations (none, 0.1, 1, 10 and 100 µg/ml).

RESULTS & DISCUSSION: All TG-VEGF doses increased vessels density up to 5-fold already after 1 week and vascularization persisted at all later time points. However, vascular in-growth reached up to 30% of the construct area in grafts with 0.1 µg/ml of TG-VEGF, whereas higher VEGF doses significantly reduced invasion by blood vessels (Fig. 1). A deeper vascular invasion in the 0.1 µg/ml condition improved human cell survival and proliferation up to the core of the graft. After 4 and 8 weeks, bone tissue development was enabled by 0.1 µg/ml of TG-VEGF as efficiently as with naïve BMSC alone. Grafts with 0.1 µg/ml of TG-VEGF also contained significantly more mature bone tissue than all other conditions. In contrast, higher VEGF doses progressively impaired bone formation, in correlation with increased osteoclast recruitment and decreased progenitor differentiation, marked by human bone sialoprotein (BSP).

CONCLUSIONS: These data suggest that VEGF effects on promoting vascularization and bone resorption are dose-dependent and that a therapeutic window exists that enables both rapid vascularization and efficient bone formation. Factor decoration of fibrin matrices provide a clinically applicable strategy, comprising no genetic modification, homogeneous and tunable factor doses and limited and controllable duration of factor delivery.



SmartCaP™: A next generation pro-angiogenic fibrin-based bone void filler

N. Kohli[1], V. Sharma[1], A. Orera[2], N. Owji[3], J. Knowles[3,4], R. Bailey[5], G. Blunn[6], E. García-Gareta[1]
Presenting Author: Nupur Kohli, kohliN@raft.ac.uk
[1] Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood UK [2]Instituto de Ciencia de Materiales de Aragón (ICMA), Universidad de Zaragoza, Zaragoza, Spain [3]Division of Biomaterials and Tissue Engineering, UCL Eastman Dental Institute, London, UK [4]Department of Nanobiomedical Science, Dankook University, Cheonan, Republic of Korea [5]Nanovision centre, School of Engineering and Materials Science, Queen Mary University of London, Mile End, LondoN, UK [6]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK

INTRODUCTION: Autografts are the standard choice of treatment for bone defects. However, their use is limited by disadvantages such as limited availability, donor-site morbidity and the risk of infection[1]. There still exists an unmet clinical need for novel bone graft substitutes that can eliminate the use of autografts. One of the main issues with current bone graft substitutes is their lack of or limited angiogenesis, which is fundamental to bone regeneration. We have developed a novel off-the-shelf, osteogenic and pro-angiogenic scaffold branded SmartCaP™ to act as a bone void filler.

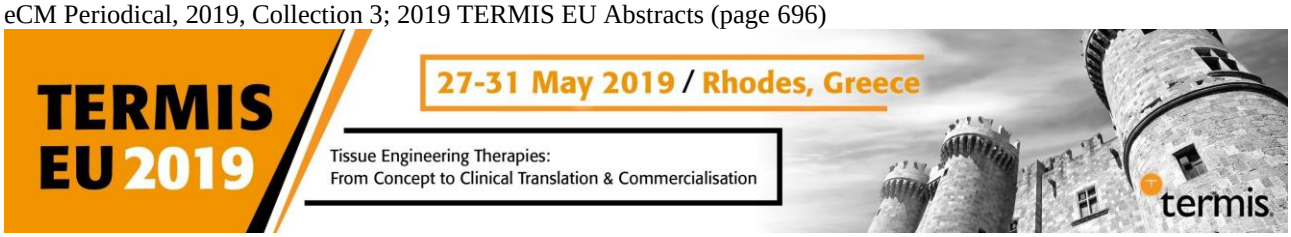
METHODS: SmartCaP™ comprises mainly fibrin in the form of a fibrous sheet, coated with intermediary precursor bone mineral phases of calcium and phosphate. SmartCaP™ was characterised using SEM, EDX, FTIR, Micro-CT, Mercury intrusion porosimetry and von kossa histological staining. Angiogenesis was tested using ex ovo CAM assays. MC3T3-E1 mouse pre-osteoblasts and human MSCs were seeded onto SmartCaP™ under osteogenic and non-osteogenic conditions over 28 days. Cell viability, proliferation and osteogenic differentiation was assessed using standard assays.

RESULTS & DISCUSSION: SEM analysis revealed that SmartCaP™ is a homogeneously porous structure comprising of a wide pore size range with an average porosity of 89%. EDX, FTIR, Micro-CT and von kossa staining confirmed the deposition of calcium and phosphate precursor phases within the biomaterial. CAM assays showed that SmartCaP™ is pro-angiogenic and supported the infiltration and growth of blood vessels. SmartCaP™ supports the growth and proliferation of MC3T3-E1 and human MSCs as well as their osteogenic differentiation, which is more marked under osteogenic conditions.

CONCLUSIONS: The data suggests that SmartCaP™ is osteogenic, osteoconductive and biodegradable. Therefore, it can act as a template to regenerate bone naturally over time. Due to the physical nature of SmartCaP™, it can be easily manipulated by the surgeons in the clinic. The future work is to test SmartCaP™ in an ovine model of cancellous bone defects. he use of such a biomaterial would eliminate the need for a second surgery to harvest autografts, significantly reducing costs and surgery times.

REFERENCES

[1] Campana V et al. J Mater Sci Mater Med. 2014;25(10):2445-2461.



Mesenchymal stem cell-derived extracellular vesicles attenuate disease severity in inflammatory arthritis

A.G. Kay[1], R. Morgan[2], M. Hyland[2], K. Treadwell[3], M. Platt[4], P. Roach[4], N. Forsyth[5], O. Kehoe[2]
Presenting Author: Oksana Kehoe, o.kehoe@keele.ac.uk
[1]Biology Department, University of York, York, UK [2]Keele University, ISTM at RJAH Orthopaedic Hospital, Oswestry, UK [3]Keele University, School of Medicine, UK [4]Loughborough University, Chemistry Department, Loughborough, UK, [5]Keele University, Guy Hilton Research Centre, UK

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune joint disease. There is no cure for RA. Novel biological therapies have revolutionised the management of RA. However, up to 30% of RA patients fail to respond to currently available biological therapeutics and 50% of those prescribed the treatment discontinue use after 2 years [1]. Mesenchymal stem cells (MSCs) possess anti-inflammatory and immunosuppressive properties that may be exploited therapeutically [2]. Extracellular vesicles (EVs) are a heterogeneous group of small membrane vesicles with key roles in cell-to-cell communications, cell signalling and immune response modulation [3]. In this project, we tested the therapeutic potential of EVs in the antigen-induced arthritis (AIA) model of inflammatory arthritis.

METHODS: EVs were isolated from conditioned medium of human bone marrow MSCs by differential ultracentrifugation. Identification of EVs was achieved using a flow cytometric analysis (MACSPlex human exosome detection kit, Miltenyi) and transmission electron microscopy. Tunable Resistive Pulse Sensing Technology from IZON Science was used to measure the size of isolated EVs. AIA was induced in pre-immunised animals by intra-articular injection of methylated BSA and EVs were injected intra-articularly 1 day post arthritis induction. Control animals were injected with EV-depleted medium. EVs effect on T cell proliferation was assessed in co-cultures.

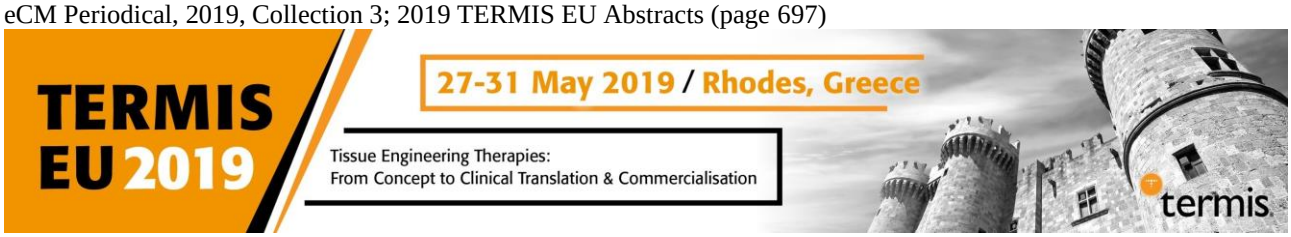
RESULTS & DISCUSSION: The positive confirmation of tetraspanin proteins CD9, CD63, CD81 was achieved by flow cytometry. The size of isolated vesicles ranged from 159 to 290 nm and the mean size and standard deviation was 198.0 ± 44.7nm. Morphological evaluation of isolated EVs revealed a consistent sphere shape of vesicles. Analysis of knee diameter as a measure of swelling showed significant reductions both 2 and 3 days post-induction of arthritis compared to control injected animals. EV-treated mice revealed a significant reduction in cartilage depletion and arthritis index, representing overall disease severity, compared to control-treated mice at day 3. EVs decrease IL-17a expression in CD4+ cells.

CONCLUSIONS: The results demonstrate a clear beneficial effect via injection of EVs into the inflamed joint. We propose EVs as a potential therapeutic approach for the treatment of inflammatory arthritis. Further studies are required to identify the molecular properties of EVs responsible for their therapeutic effects.

ACKNOWLEDGEMENTS: Research was funded by the RJAH Orthopaedic Hospital Charity and the Institute of Orthopaedics, Oswestry.

REFERENCES

[1] McInnes IB & Schett G. N. Engl. J. Med. 2011; 365, 2205–2219
[2] Prockop DJ & Oh JY. Mol. Ther. 2012; 20, 14–20
[3] Théry C et al. Nat Rev Immunol. 2009;9:581-93



Platelet-rich plasma has a positive effect on the state of hepatic tissue in experimental cirrhosis in rats

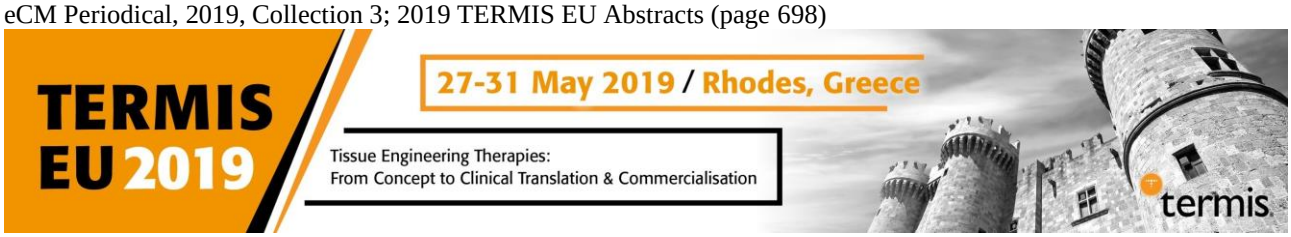
O. Kholodkova [1,2], I. Maznichenko [1,2], O. Deryabina [1,2]
Presenting Author: Olena Deryabina sonshine22@ukr.net
[1]Odessa National Medical University, Odessa, Ukraine [2]Research institute of molecular biology and genetics, Ukraine

INTRODUCTION: Despite numerous studies and discoveries in the field of the correction of diffuse liver diseases, the search for pathogenetic therapy of liver cirrhosis (LC) remains relevant today. It is known that the use of platelet-rich plasma (PRP) leads to rapid tissue repair, being one of the most promising alternatives in regenerative medicine.

METHODS: The experiment was conducted on adult male Wistar rats weighing 160-180 g, n = 50, including 10 intact animals. The following groups were singled out: I - control - animals with stimulated LC without correction, n = 20; II - rats with stimulated LC with PRP correction, n = 20. The pathology was modelled by intragastric administration of CCl_4 at a concentration of 50 % with replacement of drinking water with a solution of ethyl alcohol at a concentration of 10 %, for 120 days. The first day of PRP administration was considered the first day of the experiment. Percutaneous injection of PRP in a dose of 0.1 ml was carried out twice with an interval of 7 days. Animals were removed from the experiment on the 15th and 30th day after the last administration of PRP. Were conducted a complete blood test (GBT), biochemical studies of the activity of liver enzymes (ALT, AST) and pathological examination under a Leica DLMS light microscope, according to standard techniques.

RESULTS & DISCUSSION: In group I, on the 120th day it was revealed: GBT - the levels of erythrocytes and hemoglobin are reduced relative to the intact group - erythrocytes 3.4 ± 0.26 million, hemoglobin 107 ± 0.18 g/l; ALT activity was 4.5 times higher - 8.2 ± 0.41 units/l; AST 3.5 times higher - 4.82 ± 0.56 units/l ($p<0.05$). Histologically: violation of the histoarchitecture of hepatic tissue, expressed hyperemia, bilirubinostasis, hydropic degeneration, foci of central lobular necrosis, lympho-histiocytic infiltration with expansion of portal tracts, foci of a false and full sept formation, intralobular proliferation of connective tissue, expressed vascular sclerosis – the formed cirrhosis.
In group II, on the 15th day the cytological syndrome persisted: ALT activity was 3 times higher - 6.7 ± 0.37 units/l, AST activity was 115 % higher - 2.8 ± 0.29 units/l compared to the intact group ($p<0.05$). Histologically: the discomplexion of the hepatic beams, severe protein degeneration, areas of bridging necrosis, dilated portal tracts, anemic vessels, areas of perivascular fibrosis with areas of hyalinosis. In group II on the 30th day, it was revealed: GBT – within the normal range; ALT activity was higher by 62 % - 2.75 ± 0.35 unit/l, AST activity was 46 % higher - 1.9 ± 0.42 units/l in comparison with the intact group ($p<0.05$). Histologically: intact structure of hepatocytes, the presence of protein dystrophy, extended portal tracts with venous plethora, and mild vascular fibrosis.

CONCLUSIONS: On the 120th day of intragastric administration of CCl_4, an LC experimental animal was formed. In group I of experimental animals without PRP correction, manifestations of liver cirrhosis for all studied parameters remained during experiment. In group II, both on the 15th and 30th day after the last injection of PRP, there was a tendency to a decrease in the activity of hepatic enzymes, and the histological picture was characterized by normalization of the structure of hepatocytes, a decrease in the manifestations of inflammation and fibrosis. The use of PRP in LC has positive effect on the functional activity of hepatocytes and restoration of liver histoarhecture in rats.



Study of true morphological structure of macroporous hydrogel scaffolds using SEM and LSCM – Impact to cell growth

Olga Janouskova[1], Bohumila Podhorská[1], Miroslav Vetrik[1], Eva Chylíková Krumbholcová[1], Lucie Kománková[1], Niloufar B. Rashedi[1], Miroslav Šlouf[1], Mirka Dušková Smrčková[1]
Presenting Author: Olga Janouskova <janouskova@imc.cas.cz>
[1]Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic

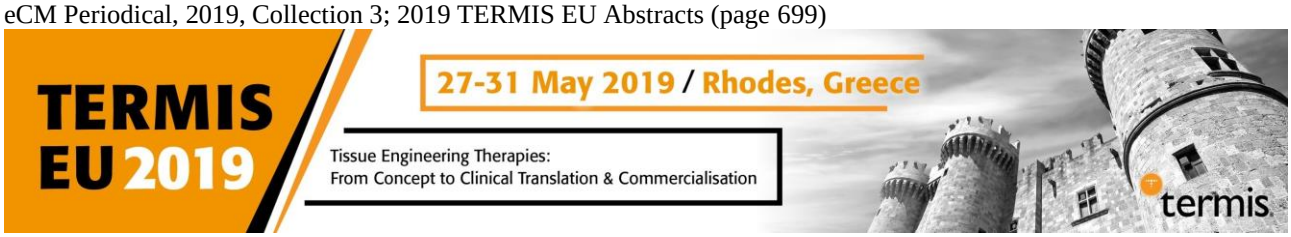
INTRODUCTION: Porous hydrogels are widely used as scaffold templates in tissue engineering. The connectivity and architecture of pores are one of the major factors governing the cell behaviour, such as cell attachment, proliferation and migration.
The scanning electron microscopy (SEM) is the most frequently technique to visualize architecture in hydrogels. The scaffold samples have to be freeze-dried for SEM. This preparation step can affect the morphology of hydrogel porous structure. Moreover, the freezing itself can greatly differ in dependence on freezing media, freezing velocity etc. Also the absence of water in hydrogels can damage the sample, above all it deforms pores, and influences pores distribution. The efficient way how to avoid these phenomena is the use of laser scanning confocal microscopy (LSCM). LSCM observing the hydrogels in their native state and allows fast assessment of hydrogel pore size distribution in the hydrated state. Moreover, LSCM enables in-situ observation of hydrogels with growing cells.

METHODS: Porous hydrogel were prepared by thermally initiated free radical polymerization of the (N-(2-hydroxypropyl) methacrylamide) (HPMA) monomer. Salt particles served as porogen. SEM were performed using TESCAN Vega PLUS TS 5135 microscope. Samples were prepared using freezing in -23ºC, -78,5ºC or -157,9ºC. LSCM were performed using Olympus laser scanning microscope FV10-ASW. The 3D computer reconstruction was done using the Software package MIMICS 8.13. Rat mesenchymal stem cells (rMSC) were cultivated in scaffolds for 5 days and cell growth were visualized by LSCM.

RESULTS & DISCUSSION: The porous non-degradable hydrogels based on covalently cross-linked poly(HPMA) were prepared. We correlated the gel morphology obtained from LSCM vs. that obtained by SEM with various freeze-transcriptional OCT-4, SOX-2, NANOG and drying cycle parameters. The tested hydrogels were made with three different sizes of porogen (0-30um, 30-50um, 50-90um). The resulting pore sizes in gels were determined from SEM images and compared with those obtained from LSCM scans. The computation 3D modelling was used to calculate pore volume and pore size distribution from LSCM pictures. Finally, the rat mesenchymal cell cultivation and growth studies were performed with all hydrogels types to evaluate the effect of hydrogel porosity architecture.

CONCLUSIONS: The LSCM is presented as a fast and useful method for real pore size distribution determination without altering the morphology of hydrogel in contrast to the SEM evaluation that revealed significant influence of the freeze-drying step. Moreover, the LSCM offered the possibility of in-situ monitoring the 3D scaffold morphology and visualization of rMSC attachment and growth in dependence of pore size.

ACKNOWLEDGEMENTS: This work was supported by the GACR project No.17-08531S and No.17-11140S.



The effect of blood-derived products on the regenerative potential of adipose-derived stem cells originated from three different fat locations

O. Kuten-Pella[1,2], M. Neubauer[2], S. Nehrer[2], Z. Lacza[1,2]
Presenting Author: Olga Kuten-Pella, olga.kuten@orthosera.com
[1]OrthoSera GmbH, Krems an der Donau, Austria [2]Center for Regenerative Medicine, Danube University Krems, Austria

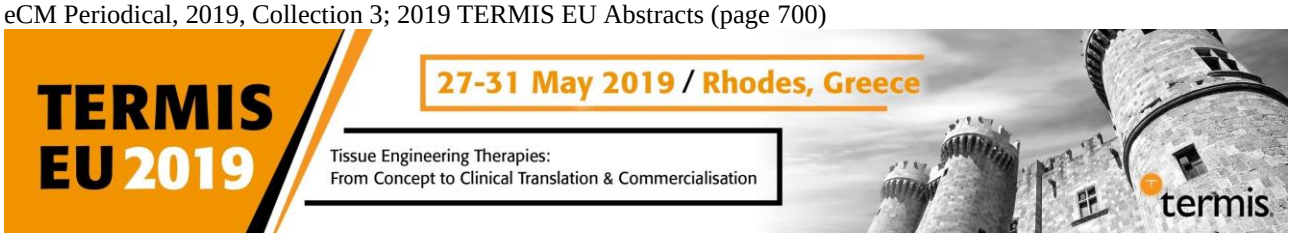
INTRODUCTION: Known limitations of autologous chondrocyte implantation (ACI) are the invasiveness of tissue harvesting and a need for two surgical procedures. In addition, known issues are fibrocartilage formation and de-differentiation of chondrocytes during cell expansion. Alternative cell sources such as adipose-derived stem cells from the soft tissues around the knee joint may offer alternative cell sources, while the patient's blood derivatives can be utilized in cell culture. The aim of the present study was to investigate the potential of adipose tissue sources and blood serum derivatives for chondrogenic and osteogenic cell culture.

METHODS: Tissue from three fat locations (i) infrapatellar fat pad (Hoffa), (ii) subcutaneous fat and (iii) distal femur/pouch were harvested from three donors during a routine surgical procedure under ethics approval. The tissue was minced with scalpel and digested in collagenase solution. After filtration and centrifugation, cells were cultured to confluence in standard growth media. The expression of specific surface markers for adipose stem cells were analysed by flow cytometry. Blood products platelet-rich plasma (PRP) and hyperacute serum were prepared from the whole blood of healthy donors. PRP was generated by two centrifugation steps in the presence of anticoagulants EDTA or sodium citrate whereas hyperacute serum was produced using the hypACT auto device according to the manufacturer´s protocol. We investigated the proliferative potential of stem cells from different fat sources, supplemented with blood-derived products, using the XTT metabolic activity assay. We induced osteogenesis and chondrogenesis with differentiation media further supplemented by either PRP or hyperacute serum. Differentiation was evaluated by alizarin red and alcian blue staining, gene expression patterns were determined by PCR at day 0, 10 and 21.

RESULTS & DISCUSSION: We observed a highly proliferative effect and enhancement of osteogenesis by hyperacute serum and citrate-PRP, while supplementation with EDTA-PRP or fetal calf serum were less effective. Highest cell viability and chondrogenic / osteogenic differentiation potential was achieved with Hoffa stem cells compared to cells from other fat tissue sources. After evaluation of chondrogenic differentiation, preliminary data revealed better potential of Hoffa stem cells supplemented with PRP with EDTA.

CONCLUSIONS: These preliminary data illustrate potentially beneficial effects of blood-derived products on the regenerative potential of adipose-derived stem cells in cartilage replacement. Choice of aniticoagulants affects the biological activity of PRP, which further supports the use of non-anticoagulated serum supplementation instead. All three fat locations can be considered as cell sources, however, best osteogenic and chondrogenic potential was observed with Hoffa stem cells.

ACKNOWLEDGEMENTS: We would like to thank our clinical partners from Evangelic Hospital Vienna for providing the tissues.



Engineering immunomodulating scaffolds by modulating particle size

O. Mahon.[1, 2], T. Gonzalez-Fernandez[2], D. Browe[2], P. Pitacco[2], D. Kelly[2], A. Dunne[1]
Presenting Author: Olwyn Mahon, olmahon@tcd.ie
[1]Molecular Immunology Group, School of Biochemistry and Immunology [2]Trinity Centre for Bioengineering, School of Engineering, Trinity College Dublin, Ireland

INTRODUCTION: Macrophages play a key role in tissue regeneration[1] and understanding their interaction with biomaterials used in tissue engineering applications is crucial to determine the success and longevity of biomaterial implants. We have previously demonstrated that micron sized HA particles directly promote M1 polarization in primary human macrophages[2]. The objective of our research is to elucidate the macrophage phenotype induced by micro and nano sized HA particles in vitro and in vivo and to determine whether macrophage phenotype and innate immune cytokines can influence bone tissue regeneration.

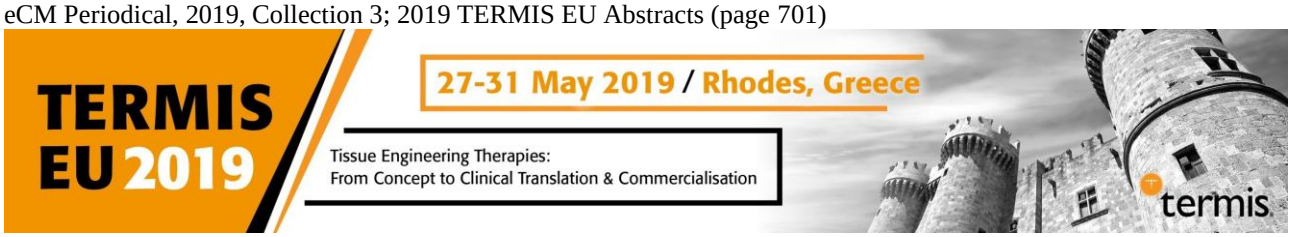
METHODS: For in vitro studies primary human macrophages were isolated from healthy blood as described previously [2] and treated with HA particles of different sizes. Immune cell profile and macrophage phenotype was determined by ELISA, Flow cytometry, RT-PCR and Western blot. For in vivo studies, a rat femoral defect model was used to assess immune cell subsets, macrophage phenotype and tissue regeneration post implantation of HA functionalized scaffolds.

RESULTS & DISCUSSION: Treatment of macrophages with nanoHA particles drove robust expression of the M2 markers, CCL13, CD206 and CD163 and specifically enhanced secretion of the anti-inflammatory cytokine IL-10. Furthermore, conditioned media from nanoHA treated macrophages enhanced expression of BMP2 and ALP and mineralization in MSCs in an IL-10 dependent manner. In our in vivo bone model, the presence of nanoHA functionalized scaffolds at the defect site was associated with reduced numbers of inflammatory T cells and neutrophils compared to micron sized HA functionalized scaffolds, while numbers of cells expressing the M2 marker $CX3CR1^{+}$ increased compared to micron HA functionalized scaffolds. Analysis of functional tissue recovery revealed enhanced mineral deposition in the nanoHA group and the presence of vWF expressing vessels.

CONCLUSIONS: We demonstrate that nanoHA particles are inherently anti-inflammatory and supportive of osteogenesis. Furthermore, we demonstrate for the first time, a novel pro-osteogenic role for the anti-inflammatory cytokine IL-10. The data herein offers compelling evidence that positions nanoHA as a prospective immune-modulating implant material that may be superior to larger particles for use in bone regeneration and may avoid the need to exogenously load cytokines into biomaterial constructs.

REFERENCES

[1] Brown, B. N. et al Biomaterials. 2009; 30, 1482–1491.
[2] Mahon, O. R. et al. Acta Biomater. 2018.65, 426–435



Developing transcript-activated coating for titanium implants

O. Fayed[1,2], M. van Griensven[1], C. Plank[2], E.R. Balmayor[1]
Presenting author: Omnia Fayed, omnia.fayed@tum.de
[1]Department of Experimental Trauma Surgery, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany [2]Institute of Molecular Immunology - Experimental Oncology, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

INTRODUCTION: Osteointegration is crucial for implant success. Bone morphogenetic protein 2 (BMP2) biomolecules (protein and nucleic acid) have been used with various biodegradable natural and synthetic polymers such as polylactides and fibrin to enhance bone healing in vitro and in vivo [1-2]. Transcript therapy using chemically modified mRNA (cmRNA) is an emerging alternative for local gene therapy resulting in efficient protein production. In this study, we aimed to utilize this technology to create BMP2 coating for Titanium (Ti) implants. For this, diverse strategies were investigated to produce different coatings. We evaluated the effect of coating type and composition on transfection efficiency, kinetics and cell viability in vitro.

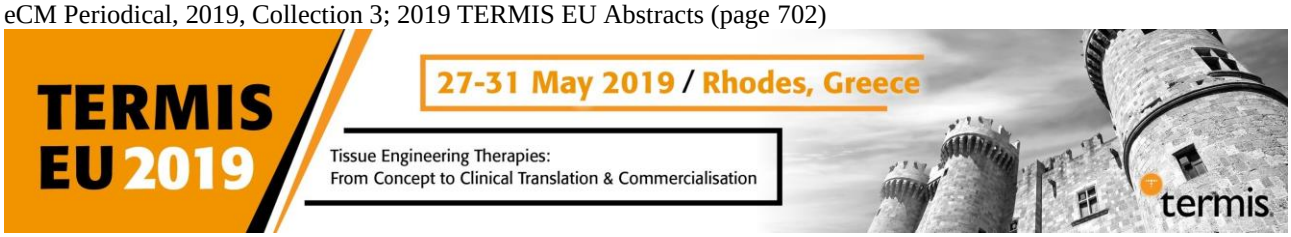
METHODS: Ti etching was done using different acids and etching times. Characterization was performed by SEM. Physical adsorption (MetLuc cmRNA-Ti) was done by air drying solutions of different concentrations of Metridia Luciferase (MetLuc) cmRNA lipoplexes on all plain Ti surfaces. Best etching conditions and cmRNA concentrations were selected for further studies. Polylactide and Fibrin coating (MetLuc cmRNA-PDLLA and -Fb): Furthermore, different concentrations of PDLLA (3, 2, 1, 0.5, 0.25 µg/well) and Fibrin (thrombin to fibrinogen final coating ratios: 1, 0.5, 0.25, 0) were used to produce further coating, by adding MetLuc cmRNA to the solutions and allowing the deposition on Ti surfaces. For Fibrin, Tissucol® was used. In this case, fibrinogen was mixed with the MetLuc lipoplexes and subsequently thrombin was added at different ratios. The coated Ti surfaces were freeze dried. Evaluation of transfection efficiency and kinetics, cell viability, and cell proliferation were done using NIH3T3.

RESULTS & DISCUSSION: Our results showed a positive transfection of MetLuc cmRNA of cells seeded on all tested Ti coatings. Interesting, the lowest transfection efficiency was obtained when MetLuc cmRNA was allowed to dry on plain Ti. A significant improvement was obtained on PDLLA coated Ti surfaces and the best results were obtained for Fibrin coated Ti. In this case, Fibrinogen-coated samples (i.e. MetLuc-F coating ratio of 0) showed the highest results in terms of transfection efficiency. Cell viability and proliferation decreased on transcript activated Ti surfaces ($p>0.05$). However, this improved significantly by PDLLA and Fibrin coating. Interestingly, fibrinogen alone (no thrombin) performed significantly better than Fibrin in terms of transfection efficiency.

CONCLUSIONS: Our obtained results highlight fibrinogen as a promising candidate for transcript therapy using coatings of Titanium implants.

REFRENCES

[1] A. Kolk et al., Biomaterials, 2011; 32(28) 6850–9.
[2] E. R. Balmayor et al., Biomaterials, 2016; 87 (5):131–146.



Swelling of hydrogels for intracranial aneurysms treatment

O. Poupart[1,3], P. Mosimann[2], C. Moser[3], P-E. Bourban[4], D.P. Pioletti[1]
Presenting Author: Oriane Poupart, oriane.poupart@epfl.ch
[1]Laboratory of Biomechanical Orthopedics, EPFL, Switzerland [2]Bern University Hospital, Bern, Switzerland [3]Laboratory of Applied Photonics Devices, EPFL, Switzerland, [4]Laboratory of Processing of Advanced Composites, EPFL, Switzerland

INTRODUCTION: Intracranial aneurysms are a major health concern and affect 3.2 % of the population [1]. Current treatments, open clipping surgery and endovascular treatment, present the drawbacks of invasive surgery and short term efficiency, respectively. The use of a photoactivated hydrogel has the potential to act as an aneurysm filler due to their suitable biological and mechanical properties. Swelling is an important feature of hydrogels and has to be controlled in order to avoid oversizing and overpressure into the aneurysm.

METHODS: 10 wt% polyethylene glycol dimethacrylate (PEGDMA) hydrogels were synthesized using two molecular weights (6 and 20 kDa) and photo-polymerized using 405 nm illumination. Hydrogels swelled in PBS at 37°C and 5% CO_2. Swelling ratio was measured in a semi-confined mold, to simulate the blood contact only at the aneurysm neck. Swelling pressure was also determined using a confined compression test. Moreover, critical swelling pressure for aneurysm rupture will be estimated by a computational model. Digital image correlation (DIC) will also be performed to assess the strains on the aneurysm wall, using 3D printed PDMS aneurysm models.

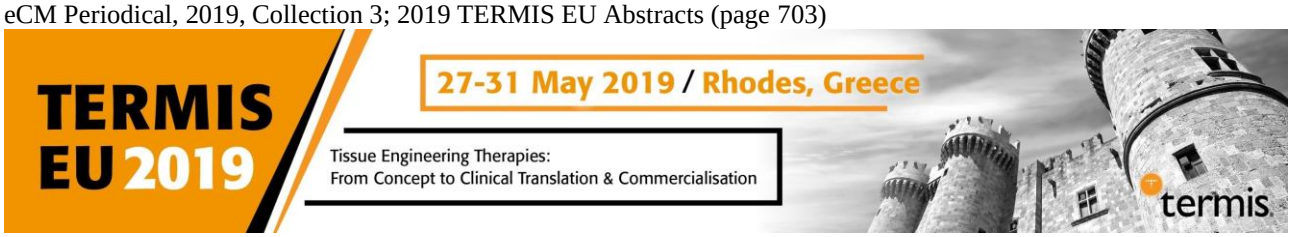
RESULTS & DISCUSSION: Swelling ratio and pressure have a different influence according to the molecular weight of PEGDMA. The swelling ratio increases when the molecular weight is higher. Moreover, the size of the 20 kDa PEGDMA hydrogels was multiplied by 3 in every direction, inducing that the hydrogels get out from the semi-confined mold. The molecular weight has less influence on the swelling pressure, ranging from 169 to 248 kPa. The maximum swelling pressure was obtained for the mixing of 6 and 20 kDa hydrogels.

CONCLUSIONS: 6 kDa PEGDMA hydrogels combine low swelling ratio and pressure, which would be appropriate to act as a filler for intracranial aneurysms. However, the simulation of blood flow into 3D printed aneurysm model using DIC will allow to estimate more accurately the swelling behavior of the hydrogels.

ACKNOWLEDGEMENTS: Financial support was received from Swiss National Science Foundation CRSII5_170992.

REFERENCES

[1] Monique H. M. Vlak et al. The Lancet Neurology 10.7. 2011; pp. 626–636.



Angiogenic signaling in bone 3D scaffolds through calcium phosphate ion release nanotechnology

O. Castano[1,2,3], J.Marti-Munoz[2,3], C. Ximenes[2,3], S. Perez-Amodio[2,3,4], E. Engel[2,3,4]
Presenting Author: Oscar Castano, oscar.castano@ub.edu
[1]Serra Hunter Fellow, Electronics and Biomedical Engineering, Universitat de Barcelona (UB), 08028 Barcelona, Spain [2]Biomaterials for Regenerative Therapies, Institute for Bioengineering of Catalonia (IBEC),The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain [3]CIBER en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain [4]Materials Science and Metallurgy, EEBE, Technical University of Catalonia (UPC), Barcelona, Spain

INTRODUCTION: Angiogenesis is needed in almost any regenerated tissue in the body. So far, many strategies have been followed to introduce the proper angiogenic signaling to control endothelial cell fate and create feasible blood vessels that colonize 3D scaffolds. However, even though their efficiency has been probed, this route implies the use of different biomolecules that have some drawbacks such as cost-efficiency ratio, storage difficulties, and a poor understanding of undesired side effects. An alternative is presented here where stimulation by calcium ions enables the cells to control their own signals, reducing side effects [1-2].

METHODS: Angiogenic nanoparticles were prepared by the sol-gel method. Nanoparticles were combined with different scaffolds prepared and optimized by 3D extrusion printing. Nanoparticles were combined with polylactic acid (PLA) as the biodegradable polymer for an ion-releasing platform. Different characterization assays were carried out to assess the microstructure and biological characterization: SEM, mechanical compression assays, ion release, pH, degradation, adhesion and proliferation with hMSCs, tubologenesis assays using hHUVECs, and the chicken CAM assay.

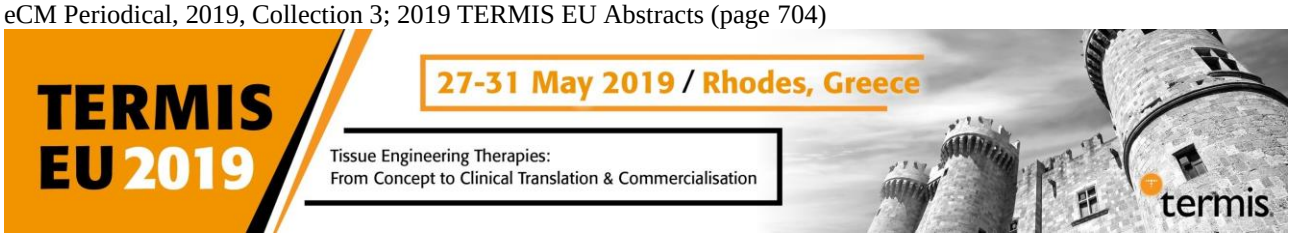
RESULTS & DISCUSSION: MSCs adhered, colonized and proliferated on the prepared 3D printed scaffolds. Measured calcium ion release had a correlation with the VEGF expressed by the MSCs. HUVECs were stimulated by this VEGF to form tubes. CAM preliminary assays also showed a higher increase of blood vessel formation and colonization inside calcium-releasing 3D scaffolds.

CONCLUSIONS: The production of 3D scaffolds combining high calcium release nanoparticles and PLA was possible. The correlation between calcium release and VEGF (among others) confirmed calcium as a promising low cost pro-angiogenic strategy.

ACKNOWLEDGEMENTS: Financial support was received from MICINU MAT2015-68906-R project.

REFERENCES

[1] Castano et al. ACS Appl. Mater. Interfaces, 2014, 6,10, 7512–7522
[2] De Oliveira et al. Acta Biomaterialia, 2016, 29, 435-445



Control of cellular focal adhesion and differentiation by topography and chemical cues of micropatterned structures and their polymeric replicas

P. Kavatzikidou[1], D. Angelaki[1,2], E. Babaliari[1,3], P. Mangana[1], L. Papadimitriou[1], A. Mimidis[1,3], V. Skoulas[1,3], S. Petrović[4], D. Peruško[4], A. Ranella[1], E. Stratakis [1,2]
Presenting Author: Paraskevi Kavatzikidou, ekavatzi@iesl.forth.gr
[1]Foundation for Research and Technology - Hellas (FORTH), Institute of Electronic Structure and Laser (IESL) [2]Department of Physics, University of Crete, Crete, Greece [3]Department of Materials Science and Technology, University of Crete, Greece [4]Vinča Institute of Nuclear Sciences, University of Belgrade, P.O.Box 522, 11001 Belgrade, Serbia.

INTRODUCTION: The extracellular matrix provides the necessary cues at micro and nano-scale for cell adhesion, alignment, proliferation and differentiation. Ultrafast pulsed laser irradiation is considered as a simple and effective microfabrication method to produce structures controlling the structure geometry and pattern regularity[1]. Such structures characterised with an anisotropy discontinuous topographical nature could enhance neuronal growth and alignment[2]. Soft lithography has been successfully used to transfer well-defined micro-sized patterns from silicon to surfaces of biomaterials allowing the recreation of controlled microenvironments and an in depth study of the influence of surface properties on cell behavior[3].

METHODS: In this study, a series of micro-patterned silicon (Si) and titanium composite structures were fabricated by using the ultrashort laser irradiation at a range of fluences, resulting in different patterns. Positive replicas on different concentrations of biodegradable polymers have been successfully reproduced from the Si structures via soft lithography. The morphological characterization of the polymeric replicas was performed by Scanning Electron Microscopy (SEM). The cytocompatibility of replicas with mouse SW10 cell line and mouse Mesenchymal Stem Cells C57BL/6 was evaluated. Immunostaining protocols assessed the cell viability (nucleus –DAPI), cell adhesion and alignment (Cytoskeleton-Phalloidin/actin), focal adhesion points (Vinculin), and early stages of differentiation (ALP Assay).

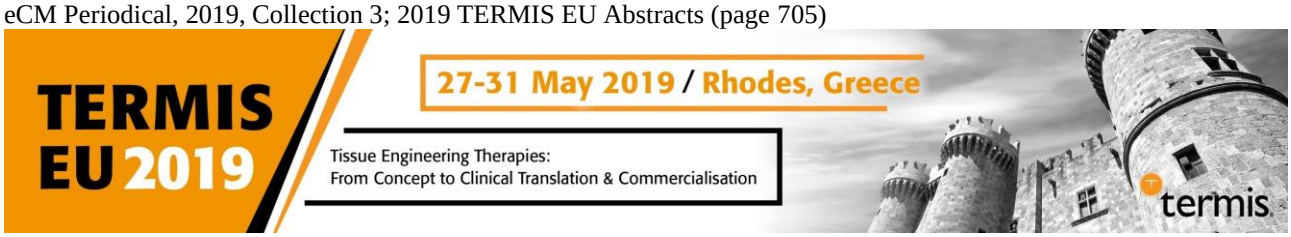
RESULTS & DISCUSSION: Figure 1: Establishment of focal adhesion on different cell types and substrates. Vinculin is shown in green on the MSCs adhered on Ti film, (right) and on the SW10 cells on Si structure (left).

CONCLUSIONS: All the topographies supported the cells' adhesion and in particular focal adhesion establishment and maturation. The surface roughness had an effect on the MSCs differentiation. Cell type played a significant role in the above findings.

ACKNOWLEDGEMENTS: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code:T1EDK-02024, MIS:5030238) ». This work was also co-financed by NFFA (EU H2020 framework programme).

REFERENCES

[1] Ranella A. et al. Acta Biomaterialia. 2010; 6: 2711
[2] Simitzi C. et al. Biomaterials. 2015; 67:115-128
[3] Babaliari E. et al. Int. J. Mol. Sci. 2018; 19(7):2053.



Application of human placenta derived amniotic epithelial cells as novel approach in cancer treatment

A. Jafari[1, 2†], P. Parhizgar[3†], H. Zali[4], B. Farhadi-Hoseinabadi[4], H. Niknejad[3]
Presenting Author: Parinaz Parhizgar, parhizgar.p@sbmu.ac.ir
[1]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Proteomics Research Center, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran [3]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran [4]School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran. [†]Co-first author

INTRODUCTION: Human amniotic membrane (hAM) is the innermost layer of fetal membranes, which surrounds the developing fetus and forms the amniotic cavity. hAM and hAM-derived cells possess unique properties that make them excellent candidates for use in clinic, such as low immunogenicity, promotion of epithelization, anti-inflammatory and antimicrobial properties as well as angio-mudulatory, anti- fibrosis and anticancer properties. We examined the effect of human amniotic epithelial cells- conditioned media (hAECs- CM) on cancerous cell lines proliferation.

METHODS: Human placentas were obtained at term pregnancy during Caesarean sections from mother with negative results for HIV-I, and hepatitis B and C. hAM was separated from the chorion by peeling and washed extensively with cold PBS. The epithelial cells were isolated enzymatically. The cell pellet was resuspended and cultured. Medium was changed once after the density of cells reached 85% confluency, and hAEC-CM was collected while cells were growing exponentially. The hAEC-CM was filtered, and used in subsequent experiments. Four different cancer cell were seeded in 96-well tissue culture plates. After 24h the culture medium was exchange with different dose of hAEC-CM. After 48h of the incubation, cell viability was determined by the MTT assay.

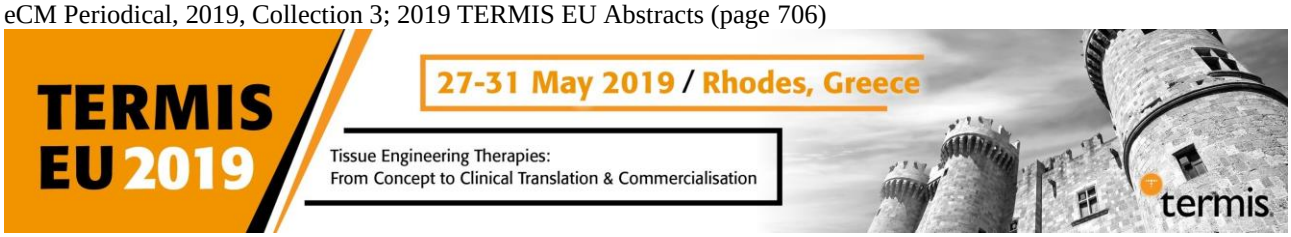
RESULTS & DISCUSSION: Data on mitochondrial activity obtained from the optical density of cell culture plates of the experimental groups were transformed in percentages in relation to the control group, considered to be 100%. Different volume of hAEC-CM (50, 100, 150, and 200 µl) reduced the viability of cancerous cell lines significantly in comparison with control group ($p<0.05$). The maximum reduction was observed in the samples with 200 µl of the supernatant.in this volume percentage cell viability of breast cancer cell lines includes MDA-MB-231, MCF-7 and BT-474 recorded by 68%, 72% and 57%, respectively. Amongst four cell lines, Hela (cervical cancer cells) showed the least viability by 37%.

CONCLUSIONS: Our results indicated that hAECs-CM is able to inhibit the proliferation of the cancer cells concentration-dependently. Taken together, these results provide strong evidence that hAECs-CM can be used as a safe and effective cancer-targeting cytotherapy for treating breast and cervical cancer.

ACKNOWLEDGEMENTS: The authors would like to thank the operating room personnel of Gandhi Hotel Hospital for their assistance in this research.

REFERENCES

[1] Kim SH et al. Tissue Cell. 2015: 1;47(1):10-6.
[2] S Bu et al Int J Oncol. 2017: 1;51(5):1405-14.
[3] Gholizadeh-Ghaleh Aziz S et al. Artif Cells Nanomed Biotechnol. 2017;45(4):765-774



Ordered assembly of cell-laden micro-scaffolds for complex hybrid tissues design and fabrication

P. Pedram[1,2], C. Mazio[2], G. Imparato[2], A. Salerno[1], P.A. Netti[1,2,3]
Presenting Author: Parisa Pedram, parisa.pedram@iit.it
[1]Department of chemical, materials and Production engineering, University of Naples Federico II, Italy [2]Centre for Advanced Biomaterials for Health Care, CABHC, Istituto Italiano di Tecnologia, Naples, Italy [3]Interdisciplinary Research Center on Biomaterials, CRIB, university of Naples, Italy

INTRODUCTION: The bottom-up realization of three-dimensional (3D) hybrid structures composed of both biomaterials and cells are one of the most interesting and novel approach for in vitro and in vivo tissue regeneration. Biocompatible and biodegradable polymers are essential elements of these bottom-up approaches. They serve as template structures for cells adhesion, proliferation and 3D assembly. Such 3D bio-hybrids have been used as possible models to study on cells differentiation and tissue vascularization. Nevertheless, there are limited studies in this regard. This study focuses on the development of a bottom-up approach enabling the construction of highly complex and precisely designed bio-hybrids by means of the ordered assembling of cell-laden micro-scaffolds.

METHODS: Porous PCL and PLGA micro-scaffolds were fabricated by fluidic emulsion/solvent evaporation technique. The morphological and structural properties of micro-scaffolds were characterized by scanning electron microscopy (SEM) and Micro-CT analysis. Then the micro-scaffolds were arranged into precisely designed layered structures and seeded with cells for the growth of the hybrid tissue. Human dermal fibroblasts were used as model cells to study of micro-scaffolds biocompatibility and tissue formation. The morphology and cell/ECM composition of bio-hybrids were assessed by SEM, immunofluorescence analysis and histology.

RESULTS & DISCUSSION: The SEM and Micro-CT results of micro-scaffolds evidenced a highly porous morphology and structure with proper pores interconnectivity. The in vitro biocompatibility tests showed that micro-scaffolds are biocompatible, enabled cells adhesion and proliferation as well as promoted the formation of hybrid self-supporting structures (Figure 1).

CONCLUSIONS: This study demonstrated that the proposed approach enabled the design and building cell/micro-scaffold hybrid systems with highly oriented pores suitable to promote and guide blood vessels formation in vitro and in vivo.

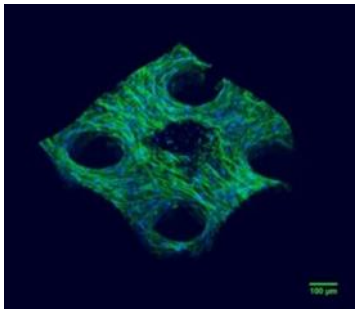
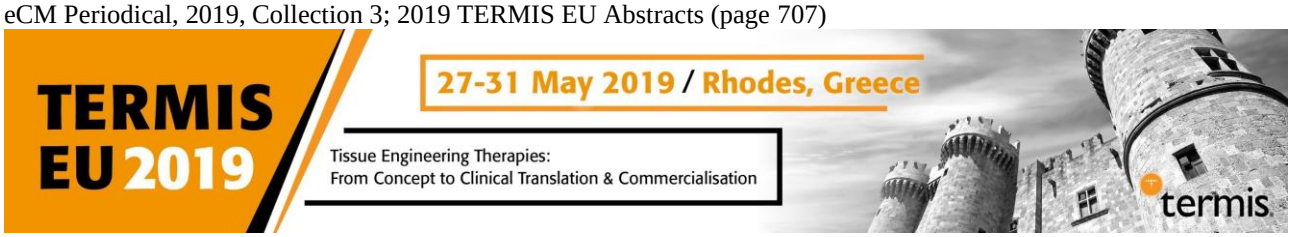


Figure 1: 3D reconstruction of a hybrid structure obtained by the ordered assembly of cell-laded micro-scaffolds. Bio-hybrid stained with DRAQ5 (green) and phalloidin (blue) after 5days; bars 100µm.



Antioxidant and oxygen releasing cryogel scaffolds for tissue engineering applications

P. A. Shiekh[1], A. Singh[1], A. Kumar[1,2,3]
Presenting Author: Parvaiz A. Shiekh, sparvaiz@iitk.ac.in
[1]Biomaterial and Tissue Engineering Group, Department of Biological Sciences and Bioengineering [2]Centre for Nanosciences [3]Centre for Environmental Sciences and Engineering, Indian Institute of Technology Kanpur, Kanpur, UP, 208016, India

INTRODUCTION: Tissue engineering strategies aim to repair and regenerate the damaged tissue. Oxygen deprivation and oxidative stress is a key regulator in number of pathological conditions such as inflammation, chronic wound healing, cardiovascular diseases such as myocardial infarction. We herein describe a novel biomaterial-based approach to attenuate oxidative stress and deliver oxygen at the damaged tissue site, thus alleviating tissue repair and regeneration.

METHODS: Antioxidant polyurethane PUAO was synthesised and characterised for biocompatibility and antioxidant potential. Oxygen releasing PUAO-CPO scaffolds were fabricated by incorporation of calcium peroxide in PUAO cryogels. Oxygen releasing cryogels were characterised for oxygen releasing capacity, biocompatibility, attenuation of free radicals and enhancement of cell survival by live/dead and MTT assay under hypoxia conditions. The effect of oxygen releasing cryogels to increase tissue survival under in-vivo ischemic conditions was evaluated in a mice skin flap model.

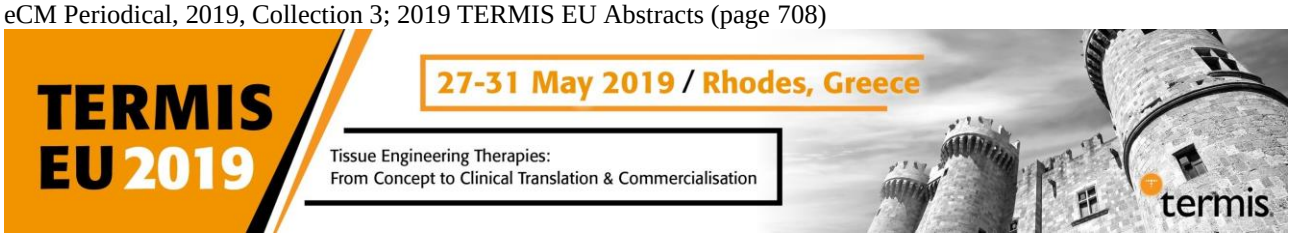
RESULTS & DISCUSSION: PUAO scaffolds were biodegradable, biocompatible and mechanically elastomeric in nature. In an in-vitro oxidative stress model system, PUAO could reduce intracellular oxidative stress significantly and can combat the menadione as well as ischemic reperfusion induced cell death in cardiomyocytes [1]. PUAO-CPO cryogels showed a sustained release of oxygen upto 10 days. Under hypoxia conditions, H9C2 cardiomyocytes have increased metabolic activity on oxygen releasing PUAO-CPO scaffolds compared to non-oxygen releasing scaffolds [2]. Similarly, H9C2 cells showed increased viability on PUAO-CPO scaffolds under hypoxic environment thus corroborating metabolic activity results. Under in-vivo conditions, antioxidant scaffolds delayed tissue necrosis whereas, oxygen releasing scaffolds prevented tissue necrosis and increased the tissue viability in severely ischemic conditions.

CONCLUSIONS: Collectively, these results demonstrated the feasibility of these scaffolds for regeneration of tissues where lack of oxygen and oxidative stress is a concern. This is an important outcome as this will pave way for development of next generation scaffolds for treatment of diseases like MI and chronic diabetic wound healing etc., where oxygen deliver is a limitation and oxidative stress is a major concern. Future studies will look into preclinical evaluation of these scaffolds in the treatment of diseases such as myocardial infarction and chronic and diabetic wound healing.

ACKNOWLEDGEMENTS: Financial support was received from Department of Science and Technology (DST), DST-UKIERI, Department of Biotechnology (DBT), Govt. of India, DBT-TATA Innovation fellowship, MHRD/ICMR (IMPRINT), and IIT Kanpur.

REFERENCES

[1] Shiekh, P. A., et al. (2018). ACS Applied Materials & Interfaces, 10(4), 3260-3273.
[2] Shiekh, P. A., et al. (2018). ACS Applied Materials & Interfaces, 10(22), 18458-18469.



The administration of β-Lapachone reverts inflammation and improves cartilage regeneration in an ex vivo model of OA

P. Diaz-Rodriguez[1], J. A. Vázquez[2], J. R. Caeiro[3], M. Landin[1]
Presenting Author: Patricia Diaz-Rodriguez, patricia.diaz.rodriguez@usc.es
[1]Dpt. of Pharmacology, Pharmacy and Pharmaceutical Technology, Universidade de Santiago de Compostela, Santiago de Compostela, Spain [2]Group of Recycling and Valorisation of Waste Materials, Marine Research Institute (IIM-CSIC), Vigo, Spain [3]Dpt. of Orthopaedic Surgery and Traumatology, Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain

INTRODUCTION: Osteoarthritis (OA) is a major musculoskeletal disease characterized by the destruction of the articular cartilage [1], which simultaneously promotes a chronic inflammatory environment that lead to the further progression of the disease. Currently, OA treatments include the intra articular administration of corticosteroids, such as dexamethasone, which decreases the inflammatory environment of OA, but shows adverse effects on tissue healing [2]. Beta-Lapachone (βLap) is a natural substrate of the NADH: quinone oxidoreductase 1 (NQO1) with well-known antitumoral activity. Recently, its therapeutic action for the treatment of fibrosis and pulmonary inflammation has also been reported [3]. The objective of this work is to evaluate the effect of βLap on the inflammatory environment and cartilage metabolism in an ex vivo model of OA.

METHODS: Human cartilage explants (disks of Ø = 6mm) were obtained from OA patients undergoing total knee replacement. Explants were placed in 24-well plates with complete media and allowed to settle for 48 hours. Explants from the same donor were subjected to all treatments: 1) no stimulation (normal media); 2) stimulation with pro-inflammatory signals (LPS and/or IFN-γ); 3) Stimulation + Dexamethasone (Dexa;1μM) or 4) Stimulation + βLap at variable concentrations (1μM-10μM). After 48 hours, media supernatant was collected and explants were cut, half was used to measure GAGs content while the other half were formalin fixed for histological analysis. Media supernatant was used to assess the secretion of PGE_2, IL-13, IL-1ra, IL-6, IL-8, MMP-13, MMP-9 and TNF-α.

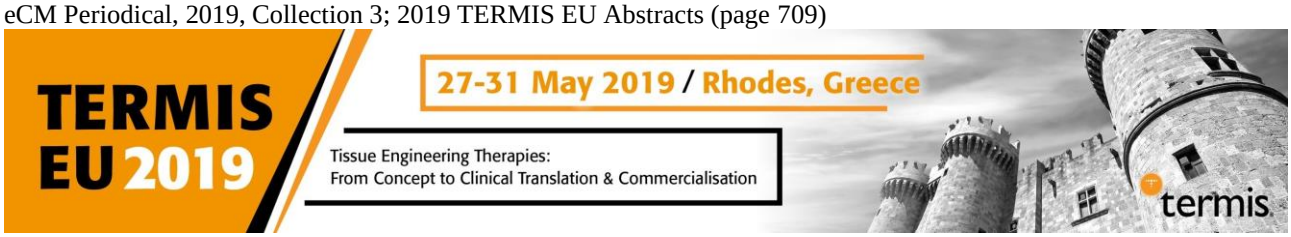
RESULTS & DISCUSSION: The addition of βLap was able to significantly decrease the secretion of pro-inflammatory cytokines as PGE_2 (Figure 1) in a similar way as Dexa control reaching levels close to non-stimulated explants. Furthermore, the addition of βLap was able to decrease the loss of GAGs on the cartilage ECM when compared to stimulated non-treated explants.

CONCLUSIONS: Results pointed out the promising activity of βLap against OA progression. This data corroborates the previously described anti-inflammatory activity of βLap. Further in vivo assessments will be performed to ensure adequate performance of βLap formulations.

ACKNOWLEDGEMENTS: Financial support was received from Interreg V-A POCTEP (0245_IBEROS_1_E) EU (FEDER).

REFERENCES

[1] Pers YM et al., Osteoarthritis Cartilage. 2015;23(11):2027-35.
[2] Slominski AT et al., J. Invest. Dermatol. 2017;137:1012-4.
[3] Oh G-S et al., Tuberc. Respir. Dis. 2016;79:257-66.



Adhesion forces of selected bone-binding motifs determined via single molecule force spectroscopy

P. Steinbauer[1,3], A. Rohatschek[2,3], O. Andriotis[2,3], P. J. Thurner[2,3], R. Liska[1,3], S. Baudis[1,3]
Presenting Author: Patrick Steinbauer, patrick.steinbauer@tuwien.ac.at
[1]Institute of Applied Synthetic Chemistry E163, Technische Universität Wien (TU Wien), Vienna, Austria [2]Institute of Lightweight Design and Structural Biomechanics E317, Technische Universität Wien (TU Wien), Vienna, Austria [3]Austrian Cluster for Tissue Regeneration, Austria

INTRODUCTION: An unmet clinical need of being able to glue small bone fractures with a biocompatible, resorbable adhesive system is frequently stated [1]. Bone adhesives have to meet several requirements for all day clinical use. Dental materials and primers influence the design of a surgically realizable bone glue. The major problem in measuring the adhesion properties of a putative bone adhesive primer is to distinguish between cohesive and adhesive forces. Our contribution directly investigates adhesion processes at molecular level via single molecule force spectroscopy (SMFS).

METHODS: A procedure to graft different adhesion motifs onto the tip of an AFM cantilever was established similar to Wildling et al. [2] In SMFS experiments, the AFM tip works as a force sensor with pN resolution. AFM chips were amino functionalized to attach a flexible PEG based linker system. Thereafter, different adhesion motifs were clicked on these spacer and pull-off forces were measured on hydroxyapatite (HAP) mimicking bone and on a TiO_2 coated silicon wafer used as implant surrogate. A specific amino acid sequence (AAS) with phosphorylated serines was chosen as adhesion motif based on its suggested binding properties to HAP in statherin [3].

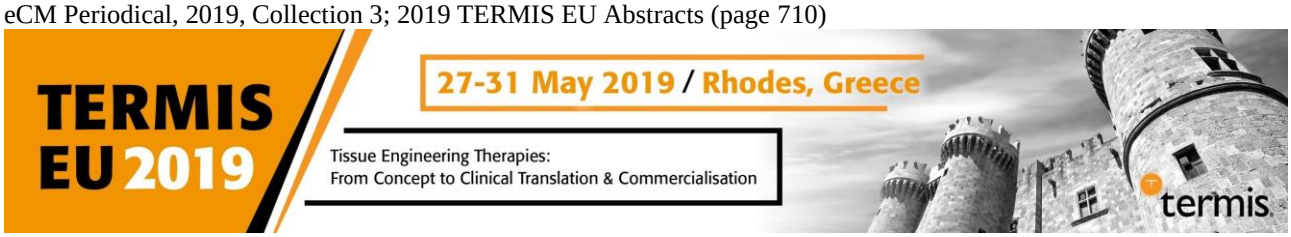
RESULTS & DISCUSSION: In order to validate tip functionalization and the experimental SMFS procedure per se, the pull-off adhesion values of unfunctionalized, amino functionalized and linker functionalized AFM tips were compared to the phosphorylated AAS sequence and the unphosphorylated sequence on HAP and TiO_2. The mean adhesion values of the phosphorylated AAS were significantly higher. It was proven that phosphonic acid primers play a decisive role in adhering on HAP and TiO_2.

CONCLUSIONS: By the establishment of a procedure to functionalize an AFM tip with adhesion motifs, it is possible to measure adhesion forces at molecular level on different substrates. The phosphorylated AAS can indeed be considered as a fully biocompatible adhesion motif for a bone glue. This is a substantial progress for designing bioadhesive systems.

ACKNOWLEDGEMENTS: The financial support by the doctoral program BioInterface (TU Wien) is kindly acknowledged (A.R.).

REFERENCES

[1] Donkerwolcke M et al. Biomaterials. 1998; 19(16): 1461-6
[2] Wildling L et al. J.Biol.Chem. 287, 105-113.
[3] Stayton PS et al. Crit. Rev. in Oral Bio. and Med. 2003;14(5): 270-6



Integrated additive design and manufacturing approach for the bioengineering of bone scaffolds for favorable mechanical and biological properties

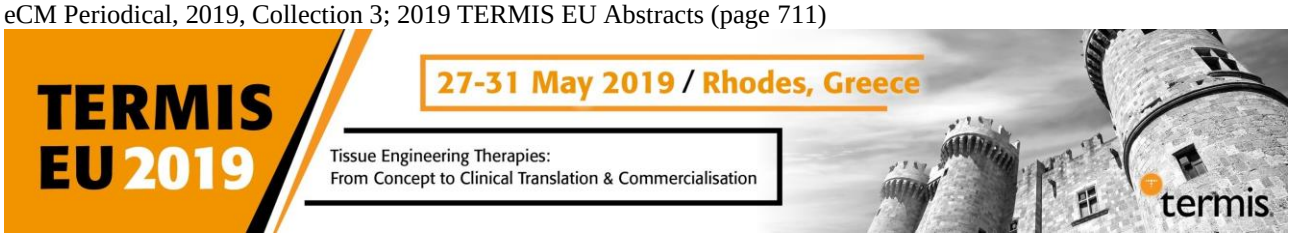
D. Valainis[2], P. Dondl[1], P. Foehr[3], R. Burgkart[3], S. Kalkhof[5,6], M. van Griensven[2], P.S.P. Poh[2, 4]*
Presenting Author: Patrina S.P. Poh, patrina.poh@charite.de
[1]Department of Applied Mathematics, Albert Ludwigs University of Freiburg, Germany [2]Experimental Trauma Surgery, Technical University of Munich, Germany [3]Clinic of Orthopaedics and Sport Orthopaedics, Department of Biomechanics, Technical University of Munich, Germany [4]Julius Wolff Institut, Charité - Universitätsmedizin Berlin, Germany [5]Institute of Bioanalysis, University of Applied Sciences and Arts of Coburg, Germany [6]Frauhofer Institute of Cell Therapy and Immunology, Department of Therapy Validation, Leipzig, Germany

INTRODUCTION: Additive manufacturing (AM) provide the possibility for the generation of scaffolds with unprecedented structural and functional designs. Particularly, scaffolds made of bioresorbable biomaterial show tremendous potential as a temporary tissue expander to support bone defect regeneration. On the contrary to the conventional scaffolds design concept, i.e. raster-angle, this study established a workflow for the generation of scaffolds with triply periodic minimal surface (TPMS) architecture, finite element (FE) analysis for scaffolds' Young's moduli and manufacturing of scaffolds by mean of melt-extrusion based 3D printer. Furthermore, the migration ability of cells in relation to the scaffolds' geometry was evaluated.

METHODS: Scaffolds with TPMS architecture, i.e., Diamond, Gyroid, and Schwarz P, were generated using customized MATLAB script. FE simulation was performed for the scaffolds' Young's modulus and validated experimentally. Melt-extrusion based 3D printer were used for the fabrication of scaffolds with polycaprolactone (PCL). Scaffolds were scanned with micro-computed tomography (µCT) for porosity characterization. After sterilization, scaffolds were seeded with human adipose-derived mesenchymal stromal cells (AdMSCs). Cells migration through the scaffold length was evaluated.

RESULTS & DISCUSSION: This study showed that the understanding of the design-to-manufacturing interface and the limitations of the 3D printer of choice is particularly important to transform digital design concept into reality. In addition, it was showed that although computer-aided generated models can be used as a input for numerical simulation of scaffolds' mechanical properties, the reliability could be impeded by the anomaly that are present in scaffolds due to the 3D printing processes. More notably, this study illustrated that the scaffolds' geometry has an influence on cell migration. Specifically, it was showed that scaffolds with a tighter curvature can better promote AdMSCs migration along the scaffolds' length.

CONCLUSIONS: The advances in AM workflow provide limitless possibilities for scaffold designs with non-non-validated functional properties. Hence, effort should drive to systematic characterization of cell behaviors in relation to scaffolds' geometries. In the future, such knowledge will be essential for the adoption of shape optimization procedures for the design of scaffolds intended for TERM with predictable functional properties.



The influence of cyclic tensile strain and collagen structure on the alignment and proliferation of multiple vascular cell types

P.S. Mathieu[1, 2], E. Fitzpatrick[1, 2], P.A. Cahill[3], C. Lally[1, 2]
Presenting Author: Pattie Mathieu, mathieup@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland [2]Department of Mechanical & Manufacturing Engineering, School of Engineering, Trinity College Dublin, Ireland [3]School of Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland.

INTRODUCTION: In-stent restenosis is characterized by excessive cell proliferation, which may be driven by the proliferation of vascular smooth muscle cells (VSMC) [1] and multipotent vascular stem cells (MVSC) [2]. In vitro, VSMC reorient perpendicular to uniaxial strain [3], however, in vivo VSMC align parallel to dominant strain direction. In order to explore the role of strain and collagen structure in directing vascular cell alignment and subsequent proliferation, rat aortic VSMC (RASMC) and rat MVSC were first exposed to uniaxial cyclic tensile strain alone. Then, the response of rMVSC was determined while seeded on decellularized arteries aligned parallel or perpendicular to the direction of strain.

METHODS: RASMC and rMVSC were cultured on PDMS and strained at 0-10%, 1Hz, uniaxial strain for 24 or 72 hr. Rat MVSC were seeded on decellularized arteries and strained at 0-10%, 1Hz, uniaxial strain parallel or perpendicular to collagen fiber direction for 10 days. Cell number and alignment, and the percentage of Ki67 positive nuclei was determined.

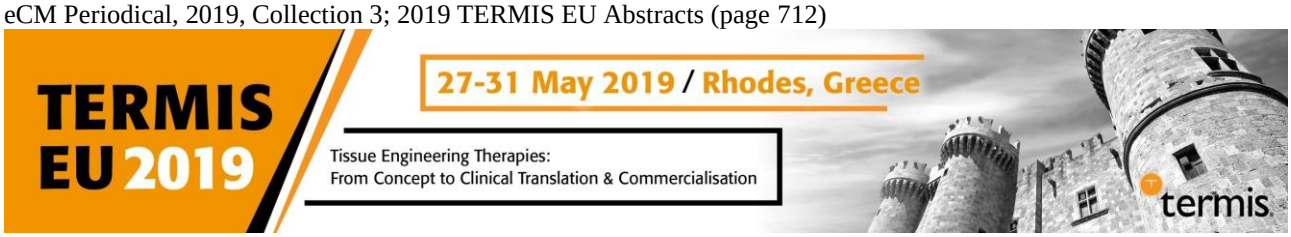
RESULTS & DISCUSSION: On cells seeded on PDMS, both rMVSC and RASMC aligned perpendicular to strain within 24hr, with rMVSC showing greater alignment at 24hr and less at 72hr. Strained cells showed decreased cell number by 72hr but not at 24hr. The rMVSC on decellularized vessels maintained alignment parallel to the fiber direction. Rat MVSC strained parallel to the fiber direction showed increased proliferation when compared to unstrained cells, but cells strained perpendicular to the fiber direction did not.

CONCLUSIONS: These are the first experiments to study the response of MVSC to uniaxial cyclic tensile strain and collagen structure. Both rMVSC and RASMC exhibit strain-avoidant behavior. Rat MVSC show a higher degree of alignment at the shorter time point, possibly indicating that these cells are critical in early strain response. These experiments have also demonstrated that underlying ECM alignment is critical to rMVSC response to strain. This suggests that any intervention that influences collagen fiber direction will change how the cells sense the strain.

ACKNOWLEDGEMENTS: Funding from Science Foundation Ireland (13/CDA/2145).

REFERENCES

[1] Newby (et al.), Toxicoll Lett 112-113:519-529, 2000
[2] Tang (et al.), Nat. Commun. 3:875, 2012
[3] Rodriguez (et al), Arterioscler Thromb Vasc Biol 35:430-438, 2015



High-porous 3D printed calcium phosphate implants capable for functionalization with mesenchymal stem cells or anticancer drugs

P.A. Karalkin[1], N.S. Sergeeva[1], I.K. Sviridova[1], V.A.Kirsanova[1], S.A.Akmedova[1], Y.D.Shanskiy[1], D.M. Zuev[2], E.S. Klimashina[2], P.V. Evdokimov[2], V.I. Putlyaev[2]
Presenting Author: Pavel Karalkin, pkaralkin@gmail.com
[1]P.A. Hertsen Moscow Oncology Research Center – branch or NMRRC, Moscow, Russia [2]Faculty of material science, Lomonosov Moscow State University, Moscow, Russia

INTRODUCTION: Substitution of critical bone defects due to injuries, osteoporosis or oncological diseases is an important problem for biomedical research [1]. The evolved promising "regenerative approach" emphasizes the creation of suitable conditions for gradual substitution of the biomaterial with a new-formed bone tissue [2]. In our work, we studied novel 3D printed calcium phosphate-based implants capable for stem cells seeding or saturation with anticancer drugs.

METHODS: Stereolithography 3D printing was used to create bioceramic implants based on non-stoichiometric β-TCP/HA biphase ceramic powder with granulometry 0.3-1 μm. Implants were printed according Kelvin 3D model modified by topological optimization. The biocompatibility and osteoconductive properties of implants were assessed in vitro using human adipose-derived mesenchymal stem cells, and in vivo in a rat tibia defect model. In addition, 3D constructs were saturated with doxorubicin and cisplatin admixed to coating gel consisted of UV-polymerized PEG diacrylate. Drug release kinetic was studied using simulating body fluid (SBF) model.

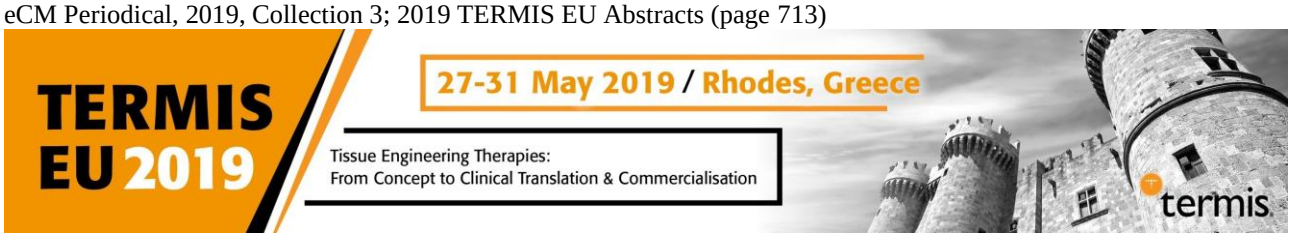
RESULTS & DISCUSSION: 3D printed constructs possessed an interconnected system of macropores (500-1000 μm in diameter), necessary for the penetration of blood vessels and nutrients inside and through the implants. The saturation of the outer PEG-DA layers with the anticancer drugs resulted in their gradual release for at least 5 days. The constructs demonstrated favourable cytocompatibility and provided suitable 3D conditions for adipose-derived MSCs seeding — after 7 days of cultivation over 80% of the surface was uniformly covered with cells according to MTT staining. In the model of the tibia defect, an intensive formation of new bone tissue at the border of the implant material and uniform colonization of the pores with bone marrow cells were shown. The gradual bioresorption of implanted 3D printed constructs exceeded the corresponded rate for calcium phosphate cement commonly used in the clinic practice.

CONCLUSIONS: Collectively, these data illustrate the possibility to create the osteoconductive calcium phosphate implants capable for bone tissue engineering and local drug delivery by means of additive manufacturing technologies.

ACKNOWLEDGEMENTS: The reported study was funded by RFBR according to the research project № 18-29-11070.

REFERENCES

[1] de Grado GF et al. J Tissue Eng. 2018;9: 2041731418776819
[2] Black CRM et al. Curr Mol Biol Reports. 2015;1(3):132-140



Orthotopic bone regeneration within 3D printed region-dependent porous bioceramic scaffolds in an equine model

P. Diloksumpan[1], R. Vindas Bolaños[2], S. Cokelaere[1], B. Pouran[3], J. de Grauw[1], M. van Rijen[3], P. R. van Weeren[1], R. Levato[3,4], J. Malda[1,3,4]
Presenting Author: Paweena Diloksumpan, p.diloksumpan@uu.nl
[1]Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University [2]Universidad Nacional, Costa Rica [3]Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, Utrecht [4]Regenerative Medicine Center, Utrecht, Utrecht University, The Netherlands

INTRODUCTION: Physiological temperature-setting, printable calcium phosphate (PCaP)-based material offers several possibilities for bone tissue engineering via both osteoconductive and osteoinductive mechanisms [1], depending on the biochemical composition and its pore network architecture. In this study, the in vivo long term performance of novel PCaP scaffolds with different internal porosities on bone regeneration was performed in large animal (equine) model.

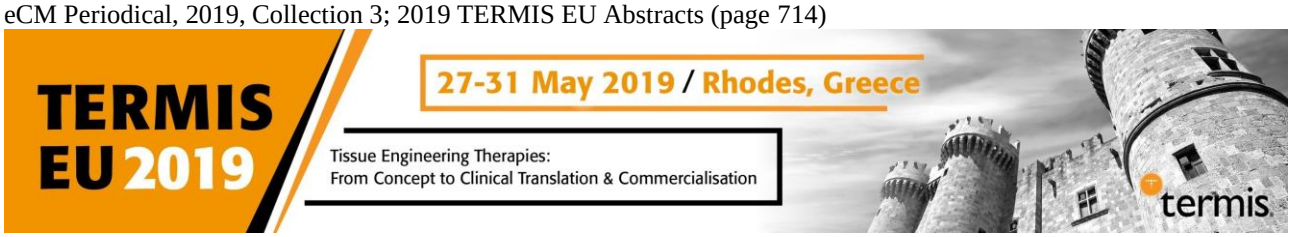
METHODS: A PCaP paste was developed by combining α-tricalcium phosphate, nano-hydroxyapatite and biodegradable crosslinkable poloxamer [2]. Two types of porous cylindrical architecture (∅ = 9.8 mm, H = 9.5 mm) were fabricated by using a pneumatic extrusion-based printer. Gradient porous scaffolds were produced with four vertically consecutive step-downs in strand-to-strand size (750, 650, 550, and 450 µm, respectively) while scaffolds with constant porosity were produced with a strand-to-strand distance of 750 µm. All scaffolds were printed with a 0-0-90-90° laydown pattern, to ensure constant lateral pore size (500 µm) and with two non-macroporous layer on top. Scaffolds with either a biomimetic gradient or a constant pore distribution were implanted after being encased in a polycarprolactone (PCL, inner ∅ = 10 mm, outer ∅ = 10.47 mm, H = 10 mm) chamber to prevent infiltration of cells from the periosteum allow bone ingrowth from single direction. Bone defects were created at tuber coxae of the ilium in eight horses (one defect per side). Each horse received both architectures (scaffold type/defect side pairs were selected randomly). The open side of PCL chamber was contacted with native bone. After 7 months of implantation, the animals were euthanized and tissues harvested for µ-CT quantification and histological analysis.

RESULTS & DISCUSSION: The defect site was divided into three main zones having equal volumes. The amount of new bone ingrowth in the constant porosity (109.80 ± 54.50 mm^3) scaffold group was significantly higher than gradient scaffold (31.49 ± 11.02 mm^3) group, particularly in the bottom and middle of scaffold. Similarly, the estimated percentage of ceramic degradation in the constant porosity group (59.26 ± 13.52%) was significantly higher compared to gradient group (38.38 ± 11.07%).

CONCLUSIONS: The macro-pore pattern of scaffolds, produced from the same composite material, exhibits the essential influences on volume and advance of new bone ingrowth, as well as on material degradation rates.

REFERENCES

[1] A. Barba et al. Acta Biomaterialia. 2018; 79: 135-147
[2] Ferry P W Melchels et al. Biofabrication 8. 2016; 1-9



Engineering phenotypically stable articular cartilage using tissue-specific extracellular matrix inspired scaffolds

P.J. Díaz-Payno[1], D.C. Browe[1], F.E. Freeman[1], J. Nulty[1], R. Burdis[1], D.J. Kelly[1]
Presenting Author: Pedro J. Díaz-Payno, diazpap@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.

INTRODUCTION: Clinical gold standards to repair damaged articular cartilage (AC) can result in chondrocyte hypertrophy with poor long-term outcomes [1]. The use of tissue-specific proteins to produce biomimetic extracellular matrix (ECM) scaffolds represents a promising strategy for regenerating phenotypically stable AC [2]. The objective of this study is to probe the matrisome of AC for key proteins that regulate the chondrocyte phenotype, and to stimulate engineered cartilage with recombinant versions of such factors in an attempt to engineer more phenotypically stable AC.

METHODS: AC and calcified growth plate cartilage (GP) from porcine knees were processed for: i) mass spectrometry (MSMS) and Matrisome and String database (DB) analysis or ii) production of pepsin-solubilised ECM. Freeze-drying was used to fabricate AC ECM scaffolds (Φ5xh3mm). Scaffolds, seeded with bone marrow cells were cultured in vitro 4-5 weeks in chondrogenic media (CM) ± Grem1. Biochemical, histological and PCR analysis were performed to determine tissue quality.

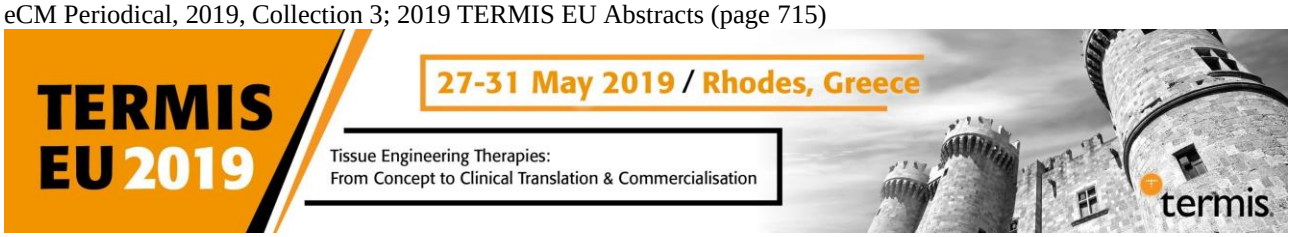
RESULTS & DISCUSSION: DB analysis demonstrated that GP and AC contained a distinct array of proteins (>800). Grem1, a known BMP antagonist [3], was identified as a AC-specific factor. Scaffolds supplemented with CM+Grem1 showed comparable levels of chondrogenesis but lower calcification (Alizarin Red staining), lower type I collagen deposition and lower COL10A1 mRNA expression than as scaffolds with CM only.

CONCLUSIONS: In conclusion, this study demonstrated Grem1 to have an anti-hypertrophic effect for cartilage engineered within AC ECM scaffolds. We are currently exploring the in vivo phenotypic stability of such in vitro engineered cartilage.

ACKNOWLEDGEMENTS: SFI.12/IA/1554, IRC.GOIPG/2015/3156 and Enterprise Ireland.

REFERENCES

[1] Makris EA et al. Nat. Rev. Rheumatol. 2015;11:21-34
[2] Cunniffe GM & Diaz-Payno PJ et al. Biomaterials 2018;188:63-73
[3] Leijten JCH et al. Arthritis Rheum. 2012;64:3302-3312



Bioinstructive Naringin micelles for driving stem cell osteodifferentiation

P. Lavrador[1], V. M. Gaspar[1], J. F. Mano[1]
Presenting Author: Pedro Lavrador, pedrolavrador@ua.pt
[1]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

INTRODUCTION: Naringin is a citrus-derived flavanone currently emerging as a promising anti-osteoporotic and osteogenic phytotherapeutic [1]. However, its bioactivity and ultimately its biomedical applications are hindered by poor in vivo bioavailability as well as lacking optimal cell internalization [1]. Nanocarrier delivery systems are well-recognized to improve entrapped therapeutics' solubility and increase their cellular uptake. In this sense, the delivery of Naringin to undifferentiated mesenchymal stem cells was explored for the first time to potentiate its biological activity over free drug [2].

METHODS: Naringin-loaded micelles were obtained by self-assembly of synthesized mPEG-PLA copolymer via nanoprecipitation. Micelle biocompatibility and cellular uptake were confirmed by metabolic activity assays and fluorescence microscopy/flow cytometry. Then, the osteogenic activity of the bioinstructive system was studied in adipose-derived mesenchymal stem cells (ASCs) through ALP, Alizarin Red S and osteopontin immunocytochemistry.

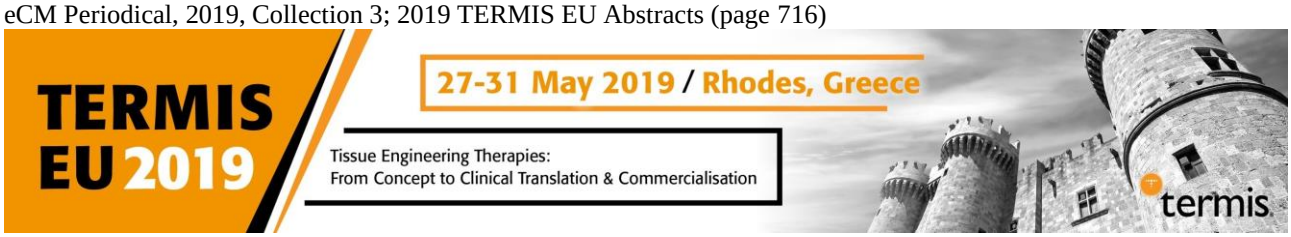
RESULTS & DISCUSSION: The amphiphilic mPEG-PLA copolymer self-assembled in water into monodisperse nanomicelles (85 ± 2 nm) with high Naringin encapsulation efficiency (87 ± 5 %). AlamarBlue metabolic activity assays confirmed the highly biocompatible nature of the Naringin-loaded nanomicelles. Fluorescence microscopy and flow cytometry showed efficient cellular uptake in ASCs. Naringin delivery improved ALP activity in earlier timepoints, accordingly 3 and 7 days. Key osteogenic markers such as osteopontin immunocytochemistry and calcium node deposition were highly augmented in the bioinstructive micelle groups. ALP activity analysis with different dose regimen also highlighted the superior osteogenic potential of Naringin-loaded micelles over free drug.

CONCLUSIONS: The biocompatible micelles internalized efficiently in ASCs and elicited an augmented osteogenic differentiation over free drug administration.

ACKNOWLEDGEMENTS: Financial support was received from an ERC grant for project ATLAS (ERC-2014-ADG-669858), FCT (SFRH/BD/141834/2018, Pedro Lavrador, SFRH/BPD/119983/2016, Vítor Gaspar), FCT/MEC for project CICECO (POCI-01-0145-FEDER-007679, FCT UID/CTM/50011/2013).

REFERENCES

[1] Lavrador P et al. Drug Disc Today. 2018; 23(6):1293-1304
[2] Lavrador P et al. Adv Healthc Mater. 2018;7(19):e1800890



Novel multimodal imaging based quantitative assessment approach to evaluate angiogenesis and vascular architecture: Combined MRI and MicroCT imaging in the CAM assay

P. Wolint[1*], A. Woloszyk[1,2,3*], A. Becker[4], A. Boss[4], W. Fath[3], Y. Tian[1], P. Giovanoli[1,5], M. Calcagni[1,5], S. P. Hoerstrup[2,6], J. Buschmann[1,5**], M. Y. Emmert[2,6**]
Presenting Author: Petra Wolint, petra.wolint@usz.ch
[1]Division of Surgical Research, University Hospital of Zurich, Zurich, Switzerland [2]Institute for Regenerative Medicine (IREM), University of Zurich, Zurich, Switzerland [3]Department of Orthopaedics, University of Texas Health Science Center San Antonio, San Antonio, TX, USA [4]Institute for Diagnostic and Interventional Radiology, University Hospital of Zurich, Zurich, Switzerland [5]Plastic Surgery and Hand Surgery, University Hospital of Zurich, Zurich, Switzerland [6]Wyss Translational Center Zurich, University Zurich & ETH Zurich, Zurich, Switzerland, *shared first authorship, **shared last authorship

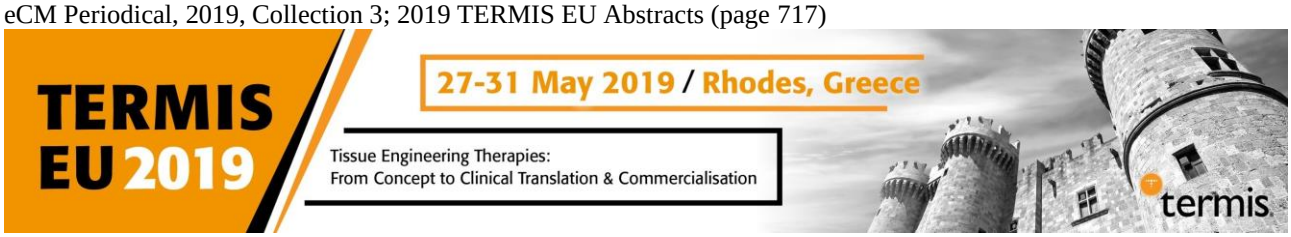
INTRODUCTION: The quantitative assessment of vessel architecture and functional perfusion capacity in biomaterials for regenerative medicine approaches requires high-tech analytical methods. Besides ultrasound, optoacoustic imaging or two-photon microscopy, magnetic resonance imaging (MRI) and microcomputed tomography (MicroCT) are prime examples to achieve a precise, reliable and robust readout. The chicken embryo model with its chorioallantoic membrane (CAM) offers an assay for evaluation of vascularization of tissue-engineered constructs with short experimental times with high grade outcome.

METHODS: In vivo perfusion capacity with MRI and ex vivo angio-architecture by MicroCT was assessed in the same scaffold. The readouts were supported by comprehensive histological analysis comprising blood vessel density and characteristics. Two different potential biomaterials, for bone regeneration, a synthetic and a natural one, were evaluated. Natural porcine collagen-based Optimaix™ (Matricel) has a laminar structure, which mimics aligned entities found in the diaphysis. Synthetic DegraPol® (Ab medica SpA), on the other hand, was chosen as its highly porous structure resembles spongy bone.

RESULTS & DISCUSSION: The newly developed pump-assisted perfusion technology for the CAM model allows for a more even, reliable and efficient distribution of contrast agent in the vessel system compared to the conventional manual injection. Perfusion capacity (MRI) and total vessel volume (MicroCT) strongly correlated for both biomaterials. In addition, a detailed analysis of branches, junctions, vessel length and radius confirmed the different vessel architectures in the two biomaterials. It was found that the highly porous DegraPol® exhibited significantly higher numbers of branches (3.5-fold), number of junctions (5-fold), total branch length (2.3-fold), however, lower number of branches per junction (0.65-fold), mean branch length (0.87-fold), and lower mean radius (0.76-fold) when compared to Optimaix™.

CONCLUSIONS: Here, we demonstrate for the first time the functional and structural analysis of the vascularization in biomaterials after one week of incubation on the CAM. Based on these results, we conclude that the two imaging methods, MRI and MicroCT, allow for a comprehensive analysis of the vascularization pattern and efficiency of biomaterials in a cost-effective, well-established and easy-to-handle immune-incompetent preclinical model.

ACKNOWLEDGEMENTS: Financial support was received from Hartmann Müller-Foundation.



Enhanced tissue integration of a composite hydrogel for load-bearing applications

P. Karami[1], C. Wyss[2], A. Khoushabi[2], A. Schmocker[3], M. Broome[4], C. Moser[3], P-E Bourban[2], D. Pioletti[1]
Presenting Author: Peyman Karami, peyman.karami@epfl.ch
[1]Laboratory of Biomechanical Orthopedics, EPFL, Switzerland [2]Laboratory for Processing of Advanced Composites, EPFL, Switzerland [3]Laboratory of Applied Photonics Devices, EPFL [4]Department of Maxillofacial Surgery, Lausanne University Hospital, Switzerland

INTRODUCTION: Integration of injectable hydrogels on biological surfaces is a key feature in minimally invasive tissue engineering approaches. However, insufficient mechanical and adhesive properties at high level of water content and the need for tissue treatment limit the clinical translation of injectable hydrogels for load-bearing tissues such as articular cartilage. Moreover, using commercial tissue adhesives such as fibrin glue and COSEAL for implantation of pre-formed scaffolds result in weak adhesion to tissues [1-4]. To address these limitations in cartilage tissue engineering, we developed a highly adhesive and injectable hydrogel with enhanced mechanical performance, which can be formed in situ without any cytotoxicity.

METHODS: The developed nanofibrillated cellulose (NFC) reinforced double-network hydrogel was synthesized by mixing PEGDMA, calcium sulphate, NFC, and Irgacure 2959 in distilled water. The mixture was homogenized at 12000 rpm and sodium alginate was added. The PEGDMA molecules were crosslinked by ultraviolet irradiation. Tissue samples were prepared from bovine articular cartilage.

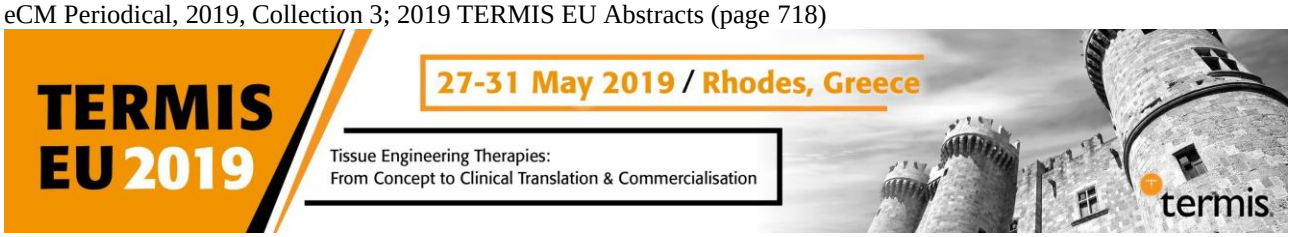
RESULTS & DISCUSSION: By implementing various toughening mechanisms in our designed biomaterial, interface integration is maintained and the crack propagation is controlled under loading. This composite double-network hydrogel significantly increases the adhesive and cohesive fracture resistance while containing ~90% water in its structure. The proposed dissipative hydrogel exhibits adhesive properties which are superior to clinically used glues. In particular, the attachment of the developed hydrogel presents ~10 times higher adhesion strength to articular cartilage than that of the currently available tissue adhesives such as Tisseel. The Cytocompatibility, enhanced mechanical properties, swelling, and injectability of the developed hydrogel were also demonstrated.

CONCLUSIONS: Considering the remarkable features of the hydrogel, our approach can facilitate development of minimally invasive adhesives for clinical translation.

ACKNOWLEDGEMENTS: This work was supported by a SNF grant # CR23I3_159301.

REFERENCES

[1] Rose S et al. Nature. 2014; 505:382-5
[2] Cui X et al. Tissue Eng Part A; 2012; 18:1304-12.
[3] Wang DA et al. Nat Mater. 2007; 6:385-92
[4] Yuk H et al. Nat Mater. 2016; 15:190-6



Tuning the composition of fibrin bioinks to enhance chondrogenesis of human MSCs in 3D bioprinted implants

P. Pitacco[1,2], F. Freeman[1,2], D.J. Kelly[1,2,3]
Presenting Author: Pierluca Pitacco, ppitacco@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland [2]Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland

INTRODUCTION: Designing a bioink often requires a compromise between formulations that support high shape fidelity bioprinting (e.g. appropriate ink viscosity & yield stress) and those that are optimal for supporting cell migration, growth, and differentiation [1]. Fibrin is a natural biopolymer commonly used as biomaterial for tissue engineering and is in widespread clinical use (typically at high concentrations, 60–120 mg/ml). Fibrin can also be made printable through the use of a gelatin-based carrier gel, and has been used to bioprint multiple tissues [2]. The goal of this study was to explore how fibrin concentration regulates chondrogenesis of human bone marrow MSCs (hMSCs) within fibrin based bioinks, and to reinforce these bioinks with networks of 3D printed PCL for their future use in endochondral bone tissue engineering.

METHODS: Two bioinks composed of gelatin, hyaluronic acid, glycerol and two different concentrations of fibrinogen (3.5 & 5 mg/ml) [2], and containing hMSCs ($5x10^6$ cells/ml), were 3D-printed inside PCL structures and crosslinked at 20 °C in a thrombin bath (20IU/mL in DMEM) for 30 minutes. Constructs were then cultured in chondrogenic media (containing TGF-β3) supplemented with 10µl/ml Aprotinin, for 3 weeks at 5% pO_2 followed by 2 weeks at 20% pO_2. Biochemical assays (DNA, sGAG and Hydroxyproline) were performed at day 0, 21 and 35. Alcian Blue, PicroSirius Red and Alizarin Red staining were also undertaken.

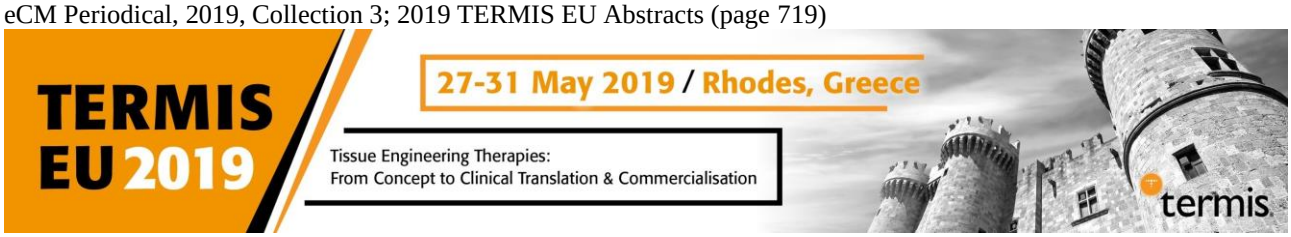
RESULTS & DISCUSSION: For both fibrin concentrations, sGAG and collagen deposition increased with time in culture, with significantly higher levels of collagen deposition observed in the 3.5% group at day 35. This was also observed histologically, with homogenous staining for sGAG and collagen observed after 35 days of culture.

CONCLUSIONS: Taken together, these data demonstrate that both fibrin bioinks support robust chondrogenesis, with the softer 3.5% fibrin supporting higher levels of collagen deposition. We have already shown with other materials [3] that in vivo degradation is a key parameter for better healing. We expect that the lower material density bioink will degrade faster in vivo, allowing better infiltration and integration with host tissue. Future studies will explore the potential of these fiber reinforced cartilage templates for the regeneration of large bone defects by recapitulating the developmental process of endochondral ossification.

ACKNOWLEDGEMENTS: Financial support was received from European Research Council (JointPrinting, n°647004); and Trinity College Dublin (Postgraduate Ussher Fellowship).

REFERENCES

[1] Malda J et al. Adv Mater 2013; 25, 5011-5028.
[2] Kang H W et al. Nat Biotechnol. 2016; 34(3), 312-319.
[3] Daly A C et al. Biomat 2018; 162, 34-46.



Innovative injectable allograft for cranio-facial bone regenerative medicine

P. Tournier[1,2,3], A. Paré[1,3], J. Veziers[1,3,4,5], A. Barbeito[2], R. Bardonnet[2], P. Corre[1,3,6], V. Geoffroy[1,3], A. Gaudin[1,3,4], J. Guicheux[1,3,4], P. Weiss[1,3,4]
Presenting author: Pierre Tournier, pierre.tournier@univ-nantes.fr
[1]Inserm, UMR 1229, RMeS, Université de Nantes, ONIRIS, Nantes, France [2]BIOBank SAS, Presles-en-Brie, France [3]Université de Nantes, UFR Odontologie, Nantes, France [4]CHU Nantes, PHU4 OTONN, Nantes, France [5]INSERM, UMS 016, CNRS 3556, Structure Fédérative de Recherche François Bonamy, SC3M facility, CHU Nantes, Université de Nantes, Nantes, France [6]CHU Nantes, Service Chirurgie maxillo-faciale et Stomatologie, PHU4 OTONN, Nantes, France

INTRODUCTION: In the cranio-maxillofacial area, bone allografts are extensively used for skeletal regeneration in several forms (blocks, powders…). To improve their use in irregular or hardly accessible sites [1], we have developed an allogenic bone paste injectable in a cohesive form, made of partially demineralized and heated bone powder. The purpose of this work is to investigate the in vivo bone regenerative capacity of this bone paste and its in vitro ability to stimulate mesenchymal stromal cells (MSC) osteogenic properties as well as polarize macrophages towards anti-inflammatory phenotype.

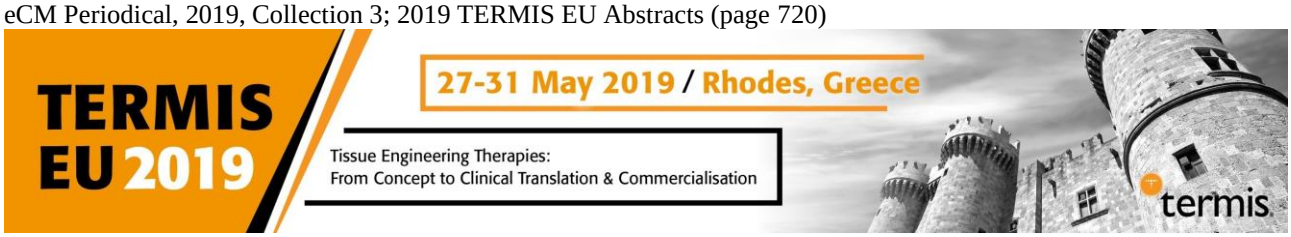
METHODS: In vivo: Critical size defects were performed on syngeneic Lewis1A rat calvaria (2x5mm per rat, n=6/condition). Defects were filled with bone powder (0.5-1mm), bone paste or left unfilled (control). After 7 weeks, the mineral volume (MV)/total volume (TV) ratio was measured by micro-CT and bone tissues was histologically analysed. In vitro osteogenic properties: human MSC from bone marrow (hBM-MSC) were grown in proliferative or osteogenic medium and changed every other day for 21 days. Afterwards, the alkaline phosphatase (ALP) mRNA expression and enzymatic activity were quantified. In vitro macrophage polarization: human monocytes isolated from circulating blood were cultured in presence of 20ng/mL of GM-CSF (inflammatory environment) or its vehicle, in contact with powder or paste for 3 days. Their resulting phenotypes were evaluated thanks to the mRNA expression of a panel of pro-inflammatory (IL-12, IL-6, TNF-α) and pro-regenerative factors (IL-10, TGF-β, VEGF).

RESULTS & DISCUSSION: After 7 weeks of implantation, histological and micro-CT analyses revealed the formation of new bone tissue in the filled defects. MV/TV was significantly higher in the defects filled with bone paste as compared to those filled with bone powder ($p<0.01$) or left empty ($p<0.001$). RT-qPCR analyses indicated that ALP mRNA expression in hBM-MSC cultured on bone paste was significantly higher that of cells cultured on bone powder ($p<0.01$). ALP enzymatic activity was similarly found to be higher in hBM-MSC cultured in contact with the bone paste ($p<0.01$). Interestingly, macrophages in contact with bone paste showed increased pro-regenerative factors mRNA expression (IL-10 and VEGF) in comparison with bone powder.

CONCLUSIONS: Our data demonstrated the substantial in vivo bone regenerative capacity of the bone paste. We also demonstrated that bone paste is able to support the in vitro osteogenic differentiation of hBM-MSC and contribute to favors the switch of inflammatory macrophages to pro-regenerative ones. Taken together, these data highlight the potential of this innovative bone paste as an injectable allogeneic bone substitute.

REFERENCES

[1] Wang, W. et al. Bioactive Materials 2, 224–247 (2017)



Decellularized caprine ear cartilage in osteochondral defects repair

P. Das[1], B. Devi[2], R. Kanikerajesh[3], B. Kundu[4], D. Lahiri[3], P. Basak[1], M. Roy[2], S. K. Nandi[5]
Presenting Author: Piyali Das, piyalidas.mcb@gmail.com
[1]School of Bioscience and Engineering, Jadavpur University, Kolkata, India [2]Department of Metallurgical and Materials Engineering, Indian Institute of Technology-Kharagpur, WB, India [3]Department of Metallurgical and Materials Engineering, Indian Institute of Technology Roorkee, Uttarakhand, India [4]Bioceramics and Coating Division, CSIR-Central Glass and Ceramic Research Institute, Kolkata, India [5]Department of Veterinary Surgery and Radiology, West Bengal University of Animal and Fishery Sciences, Kolkata, India

INTRODUCTION: Reconstruction and repair of cartilage is difficult due to its inability to self-healing and regeneration. Until today a variety of replacement matrices and scaffolds with suitable microenvironment is abandoned. However, this study concentrates on developing acellular xenogenic cartilage matrices as an alternative in biomedical and clinical applications.

METHODS: The full thickness ear cartilage derived from caprine was decellularized post slaughter with Triton X-100 and hypertonic-hypotonic solution [1, 2]. The effect of decellularizing agents on cells and extracellular matrix (ECM) was analyzed by H&E staining, DNA content, scanning electron microscopy (SEM), proteoglycan, collagen, elastic fiber staining and biochemical quantization, nanoindentation study (mechanical property), swelling behavior and in vitro hemocompatibility study. The developed materials were further characterized for in vivo biocompatibility (rabbit) subcutaneously and later on analyzed by gross observation, histology and biochemical study. Ultimately, acellular cartilages were implanted in osteochondral defects in rabbit for 3 months followed by histology and micro-CT study.

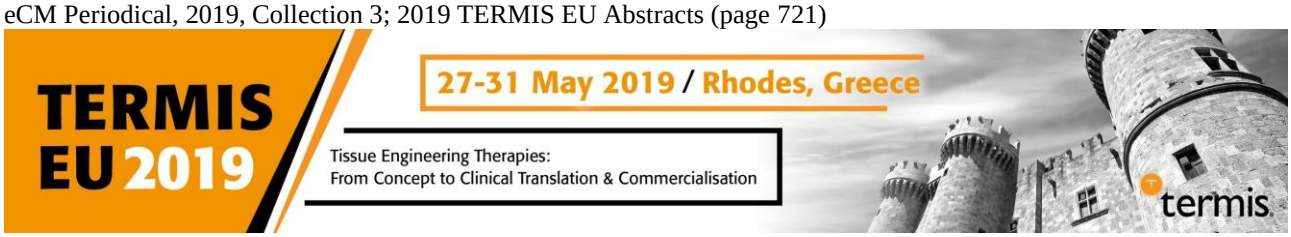
RESULTS & DISCUSSION: Complete cellular loss was ascertained by H&E staining and DNA ($p \leq 0.5$). However proteoglycan was significantly reduced ($p \leq 0.5$). Additionally, the acellular materials were confirmed to be non- reactive and non hemolytic biomaterials as assessed by in vitro hemocompatibility study. The in vivo experiment revealed that the developed acellular cartilages are biocompatible. Upon implantation in rabbit osteochondral defects, the gross observation, histological and micro-CT studies depicted that these acellular matrices significantly boosted the regeneration of cartilage along with the subchondral bone tissue.

CONCLUSIONS: Therefore, the successful outcome opens a new approach to design cost effective acellular xenogenic cartilage biomatrices with versatile physical characterizations that can be applied to numerous areas of tissue engineering.

ACKNOWLEDGEMENTS: We are thankful to DBT, Govt. of India for funding and the Vice Chancellor of West Bengal University of Animal and Fishery Sciences, Kolkata, India.

REFERENCES

[1] Crapo PM et al. Biomaterials, 2011; 32(12). 3233-3243
[2] Elder BD et al. Biomaterials, 2009; 30, 3749–3756



Low-intensity LED irradiation improves cell viability in 3D scaffolds

P.Y. Bikmulina [1], N.V. Kosheleva [2,3], A.I. Shpichka [1], P.S. Timashev [1], Y.A. Rochev [1,4]
Presenting Author: Polina Y. Bikmulina, polina_bikmulina@mail.ru
[1]Institute for Regenerative Medicine, Sechenov University, Moscow, Russia [2]Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia [3]FSBSI Institute of General Pathology and Pathophysiology, Moscow, Russia [4]National University of Ireland, Galway (NUI Galway), Galway, Ireland

INTRODUCTION: The development of 3D scaffold systems for regenerative medicine is limited due to the lack of oxygen and nutrients in macrostructures. One of the promising approaches to stimulate cell metabolism, proliferative activity and viability via mitochondrial activation is low-intensity LED irradiation (LILI). Nevertheless, the mechanism of these LILI effects may vary among different cell types. This work aims to reveal LILI effects on viability and metabolic activity of human mesenchymal stem cells (MSC), human neuroblastoma (Sk-N-BE(2)), and mouse embryonic fibroblasts (3T3) immobilized within 3D hydrogels.

METHODS: Three cell types were used – primary MSC culture derived from the bone marrow, neuroblastoma cell line Sk-N-BE(2), fibroblast cell line 3T3. They were cultured in full growth medium (DMEM/F12 supplemented with 10% FBS (HyClone, USA)). For 3D culture, cells were stained with Hoechst 33258 (Sigma, USA) and MitoTracker Green FM (ThermoFisher, USA) and encapsulated in the PEGylated fibrin hydrogel [1, 2] at a concentration of 1×10^4cell per 200 ml of gel, the resulting gel thickness was 1.5 or 3 mm. Continuous non-thermal LILI was performed using two LED types: 1) 633nm, 1200s, 22kJ/m^2 and 2) 840nm, 600s, 21kJ/m^2. Cultures were monitored via time-lapse microscopy using CellInsight CX7 (ThermoFisher, USA). Live/Dead, MTT, and Alamar blue assays were used to study cell viability. PicoGreen assay were applied to reveal the amount of cells. Morphology in 3D cell culture was studied via scanning electron microscopy.

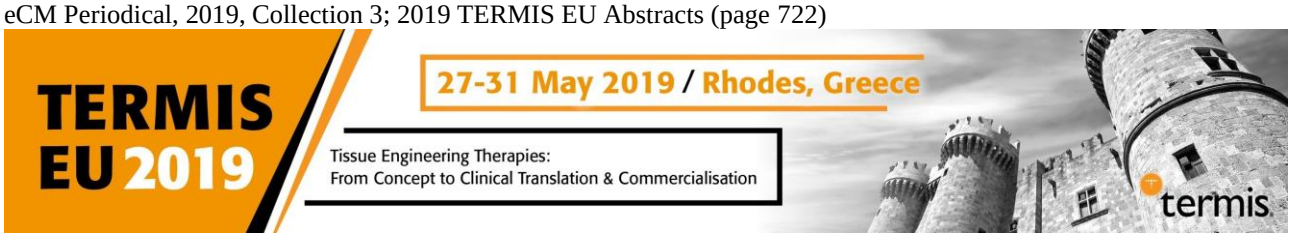
RESULTS & DISCUSSION: Cells encapsulated with the gel had the reduced proliferative activity. For instance, after 24 hours of cultivation, the proliferation of MSC was 35% (in 1.5 mm gels) or 60% (in 3 mm gels) less than that in 2D cultures. When irradiated during encapsulation, MSC proliferated only 15% and 25% less than those in 1.5 mm and 3 mm gels, respectively. The use of LILI during hydrogel polymerization increased cell viability by 10% for all cell cultures. Moreover, the irradiation of cells immobilized within the hydrogel resulted in the increase in mitochondrial activity (15%).

CONCLUSIONS: We showed that LILI of cells suffering from oxygen and nutrient starvation in 3D culture increased both their viability and metabolic activity. The developed approach to improve cellular activity in 3D scaffolds can be widely applied in tissue engineering.

ACKNOWLEDGEMENTS: The study was supported by the Russian Science Foundation (grant № 15-15-00132, hydrogel formation) and the Russian Foundation for Basic Research (№ 17-02-01248, cell viability assays; № 17-02-00832, LED irradiation).

REFERENCES

[1] Shpichka et al. Cell and Tissue Biology. 2017;58(10):785-791.
[2] Gorkun A et al. Biomedical Materials. 2018;13(4): 044108



Architecture and composition of natural polymers affect adipose derived stem cells behaviour: An in vitro and in vivo study

P. Sawadkar[1], N. Mandakhbayar[2], S. Brown[1], P. Rajasekar[3], B. Rahmani[4], JH. Lee[2], HW. Kim[2], J. Knowles[5], E. García-Gareta[1]
Presenting Author: Prasad Sawadkar sawadkarp@raft.ac.uk
[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, UK [2]Institute of Tissue Regeneration Engineering (ITREN), Dankook University, South Korea [3]School of Medicine, University of Nottingham, UK [4]Mechanical engineering department. UCL, UK [5]Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, UCL, UK

INTRODUCTION: Organ failure leads to decreased quality of life and morbidity. Current gold standards for organ transplantation have inherent weaknesses like donor site morbidity, donor shortage, and immuno-compatibility. A tissue engineering approach by using biomaterials fabricated from natural polymers and seeded with autologous stem cells has potential to restore functional organs as they are bio- and immuno-compatible, and stem cells have the capacity for self-renewal and differentiation into specific linages. However, to fabricate these functional tissue equivalents there is limited understanding about interaction between stem cells with natural polymers present in the extracellular matrix (ECM) like collagen, fibrin and elastin[1,2,3]. Therefore, the aim of our study was to investigate how different 3D environments made of proteins found in the natural ECM affect cellular behavior of human adipose derived stem cells (hADSCs) as a preliminary step to design 3D constructs for tissue and organ regeneration.

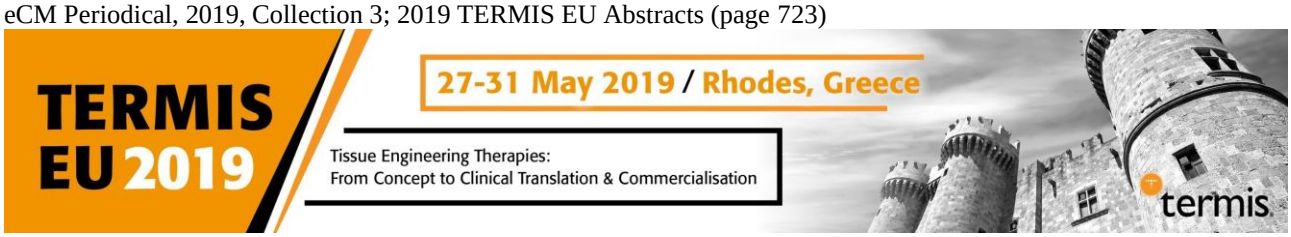
METHODS: Scaffolds from natural polymers (collagen, fibrin and elastin) were fabricated by custom designed processes and their material properties thoroughly characterised. Scaffolds were seeded with hADSCs and implanted in 9 SD rats. In vitro cell studies were carried out where survival, proliferation, and phenotype were investigated. In vivo, output was carried out by haematology, µCT and immuno-histology.

RESULTS & DISCUSSION: Collagen and fibrin scaffolds had fourfold higher swelling ratio than elastin, but elastin was the most stable polymer in culture conditions of all scaffolds. Differentiation results in scaffolds indicated significantly higher ($P < 0.05$) adipogenic phenotype (PPARg and CEBP) than osteo, chondro-genic phenotypes by day 28. In vivo results showed a higher vascular network on collagen and fibrin scaffolds than the elastin scaffold.

CONCLUSIONS: Collagen had higher cell adhesion and proliferation in vitro and integration properties in vivo whereas fibrin had less cell adhesion in vitro but higher vascularization in vivo. Cells on elastin maintained its undifferentiated state in vitro but in vivo elastin was an structurally unstable scaffold. These findings about how polymers present in the natural ECM affect hADSCs behaviour in vitro and in vivo can help designing 3D constructs for tissue and organ regeneration.

REFERENCES

[1] Zhang and Kohn; Birth Defects Research (Part C) 96:63–81, 2012
[2] Daamen E et al., Biomaterials;28(30):4378-98, 2007
[3] Buttafoco L et al., Biomaterials; 27(5):724-34 2006



Modification of mechanical environment to control vascular organization within developing chicken embryo

P. Padmanaban[1], A. Chizari[2], W. Steenbergen[2], J. Rouwkema[1]
Presenting Author: Prasanna Padmanaban, p.padmanaban@utwente.nl
[1]Department of Biomechanical Engineering, Technical Medical Centre, University of Twente, 7522NB Enschede, The Netherlands [2]Biomedical Photonic Imaging Group, Faculty of Science and Technology, University of Twente, 7522NB Enschede, The Netherlands

INTRODUCTION: Vascular tree formation and their network organization has previously been shown to be sensitive to the mechanical environment in vitro, yet the specific relationship between the mechanical factors such as geometry, shape, pressure, flow characteristics and vascular organization in vivo is not well understood. The present study aimed to investigate the effect of yolk shape on vascular network organization, within developing chicken embryos cultured in containers with different geometries, resulting in a different mechanical environment of the egg yolk. Using both laser speckle contrast (LSCI) and laser doppler perfusion imaging (LDPI) techniques, the spatio-temporal changes of heart rate and flow velocity in the vascular networks within developing chicken embryos were determined.

METHODS: PDMS based artificial egg shell systems: 3D geometric (cube, cylinder and triangular prism shaped) containers based on oxygen permeable thin polydimethylsiloxane (PDMS) membranes were assembled using soft lithographic templates. 3D printed poly(lactic acid) frames were used as mechanical support. Fertilized white leghorn chicken eggs were incubated with 38°C and 65% humidity under regular rotation. After 3-days of incubation, chicken embryos were transferred to the artificial geometric culture systems as shown in the figure.

RESULTS & DISCUSSION: Results showed that the heart beat rate (figure -right) and vascular network density were influenced by changing the local mechanical environment of egg yolk. Further, LSCI revealed changes in the perfusion rates within the chick vasculature. Changes in vascular organization with respect to differences in the local microenvironment of the yolk were observed.

CONCLUSIONS: With the ultimate goal to understand in vivo vascular organization, fluid flow and growth factor gradient patterns adjacent to the developing chicken blood vessels will next be introduced in the PDMS systems. This 3D integrated platform offers the possibility to evaluate the effect of multiple signals towards vascular organization in a single system.

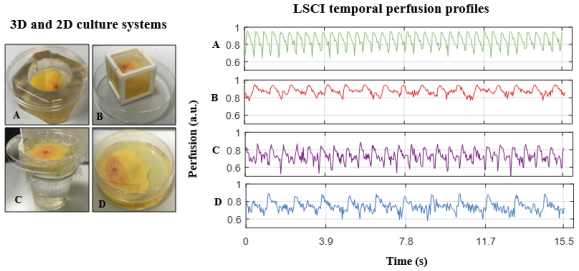
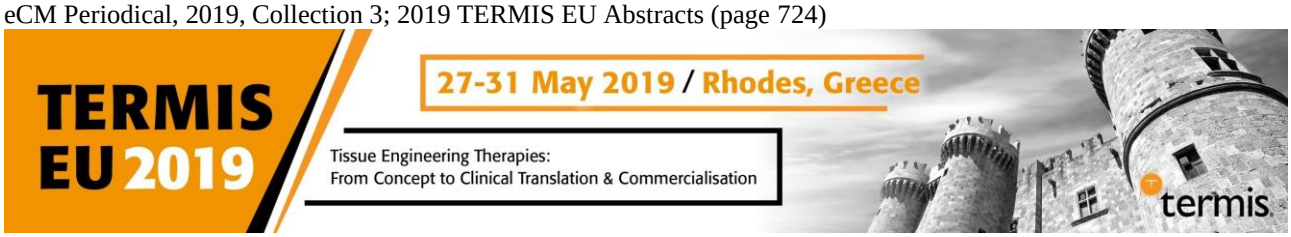


Figure1: (left) chicken embryos exposed to different mechanical environments and (right) variations in perfusion rates of chick vasculature subjected to different mechanical environments

ACKNOWLEDGEMENTS: This work is supported by an ERC Consolidator Grant under grant agreement no 724469 and NWO Dutch funding with project number 14538.



Electrospun nanofibrous scaffolds modified with collagen-I and fibronectin with LX-2 cells to study liver fibrosis in vitro

P. Das[1,2], Jason A. Wertheim[3], L.P. Tan[1,2]
Presenting Author: Prativa Das, PRATIVA001@e.ntu.edu.sg
[1]Interdisciplinary Graduate School, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore [2]School of Materials Science & Engineering, College of Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore [3]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, United States

INTRODUCTION: Three-dimensional microenvironment is a need to study the event cascades of liver fibrosis in vitro [1]. In this study, we fabricate a series of three-dimentional (3D) electrospun scaffolds by wet electrospinning process modified with different ratios of collagen-I to fibronectin to achieve optimized distribution of these two ECM (Extracellular matrix) proteins on the fiber surface to create an in vitro liver disease model by using LX-2 cells.

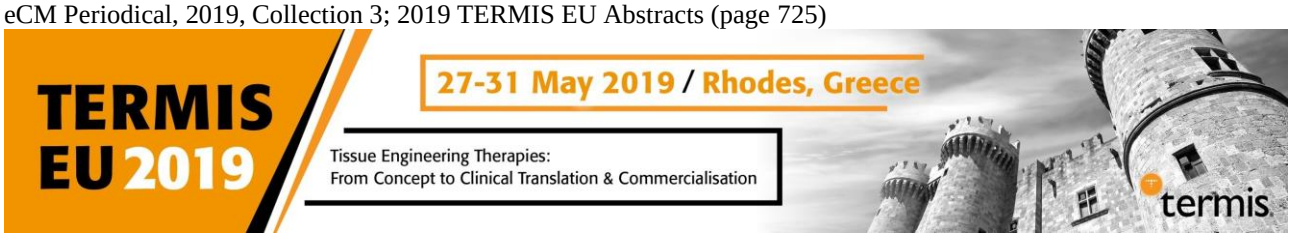
METHODS: 3D electrospun PLGA (poly(lactic-co-glycolic)acid) fibrous scaffolds were modified with rat-tail Collagen-I and plasma fibronectin (Life Technologies, Carlsbad, California, USA) at five different ratios by using chemisorption process (C:F_1:0; C:F_0:1; C:F_1:1; C:F_3:1; CFF_9:1). We have cultured LX-2 cells, a human hepatic stellate cell line on 3D electrospun scaffolds for 14 days. Matrigel culture was used as a negative control for activated hepatic stellate cells whereas TCP (tissue culture plate) culture used as a positive control to the activated phenotype.

RESULTS & DISCUSSION: A ratio of 3:1 of collagen-I to fibronectin was found to be optimum for surface modification of electrospun poly(lactic-co-glycolic acid) (PLGA) fibers by chemisorption process. In 3:1 collagen-I to fibronectin modified scaffolds the total protein content increased by ~2 fold compared to collagen-I modified and ~1.5 fold compared to 1:1/9:1 collagen-I to fibronectin modified scaffolds. We have cultured LX-2 cells on this scaffold over 14 days and found that LX-2 cells acquire more quiescent phenotype throughout the culture period and shown significantly ($p<0.001$) lower expression of alpha smooth muscle actin and collagen-I.

ACKNOWLEDGEMENTS: Financial support was received NTU-Northwestern Institute for Nanomedicine (Nanyang Technological University, Block N3.1, #01-03, 50 Nanyang Drive, Singapore 637553).

REFERENCES

[1] Brown JH et al. Acta biomaterialia 2018;73:217-27.



Use of short phosphate glass fibre reinforcement to promote bone mineralisation on PLLA composites

P. Melo[1], E. Tarrant[2], T. Swift[3], A. Townshend[4], M. German[4], A.M. Ferreira[1], P. Gentile[1], K.W. Dalgarno[1]
Presenting Author: Priscila Melo, P.Melo2@newcastle.ac.uk
[1]School of Engineering, Newcastle University, Claremont Road, NE1 7RU, Newcastle Upon Tyne, United Kingdom [2]Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH UK [3]School of Chemistry and Biosciences, Faculty of Life Sciences, University of Bradford, Bradford, BD7 1DP [4]Centre for Oral Health Research, School of Dental Sciences, Newcastle University, Newcastle Upon Tyne, NE2 4BW, UK

INTRODUCTION: Composites have been introduced for clinical application for their ability to blend the mechanical and chemical properties of multiple materials. In this study medical grade Poly(L-lactic) acid (PLLA) was reinforced with short Ca-doped phosphate-based glass fibres to assess the influence in human mesenchymal stromal cells differentiation due to Ca deposition.

METHODS: The materials were initially mixed by melting PLLA granules with the short fibres, before being extruded to form a homogenous filament, which was pelletized and used as feedstock for compression moulding. Mechanical properties and degradation profile were studied along 8 weeks. Human mesenchymal stromal cells were cultured on the surfaces of scaffolds, and the metabolic activity, alkaline phosphatase production and mineralisation monitored over a three week period[3].

RESULTS & DISCUSSION: As made the composite materials had a bending strength of 51MPa±5, and over the course of eight weeks in PBS the average strength of the composite material was in the range 20-50 MPa. The short fibre reinforcement made no significant difference to cell proliferation or differentiation, but had a clear and immediate osteoinductive effect, promoting mineralisation by cells at the material surface.

CONCLUSIONS: It is concluded that the PLLA/PGF composite material offers a material with both the mechanical and biological properties for potential application to bone implants and fixation, particularly where an osteoinductive effect would be valuable.

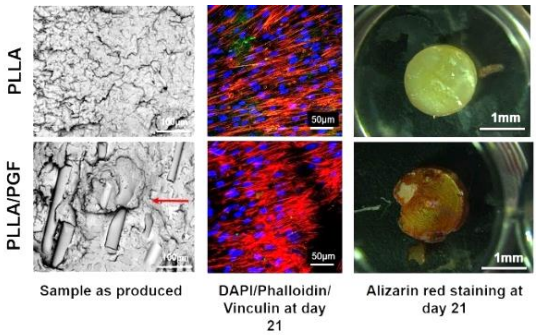
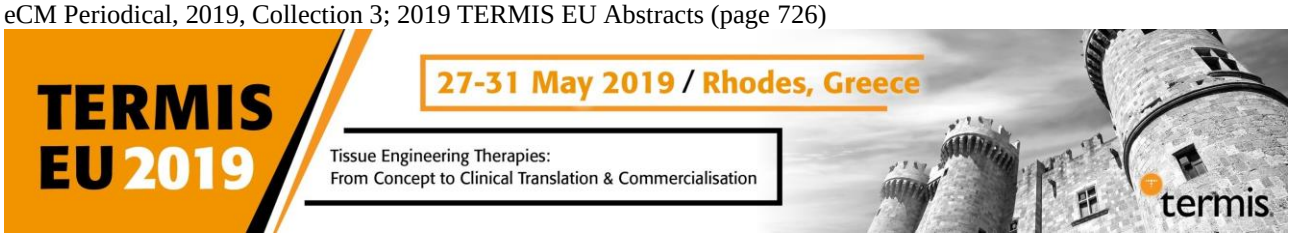


Figure 1: From right to left: SEM image of compression moulded samples; Confocal image of cell morphology and attachment. Blue staining for nucleus (DAPI), red for cell body (Phalloidin) and green the actin filaments representing adherence (Vinculin); Alizarin red staining of cell-seeded PLLA and PLLA/PGF discs.



Engineered bridge proteins with dual affinities for bone morphogenetic protein (BMP)-2 and collagen improve controlled release of BMP-2 for bone regeneration

P.S. Briquez[1], A. Abu-Khalil[1], J.A. Hubbell[1]
Presenting Author: Priscilla S Briquez, pbriquez@uchicago.edu
[1]Institute for Molecular Engineering, University of Chicago, Chicago, USA

INTRODUCTION: BMP-2 is a potent growth factor (GF) to promote bone regeneration. Particularly, it is currently used in the clinic for spine fusion, under the product InFuse® Bone Graft, by Medtronic. However, the clinical safety of BMP-2 has been questioned, due to serious side effects in treated patients, such as ectopic bone formation or nerve injuries. These side effects can be partially imputed to the supra-physiological dose used as well as to the lack of appropriate delivery system [1]. Here, we engineered bridge proteins with strong affinities for both BMP-2 and collagen matrix to control the release of BMP-2, for application in bone regeneration.

METHODS: Bridge proteins were designed as chemical conjugation or recombinant fusion of an anti-collagen I Fab with a GF-binding domain, displaying strong affinity for BMP-2. Release tests of BMP-2 from collagen gels (PureCol, Adv. BioMatrix) were performed by incorporating 500 ng/mL of BMP-2 into collagen gels, with or without bridge proteins (10:1 molar ratio). Release buffer was taken and refreshed every day for 6 days, and the amount of released BMP-2 was quantified by ELISA.

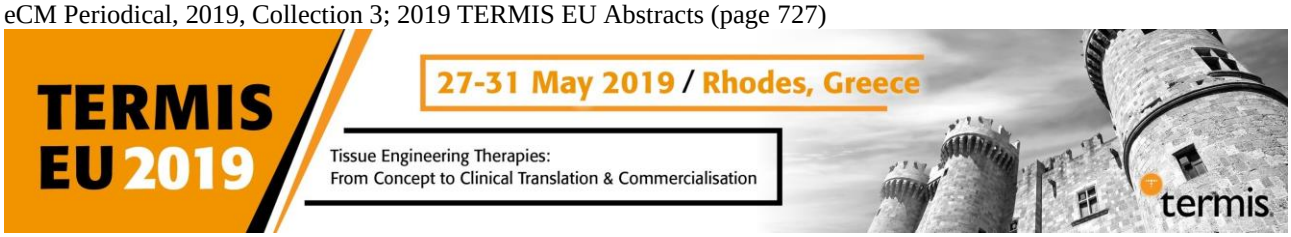
RESULTS & DISCUSSION: Bridge proteins were produced and purified to high quality, as assessed by SDS-PAGE and western blot analysis. Affinities of the bridge proteins to collagen I was confirmed by ELISA-based binding assays, and dissociation constants K_D were determined to be at 1-3 nM, which is considered as high-affinity. Similar methods were used to demonstrate binding of BMP-2 to the bridge proteins, with affinities in range of 50 nM. Further release assays showed that bridge proteins allow strong retention of BMP-2 into collagen matrices, with about 20% released after 6 days, in contrast to BMP-2 alone (i.e. without bridge proteins) that is fastly released at about 70% after 1 day and 80% at 6 days. Increased retention was additionally visualized by immunohistochemistry of retained BMP-2 into the collagen matrices. In vivo efficacy studies on bone regeneration are ongoing in the critical-size calvarial defect model in mice.

CONCLUSIONS: In this study, we engineered bridge proteins able to increase the retention of BMP-2 into collagen matrix. Increased local delivery of BMP-2 is expected to increase local efficacy and lower side effects in vivo. Should this be confirmed in our case, our proposed delivery system is particularly interesting in terms of clinical translation as it does not require modification of BMP-2 or of the collagen, both being approved in the clinic.

ACKNOWLEDGEMENTS: Financial support was received from the University of Chicago.

REFERENCES

[1] Martino MM et al. Adv Drug Deliv Rev. 2015.



Chemo-radiotherapy screening on a novel 3D pancreatic tumor model

S. Totti[1], P. Gupta[1], A. Nisbet[2,3], R. Webb[4], G. Schettino[2,5], E. G. Velliou[1]
Presenting Author: Priyanka Gupta, m10749@surrey.ac.uk
[1]Bioprocess and Biochemical Engineering group (BioProChem), Department of Chemical and Process Engineering (CPE), University of Surrey, UK [2]Department of Physics, University of Surrey, UK [3]Royal Surrey County Hospital, NHS Foundation Trust, UK [4]Advanced Technology Institute, Ion Beam Centre, Physics, University of Surrey, UK [5]National Physical Laboratory, UK

INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) has an extremely low survival rate [1], with its resistance to most current treatment methods being one of biggest therapeutic challenges. Traditional 2D systems for therapeutic analysis is unable to mimic the in vivo tumour niche and it has been shown that 3D systems respond to drugs differently than 2D systems. Several groups have developed spheroid systems with different cell types, to study the effect of radio and chemotherapy for PDAC treatment [1]. However, spheroid systems have several disadvantages including lack of long term maintenance, difficulty in structural tuning. To tide over these, we have previously developed and novel scaffold based PDAC tumour model [2]. In this work we present for the first time, we study the effect of radio and chemotherapy on our novel 3D PDAC model.

METHODS: PANC-1 cells were cultured in our novel scaffolds for 46 days, which is the longest in vitro reported culturing period. Based on our previous work, on day 29 cells in the scaffolds have formed a physiologically relevant phenotype. Therefore, on day 39, we applied chemotherapy gemcitabine (GEM) at concentrations of 10, 50 and 100µm, radiotherapy (dose range of 2-8 Gy) and a combinatory treatment (10µm GEM & 6 Gy). The tumour inactivation kinetics were monitored and quantified up to 17 days post-treatment with live/dead staining and in situ quantification of caspase-3/7 apoptotic marker with confocal laser scanning microscopy (CLSM).

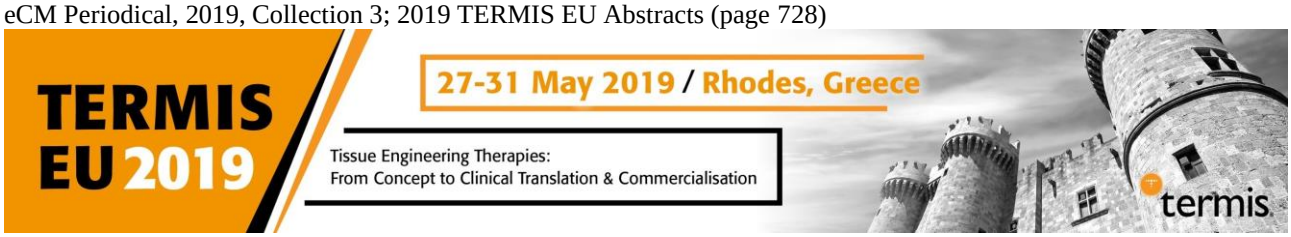
RESULTS & DISCUSSION: Our findings show that the cell viability in the 3D scaffolds remained high up to 17 days post treatment and then significantly decreased. The inactivation kinetics were dose and treatment dependent with the combined chemo-radiotherapy treatment inducing higher cell death. The caspase-3/7 activation followed a similar pattern for all the treatment conditions. Similar post treatment induced apoptosis trends in a dose dependent manner have been previously reported for PDAC xenografts.

CONCLUSIONS: We present here for the first time, chemo and radiotherapy screening on our novel scaffold based PDAC in vitro model. We highlight the positive effect of combinatorial treatment and the effect of dosage for both chemotherapy and radiotherapy. Our microscopic observations of dose dependent changes in apoptotic marker and cell viability match with previously published data of PDAC xenografts, highlighting the feasibility of using this model to accurately study PDAC and its treatments in vitro, taking us closer to an animal free research.

ACKNOWLEDGEMENTS: This work was supported by CPE, University of Surrey, an Impact Acceleration Grant (IAA-KN9149C), an IAA –EPSRC Grant (RN0281J) and the Royal Society. P.G is funded by Commonwealth Rutherford Fellowship.

REFERENCES

[1] Totti, S. et al. Drug discovery today. 2017; 22(4): 690-701.
[2] Totti, S. et al. RSC Advances. 2018; 8(37): 20928-20940.



A polyhydroxyalkanoate patch for the delivery of pluripotent stem cell-derived cardiomyocytes and endothelial cells to the infarcted heart

Q. A. Majid[1], G. Foldes[1], T. Kodagoda[1], P. Basnett[2], C. L. Grigsby[3], M. M. Stevens[3, 4], I. Roy[1, 2], S. E. Harding[1]
Presenting author: Qasim A. Majid, qam15@ic.ac.uk
[1]National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, United Kingdom [2]School of Life Sciences, College of Liberal Arts and Sciences,University of Westminster, United Kingdom [3]Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden [4]Department of Materials, Faculty of Engineering, Imperial College London, United Kingdom [5]Department of Bioengineering, Faculty of Engineering, Imperial College London, London, United Kingdom

INTRODUCTION: Myocardial infarction (MI) results in the necrosis of cardiomyocytes often leading towards heart failure (HF). Although cellular therapies aim to replace these cells, their efficacy remains challenging [1]. Cardiac tissue engineering (CTE) utilising human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) may offer a solution however the relative immaturity of these cells is a concern [2]. In this study, we aimed to generate reproducible, anisotropic scaffolds with structural cues capable of maturing hPSC-CMs in-vitro and delivering these to the infarcted heart in addition to human pluripotent stem cell-derived endothelial cells (hPSC-ECs) to develop a primitive vasculature that could aid retention of delivered cells.

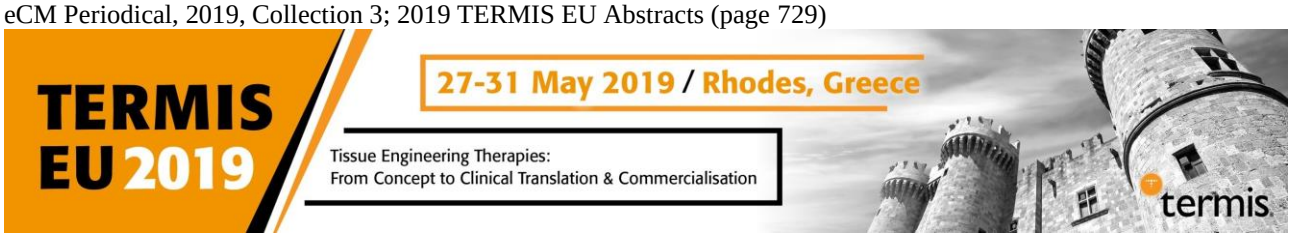
METHODS: Medium-chain-length polyhydroxyalkanoates (MCL-PHAs), a family of bioresorbable polymers with mechanical properties conducive to CTE were generated via bacterial fermentation. These polymers were electrospun to generate reproducible scaffolds. hPSC-CMs generated via a small molecule differentiation protocol were seeded onto PHA scaffolds for 1 week. Gene expression and optical mapping studies revealed hPSC-CMs remained functional on the PHA scaffolds. Morphological assessment conducted after 1 week showed organised α-actinin alignment as compared to hPSC-CMs cultured on fibronectin ($P \leq 0.01$). Additionally, hPSC-CMs cultured on PHA scaffolds were shown to be highly anisotropic as compared to hPSC-CMs cultured on fibronectin. Scanning electron microscopy was completed to identify topographical cues that may be responsible for this alignment.

RESULTS & DISCUSSION: hPSC-ECs were generated via a growth factor protocol and were seeded onto PHA scaffolds without prior preconditioning resulting in a functional monolayer with angiogenic properties comparable to collagen culture. Viable co-culture conditions conducive to hPSC-EC proliferation and tube formation were identified and used to co-culture hPSC-ECs and hPSC-CMs on the PHA scaffolds. Finally, initial in-vivo studies show myocardial compatibility of the PHA scaffold itself with subsequent studies set out to investigate the functional benefits of the complete cell-seeded cardiac patch.

CONCLUSIONS: Here we have developed a new delivery system capable of aligning and maturing hPSC-CMs whilst maintaining hESC-EC function thus this scaffold has the potential to promote angiogenesis and facilitate retention of the delivered cells.

REFERENCES

[1] Malliaras K et al. Clin Pharmaol Ther. 2011; 90:532-541
[2] Veerman C. C et al. Stem Cells Dev. 2015; 24:1035–1052



Engineered humanized bone marrow microenvironments for investigating breast cancer metastasis in vivo

Q. Vallmajo-Martin[1,2], A.E. Chiou[3], M. Whitman[3], I. Martin[4], C. Fischbach[3], M. Lütolf[2], M. Ehrbar[1]
Presenting Author: Queralt Vallmajo-Martin, queralt.vallmajomartin@usz.ch
[1]Laboratory for Cell and Tissue Engineering, University Hospital Zürich, Switzerland [2]Laboratory of Stem Cell Bioengineering, EPFL, Lausanne, Switzerland [3]Biomedical Engineering, Cornell University, USA [4]Department of Biomedicine, University Hospital Basel, Switzerland

INTRODUCTION: Though it is well known that bone is the preferential homing site for breast cancer metastasis, the disease remains largely incurable with only palliative treatment options. This clinical void can be partially attributed to the lack of suitable animal models that mimic key features of metastasis of human tumor cells to the human bone microenvironment. To address this, we designed a humanized tissue engineered bone model. This model holds tremendous promise for further investigation into the factors involved in osteotropic metastasis of breast and other cancers.

METHODS: Polyethylene glycol (PEG) hydrogels (PEG was selected as a defined non-inductive 3D environment) [1] were seeded with various combinations of human mesenchymal stem cells (hMSCs) from diverse sources and bone morphogenetic protein-2 (BMP-2). These constructs were subcutaneously implanted in mice. After bone formation, human breast cancer cells (MDA-MB-231) were subsequently introduced to the system and their dissemination to the humanized ossicles could be tracked via bioluminescent imaging. Cancerous lesions in the bones were monitored via both microCT and histological evaluation.

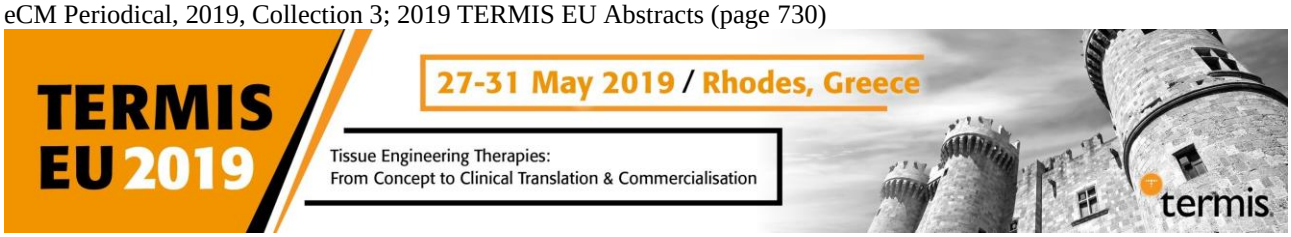
RESULTS & DISCUSSION: As a first step, the parameters were optimized for maturation of the humanized bone marrow constructs. PEG hydrogels were seeded with various combinations of human mesenchymal stromal cells (hMSCs) from diverse sources and bone morphogenetic protein-2 (BMP-2). We first determined the minimum concentration of BMP-2 and minimum cell density for bone marrow-derived hMSCs from healthy donors. Strikingly, we found that 20 million hMSCs/ml without presence of BMP-2 could remodel PEG gels into ossicles, as shown by microCT analysis and histology. Moreover, these implants not only still contained hMSCs, but were also highly infiltrated by murine osteogenic, endothelial and hematopoietic cells as demonstrated by FACS analysis. Importantly, tissue type and donor health significantly affect hMSC potential. Cancer cells added at different times of bone development disseminate to the ossicles within 2 weeks, and reduced bone formation by increasing osteoclasts activity.

CONCLUSIONS: Taken altogether, this intricate xenograft model featuring humanized bone microenvironments and human tumor cells has potential to be a powerful tool for studying osteotropic metastasis. It can be utilized to study the molecular cargo that may be involved in homing cancer cells to bone, priming the pre-metastatic site for secondary lesions, and mapping out morphological changes in the bone microenvironment that contribute to disease progression. Furthermore, it may prove valuable as preclinical tool for screening novel therapeutics that combat metastatic processes allowing for earlier intervention and vastly improved patient outcomes.

ACKNOWLEDGEMENTS: Swiss National Science Foundation grant 153316.

REFERENCES

[1] M. Ehrbar et al. Biomaterials 2007; 28:3856-66.



Development of a controlled drug release system of Cripto for muscular dystrophies therapy

R. Lev[1], O. Kossover[1], Y. Lati[1], O. Guardiola[2], G. Minchiotti[2], D. Seliktar[1]
Presenting Author: Rachel Lev, rachelev@campus.technion.ac.il
[1]Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel [2]Stem Cell Fate Laboratory, Institute of Genetics and Biophysics "Adriano Buzzati-Traverso", Naples, Italy

INTRODUCTION: Muscular dystrophies (MDs) are inherited disorders that manifest as progressive muscle wasting and weakness [1]. Cripto protein has been recently found to have therapeutic value in alleviating muscle myopathies by regulating muscle regeneration and satellite cell progression through myogenesis [2]. In this study, we establish a highly reliable methodology for producing large quantities of the Cripto protein and further clarify the strategy in which we can encapsulate the Cripto into the microgel carrier for the purpose of controlled release. PEG-fibrinogen (PF) hydrogels provide the substrate for protein production. For the purpose of controlled release in situ delivery vehicle, both well-established PF and preliminary but promising Pluronic®F127 fibrinogen (FF127) hydrogels are attractive and potentially applicable.

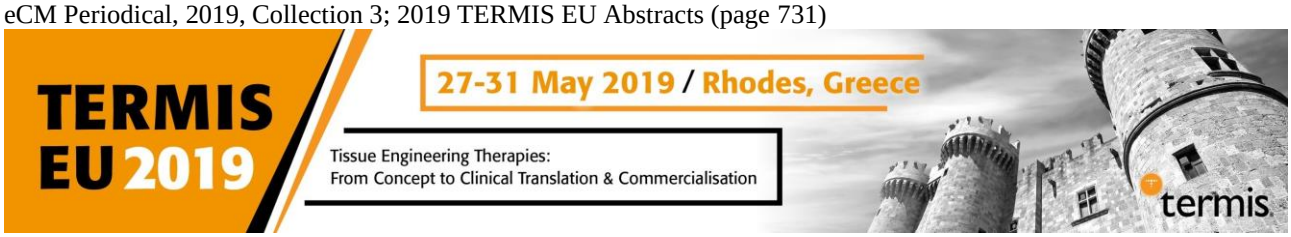
METHODS: In order to attain efficient production of Cripto, PF hydrogel-based microcarriers were designed to enable mammalian cell survival and proliferation, as well as the secretion of large amounts of therapeutic proteins. Cripto-overexpressing HEK 293T cell lines were encapsulated in the PF microcarriers and cultivated in stirred suspension bioreactors. PF microcarriers were prepared to possess high mechanical strength in order to resist the shear forces and biodegradation associated with being immersed in a long-term suspension culture in the bioreactors. Subsequently, two distinct injectable controlled-release systems were designed for the encapsulation of the Cripto: PF hydrogel-based pre-formed microspheres, and in situ formed FF127 hydrogel-based implants.

RESULTS & DISCUSSION: The yield of Cripto proteins produced using this system was significantly higher than the traditional two-dimensional (2D) system. This result was in accordance with previous production cycles of other proteins performed in our laboratory. Likewise, both injectable systems exhibited similar sustained-release profiles of Cripto for up to 27 days, though with a prominent difference in encapsulation rate in favor of FF127. The Cripto that was released was biologically active and promoted the proliferation of mouse myoblasts. We further demonstrated that the release rate of Cripto from PF-hydrogel microspheres could be controlled by tuning the crosslinking density of the hydrogel, which was controlled by varying the concentration of poly(ethylene glycol) diacrylate (PEG-DA).

CONCLUSIONS: Concurrent with the optimization of both delivery systems in vitro, it is intended that the significance of these results be further investigated by means of follow-up in vivo trials.

REFERENCES

[1] Mercuri E, Muntoni F. Lancet 381:845-860, 2013.
[2] Guardiola O et al. Proc Natl Acad Sci USA 109:E3231-E32,2012.



Designing a novel bioengineered substrate as a treatment for AMD

R. McCormick[1], I. Pearce[2], A. Haneef[1]
Mccormick, Rachel, R.Mccormick@liverpool.ac.uk
[1]Eye and vision department, Institute of Ageing and Chronic Disease, University of Liverpool, William Duncan Building, 6 West Derby Street, Liverpool L7 8TX [2]Royal Liverpool University Hospital, St Paul's Eye Unit, Prescot Street, Liverpool, United Kingdom

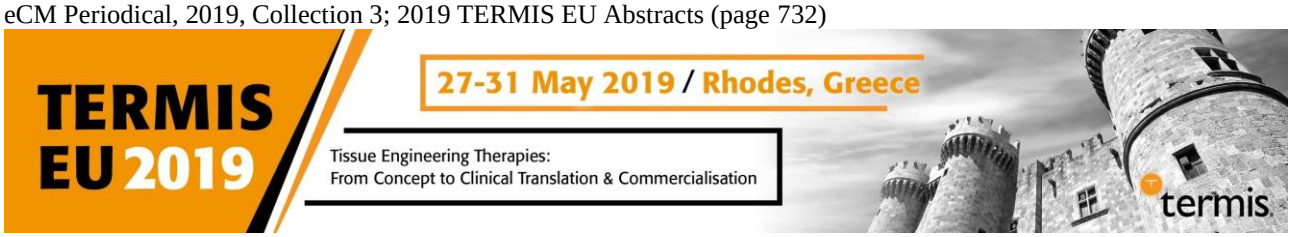
INTRODUCTION: Age related macular degeneration (AMD) is the third most prevalent cause of blindness worldwide, and the leading cause of blindness in industrialised countries. In AMD, the Bruch's membrane, a section of the retina, thickens with age, resulting in impaired waste/nutrient exchange between the retinal pigment epithelial (RPE) cells and the choroid. This in turn leads to the photoreceptor death and eventually permanent central vision loss. There are two types of AMD, wet and dry. Dry AMD, also known as geographic atrophy, is the most common affecting 90% of AMD patients and there is currently no effective treatment. Considering there is no current treatment for atrophic AMD and with its prevalence anticipated to rise due to increasing life span, a treatment for this debilitating condition is essential. Here we have optimised the production of a bioengineered substrate to act as an artificial Bruch's membrane, suitable for growing RPE cells as well as a bioactive layer that will deliver active molecules at a controlled rate to break down the diseased native Bruch's membrane.

METHODS: 25% w/v PET (polyethylene terephthalate) dissolved in hexafluro-2 propanol (HFIP) scaffolds, capable of growing RPE cells in their native role, were produced through electrospinning. RPE cells were plated on 2cm^2 scaffolds at a density of 50,000 cells. Electrospun PET was also subjected to tensile testing. For nanoparticle production PGLA (polyglycolic-lactic acid, 10% HFIP) and polyglycolic (PGA, 10% acetone) were subjected to different working distances and voltages to ensure the formation of nanoparticles, using SEM images as a confirmation. Following on from this they were then tested for their ability to encapsulate, protect and release substrates.

RESULTS & DISCUSSION: Our results have shown that electrospinning, 25% PET can produce nanoscale fibres and confirm that electrospun PET is a suitable scaffold for RPE cells. Tensile testing of PET fibres show electrospun PET has a high elasticity, compared with cast PET sheets which are extremely brittle. We have also shown that electrospraying of biodegradable compounds (PGA, PLGA) is able to form reproducible nanoparticles that are capable of encapsulating biological moieties. Degradation studies using FITC (fluorescein isothiocyanate) tagged bovine serum albumin (BSA) and enzymes have shown that these nanoparticles are capable of releasing BSA and enzymes (lipases, collagenases), at a controlled rate. Gel enzyme assays show the nanoparticles do not affect the function of the enzymes and are likely to protect the enzymes from degradation.

CONCLUSSIONS: This work shows that PET is a suitable scaffold for RPE cells and that electrosprayed biodegradable nanoparticles are able to encapsulate, protect and sustain the release of biological enzymes. Future work will focus on the optimisation of the specific cocktail and release of enzymes suitable for the breakdown of drusens for apical side of replacement RPE and Bruch's membrane, as a treatment for dry AMD.

ACKNOWLEDGEMENTS: Financial support was received from ESPRC.



Anabolic effects of L51P and bone morphogenetic protein 2 on human intervertebral disc cells

R.D. May[1], D.A. Frauchiger[1], C.E. Albers[2], L.M. Benneker[2], W. Hofstetter[1], B. Gantenbein[1,2]
Presenting Author: Rahel May, rahel.may@dbmr.unibe.ch
[1]Department for BioMedical Research, University of Bern, Switzerland, [2] Department of Orthopaedic Surgery & Traumatology, Bern University Hospital, University of Bern, Bern, Switzerland

INTRODUCTION: In clinics, Bone Morphogenetic Protein 2 (BMP2) was applied for the support during spinal fusion. Further BMP2 was tested in IVD models and showed potential for IVD regeneration. The aim of this study is the investigation of BMP2 and the BMP2 analogue, L51P, on different cell types of the human IVD in 3D alginate beads, particularly their plasticity to undergo bone formation.

METHODS: Human nucleus pulposus (NPC), annulus fibrosus (AFC) and cartilaginous endplate cells (CEPC) were each encapsulated in 1.2% alginate at a density of 4 Mio/mL. NPC, AFC, and CEPC beads were then cultured in α-MEM or osteogenic medium (OM) supplemented with 10% FBS and 100 ng/mL BMP2 and/or L51P for 21 days. Medium supplemented with cytokines was refreshed twice per week. Beads were snap frozen with liquid nitrogen after 7 days for mRNA analysis of Aggrecan (ACAN), Collagen type1 (COL1), Collagen type 2 (COL2) and runt-related transcription factor 2 (RUNX2) qPCR. Further, beads were stained with Alcian Blue after 21 days.

RESULTS & DISCUSSION: ACAN expression was the highest up-regulated in IVD cells stimulated with OM and 100 ng/mL BMP2 and L51P compared to negative control (basal medium only) in NPC, AFC and CEPC (mean ± SEM NP: 18.95 ± 15.65). The same was true for COL2 expression (NP: 72.47 ± 62.95). COL1 remained unaffected (N=2).

CONCLUSIONS: In this study we showed the trend of an increase in ACAN and COL2 gene expression in stimulated cells. Interestingly the co-treatment of BMP2 and L51P showed a cumulative effect towards an increased ECM production.

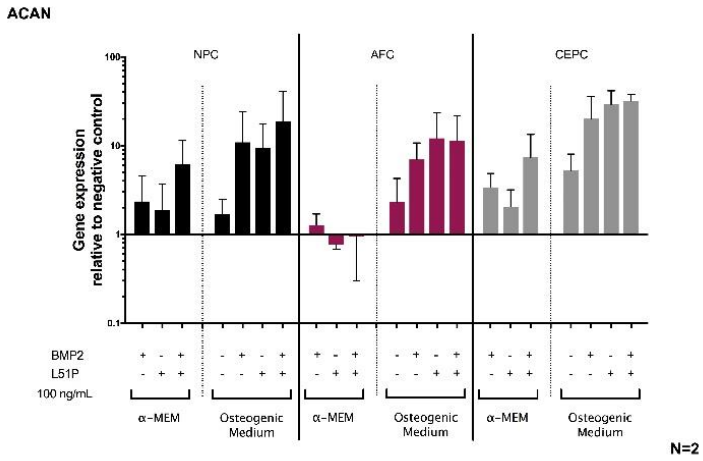
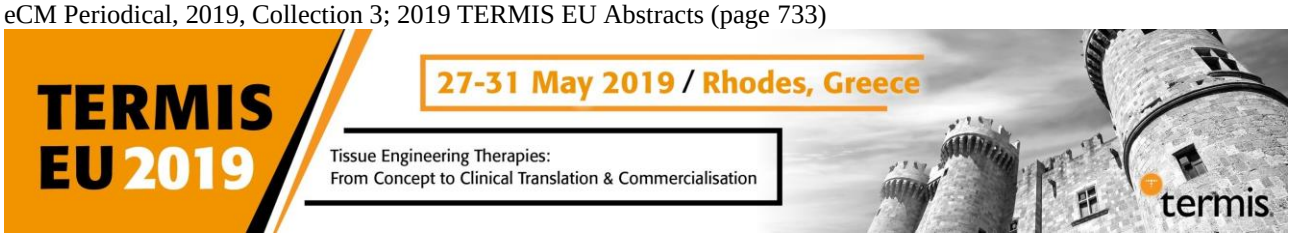


Figure 1: Gene expression of ACAN, in NPC, AFC and CEPC cultured in 3D alginate culture. Results are presented as mean ± SEM. N=2

ACKNOWLEDGEMENTS: Clinical Trial Unit, Inselspital, Bern University Hospital, and by a grant of the Swiss Society of Orthopaedics and Traumatology to C. E. A.



Biochemical gradients to form a 3-D osteochondral in vitro model

R. F. Canadas[1,2,4], T. Ren[4], A. P. Marques[1,2,3], J. M. Oliveira[1,2,3], R. L. Reis[1,2,3*], U. Demirci[4,5*]
Presenting Author: Raphaël F. Canadas, raphael.canadas@i3bs.uminho.pt
[1]3B's Research Group – Biomaterials, Biodegradable and Biomimetics, Headquarters of the European Institute of Excellence on tissue Engineering and Regenerative Medicine, AvePark, 4805-17 Barco, Guimarães, Portugal [2]ICVS/3B's- PT Government Associate Laboratory, University of Minho, Guimarães-Braga, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Guimarães, Portugal [4]Bio-Acoustic MEMS in Medicine Laboratory, Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Palo Alto, California 94304, USA [5]Electrical Engineering Department by courtesy, Stanford University, Stanford, California 94305, USA

INTRODUCTION: Gradients of 3-D complex functional patterns at micro-scale are common in nature [1]. While diverse fabrication techniques based on material science, micro-scale engineering, and microfluidics have been used to synthesize biomimetic and tailored microenvironments [2,3], a great need is still present for improved methods of gradient tissue engineering. Here, we developed the precise spatial control over both cell/tissue phenotype and pre-vasculature formation, which opens-up possibilities for the study of complex tissues interfaces, with broader applications in drug testing and regenerative medicine.

METHODS: The fabrication of microparticles gradient was performed combining methacrylated gelatin (GelMA), gellan gum (GG), and the hydroxyapatite microparticles (HAp). The temperature was controlled to mix the interface of two polymeric layers, being the second added at 10ºC higher than the first one. The promoted convection was used to drive the microparticles forming the gradient. After crosslinking, the freezing step was tuned using an external cover of styrofoam forcing the ice crystals to grow linearly. A bioreactor device was designed to culture fat pad adipose-derived stem cells and microvascular endothelial cells in 3-D biochemical gradients.

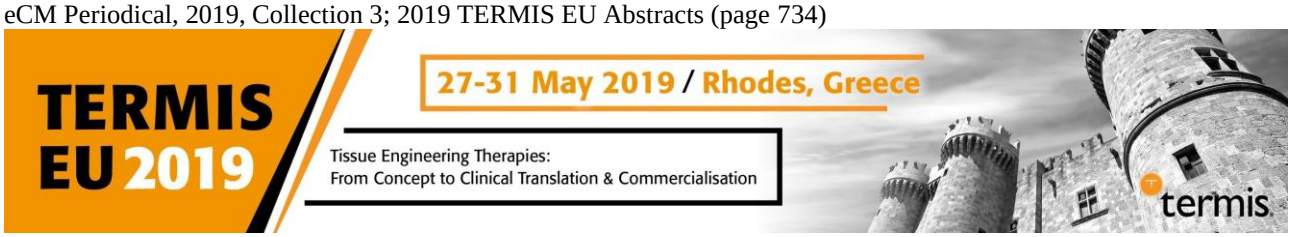
RESULTS & DISCUSSION: Using control over temperature and crosslinking, hydrogel-like structures were built in 3-D isotropic and anisotropic HAp gradients. Finally, an in vitro osteochondral tissue model was obtained. Results showed a significant difference ($p < 0.05$) of SOX9 and bone markers from top to bottom regions of the 3-D gradient structures under dynamic conditions using a custom dual-chamber bioreactor.

CONCLUSIONS: In the present work, microparticle and biochemical gradients were combined into iso- and anisotropic architectures. Overall, these results enable the precise control of 3-D gradients anisotropy with broad applications in interfaced tissue engineering and drug testing with special focus on regenerative medicine.

ACKNOWLEDGEMENTS: The authors are grateful for the FLAD and FCT funding (SFRH/BD/92565/2013).

REFERENCES

[1] Gurdon J B et al. Nature. 2001; 413:797-803
[2] Canadas R F et al. Biomat. 2018; 181:402-414
[3] Polacheck W J et al. PNAS. 2014; 111:2447-2452



Multi-nozzle inkjet and its potential for the industrialization of bioprinting

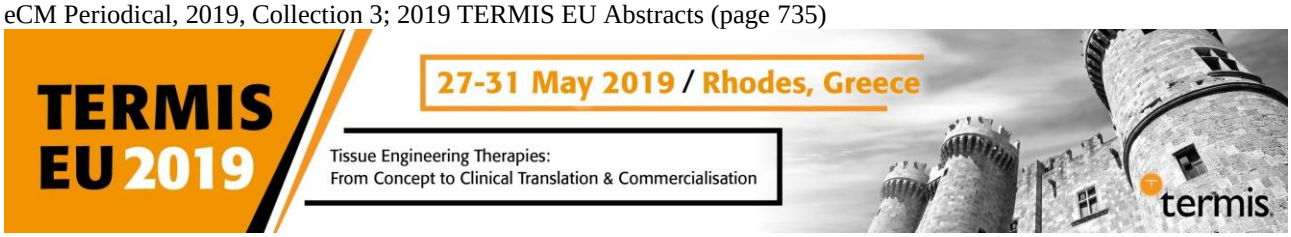
R. Wenger[1], M. Mauron[1], F. Bircher[1], R. Nussbaumer[1]
Presenting Author: Raphael Wenger, raphael.wenger@hefr.ch
[1]iPrint Institute, School of Engineering and Architecture of Fribourg, Member of the University of Applied Sciences of Western Switzerland, Switzerland

INTRODUCTION: Bioprinting, thanks to the unique flexibility and spatial accuracy it offers, has a promising future for the deposition of cells and extracellular matrices toward the fabrication of physiologically relevant 3D tissue models. Most bioprinting platforms offer switching single-nozzle printheads. In contrast, industrial inkjet printheads feature hundreds of nozzle in parallel while enabling the deposition of drops in physiologically relevant resolution. We hypothesize that this high-throughput industrial technology will enable the cost-effective fabrication of 3D tissue models, potentially leading to the industrialization of bioprinting. To test our hypothesis, we are investigating the reliability of the printing process, its throughput and its impact on cells.

METHODS: A 3D Bioprinting platform was developed and set up with a special Xaar 128 printhead compatible with water-based inks. Bioinks based on culture media with viscosity modifiers were prepared and characterized to improve the stability of the cell suspensions while remaining liquid enough for inkjet printing (<15mPa*s). 3T3 mouse fibroblast, A549 human alveolar basal epithelial and HUVEC were cultivated and suspended in bioinks (3x106cells/mL = 100% concentration) and their sedimentation rate measured. 7mL of the cell-loaded bioinks were fed into the ink system and were kept there for up to 35min of alternating sequences of purging, printing and settling at room temperature. Batches of cell-loaded bioinks were printed as one big drop in a petri dish that was subsequently divided into n=3 wells for culture. To quantify the stability of the process, cell concentration was measured after each batch printing. To measure the impact of the process on the cells, a viability test (Trypan Blue exclusion assays) was performed.

RESULTS & DISCUSSION: When using DMEM with 10%FBS as a bioink, suspended cell concentration in the printed batches dropped to 31% after 5min settling and sedimented cells were observed to clog the nozzles. Increasing the viscosity of the bioink by adding 5% Ficoll PM400 led to an increase of the cell concentration to 81% after 5 minutes settling while limiting the clogging of the nozzles. This indicates that the rheological properties of the bioink can be tuned to improve the stability of the printing process. Viability directly after printing and for up to 4 days culture remained over 85%, suggesting that the printing process and the addition of Ficoll has a limited impact on the cells.

CONCLUSIONS: Our study demonstrates that high-throughput and reliable cell deposition can be achieved through industrial inkjet by modifying the rheological properties of the bioink.



The use of skeletal muscle-derived pericytes in tissue engineering of skeletal muscle graft based on 3D pluronic-fibrinogen (FF-127) hydrogel environment

R. Hamami[1], D. Seliktar[1]
Presenting Author: Raziel Hamami, Razielh@campus.technion.ac.il
[1]Department of Biomedical Engineering, Technion Israel Institute of Technology, Haifa 3200003, Israel

INTRODUCTION: Muscular dystrophies (MDs) are genetic disorders characterized by progressive muscle wasting, leading to limitations in motor capacity and in many cases to progressive paralysis and death. In this present study, we aim to rejuvenate skeletal muscle function by developing an injectable approach for intra-arterially delivery of cells to the injury site using biomaterials combined with cell therapy.

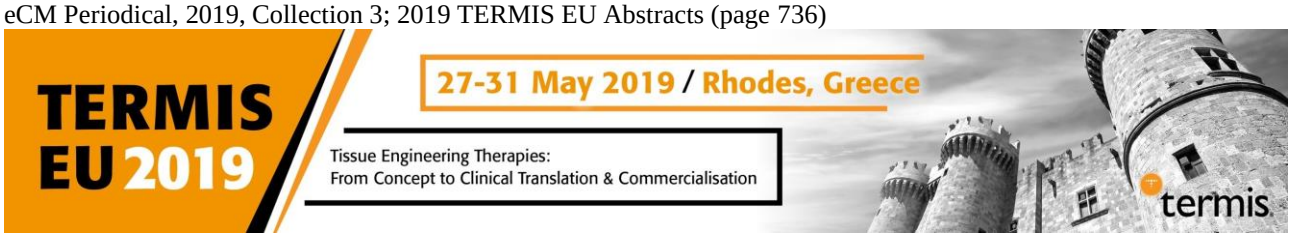
METHODS: For physically cross-linked cell-seeded microcapsules, a cell suspension 3×10^6 cells/mL polymer mixture was dripped through a droplet-based microfluidic device into a warm (37 °C) gelation bath containing continually stirred media, forming micro-carriers with a diameter ranging from 150 to 500 μm.

RESULTS & DISCUSSION: In the first experiments, FF-127 micro-carriers were seeded with skeletal muscle-derived pericytes (MP) using a new droplet-based microfluidic device, as the basis of our skeletal muscle repair strategy. Guided by the fact that cell viability directly correlates to the success of cell transplantation therapies, viability of encapsulated cells were investigated 1, 4, 7, 14 and 21 days after culture. Viability, morphology, proliferation and cell recovery yields were shown to be higher than photo-chemically cross-linked over physical crosslinking micro-carriers made from a similar material, especially in the MPs. Moreover, throughout syringe needle flow, cells experience three types of mechanical forces that can lead to cell disruption: (i) a pressure drop across the cell, (ii) shearing forces due to linear shear flow, and (iii) stretching forces due to extensional flow. In this study, our proposed system is cell-compatible as harsh mechanical or crosslinking processes such as UV and aggressive chemical crosslinking mechanisms are avoided.

CONCLUSIONS: Our studies demonstrate a novel strategy for injectable FF-127 cell-seeded micro-carriers production using an effective microfluidic device. These micro-carriers successfully preserved the viability and concentration of human MPs required for positive transplantation later on. Using fewer cells to achieve a similar number of transplanted, viable cells would greatly reduce the cost, time, and effort required to restore lost skeletal muscle function using transplantation protocols.

REFERENCES

[1] J Cell Biol 1999,144(6):1113–1122.
[2] StemCellRes. 10, 67–84.doi:10.1016/j.scr.2012. 09.003
[3] Front. Physiol. 5:203. 10.3389/fphys.2014.00203.



Novel surface coatings as biocompatible reservoirs to deploy BMP-2 for bone regeneration

R. Anouz[1], A. Repanas[1], E. Schwarz[2], T. Groth[1,3]
Presenting Author: Reema Anouz, reema.anouz@student.uni-halle.de
[1]Department Biomedical Materials, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Germany [2]Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Germany
[3]Interdisciplinary Center of Materials Science, Martin Luther University Halle-Wittenberg, Germany

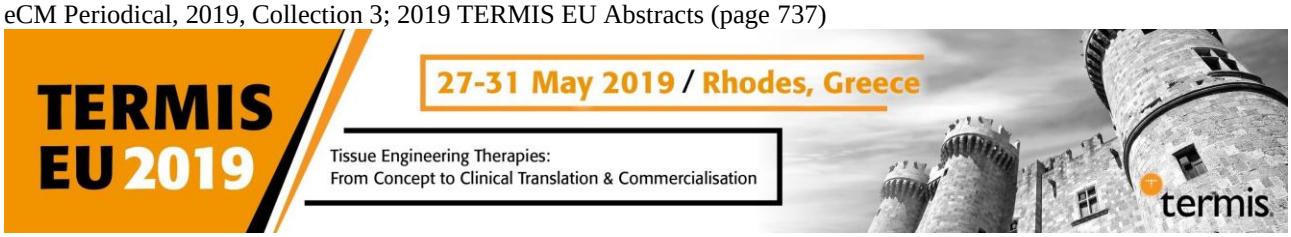
INTRODUCTION: Layer-by-Layer (LbL) is a technique that permits the formation of nanostructured surface coatings useful for delivery of bioactive drugs and proteins, like growth factors (GF). Bone morphogenetic protein-2 (BMP-2) promotes osteogenic differentiation of mesenchymal and other cells and is used in the treatment of non-healing bone fractures. However, bolus injections of BMP-2 are rapidly cleared-off and lose their activity rapidly. Hence, high dosages are needed, which can result in inflammation, increased cancer incidence and further complications, in addition to high costs of treatment. Thus, the need for controlled release systems lowering therapeutic concentrations of GF are badly needed.

METHODS: Heparin, chondroitin sulfate and their oxidized forms as polyanions were combined with chitosan and collagen I as polycations to form various multilayer coatings on model materials with getting advantage of the intrinsic cross-linking formed between oxidized glycosaminoglycans (GAGs) and polycations to improve multilayer stability and affect the release of BMP-2. The myoblast cell line C2C12, which can differentiate into osteoblasts was seeded on 5 µg/mL BMP-2 loaded multilayers. Cell viability was investigated by Qblue assay; adhesion using immunohistochemical staining, osteogenic differentiation by alkaline phosphatase (ALP) assay and alizarin red-S staining. In addition, studies on release of BMP-2 were done by ELISA.

RESULTS & DISCUSSION: C2C12 cells cultured directly on the top of multilayers showed that particularly BMP-2 loaded multilayers made of oxidized GAGs promoted an osteogenic differentiation that was nearly comparable to the positive control, when 5 µg/mL BMP-2 was added directly to the medium. Interestingly, the BMP-2 had synergistic effect on cell adhesion and spreading. BMP-2 in oxidized chondroitin sulfate multilayers was successfully loaded to the layers, sustainably released over time and affected cell differentiation more than the soluble BMP-2.

CONCLUSIONS: The results show that oxidized GAGs forming intrinsically cross-linked multilayers are useful as reservoirs for sustained release of BMP-2 in which the intrinsic cross-linking affected BMP-2 release, improved multilayers stability due to the resulting stiff surface compared to the native ones, supported cell adhesion, proliferation and subsequent differentiation. This can pave the way for coating implants and scaffolds for repair and regeneration of bone fractures.

ACKNOWLEDGEMENTS: This work was supported by Bundesland Sachsen-Anhalt in the frame of Leistungszentrum Bio-und Systemtechnik and the International Graduate School AGRIPOLY.



Regulation of inflammatory response by novel herbal small molecules in human osteoarthritic chondrocytes

R. Ziadlou[1,2], S. Grad[1], M. Stoddart[1], X. Wang[3], L. Qin[3], A. Barbero[2], I. Martin[2], M. Alini[1]
Presenting Author: reihane.ziadlou@aofoundation.org
[1]AO Research Institute Davos, Davos, CH [2]Department of Biomedicine, University Hospital Basel, University of Basel, Basel, CH [3]Translational Medicine R&D Center, Shenzhen Institutes of Advanced Technology

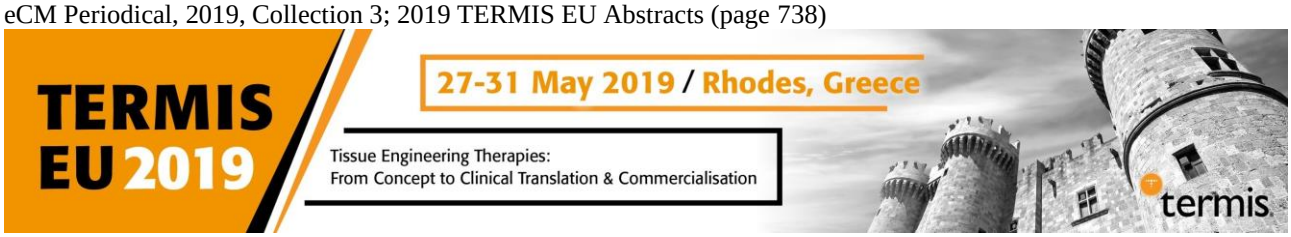
INTRODUCTION: Osteoarthritis (OA) results from a combination of genetic and environmental factors which cause imbalance between anabolism and catabolism and affect articular cartilage. To tackle the problem, inhibition of pro-inflammatory cytokines which are excessively expressed in OA joint is necessary. After screening of 40 Traditional Chinese Medicine (TCM) compounds, the bioactive ingredients with most potent effect for treatment of OA were further evaluated.

METHODS: The anabolic effects of 40 TCM compounds in a 3D pellet culture model, were assessed biochemically (GAG/DNA) using human OA chondrocytes. The most chondrogenically active compounds were tested in an inflammatory model consisting of 3 days of inflammatory induction with cytokines (IL-1β/TNF-α) with and without supplementation of TCM compounds. The anti-inflammatory effects of the compounds were assessed transcriptionally (q-PCR and RNA sequencing), biochemically and histologically.

RESULTS & DISCUSSION: From the 40 compounds tested, several showed significant anabolic effects. After induction of inflammation, the pro-inflammatory and catabolic marker genes were up-regulated, and GAG/DNA ratio was significantly decreased; while several compounds had anti-catabolic effects, by down-regulating pro-inflammatory markers including MMPs and COX-2. After 2 weeks of treatment with the compounds following inflammation, the GAG/DNA ratio was restored by Psoralidin, (Ps), Vanilic acid (VA), Protocatechuicaldehyde (PCA), Epimedin C (Epi C), 4-Hydroxybenzoicacid (4-HBA) treatment. Immunohistochemistry and Safranin-O staining confirmed the accumulation of cartilaginous matrix in compound treated pellets.

CONCLUSIONS: Of the 40 TCM compounds tested, Ps, PCA, VA, 4-HBA and Epi C, showed promising anabolic and anti-inflammatory effects. A local drug delivery system for the bioactive compound is envisioned and their efficacy for cartilage repair will be tested.

ACKNOWLEDGEMENTS: Funded by Swiss National Science Foundation (SNF) under the SSSTC program with grant number 156362.



Microencapsulated human induced pluripotent stem cells (hiPSC) for the cell and molecular therapy of type 1 diabetes mellitus (T1D): Preliminary in vitro and in vivo data

G. Basta, P. Montanucci, T. Pescara, R. Calafiore
Presenting Author: Riccardo Calafiore, riccardo.calafiore@unipg.it
Laboratory for Cell Transplants and Biohybrid Organs, University of Perugia, Perugia, Italy

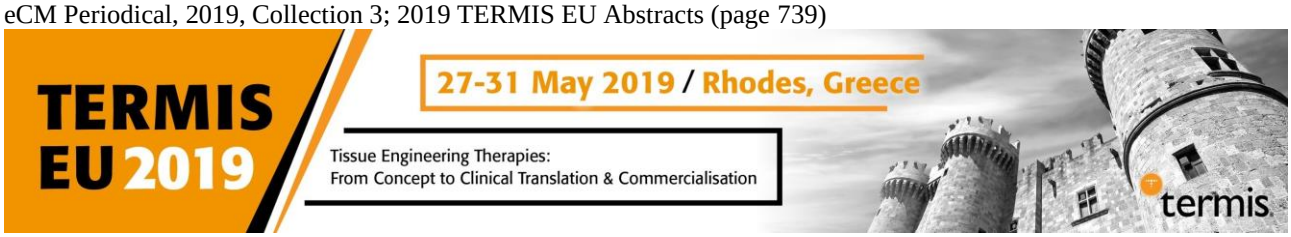
INTRODUCTION: Replacement of destroyed pancreatic islet beta-cells with functionally competent insulin-secreting cells, may represent the final cure for T1D. hiPSC committed to differentiation into beta-like cells could provide for virtually unlimited source of insulin producing cells. We then aimed at evaluating differentiation pathways of hiPSC towards beta-like cell phenotypes, able to secrete insulin in vitro, and in vivo, in experimental animal models of T1D, upon microencapsulation in human recombinant elastin monomers (ELR).

METHODS: hiPSC underwent in vitro differentiation protocols We carefully characterized the obtained cell hiPSC spheroids, in terms of morphology, viability and cell phenotyping, by molecular biology, electron microscopy and immunofluorescence technical procedures. Spheroids underwent triple ELR coating. Coated spheroids were analyzed in terms of both, in vitro and in vivo retention of viability and function. In vitro, the spheroids were exposed to glucose at different concentrations to determine insulin secretion and content. In vivo, the coated spheroids survival was evaluated upon intraperitoneal graft into immunoincompetent NOD/scid (n=4) mice and immunocompetent CD-1 mice (n=4). Cell/tissue reaction to graft was examined upon peritoneal lavage and flow cytometry analysis of spleen and lymph nodes cell phenotypes. Graft of ELR-coated (vs. uncoated) spheroids was performed in either normal or Alloxan-induced diabetic mice.

RESULTS & DISCUSSION: At term of in vitro differentiation, hiPSC spheroids showed good viability, as assessed by staining with ethidium bromide and fluorescence diacetate, and fair insulin content, upon staining with diphenyl-thiocarbazone. Expression of hormones typical of pancreatic islet cells (insulin, glucagon, somatostatin) was qualitatively comparable to that of differentiated islets. ELR coating of the spheroids, upon transmission electron microscopy examination, was clearly detectable and it did not alter viability of the encapsulated sub-cellular organelles and hormone granules of the hiPSC spheroids. In vitro metabolic data showed insulin secretory patterns and content, consistent with the presence of differentiated beta-like cells. At two weeks of graft, peritoneal lavage in NOD/scid mice was associated with 90% viability retention of the retrieved coated vs. control uncoated spheroids. At the same post-transplant time period, and using the same procedure, CD-1 mice showed viability that was higher for the coated vs. control uncoated spheroids. Peritoneal cellular response to graft was incomparably lower for coated vs. uncoated hiPSC spheroids. Two diabetic mice grafted with ELR-coated hiPSC spheroids showed partial decline of blood glucose, as compared to controls, but only in fasting conditions. New transplant experiments are in progress.

CONCLUSIONS: These very preliminary data seems to support the possibility that hiPSC may serve for beta-like cell surrogates, for beta-cell substitution therapy in T1D. ELR coatings are associated with tangible immunoprotection properties. Implementation of hiPSC maturation pathways may likely improve the metabolic outcome in the next series of experimental transplant studies.

ACKNOWLEDGEMENTS: Financial support was received from Elastislet, H2020-NMP-2014-2015 – 646075.



Electrically conductive graphene-polymer composites for cardiac tissue engineering

R. Balint[1], L. A. Hidalgo-Bastida[2]
Presenting Author: Richard Balint, richard.balint@manchester.ac.uk
[1]Manchester Institute of Biotechnology (MIB), University of Manchester, United Kingdom [2]Centre for Biomedicine, Manchester Metropolitan University, United Kingdom

INTRODUCTION: Electrically conductive biomaterials are of great interest for the engineering of excitable tissues, and as electrodes for implant devices. Metals, such as gold or platinum, are highly conductive and chemically inert, but are expensive and mechanically incompatible with tissue. As alternative, conductive polymers have been explored, however, these are difficult to process after synthesis and lose their conductivity after repeated cycles of electrical stimulation [1]. In this study the possibility of developing a conductive, biocompatible, inexpensive and easy-to-make substrate for excitable tissue engineering (with the main focus being on cardiac applications) was examined through compositing polyurethane or polycaprolactone with graphene.

METHODS: Four types (normal, large flake, high surface area and hydrophilic) of commercially available graphene were composited and solvent cast with polycaprolactone and medical grade polyurethane. Graphene weight percentages between 10% and 66% were tested. The formed composites were assessed for electrical conductivity, wettability, surface topology (SEM), and mechanical properties (nano-indentation). Biocompatibility was tested using C2C12 mouse myoblast cells, RN22 rat Schwann cells, and primary human cardiac myocytes through the PicoGreen assay and fluorescent microscopy with Vybrant DiD live cell staining. An inexpensive and autoclavable bioreactor system for the effective delivery of electrical stimulation to cells through the graphene composites was designed and custom built. The level and distribution of electrical field strength and current density within the bioreactor chamber was determined through FEM computer simulation. Monophasic pulsed electrical stimulation was delivered to human primary and human iPSC derived cardiac myocytes cultured upon the composites, either in monolayer or in a 3D collagen gel. The effect of electrical stimulation on cell growth, morphology and distribution and cardiac gene expression profile was determined.

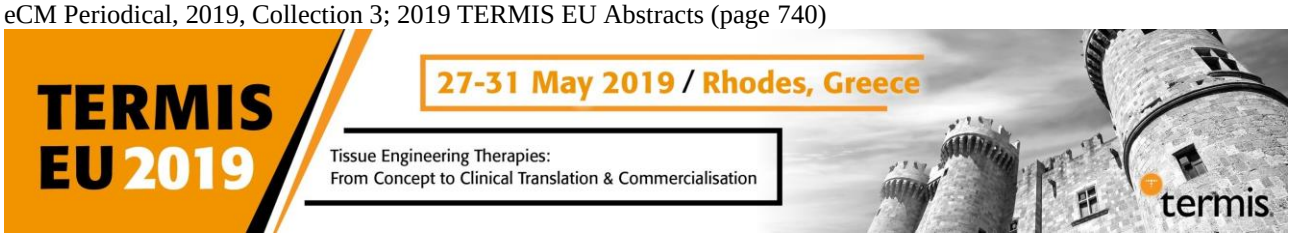
RESULTS & DISCUSSION: Computer simulations showed that 1.7 S/m is the critical conductivity for the substrates. All four graphene-polymer types were able to achieve greater conductivities, the highest value being 64 S/m. Conductivity was not decreased by 6 days of continuous electrical stimulation Addition of hydrophilic graphene decreased contact angle to approx. 60°, while all other types increased it. Material roughness, porosity, reduced modulus and hardness were also increased by the addition of graphene. C2C12 and RN22 cells adhered and proliferated on hydrophilic graphene composites to an equivalent or greater extent than on pure polymer substrates. Hydrophilic composites also performed well with cardiac myocytes in bioreactor experiments.

CONCLUSIONS: This study demonstrates that graphene-polymer composites offer a valuable alternative to conductive polymers in engineering of cardiac tissues.

ACKNOWLEDGEMENTS: We thank the EPSRC Doctoral Prize Fellowship and the EPSRC Postdoctoral Fellowship (EP/P016898/1) funding for financial support.

REFERENCES

[1] Balint R et al. Acta Biomater. 2014; 10:2341-53



Tissue engineering to enhance cell therapy for traumatic spinal cord injury

R.D. Bartlett[1,2,3], V. Roberton[1,3], D. Choi[2,3], J.B. Phillips[1,3]
Presenting Author: Richard D Bartlett, richard.bartlett.11@ucl.ac.uk
[1]Department of Pharmacology, UCL School of Pharmacy, University College London [2]Brain Repair and Rehabilitation, Institute of Neurology, University College London [3]UCL Centre for Nerve Engineering University College London, United Kingdom

INTRODUCTION: Olfactory ensheathing cells (OECs) demonstrate convincing efficacy in animal models of spinal cord injury and they can be safely harvested from human patients to facilitate autologous transplant. However, only 1% of OECs remain viable at the lesion site and the spinal cord microenvironment lacks the structural guidance cues required for effective regeneration. Such factors limit the current effectiveness of OEC transplant and provide opportunities for significant improvement. We aimed to optimize gel formulation and cell alignment to generate cellular OEC hydrogels suitable for spinal cord repair.

METHODS: OECs were extracted from Sprague-Dawley rats and cultured for 14 days. Cells were then seeded into various collagen and fibrin hydrogels. Gels were incubated for 16 hours in vitro, after which time live-dead assay or immunocytochemistry were performed. OEC-seeded gels were tested in vivo using a rat dorsal resection model of spinal cord injury. Forepaw posturing was quantified and post-mortem histology performed. The effect of OEC alignment was assessed using dorsal root ganglia neurons.

RESULTS & DISCUSSION: The survival of OECs differed between the various collagen and fibrin hydrogel formulations tested ($p<0.001$ one-way ANOVA, $n = 17$). 10% v/v fibrin conferred the best cell survival with 85% of OECs remaining alive. Collagen hydrogels promoted the highest proportion of $p75^{NTR}$ immunopositive cells (78%, $p<0.0001$ one-way ANOVA, $n = 24$). Compared with OEC collagen hydrogels, fibrin OEC hydrogels resulted in significant improvements in rat forepaw posturing at 14 days post-injury ($p<0.05$, independent t-test, $n = 5$). Highly-aligned OEC constructs guided the linear extension of dorsal root ganglia neurites.

CONCLUSIONS: Collagen and fibrin hydrogels promote favorable OEC survival and increase the proportion of cells expressing a key repair marker. Collagen hydrogels can also be used to create highly-aligned OEC constructs, which guide neurites and may help ensure organized axon regeneration across the spinal cord lesion site in the future. This work marks important progress to improve the survival, phenotype and organization of OECs for spinal cord repair.

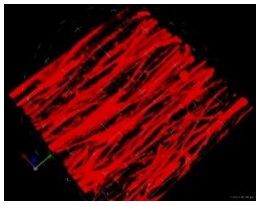
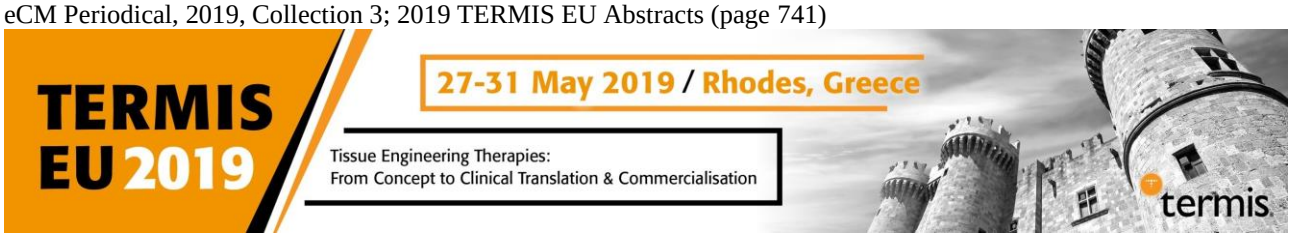


Figure 1: Confocal micrograph of engineered cellular tissue containing highly-aligned OECs capable of guiding neurite regeneration.

ACKNOWLEDGEMENTS: Kindly supported by the UCL MB/PhD programme, the Sackler Fund, the Wellcome Trust and the UCL/UCLH NIHR Biomedical Research Centre.



The development of a novel 3D bioprinted smart responsive dressing to treat skin scars

R. Ibanez[1], R.M.F.M. Lemoine[1-3], R.L. Reis[4-5], A.P. Marques[4-5], F.J. O'Brien[1-3]
Presenting Author: Rita Ibanez, ritaibanez@rcsi.com
[1]Tissue Engineering Research Group, Royal College of Surgeons in Ireland, Ireland [2]Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Ireland [3]Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI & TCD, Ireland [4]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Avepark 4805-017 Barco, Guimarães, Portugal [5]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

INTRODUCTION: Current treatments for skin injuries are limited by the prevalence of bacterial infection[1].Herein we propose a bi-layered antimicrobial scaffold-based dressing that mimics the epidermal and dermal layers of skin, potentially enhancing wound healing while limiting bacterial infection. The aim of the study was to use 3D printing technology to print a gelatin-methacrylate (GelMA) porous scaffold representative of the dermal layer combined with a collagen-chitosan film with antimicrobial potential to represent the epidermal surface.

METHODS: 3D GelMA constructs with varying inter-filament distances (0.6 mm – 2 mm) were printed with a Biobots 2 (Allevi, USA) 3D printer. Construct biocompatibility was determined culturing primary human fibroblasts on the scaffolds and analysing with Picogreen and live-dead staining. Collagen, chitosan and Collagen:Chitosan (Col\Cht) 1:1 v/v films were prepared. Mechanical tensile testing of the films was performed. An antimicrobial assay was conducted using Staphylococcus Aureus (SA). Biocompatibility and proliferation were assessed using a Human keratinocyte cell line (HaCat) and analysed with Alamar blue and Picogreen.

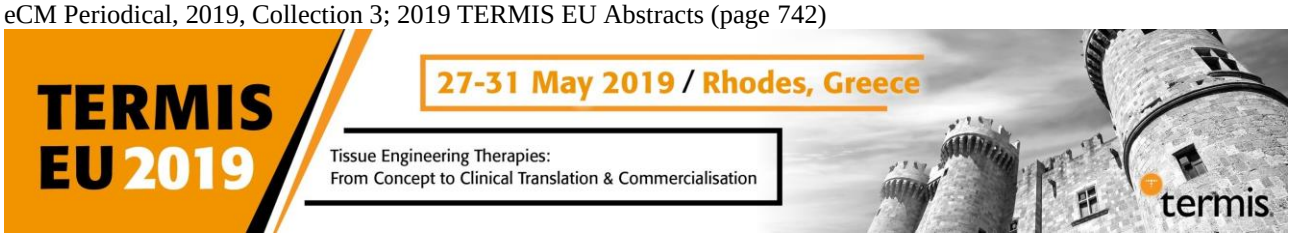
RESULTS & DISCUSSION: High resolution scaffolds were obtained with 3D printed GelMA; different sized pores (272 µm - 898 µm) were achieved by altering inter-filament distance ($p<0.05$). Col\Cht films present mechanical characteristics similar to native skin[2] with a significant increase in ultimate tensile strength (UTS) compared with chitosan alone ($p<0.05$) while no difference was observed on the Young's moduli (data not shown). The films also show remarkable antimicrobial activity against SA ($p<0.05$). The film support HaCat viability (data not shown).

CONCLUSIONS: We have shown that it is possible to print a versatile GelMa scaffold targeted towards dermal regeneration and we also produced an epidermal film that is both antibacterial and capable of supporting mammalian cell growth. The development of this new antimicrobial bi-layered scaffold-based dressing may lead to an effective new treatment for burn injuries that may have a significant impact on patients, clinicians and healthcare systems.

ACKNOWLEDGEMENTS: Science Foundation Ireland, EUH2020 M-ERA.NET programme (16/M-ERA/3420)

REFERENCES

[1] Groeber F et al, Adv Drug Deliv Rev. 2011,(4–5):352–66
[2] Ni Annaidh A et al, Mech Behav Biomed Mater. 2012, 5(1):139-48



Functionalised electro-spun meshes for abdominal wall wounds

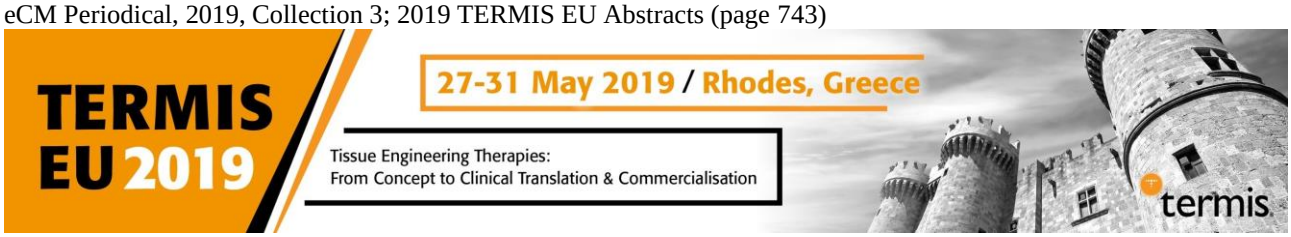
R. Peixoto[,2], D.I. Zeugolis[1,2]
Presenting Author: Rita Peixoto, r.peixoto1@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway [2]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

Wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner become chronic wounds. These non-healing wounds represent a challenging and escalating problem which exposes patients to additional complications, decrease in their quality of life and long-term treatment with high costs [1,2]. Herein, we develop a drug-controlled release scaffold as a wound healing treatment. A functionalized scaffold was fabricated as a prophylactic wound healing therapy. The fabrication and functionalisation were then followed by morphological, mechanical, thermal and biochemical characterization of the produced scaffolds, including the release profile of the loaded drugs. A drug loaded scaffold has potential value as a wound healing treatment for abdominal wall surgical wounds.

ACKNOWLEDGEMENTS: The authors would like to thank Science Foundation Ireland and the European Regional Development Fund (Grant Agreement Number: 13/RC/2073) for providing financial support to this project.

REFERENCES

[1] Abrigo M. et al. Macromol Biosci. 2014; 14(6):772-92
[2] Hassiba A.J. et al. Nanomedicine. 2016; 11(6):715-37



A human bone-derived 3D scaffold for studying MSC interactions within the skeletal niche

AR. Pereira[1,2], D. Gehweiler[3], D. Trivanović[1,2], T. Kreuzahler[1,2], M. Herrmann[1,2]
Presenting Author: Rita Pereira, r-pereira.klh@uni-wuerzburg.de
[1]IZKF Group Tissue Regeneration in Musculoskeletal Diseases, University Hospital Wuerzburg, Germany [2]Orthopedic Center for Musculoskeletal Research, University of Wuerzburg, Germany [3]AO Research Institute Davos, Switzerland

INTRODUCTION: Approximately 10% of individuals with bone fractures fail to heal under the usual treatments, where the underlying reasons are currently not totally understood [1]. It is well established that mobilization and activation of resident mesenchymal stem/stromal (MSCs) and/or adjacent tissue' cells are involved in triggering of bone healing. This process is strictly controlled by complex interactions with the physical and chemical environment. We here aim to develop a meaningful in vitro model where the response of MSC to individual signals can be studied in a naïve microenvironment resembling naïve skeletal stem cell niche.

METHODS: Human femoral heads were collected after patient's total hip arthroplasty with informed consent and agreement of the local ethics committee. Bone slices of 3 mm thickness were decellularized and decalcified. Cylindrical-shaped scaffolds with 5 mm diameter were prepared and lyophilized. The efficiency of the decalcification and decellularization protocol was confirmed by x-ray imaging of scaffolds' bone mineral density and qualitative histology confirmation of nuclei absence, respectively. Information of the decellularized scaffold structure was obtained by micro computed tomography (m-CT). MSCs were isolated from human bone marrow of femoral heads, as previously described, and seeded in passage 2-5 into the scaffolds at different cell densities. Their morphology and distribution through the scaffold was assessed using CellTracker™ Green labelling. MSCs' metabolic activity was studied throughout culture time using reduction of resazurin based-assays.

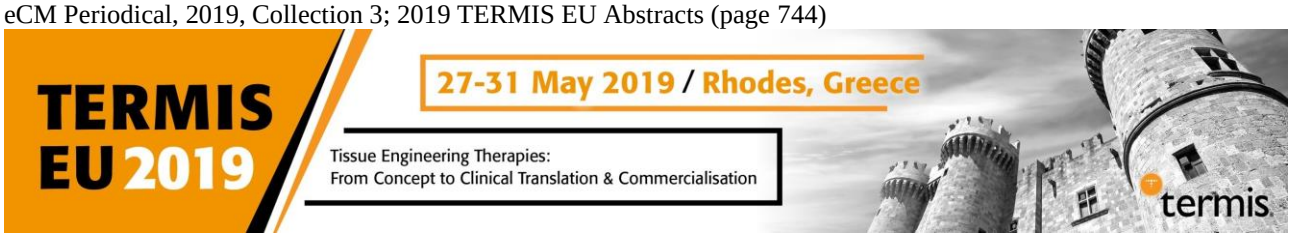
RESULTS & DISCUSSION: The here established protocol resulted in homogenous and regular decellularized and decalcified bone scaffolds. The scaffolds size did not present significant inter- or intra- donor variation. The conservation of human native bone ultra-structure was illustrated by histology of decellularized scaffolds and confirmed with m-CT quantitative analysis of connectivity and trabeculae features. Seeding of green labelled MSCs in the scaffolds revealed attachment of cells to porous surfaces after 24h as well as homogeneous distribution in the scaffold volume over time. Viability of MSCs and their sustained metabolic activity was assessed through the reduction of resazurin in culture media over time, showing that viable MSCs persist over long culture times. Increase of metabolic activity over time was slower when compared with 2D monocultures.

CONCLUSIONS: Our results show that meaningful human bone-derived 3D in vitro models could successfully be produced and support MSCs viability. Future studies will further investigate the MSC phenotype in the scaffolds as well as their response to microenvironmental cues.

ACKNOWLEDGEMENTS: This work is supported by the Interdisciplinary Center for Clinical Research (IZKF) at the University of Wuerzburg (Project D-361).

REFERENCES

[1] Holmes D. Nature. 2017; 550: 193



Circulating BST2+ cells are functionally activated by the injury-regulated systemic factor HGFA

C. Lo Sicco[1], D. Reverberi[1], F. Villa[1], U. Pfeffer[1], R. Cancedda[2], R. Tasso[1,2]
Presenting Author: Roberta Tasso, roberta.tasso@unige.it
[1]Ospedale Policlinico San Martino, Genova, Italy [2]University of Genova, Genova, Italy

INTRODUCTION: Restoration of damaged tissues through the activation of endogenous progenitors is an attractive therapeutic option [1]. A deep evaluation of the intrinsic stem/progenitor cell properties as well as the reciprocal interactions with injured environments is of critical importance. In this context, it has been recently shown that a population of circulating progenitors, defined as circulating healing (CH) cells, characterized by a lineage-negative/CD45-negative (Lin-CD45-) profile, possess a strong chemotactic potential, being responsive to the signals released by an injured environment as the one generated by a bone fracture [2]. These stimuli directed their migration, with subsequent engraftment and differentiation into specific cells belonging to the affected tissue [2].

METHODS: A distinctive core signature characterizes CH cells. In particular, genes encoding for cell surface antigens were deeply analyzed and Bone Marrow Stromal Cell Antigen 2 (BST2) was identified as an efficient CH cell marker. Taking advantage of a mouse model of stabilized transverse femoral fracture we demonstrated the bone marrow (BM) origin of BST2+ CH cells, their homing capacity toward the lesion site as well as their specific activation under injury conditions.

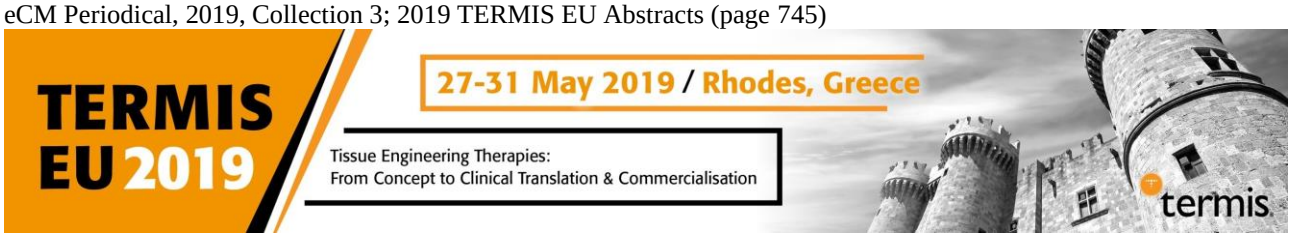
RESULTS & DISCUSSION: We here report for the first time the existence of a rare population of BM-derived progenitors co-expressing LepR and BST2, traceable in the circulation of naive mice in response to normal physiological stimuli, and more importantly, activated under injury conditions. CH cells share the distinctive property of many adult progenitors to be retained in a quiescent, non-cycling state, until they are needed for maintaining tissue homeostasis or enhancing tissue repair. In our experimental model, during
the acute phase response of the host to bone fracture, BM-derived CH cells start to proliferate and over-express specific genes related to motility pathways, leading, in the later phases of the healing process, to an efficient cell engraftment within the hard callus and the articular cartilage of injured mice. The proof that injury induces the release of high levels of HGFA in the serum and that CH cells significantly over-express the gene encoding for the HGF-receptor (Met) when they circulate in physiologic conditions or when they are present in the bone marrow of injured mice has suggested that HGFA could be also involved in their priming. Indeed, the administration of a single dose of rHGFA mimicked the fracture effects, prompting the transitioning of CH cells to an alerted state and the functional changes implicated in the complex events underlying cell migration and activation.

CONCLUSIONS: Taken together, these results could pave the way for the identification of new strategies to enhance and potentiate endogenous regenerative mechanisms for future therapies.

ACKNOWLEDGEMENTS: Financial support was received from the Italian Ministry of Health ("Young Investigator Grant"—GR-2013-02357519).

REFERENCES

[1] Miller F et al. Cell Stem Cell. 2012;10(6):650-652.
[2] Lo Sicco C et al. Sci Rep. 2015;5:16574.



Real-time measurements of 3D cardiac microtissue electrical activity integrated in a beating heart-on-chip

R. Visone[1], G.S. Ugolini[1], D. Cruz-Moreira[1], P. Occhetta[2,3], M. Rasponi[1]
Presenting Author: Roberta Visone, roberta.visone@polimi.it
[1]Department of Electronics, Information and Bioengineering, Politecnico di Milano [2]Departments of Surgery and of Biomedicine, University Hospital Basel, [3]BiomimX Srl, Milano, Italy

INTRODUCTION: Drug discovery is a long and expensive process, whose efficiency is often limited due to the poor predictivity of in vitro models currently exploited in the pre-clinical phases [1]. Among others, cardiac toxicity represents a prevalent adverse effect preventing the drug to reach the market. Here we present a new beating heart-on-chip platform able to generate functional 3D human cardiac microtissues, whose electrophysiological signals can be directly extracted during culture by means of an integrated electrical measurement system. The proposed microfluidic platform represents a powerful pre-clinical cardiac model to screen the cardiotoxic effects of new compounds.

METHODS: The platform design builds upon our previous beating-heart-on-chip [2]. 3D cell laden hydrogels are conditioned through a combination of biochemical and mechanical (uniaxial strain 10-12% at 1Hz) stimulations. Microtissue electrophysiological measurements are recorded by means of an integrated electrical system consisting of paired electrodes inserted in specific positions through dedicated guide microchannels. Neonatal rat cardiomyocytes (NRCMs) or human induced pluripotent stem cell derived cardiomyocytes (h-iPSC-CMs) were embedded at $100 \cdot 10^6$ cells/ml in fibrin gel (20mg/ml) and cultured within the platform for up to 10 days. Microtissue electrical activity, named field potential, was recorded after the onset of a spontaneous and synchronous beating of the whole cardiac construct (between day 6 and day 10). The effects of compounds known to alter the cardiac electrical activity (Verapamil, Terfenadine, Sotalol) were evaluated by evaluating the variations in the field potential morphology induced after 10 min of administration at incremental concentrations.

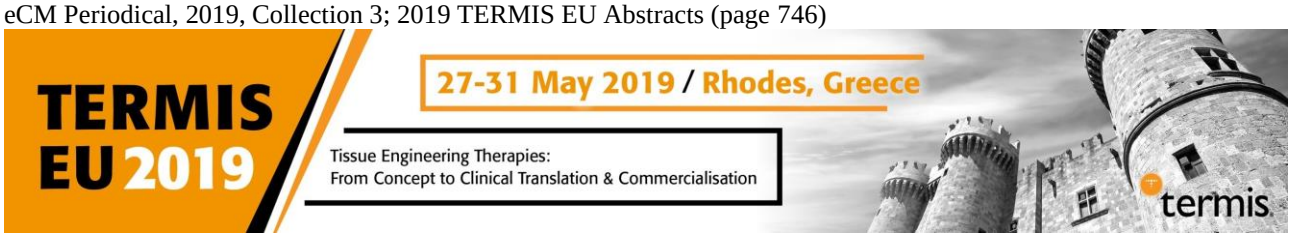
RESULTS & DISCUSSION: Cardiac microtissues from NRCMs and h-iPSC-CM showed spontaneous beating after 3 days of mechanical stimulation within the platform, which was enhanced and synchronized during time (day 6-10). Field potential measurements from rat microtissues revealed that Verapamil prolongs the repolarization time in a dose-dependent manner (i.e. 1-1000nM). Terfenadine resulted to prolong the repolarization time of human cardiac microtissue at high concentrations (i.e. 100-1000 nM) and Sotalol showed to prolong the repolarization wave peak in both rat and human- cardiac microtissues.

CONCLUSIONS: The beating heart-on-chip generates functional cardiac microtissues responding to drugs in a physiological manner [3]. The innovative integrated recording system makes the platform a unique tool for direct on-chip cardiotoxicity drug screening.

ACKNOWLEDGEMENTS: This work was supported by Fondazione Cariplo (grant2012-0891), and by the European Commission Horizon 2020, MSCA-ITN-ETN European Training Networks (Project 642458).

REFERENCES

[1] Stevens, Drug Discov Today, 2009;14:162-7
[2] Marsano A et al. Lab Chip. 2016; 16(3):599-610
[3] Ando H et al. J Pharmacol Toxicol Meth. 2017; 84:111-127



Surface modification of biodegradable scaffolds via diazonium chemistry for tissue engineering: A piezoresponse and wettability study

R.V. Chernozem[1,2], O.A. Guselnikova[1], M.A. Surmeneva[1], P.S. Postnikov[1], A.G. Skirtach[2], R.A. Surmenev[1]
Presenting Author: Roman Chernozem, r.chernozem@mail.ru
[1]Tomsk Polytechnic University, Tomsk, Russia [2]Ghent University, Ghent, Belgium

INTRODUCTION: Biodegradable polymers, such as polycaprolactone (PCL) and polyhydroxybutyrate (PHB), are of great importance in tissue engineering. Moreover, PHB possesses piezoelectric properties allow to delivering directly electrical and electrochemical stimuli to cells. Recently, it has been reported that diazonium chemistry can be successfully utilized to improve wettability of polyvinylidenefluoride/polymethylmethacrylat. However, its influence on the piezoelectric response is still not studied in sufficient details. Therefore, the aim of this study is to improve the wettability and preserve the piezoelectric properties of biodegradable scaffolds based on PCL and PHB using diazonium chemistry for tissue engineering.

METHODS: The fibrous PCL and PHB scaffolds were fabricated via the electrospinning technique. The prepared samples were soaked in 20 mM solutions of 3,4-dicarboxybenzenediazonium tosylate. After immersion, the samples were treated by UV-light using a Sylvania G15T8 15W (254 nm) UV-lamp. A sessile drop method and Wide-range d_{33} meter were used to investigate the wettability and piezoelectric properties of the samples before and after surface modification.

RESULTS AND DISCUSSION: Figure 1 presents the results of the wettability and piezoelectric charge constant d_{33} measurements before and after polymer scaffolds surface modification. It can be seen that water contact angle is significantly decreased for PHB and PCL scaffolds after surface modification (Fig. 1A). Besides that, almost unchanged d_{33} coefficient was observed after treatment of PHB scaffolds (Fig. 1B). The slight deviation can be explained taking into account a thin molecular COOH layer onto the fiber surface. The piezoelectric properties of PHB scaffolds are in good agreement with literature.

CONCLUSIONS: The diazonium chemistry allows to significantly improve surface wettability while maintaining the piezoelectricity of the polymer scaffolds due to the formation of the COOH-functionalized molecular layer. The PCL and PHB scaffolds modified with diazonium salt can be useful for different tissue engineering applications.

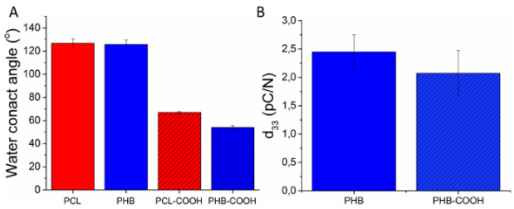
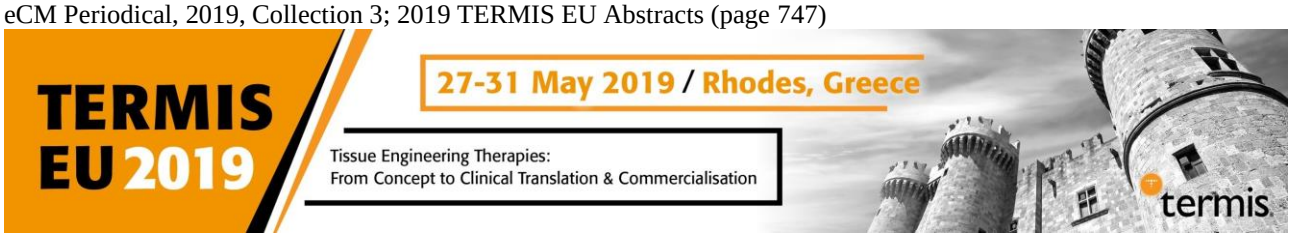


Figure 1: (A) Wettability and (B) piezoelectric d_{33} charge coefficient of the biodegradable fibrous scaffolds based on PCL and PHB before and after surface modification

ACKNOWLEDGEMENTS: This research is supported by the Special Research Fund (BOF) of Ghent University (BOF16/FJD/029). The present study was supported by Russian Science Foundation (18-73-10050).



Adult neural crest-derived stem cells from human hair follicle and skin dermis for regenerative medicine applications

R. Vasyliev[1,2], O. Gubar[2,3], I. Gordiienko[2,4], L. Litvinova[5], A. Rodnichenko[1,2], V. Shupletsova[5], A. Zlatska[1,2], K. Yurova[5], N. Todosenko[5], V. Khadzhynova[2], M. Shulga[2], S. Novikova[1], D. Zubov[1,2]
Presenting Author: RomanVasyliev, rvasyliev.ilaya@gmail.com
[1]State Institute of Genetic & Regenerative Medicine NAMSU, Kiev, Ukraine [2]Medical company ilaya®, Kiev, Ukraine [3]Institute of Molecular Biology & Genetics NASU, Kiev, Ukraine [4]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NASU, Kiev, Ukraine [5]Immanuel Kant Baltic Federal University, Kaliningrad, Russia

INTRODUCTION: The adult neural crest-derived stem cells (NCSCs) have significant potential for regenerative medicine [1]. The promising sources for the isolation of adult NCSCs are the skin dermis (SD) and hair follicle (HF) due to the come-at-able and low invasive biopsy procedure. The main purposes of the study were an isolation, large-scale expansion and comparative characterization of HF- and SD-derived NCSCs.

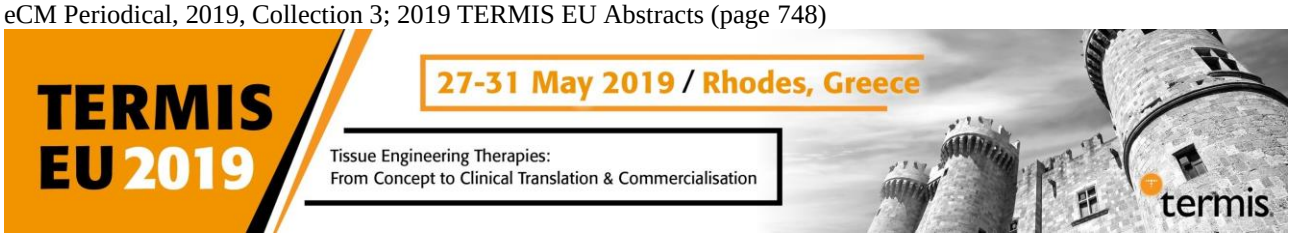
METHODS: The HF NCSCs were obtained by explant method [2] in our modification [3]. The SD NCSCs were isolated by original method combining enzymatic dissociation, pre-plating and cultivation under selective conditions. The SD and HF NCSCs characterization was provided by flow cytometry, karyotyping, ICC, qPCR and Bio-Plex multiplex assay. Also a frequency of CFUs, ability to self-renewal, spherogenesis and directed multilineage differentiation were determined.

RESULTS & DISCUSSION: Adult SD and HF NCSCs were successfully obtained from a same skin samples. For cell isolation two skin specimens ø4 mm per donor (n = 10) were used. HF and SD NCSCs had slightly different morphology (elongated vs compact polygonal). SD NCSCs showed a higher growth rate than HF NCSCs during expansion under standard 2D (PDT 30.2±3.8 vs 34.4±8.0 h) and 3D fibrin hydrogel (PDT 20.8±0.5 vs 21.8±0.8 h) conditions. SD NCSCs also showed a higher CFUs frequency: 34±3% compared to 25±5%. HF and SD NCSCs have similar phenotype: $SOX10^+$ $CD271^+$ $NESTIN^+$ $SOX2^+$ $CD73^+$ $CD90^+$ $CD105^+$ $CD140a^+$ $CD140b^+$ $CD349^+$ $CD34^-$ $CD45^-$ CD56 $CD117^-$ $HLA\text{-}DR^-$. Importantly, after large-scale expansion the population of SD NCSCs contains more SOX10, CD271, CD105, CD140a, CD146 and CD349 positive cells. HF and SD NCSCs expressed at similar level epy following genes: OCT3/4, SOX2, KLF4, C-MYC, NANOG, SOX10, TFAP2A, LNGFR, NESTIN, SNAIL1, SNAIL2, TWIST1, VEGFA, FGF2, NGF, BDNF, GDNF, NTF3, and NTF4/5. Significantly elevated expression of SOX9 was specific for HF NCSCs. On protein level HF NCSCs produced more IL-6, IL-8, IL-16, sIL-2Ra, MCP-1, M-CSF and HGF, while SD NCSCs produced more IL-3, CTACK, SDF-1a, GM-CSF, SCGF, VEGF-A, FGF-2 and NGF. It was shown the ability of adult HF and SD NCSCs to directed differentiation into the neurons, Schwann cells, adipocytes and osteoblasts.

CONCLUSIONS: The HF and SD are useful sources for large-scale expansion of adult NCSCs with similar biological properties. SD NCSC demonstrated higher proliferation rate and formed more homogenous population. SD NCSC isolation method is cheaper, easier and minimal time consuming.

REFERENCES

[1] Achilleos A et al. Cell Res. 2012;22(2):288-304
[2] Sieber-Blum M. et al. Dev Dyn. 2004;231(2): 258-269
[3] Vasyliev R et al Exp. Oncol. 2017;39(3):171-180



Toward building cartilage by mechanotransduction: Evidence that primary OA chondrocytes can transduce mechanical loads through central metabolism

D. Salinas[1], B. Mumey[2], R. Carlson[3], R. June[4]
Presenting Author: Ron June, rjune@montana.edu
[1]Computer Science Department, Westminster College [2]Gianforte School of Computing [3]Chemical & Biological Engineering [4]Mechanical & Industrial Engineering and Cell Biology & Neurosciences. Norm Asbjornson College of Engineering, Montana State University, USA

INTRODUCTION: Mechanical stimulation has long been known to affect chondrocyte synthesis of matrix molecules [1]. Matrix molecules are largely composed of non-essential amino acids. Non-essential amino acids are synthesized from central metabolism. The objective of this study is to determine if chondrocytes can transduce mechanical loads through central metabolism in a manner consistent with synthesis of non-essential amino acids.

METHODS: Primary human chondrocytes from Grade IV OA patients were expanded for 1 passage, encapsulated in 4.5% v/v agarose, and stimulated with 5±1.9% sinusoidal compression for 0, 15, or 30 minutes [2]. Metabolites of central metabolism were analyzed using LC-MS, and central energy metabolism was modeled using metabolic flux analysis [3].

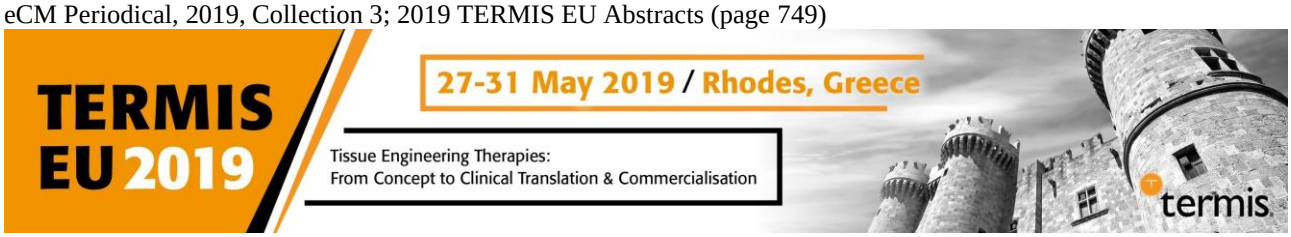
RESULTS & DISCUSSION: Compression induced significant changes in the metabolites. These metabolite changes resulted in reaction fluxes for central metabolism for both intervals (0-15 and 15-30 min). These fluxes depended on the donor, but all donors responded to compression.

CONCLUSIONS: The greatest magnitude and variation in metabolic fluxes was encompassed by the reactions modeling the enzymes adenylsuccinate lyase and adenylsuccinate synthetase isozyme 1, aspartate decarboxylase, aspartate oxidase, lactate dehydrogenase, fumarase, and malate dehydrogenase (ASal, ASde, ASox, PYLAC, TCA7, and TCA8). These reactions contribute to producing non-essential amino acids necessary for matrix production. For example, the protein sequence of collagen (COL2A1) includes 62 aspartate residues which are directly produced by TCA7 and TCA8. These data indicate that tissue engineering solutions to cartilage repair may benefit from directed interventions that enhance metabolic production of amino acid precursors by mechanotransduction.

ACKNOWLEDGEMENTS: Financial support from the NSF (1342420, 1554708, 1542262) and NIH (P20GM103474).

REFERENCES

[1] Sah RL et al. J Orth Res. 1989;7(5):619-36
[2] Zignego DL et al. 2015 Dec 16;48(16):4253-61
[3] Salinas D et al. Biomech Model Mechanobiol (2018)



Incorporation of platelet-rich plasma into collagen-glycosaminoglycan scaffolds for wound repair

R.J.F.C. do Amaral[1,2], N.M.A. Zayed[1,3], E.I. Pascu[1], B. Cavanagh[4], F.J. O'Brien[1,2,5,6], C.J. Kearney[1,5,6]

Presenting Author: Ronaldo do Amaral, ronaldoamaral@rcsi.ie

[1]Kearney Lab and Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Centre for Research in Medical Devices (CURAM), National University of Ireland Galway, Galway, Ireland [3]Khalifa University, Abu Dhabi, United Arab Emirates [4]Cellular and Molecular Imaging Core, RCSI, Dublin, Ireland [5]Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI, Dublin, Ireland [6]Trinity Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland

INTRODUCTION: Collagen-glycosaminoglycan (CG) scaffolds [1] are a proven biomimetic regenerative dermal template, which lack growth factors (GFs) that could improve their regenerative potential. Those could be provided by platelet-rich plasma (PRP) [2] - an autologous source of GFs. Therefore, we incorporated PRP into CG scaffolds and evaluated its effects for wound repair.

METHODS: After incorporation of PRP into CG scaffolds, the resulting scaffolds (PCG) were analyzed by histological techniques, mechanical testing (5N load cell), ELISA for GFs release (e.g., PDGF-BB, bFGF and VEGF) and scaffolds an in vitro vascularization assay [2].

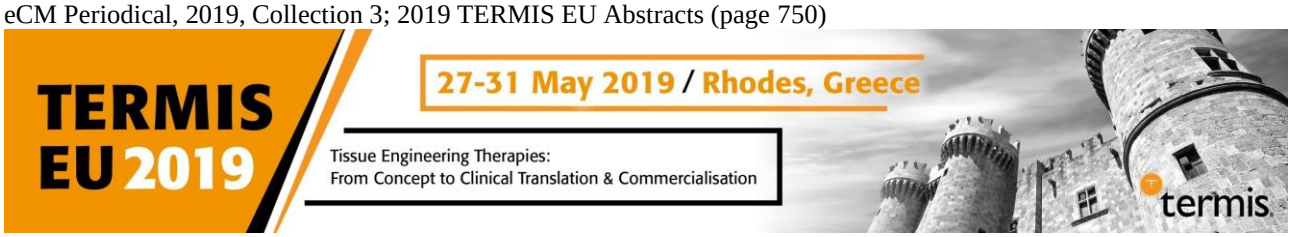
RESULTS & DISCUSSION: PRP was homogenously incorporated into CG scaffolds. PCG scaffolds had a tensile modulus 3 times higher than that of the PRP clot alone. GFs were successfully released from PCG for up to 14 days and the novel scaffolds elicited a 2-fold increase in vessel formation over CG scaffolds in vitro.

CONCLUSIONS: Fabrication techniques were developed to homogeneously incorporate PRP in CG scaffolds (PCG). These novel scaffolds had improved mechanical properties, released GFs for up to 14 days and demonstrated enhanced vascularization over control scaffolds in vitro. To fully confirm the effectiveness of PCG for wound repair, in vitro and in vivo dermal and epidermal studies are being performed

ACKNOWLEDGEMENTS: European Region Development fund/SFI (13/RC/2073); ERC StG: BONDS (758064); EUH2020 Marie Curie (713690); and Science Foundation Ireland (SFI/12/RC/2278).

REFERENCES

[1] McFadden T et al. Acta Biomat. 2013; 9: 9303-9316.
[2] do Amaral R J F C et al. InTech. 2015; 11: 221-237.



High-throughput biofabrication of organoids for osteochondral tissue engineering

R. Burdis[1,2], D. Kelly[1,2]
Presenting Author: Ross Burdis, burdisr@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland [2]Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Ireland

INTRODUCTION: Cellular self-assembly (SA) recapitulates distinct phases of articular cartilage development [1] and can be used to engineer cartilage comparable to the native tissue in vitro [2]. SA has also been used to produce hypertrophic cartilage templates capable of forming a bone ossicle in vivo via endochondral ossification [3]. Leveraging the benefits associated with self-assembled µtissues from MSCs, our goal is to biofabricate an osteochondral implant using such organoids as biological building blocks. The objective of this study was to develop a high-throughput (HT) method for producing cellular µtissues that could be used in 3D bioprinting applications, and use self-organisation to generate hyaline cartilage via fusion of these µtissues.

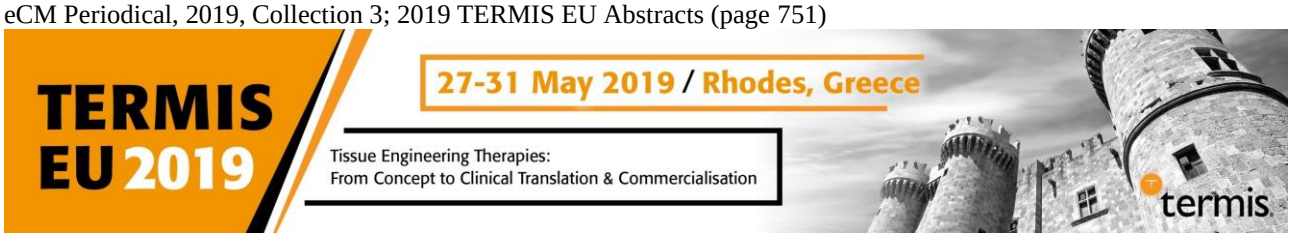
METHODS: HT-SA: Molten agarose (4 % w/v) was cast into a custom microwell array. Each array consisted of 401 x 1 mm Ø round bottom wells. MSCs and chondrocytes (3:1 ratio) were seeded to create four pellet sizes, doubling in density from 0.1 x 10^4 to 1.6 x 10^4 cells/pellet. µtissue growth was evaluated over 7 days, 4 days in expansion medium (+ FGF-2) and 3 in chondrogenic medium (CDM+). Self-Organisation: Primed µpellets were seeded into 3 mm Ø agarose wells (2 % w/v). The self-organised (SO) tissues were evaluated visually, mechanically, biochemically, and histologically over 4 weeks (in CDM+). Bioprinting µTissues: Prior to printing, an alginate hydrogel was pre-crosslinked using 40 mM calcium chloride solution, yielding a printable bioink (2.45 % w/v LVG alginate). The µpellet laden bioink was extruded through a piston assisted pneumatic microextrusion system (3D Discovery, RegenHU). Live/dead imaging was used to evaluate the cell viability after 3 days. 3D Printing an Anatomically Accurate Implant: µCT scans were used to determine the geometry of the lapine proximal humeral head. The implant was fabricated from a biodegradable polymer, polycaprolactone (PCL), using a FDM system (3D Discovery, RegenHU).

RESULTS & DISCUSSION: µtissues were successfully generated via SA using a range of starting cell densities. The diameter of these µtissues significantly increased with the addition of CDM+. Harvested cartilage spheroids were shown to SO after 2 days, forming interconnections which resulted in a unified neo-tissue. The viability of bioprinting as a means of spatially organising µtissues was demonstrated by maintaining cell viability post extrusion. Finally, a PCL implant, designed using medical images, has been 3D printed using FDM as a putative structural support for bioprinted µtissues.

CONCLUSIONS: Collectively, a high-throughput method for the generation of self-assembled chondrogenic µtissues, amenable to biofabricating scaled-up osteochondral tissues via bioprinting, has been demonstrated. The resultant construct is intended as a patient specific biological implant for total joint replacement.

REFERENCES

[1] Athanasiou (et al.), Rev. Biomed. Eng. 2013; 15, 115-136
[2] Bhumiratana (et al.), Proc. Natl. Acad. Sci. 2014; 111, 6940–6945
[3] Scotti (et al.), Proc. Natl. Acad. Sci. 2013; 110, 3997–4002



A combined design and fabrication strategy to engineer biomimetic implants for osteochondral tissue engineering

R. Schipani[1], S. E. Critchley[1], D.R. Nolan[1], E. Alsberg[2], C. Lally[1], D. J. Kelly[1]
Presenting Author: Rossana Schipani, schipanr@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland [2]Case Western Reserve University, Cleveland, OH, USA

INTRODUCTION: Finite Element (FE) modelling integrated with 3D bioprinting offers a powerful tool for the development of functional tissue replacements [1]. The aims of this study were to use FE models to design mechanically functional 3D printed polycaprolactone (PCL) scaffolds and to combine these with region-specific cell-laden bioinks to obtain a biphasic implant for osteochondral tissue engineering.

METHODS: The geometry of PCL scaffolds was modulated to obtain two designs with printed filaments that were either Aligned with the previous layer or offset in two planes (termed Double Offset). ABAQUS was used to develop FE models of the scaffolds. The compressive modulus and element fraction experiencing stresses greater than 17MPa (PCL yield stress) were predicted. To validate the FE model, scaffolds compressive modulus and permanent deformation was assessed experimentally. Biphasic implants were printed with two distinct PCL geometries: Double Offset for the chondral phase and a stiffer Aligned configuration for the osseous phase. The chondral phase was filled with a gelMA-alginate bioink containing a co-culture of MSCs and chondrocytes (3:1). The osseous phase was filled with a RGD-y irradiated-alginate bioink containing only MSCs. Constructs were cultured in media supplemented with TGF-ß3 for 42 days. Biochemical, histological and immunohistochemical analysis was performed.

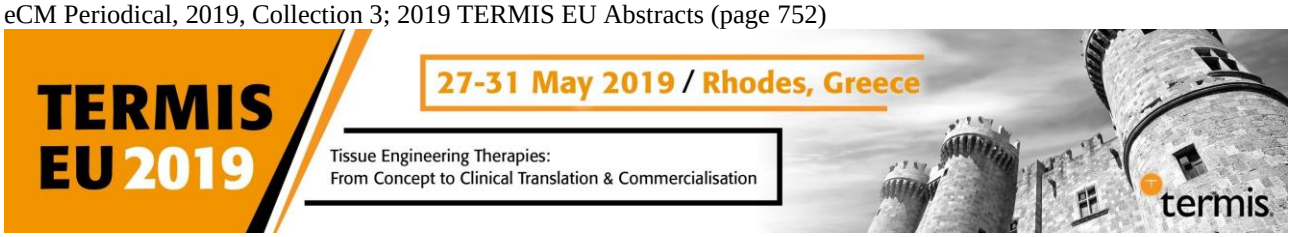
RESULTS & DISCUSSION: FE modelling of different PCL architectures was used to develop scaffold designs with compressive properties comparable to articular cartilage. The plastic deformation experienced by the scaffolds was also tailored. Computational predictions were verified experimentally. GelMA-alginate and RGD-y irradiated-alginate bioinks facilitated the bioprinting of a spatially defined osteochondral-like template. sGAG and collagen levels were higher in the chondral phase of the construct, with more evidence of mineralization and collagen type X observed in the osseous phase.

CONCLUSIONS: CAD based-FE models and 3D printing were successfully used to engineer cell laden, fibre reinforced composites with mechanical properties comparable to native tissues.

ACKNOWLEDGEMENTS: Funded by the ERC grant 204144.

REFERENCES

[1] Hendrikson et al., Front. Bioeng. Biotechnol 5, 1-13 (2017).



Design of polyurethane-based temperature- and pH-responsive hydrogels

R. Laurano[1], M. Boffito[1], M. Abrami[2], M. Grassi[2], G. Ciardelli[1], V. Chiono[1]
Presenting Author: Rossella Laurano, rossella.laurano@polito.it
[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy
[2]Department of Engineering and Architecture, Università di Trieste, Trieste, Italy

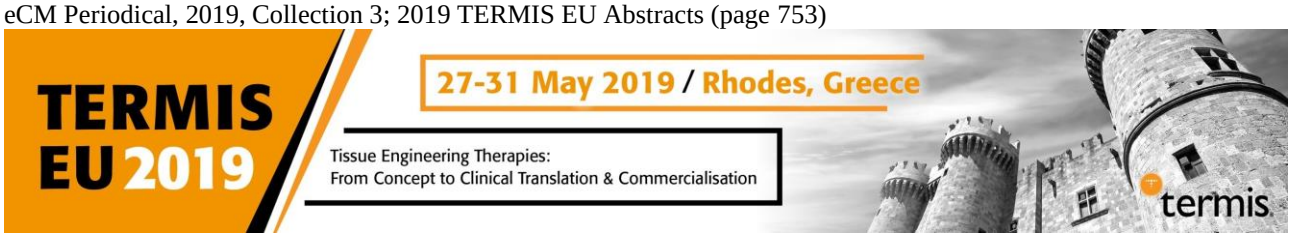
INTRODUCTION: Polyurethanes (PUs) are very tunable polymers due the high versatility of their chemistry that allows the synthesis of materials with very different properties by simply changing their building blocks. With the final aim to design thermo- and pH-responsive hydrogels for injectable or 3D-printing applications, an amphiphilic PU was first synthesized and its powder were then plasma-treated in the presence of acrylic acid (AA) vapor to graft acid groups along polymer backbone.

METHODS: An amphiphilic PU was synthesized according to [1] and its powder with controlled diameter were plasma-treated with Ar and AA vapor to expose –COOH groups (P-PU), thus enhancing hydrogel sensitivity to alkaline pH. Chemical characterization was performed by Size Exclusion Chromatography (SEC), Infrared (IR) Spectroscopy, Toluidine Blue O (TBO) assay and Proton Nuclear Magnetic Resonance (^{1}H-NMR). PU and P-PU gels (15% w/v) were characterized in terms of sensitivity to temperature (tube inverting and gelation time tests at 37°C, rheology) and external pH (hydrogel pH change in response to external pH, swelling test in contact with buffers at pH 5 or 8). Finally, an innovative investigation of hydrogel thermo- and pH-sensitivity was conducted by Low-Field NMR.

RESULTS & DISCUSSION: SEC and IR assessed the successful PU synthesis, while TBO assay, ^{1}H-NMR and IR demonstrated the grafting of –COOH groups to PU chains and evidenced a high intra- and inter-synthesis repeatability of the plasma treatment. Tube inverting, gelation time and rheological tests showed the ability of both PU and P-PU solutions (15%w/v) to undergo a sol-to-gel transition at about 27 °C. However, frequency sweep tests showed different storage and loss moduli crossover frequencies at each analyzed temperature (58 vs 82 rad/s, 4 vs 6 rad/s and 0.25 vs 0.15 rad/s for PU and P-PU gels at 25, 30 and 37 °C, respectively), suggesting that the exposure of -COOH groups slightly slowed down gelation kinetics. Concerning sensitivity to basic pH, P-PU gels transferred alkaline pH from the external medium to the gel core with a significantly faster kinetics, resulting from an increased sensitivity of almost the 60% compared to PU gels. Conversely, both systems behaved similarly in the presence of acid buffers. Swelling test against an alkaline buffer confirmed the improved absorption ability of P-PU gels compared to the control (3.3% vs 1.1%, respectively). These results were further proved by LF-NMR that allowed the investigation of hydrogel structural changes at the micro/nano-scale in response to temperature and pH changes in terms of micelle nucleation and organization and their interaction with water molecules.

CONCLUSIONS: PUs are promising candidates for the design of stimuli-responsive hydrogels, as their properties can be easily tuned by changing their building blocks. In this work, stimuli-sensitive PU-based hydrogels were successfully designed and their thermo- and pH-responsiveness was thoroughly investigated.

ACKNOWLEDGEMENTS: Financial support from the H2020 EU funding for Research & Innovation project "MOZART" (MesopOrous matrices for localiZed pH-triggered releAse of theRapeuTic ions and drugs)(H2020-NMP6-2015) is acknowledged.



Self-assembling Laponite hydrogel scaffolds with spontaneous 3D micropatterning of bioactive factors for bone tissue regeneration

R.S. Ramnarine-Sanchez[1], N. Evans[1], R. Oreffo[1], J. Dawson[1]
Presenting Author: Roxanna S Ramnarine-Sanchez, rsrs1g15@soton.ac.uk
[1]Bone & Joint Research Group, Centre for Human Development, Stem Cells & Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton, SO16 6YD, United Kingdom

INTRODUCTION: Biomimicry is an important principle underlying a number of tissue engineering (TE) strategies. Emulating the three dimensional (3D) hierarchical organization of physical and/or biochemical cues of the native cellular microenvironment is likely to be key to building scaffold materials with higher levels of functionality[1]. Despite advances in TE, true 3D patterning of biochemical cues has proved difficult[2]. Clay nanoparticle gels offer potential in TE for their ability to sequester proteins for sustained localized function[3]. The current study reports a simple and biomimetic approach to applying self-assembling clay hydrogels for spontaneous 3D micropatterning of proteins under physiological conditions.

METHODS: Hydrous suspensions of Laponite, a synthetic smectite clay were added to a master solution containing defined concentrations of biomolecules and ions present in blood plasma to initiate a diffusion-reaction mediated self-assembly process. The structures were analysed using a range of microscopy techniques and tested for their ability to pattern model proteins (serum albumin, avidin, streptavidin, immunoglobulin G and casein) and localize the activity of bone morphogenetic protein in vivo.

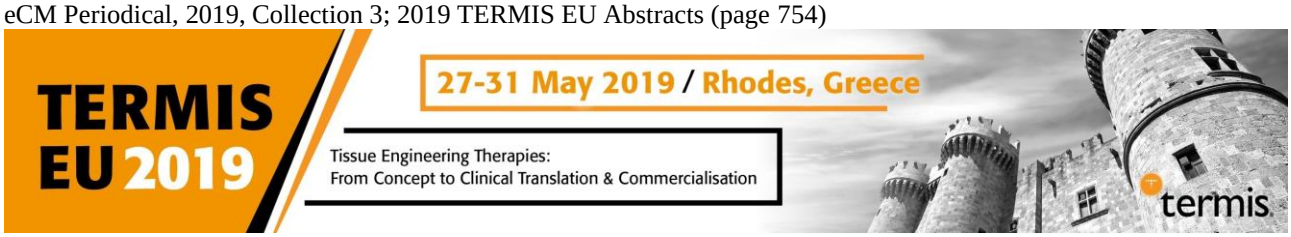
RESULTS & DISCUSSION: Laponite/protein scaffolds possessed an internal degree of order able to template concentration gradients of fluorescently labelled model proteins. Characterization of the structures using confocal scanning laser microscopy demonstrated micron resolution control of 3D patterning according to assembly time, temperature and concentration. Non-self-assembled Laponite gel with blood plasma components adsorbed model proteins on gel surfaces[3]. Polarized light imaging revealed radial birefringence patterns indicating a nanoscale periodical arrangement, which changes as function of time at physiological temperature. Scanning Electron Microscopy and rheological studies show rearrangement of the nanoparticle upon interaction with proteins. Scaffolds loaded with BMP 2 achieved formation of ectopic and localized bone in a murine subcut model.

CONCLUSIONS: This study reveals, for the first time, the possibility to harness interactions between clay nanoparticles, biomolecules and ions present in physiological fluids to trigger the assembly of supramolecular structures of physical and biochemical cues. This bottom up approach affords new opportunities for 3D protein micropatterning and delivery of growth factors for tissue engineering of hard and soft tissues.

ACKNOWLEDGEMENTS: Funded by EPSRC (EP/L010259/1) and University of Southampton, Faculty of Medicine and Faculty of Engineering & Environment postgraduate awards to JI Dawson.

REFERENCES

[1] Chow LW et al. Exp. Biol. Med. 2015; 241: 1025-1032
[2] Wylie RG, et al. Nat. Mater. 2011; 10: 799-806
[3] Dawson JI, et al. Adv. Mater. 2013; 23: 3304-3308



Multilayer, multifunctional core-shell airbrushed nanofiber membrane for periodontal regeneration

R. Singh[1], J. Giri[1]
Presenting Author: Ruby Singh, bm16resch01002@iith.ac.in
[1]Indian Institute of Technology (IIT) Hyderabad, India

INTRODUCTION: Currently used guided tissue regeneration (GTR) mainly act as barrier membrane to prevent down growth of epithelial and gingival tissue without any regenerative functionality, have limited success in the clinic. There is a need to develop new biomimetic, tissue adaptive nanofibers membrane for effective regeneration of periodontal tissues. Electrospinning, most common method of nanofibers fabrication suffers several limitations such as low deposition rate, costly equipment etc. Conversely, our recently developed coaxial airbrushing is a cost effective alternative to electrospinning overcoming its limitations, offering rapid fabrication of coaxial fibers directly on the target of interest with a simple set-up. The objective of this study is to develop a new multilayered (two layers) functional membrane, which not only act as barrier membrane but also promote specific periodontal tissues regeneration by providing microenvironment of chemical cues (Growth Factors; GFs), physical cues (nanofiber topography) and tissue adaptive degradation properties for potential GTR application.

METHODS: In-house built co-axial airbrushing technique was used to fabricate GFs encapsulated two-layer core-shell nanofiber composite membrane with core-shell nanofibers (PCL-PEO blends) of tailor degradation properties. Top layer loaded with bFGF has least porosity whereas bottom layer with VEGF+PDGF is highly porous to promote cell recruitment and migration. Composite nanofibers were characterized by various techniques. Release profiles of different GFs were measured using specific ELISA kits. Three different cells, human gingival fibroblast (HGF), HUVEC and hBMSC cells were used to test the biocompatibility properties as well as specific regenerative functionality of different membrane.

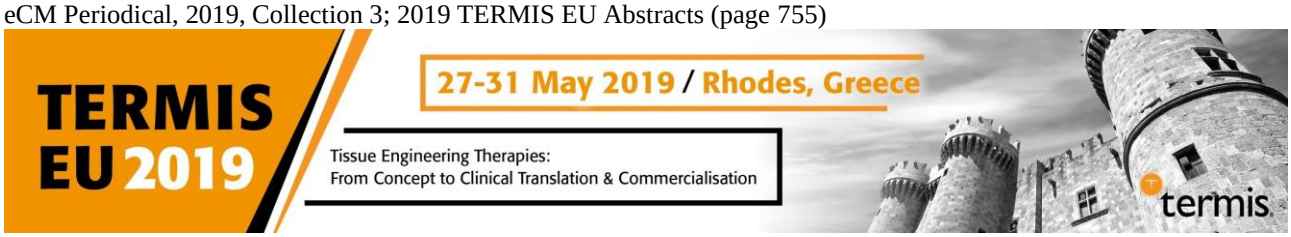
RESULTS & DISCUSSION: For the first time GF encapsulated multilayered nanofibrous scaffold was fabricated by coaxial airbrushing method. Core-shell composite nanofibers membrane shows sustained release of different GF (up to 30 days) to promote the specific biological response. Cells showed extended morphology and higher cell proliferation in GF loaded fibers as compared to control. Top layer promotes proliferation of HGF and showed limited penetration of cells into membrane. Bottom layer, promote proliferation, migration and differentiation of HUVEC and hBMSC.

CONCLUSIONS: This study confirms that the novel multilayer core shell nanofiber scaffolds, fabricated by airbrushing technique is biocompatible, cost effective and have huge potential for periodontal tissue regeneration.

ACKNOWLEDGEMENTS: Financial support was received from DST, Nanomission (SR/NM/-NS-1364/2014(G).

REFERENCES

[1] Singh R et al. ACS Appl. Mater. Interfaces, 2018, 10 (49), pp 41924–41934.
[2] Lee BS et al. Carbohydr Polym. 2016 Oct 20;151:790-802.



Magneto-mechanical actuation of magnetic responsive fibrous scaffolds boosts tenogenic commitment of human adipose stem cells

A.R. Tomás[1,2], R.M.A. Domingues[1,2,3], A.I. Gonçalves[1,2], R.L. Reis[1,2,3], M.E. Gomes[1,2,3]
Presenting Author: Rui MA Domingues, rui.domingues@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal.

INTRODUCTION: The functional behavior of tendons is highly related with their ECM anisotropic alignment and hierarchical organization. As mechanosensitive tissues, the combination of tendon mimetic scaffolds with mechanical stimuli might synergistically contribute for their regeneration. We previously developed 3D fibrous scaffolds that induced stem cell tenogenic commitment and prevented the phenotypic drift of tenocytes [1]. We hypothesize that by combining it with superparamagnetic nanoparticles as remote actuators to mechano-magnetic stimulation, we can further boost their functional regenerative potential.

METHODS: Electrospun nanofiber threads were assembled into yarns and these into woven hierarchical 3D scaffolds [1]. The threads based on PCL were mechanically reinforced with cellulose nanocrystals decorated with iron oxide nanoparticles and coated with polydopamine grafted with hydrophobic dodecanethiol (DT-NP). The tenogenic commitment of hASCs cultured on the magnetic responsive systems was assessed.

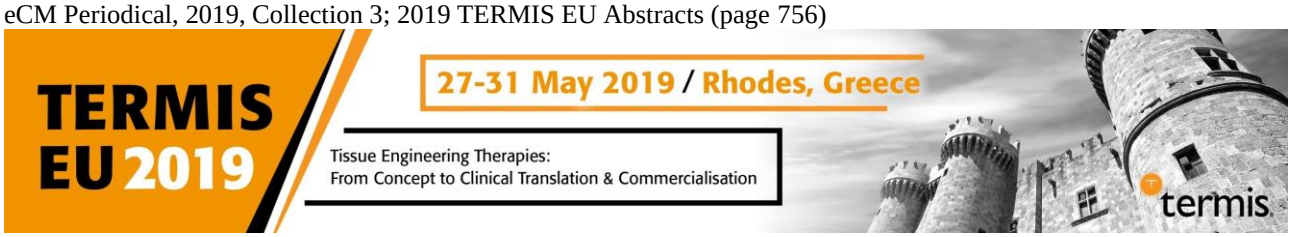
RESULTS & DISCUSSION: Incorporation of DT-NP (0-5wt.%) significantly improved the mechanical properties of the nanofiber yarns, simultaneously endowing them magnetic responsiveness. Magneto-mechanical stimulation of hASCs promoted activation of the mechanosensitive pathway YAP/TAZ, increasing cell cytoskeleton anisotropic organization. As feedback, hASCs showed higher gene and protein expression of tendon-related markers, such as TNMD and SCX, when compared to non-stimulated conditions.

CONCLUSIONS: The engineered magnetic nanofiber yarns were able to mechanically stimulate cells by remote actuation. The scaffolds architecture and stimulation synergistically contribute to boost the tenogenic commitment of hASCs.

ACKNOWLEDGEMENTS: SmarTendon-PTDC/NAN-MAT/30595/2017, ERC CoG MagTendon nr772817, NORTE-01-0145-FEDER-000021, SFRH/BPD/112459/2015, Hospital da Prelada (Porto, Portugal).

REFERENCES

[1] Laranjeira M et al. Small. 2017; 13: 1700689.



AI-guided morphology-based non-invasive cell quality control system for enhancing cell manufacturing consistency

R. Kato[1, 2]
Presenting Author: Ryuji Kato, kato-r@ps.nagoya-u.ac.jp
[1]Department of Basic Medicinal Sciences, Graduate School of Pharmaceutical Sciences, Nagoya University [2]Division of Micro-Nano Mechatronics, Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University, Japan

INTRODUCTION: By the growing expectations toward cellular products, the size and quantity of cells required for applications are exponentially growing. However, manufacturing process for cells for therapy comprises too many parameters that are still not clearly understood, therefore it is still difficult to control its consistency. Especially from the aspect of product quality control, cellular products are difficult to monitor its status to manufacture intact cells. In such situation, as the most practical and historical technique, cellular status monitoring had been achieved by manual microscopic observations. Our group has been proposing the combination of image processing and artificial intelligence (AI)-related machine learning for non-invasive cell quality evaluation technology to monitor cellular status for entire cell culture process [1, 2, 3]. Here we show that cellular morphological profiling guided by AI prediction can be applied to support the quality control of consistent cell manufacturing.

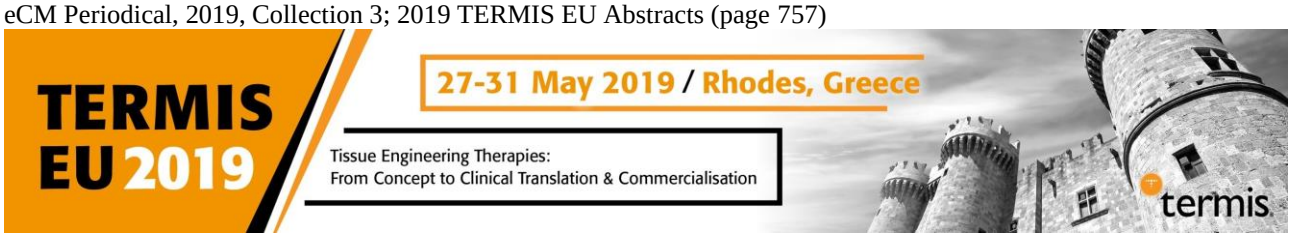
METHODS: Mesenchymal stem cells (MSCs), neural stem cells (NSCs), and induced pluripotent stem cells (iPSCs) are cultured under various conditions, and their phase contrast microscopic images were acquired with automatic image analysis system. Culture conditions were designed to mimic the cell culture failures. From the time-course cellular images, multiple morphological parameters were extracted as fingerprint signature. These parameters were then tagged with the experimentally assayed cellular status and applied to construct machine learning models.

RESULTS & DISCUSSION: By our multiple morphological parameter profiling, which reflects the heterogeneity of cellular population, the small differences between different cell culture conditions could be visualized. The constructed computational models which predicts the undifferentiation/differentiation status of cells were found to have high performances with different stem cell types. However, the most important point that was clarified through our investigation was that such performance of morphology-based quality evaluation by AI models were only achieved by collection technique for qualified and non-biased image data.

ACKNOWLEDGEMENTS: Our research was funded by the JST Program for Creating STart-ups from Advanced Research and Technology (START) program, and NEDO AI project.

REFERENCES

[1] Imai Y., et al, Regen. Therapy, 2018; 9: 15-23.
[2] Fujitani M., et al. J. Biosci. Bioeng., 2017; 124:351-358
[3] Kato R, et al., Sci. Rep. 2016; 6: 34009



A new model of vascularized reconstructed skin

S. Salameh1, N. Tissot1, I. Suzuki1, P.A. Marinho1, J. Demaude1, M. Rielland1, J. Soeur1, S. Takeuchi3, S. Germain2, L. Breton1
Presenting Author: Sacha Salameh, sacha.salameh@rd.loreal.com
[1]L'Oréal Research and Innovation, Aulnay-sous-Bois, France [2]Center for Interdisciplinary Research in Biology, Collège de France, Inserm U1050, CNRS UMRS 7241, Paris, France [3]Center for International Research on Integrative Biomedical Systems (CIBiS), Institute of Industrial Science (IIS), The University of Tokyo, Japan

INTRODUCTION: Many advances have been made in tissue engineering but challenges still exist for constructing thick or complex tissues with all in vivo functions. One of the main challenges is vascularization. In human body, cells are fed with nutrients and oxygen from capillaries located not further than 100-200 μm range. Therefore, skin constructs thicker than 200 μm have a diffusion limitation [1,2]. To address this issue, we developed a full perfusable vascularized reconstructed skin.

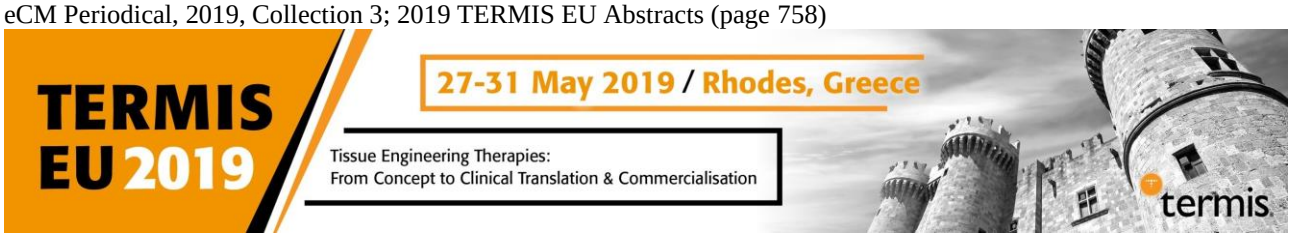
METHODS: Our technology consists of reconstructing the skin-equivalent in a culture device where nylon wires are strung across connectors to form molds inside a dermis-like layer composed of a mix of human fibroblasts and collagen hydrogel. Once hydrogel contracts, nylon wires are removed and channels are seeded with endothelial cells to form microvessels perfused with an external peristaltic pump. After human keratinocytes seeding, skin-equivalent can be lifted to air-liquid interface to allow the stratification of the epidermis [3].

RESULTS & DISCUSSION: Histological analysis and immunostaining of Collagen IV, Perlecan, CD31 and Vimentin showed that endothelial cells are organized in microvessels with accumulation of fibroblasts near microvessel structure. K14, K10 and Involucrin confirmed epidermis is correctly differentiated compared to normal human skin. The functional significance of the vascular structures was evaluated with fluorescent dextran diffusion.

CONCLUSIONS: After optimization of device' surface treatment, fibroblast and endothelial cell concentrations, flow rate and media, successful development of a full thickness skin with perfusable microvessels was achieved. Perfusable skin models can be used for relevant systemic evaluation of actives and drugs in cosmetic and pharmaceutical research.

REFERENCES

[1] Liu J et al. Int J Mol Sci.2015 16(7):15997-6016
[2] Rouwkema et al. Trends Biotechnol.2016 34(9):733-45
[3] Mori N et al. Biomaterials. 2017 116:48-56



Mimicking the osteon structure by biofabrication techniques

M. Trujillo Miranda, J. Uribe Gomez, T. Scheibel, L. Ionov, S. Salehi*
Presenting Author: Sahar Salehi, sahar.salehi@bm.uni-bayreuth.de
[1]Department of Biomaterials, University of Bayreuth, Bayreuth, Germany

INTRODUCTION: Ink-printing has been used to deposit living cells, hydrogels (3D-ink) and other biomaterials in user-defined structures to build complex tissue constructs "as a bottom up approach."[1] Natural cortical (compact) bone is composed of tightly packed units as concentric layers called osteons. Osteons are oriented parallel along to the axis of the bone and are made of mineralized collagen fibers around central Haversian canal, where vasculature and nerves are localized. The goal of this study was to mimic this 3D concentric layers "Osteon" using self-rolling constructs made of a composite hydrogel reinforced with bioceramics particles. The printed construct reinforced with particles will be able to provide mechanical stability and bioactivity which is required for the biomineralization of the system [2]. This platform can be further developed similar to the native osteon with vascular system and neuronal network.

METHODS: The nano particles of forsterite (Mg_2SiO_4) with high surface area were synthesized using sol-gel technique [2] and printing parameters and shape fidelity was evaluated using different concentration of hydrogel as well as particles concentrations (up to 5%wt nFo). Bioactivity of composite hydrogels, was also investigated during 14 days incubation in simulated body fluid (SBF) prepared according to Kokubo protocol [3]. Scanning electron microscopy-EDX, FTIR and elemental analysis was used for further analysis. The mechanics of the hydrogels were tested using rheometer.

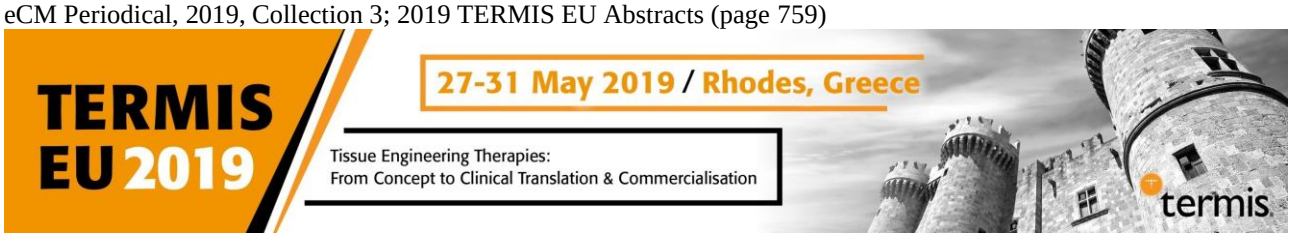
RESULTS & DISCUSSION: The results showed by controlled crosslinking and optimized amount of particles up to 1 wt%, a flexible and elastic nFo-alginate printed structure is achievable. By increasing the amount of the particles the higher bioactivity was observed which was confirmed by higher apatite precipitations on the surface of cross-linked hydrogels during 14 days. The elemental analysis also confirmed the higher release of Mg and Si ions from the composite inks containing 5wt% nFo. These results shows the suitability of this composite as an effective bone graft substitute.

CONCLUSIONS: In conclusion, this study demonstrates optimization of material parameters for 3D printed nFo-Alginate scaffolds and enhancement of mechanical properties and bioactivity by incorporation of forsterite particles.

ACKNOWLEDGEMENTS: Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 326998133 – TRR 225 (subproject B03).

REFERENCES

[1] Turnbull G. et al. Bioactive Mater, 2018, 3, 278.
[2] Sanosh K. et al. J Alloys Comp, 2010, 113-115.
[3] Kokubo T., et al. Biomaterials, 2006, 2907-2915.



Explore the effect of Sharklet® and channels microtopographies to control cellular responses on peripheral nerve interface

S. Mobini[1, 2,5], C.A. Kulasha[3, 5], J. W. Judy[3,5], K.J. Otto[2,5], C.E. Schmidt[2,5], A.B. Brennan[2,4,5]
Presenting Author: Sahba Mobini, sahba.mobini@csic.es
[1]Instituto de Micro y Nanotecnología IMN-CNM, The Spanish National Research Council (CSIC), Spain [2]Crayton Pruitt Family Department of Biomedical Engineering [3]Electrical and Computer and Engineering Department [4]Materials Science and Engineering Department, and [5]Nanoscience Institute for Medical and Engineering Technology, University of Florida, USA

INTRODUCTION: There is growing interest in peripheral nerve interfaces (PNIs) to create long-lasting nerve/machine connection to improve prosthetic robotic limbs for amputees [1]. Undesirable chronic cellular responses to PNIs is a challenge that must be overcome. To improve PNIs function, we propose to use tissue engineering techniques that provide advanced microarchitecture to stimulate healthy Schwann cell (SC) migration and elongation while inhibiting fibrotic encapsulation in order to promote natural axonal regeneration. We designed various microtopographies including channels (CH) and Sharklet® (SK), and then screened them against fibroblasts and SCs in vitro. SK, inspired by the dermal denticles of shark skin, is effective at modulating the fouling response of a wide range of fouling vectors including bacteria, marine organisms, and eukaryotic cells [2].

METHODS: Silicon wafers were patterned containing the inverse of nineteen separate microtopographies with dimensions ranging from 2 to 20 μm using photolithography and etched up to 3.7 μm deep using deep reactive ion etching. Individual polyimide disks were formed by spin coating and curing polyimide (U-Varnish S, UBE Ind.) onto etched wafers and isolated from the cured film by O_2 plasma dry etching. Samples were adhered onto disk coverslips, placed in 24-well cell culture plates, sterilized, and seeded with either rat SCs or fibroblasts. Metabolic activities of the cells were assessed and quantified using Alamar blue Assay at day 1, 3, and 7 and compared to the smooth control (n=3). The cell cytoskeleton and cell nuclei were visualized and imaged to study the changes of the cell shape and morphology in response to microtopography shape and geometry.

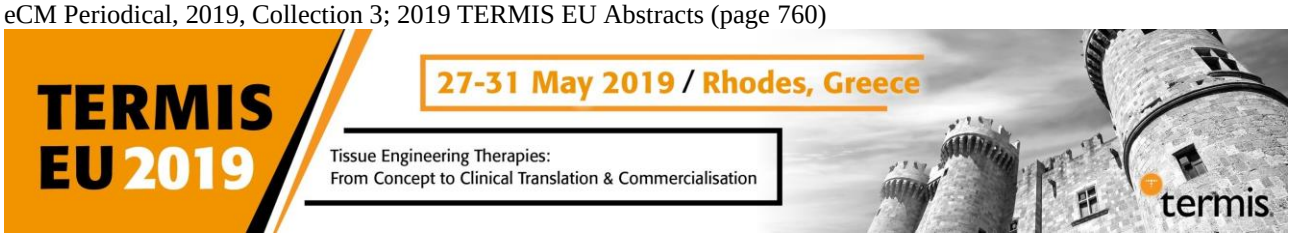
RESULTS & DISCUSSION: Engineered microtopographies were characterized by profilometry and SEM (Figure 1). Cell proliferation was normalized against smooth controls, and results show a strong correlation between pattern dimension and geometry with a mix of inhibitory and promoting patterns. Interestingly SK 20×2 inhibits fibroblast adhesion and proletarian while promoting SC proliferation. Staining shows how cells are changing the shape of their body and nuclei in interaction with patterns.

CONCLUSIONS: In vitro, engineered microtopographies can control the cellular response of fibroblasts and SCs. SK 20×2 can be used to inhibit fibroblasts and promote SCs in PNIs. We also performed limited in vivo tests, which for future applications need to be expanded.

ACKNOWLEDGEMENTS: Financial support was received from: USA Defense Advanced Research Projects Agency (DARPA), Biological Technologies Office, Electrical Prescriptions (ElectRx) Pacific Cooperative Agreement: No. HR0011-15-2-0030.

REFERENCES

[1] Spearman BS et al. Adv. Funct. Mater. 2018; 28, 1701713
[2] Carman ML et al. Biofouling. 2005; 22, 11-21



Poly ethylene(glycol) hydrogels to mimic the stem cell bone marrow niche

S. Donnelly[1], S. Trujillo-Munoz[1], E.R. Cross[2], E.R. Draper[2], M. Salmeron-Sanchez[1], M.J. Dalby[1]
Presenting Author: Sam Donnelly, s.donnelly.1@research.gla.ac.uk
[1]Centre for the cellular microenvironment University of Glasgow [2] Department of Chemistry University of Glasgow, Scotland, UK

INTRODUCTION: Poly ethylene(glycol) (PEG) is a bioinert and biocompatible substance that can be used to form hydrogels for 3-dimensional cell culture [1,2]. By using PEG, we can control gel physico-chemical properties and composition, such as stiffness and degradability, through the addition of a degradable crosslinker. Fibronectin (FN) can also be incorporated into the gel aiding cell adhesion and binding growth factors (GFs). Here, we tested how these matrices capture GFs that are involved in maintaining the bone marrow stem cell niche, including C-X-C motif chemokine 12 (CXCL12), fms-like tyrosine kinase 3 ligand (Flt-3) and stem cell factor (SCF).

METHODS: Three different gel compositions were used to assess the retention of GFs over a period of 48 hours. PEG gel with no FN, PEG gel with FN and PEG gel with 25% degradable crosslinker and FN. All gels were made up to 50ul and were 5 wt. % 4-arm PEG maleimide with a PEG diThiol crosslinker and a custom-synthesised protease-degradable peptide crosslinker (GCRDVPMSMRGGDRCG, VPM peptide). GFs (CXCL12, Flt-3 and SCF, 10 μg/mL) were added to the gels before gelation, allowing them to be encapsulated into the gels.

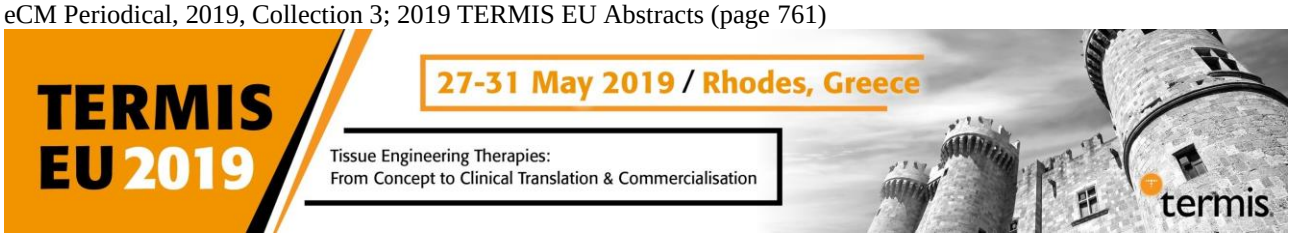
RESULTS & DISCUSSION: Analysis of GF release using the Kruskal-Wallis statistical test illustrated some significant differences between PEG only gels and gels containing FN. When studying CXCL12 release, PEG only hydrogels released close to 100% of the CXCL12 initially added, whereas the condition of PEG with FN ($p \leq 0.05$) released around 70% of the GF incorporated. The release of Flt-3 showed similar results, where the PEG only condition did not retain any GF at 48 h but the PEGVPM with FN ($p \leq 0.05$) retained up to 50% of the Flt-3 encapsulated. For SCF release, PEG only hydrogels released 60% of the GF added and the PEG with FN only 40% ($p \leq 0.01$) at the same timepoint. Moreover, no differences were found when adding degradable crosslinker to the PEG with FN hydrogels for all three growth factors tested, suggesting that FN plays an active role in growth factor retention independently of the crosslinker added.

CONCLUSIONS: Collectively, these data clearly show the increased retention of GFs relevant to the bone marrow niche in gels containing FN.

ACKNOWLEDGEMENTS: We thanks EPSRC for funding S. Donnellys PhD. We also thank Carol-Anne Smith for technical assistance.

REFERENCES

[1] Zhu J. Biomaterials. 2010; 31(17): 4639-4656.
[2] Raeber G et al. Biophysical Journal. 2005; 89(2): 1374-88



Fluidized bed bioreactor for the potential scale-up of an adult erythroid cell line

S. Moise[1], S. Pellegrin[2], J Frayne[3], A. M. Toye[2], M. J. Ellis[1]
Presenting Author: Sandhya Moise, s.moise@bath.ac.uk
[1] Centre for Integrated Bioprocessing Research (CIBR), Dept. of Chemical Engineering, University of Bath, Bath, United Kingdom [2]School of Biochemistry, University of Bristol, Bristol, United Kingdom [3]NIHR Blood and Transplant Research Unit in Red Cell Products, University of Bristol

INTRODUCTION: The efficient production of laboratory-manufactured reticulocytes (that differentiate into red blood cells in vivo) could provide an alternative source of blood for transfusion. The immortalized erythroid progenitor cell line BEL-A [1], is highly proliferative, can be gene edited to remove blood groups [2] and also differentiate into functional reticulocytes offering a potential sustainable reticulocyte source for in vitro manufacture. In this work, we have begun to engineer a fluidized bed bioreactor (FBB) system to culture and eventually scale up the expansion and differentiation of the BEL-A cells in vitro. FBBs are attractive bioreactor technologies due to their superior mass transfer characteristics compared to traditional stirred tank reactors and hollow-fibre systems [3].

METHODS: $1 – 3 \times 10^4$ BEL-A cells/ml were cultured in suspension within FBB glass columns (10 and 25 mm internal diameter) and cell-culture media perfused ($2 – 7 \times 10^{-6}$ m s^{-1}) through the column to fluidize the cells. The fluid velocity at which the bed fluidizes was determined by measuring the change in the pressure drop across the bed of cells. The viability and differentiation of cells within the FBB were compared to static 2D culture. Viability was measured via Trypan blue-assisted cell counts and DNA measurements (Picogreen assay). Differentiation was assessed by measuring marker proteins GAP and Band-3 via flow cytometry. The dO_2 levels were measured using in situ oxygen sensors while glucose and lactate levels measured using HPLC.

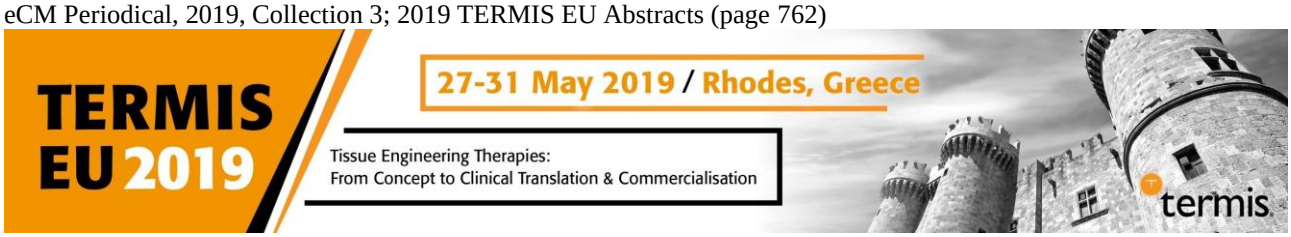
RESULTS & DISCUSSION: Compared to the 10 mm column, the fold change in cell numbers was higher in the 25 mm column (5.8 vs 3.4 in 10 mm) although lower than in 2D static culture (6.7) after 5 days in culture. The larger column, for the same velocity of fluid flow to the 10 mm column, permits a higher flow rate (difference in surface area) and hence has improved nutrient and dO_2 supply. This promoted cellular proliferation and additionally reduced stimulating differentiation of the BEL-A cells towards the erythroid lineage.

CONCLUSIONS: By designing the FBB to culture BEL-A cells at the optimum cells per unit volume and reducing shear while maximizing supply of nutrient, it is possible to achieve higher proliferation with reduced induction of cellular differentiation. In addition, the design can be easily adapted for cellular differentiation whilst allowing easy harvest of the final cells of interest (reticulocytes). Future work will focus on optimizing the operating conditions to scale-up the manufacturing process.

ACKNOWLEDGEMENTS: The authors would like to thank NIHR funding of the Blood and Transplant Research Unit (NIHR BTRU) in red cell products (University of Bristol

REFERENCES

[1] Trakarnsanga K et al. Nat Commun. 2017; 8:14750
[2] Hawksworth J et al. EMBO Mol Med. 2018; 10(6): e8454
[3] Lu J et al. PloS One. 2016; 11(2):e0147376



Janus 3D printed scaffolds for ultrasound stimulated bone regeneration

S. Camarero-Espinosa[1], L. Moroni[1]
Presenting Author: Sandra Camarero-Espinosa, s.camarero-espinosa@maastrichtuniversity.nl
[1]MERLN Institute for for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands.

INTRODUCTION: Mechanical stimulation is known to promote an increased bone mass formation during fracture healing processes. Mechanical stimulation via application of ultrasounds is a clinically used method for this purpose [1]. However, healing of long fractures still requires the use of autologous tissue grafts. To overcome this issue we have fabricated self-assembled Janus 3D printed scaffolds from common biomaterials [2]. We hypothesized that these structures will behave as transducers, with a "reacting" and a "backing" material. Here, we report on the potential of these scaffolds as substrates to promote the differentiation of human mesenchymal stem cells (hMSCs) and the formation of bone.

METHODS: Polycaprolactone (PCL) and polylactide (PLA) were melt-blended at different ratios and their phase behavior upon 3D printing was analyzed by scanning and transmission electron microscopies. hMSCs were seeded at 10,000 cells/cm^2 on Janus scaffolds fabricated from a 50:50 PCL:PLA polymer blend and neat PCL and PLA scaffolds. hBMSCs were cultured for 7, 14 or 21 days under daily pulsed-ultrasound stimulation at 0, 15, 30 or 45 MHz during 30 minutes. hMSCs behavior was studied in terms of proliferation, matrix deposition and differentiation potential and the role of ultrasound stimulation in mechanotransduction was studied in terms of formation of focal adhesions.

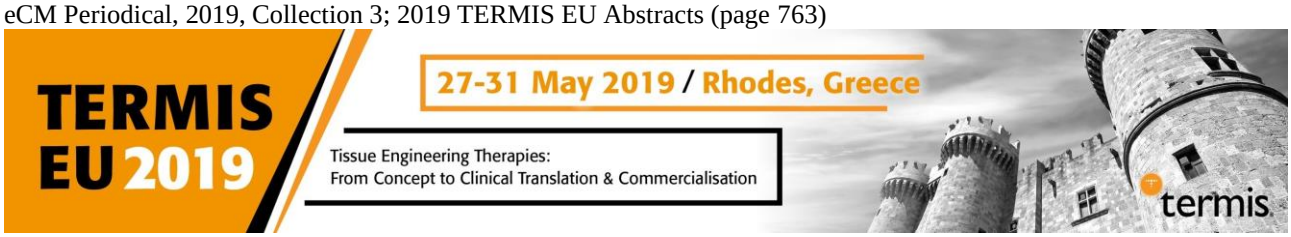
RESULTS & DISCUSSION: 3D printing of PCL and PLA resulted in self-assembled scaffolds presenting different phases depending on the ratio of the polymers. At a 50:50 PCL:PLA Janus-fibered scaffolds were formed. Ultrasound stimulation of hMSCs seeded on Janus scaffolds showed an increased cell number dependent on the applied frequency. Stimulation at 45 MHz results in an increased cell proliferation, matrix deposition and osteogenic differentiation as shown by immunohistological and polymerase chain reaction (PCR) experiments. A study on the mechanism driving osteogenic differentiation of hMSC points out specific mechanotransduction pathways.

CONCLUSIONS: Our findings describe a method for the fabrication of Janus scaffolds and their utilization as bone regeneration platforms under ultrasound stimulation. A define mechanism driving cell differentiation is also proposed.

ACKNOWLEDGEMENTS: This work was supported by the ERC starting grant "Cell Hybridge" under the Horizon2020 framework program (Grant # 637308)

REFERENCES

[1] Padilla F et al. Ultrasonics. 2014; 54 (5):1125-45
[2] Camarero-Espinosa S et al. Abstr. Pap. Am. Chem. Soc.. 2018; 255



3D-printed PCL/PPy conductive scaffolds for neural tissue engineering

S. Vijayavenkataraman[1], K. Sathya[1], G. Sriram[2], T. Cao[2], J.Y.H. Fuh[1], W.F. Lu[1]
Presenting Author: Sanjairaj Vijayavenkataraman, vijayavenkataraman@u.nus.edu
[1]Department of Mechanical Engineering, National University of Singapore (NUS), Singapore
[2]Faculty of Dentistry, National University of Singapore (NUS), Singapore

INTRODUCTION: Conductivity is a desirable property of an ideal Nerve Guide Conduit (NGC) that are being considered for use in peripheral nerve injury treatment. Most of the conductive polymers reported in use for fabrication of tissue engineering scaffolds such polypyrrole (PPy), polyaniline (PANI), polythiophene (PTh) and poly(3,4-ethylenedioxythiophene) (PEDOT) are non-biodegradable and possess weak mechanical properties to be fabricated into 3D structures [1]. In this study, a biodegradable and conductive block copolymer of PPy and PCL (PPy-b-PCL) was used to fabricate 3D porous NGCs using a novel Electrohydrodynamic Jet 3D Printing process.

METHODS: An in-house built EHD jetting system was used for the fabrication of scaffolds [2]. Three different concentrations of PPy-b-PCL (0.5%, 1% and 2% v/v) are mixed with PCL (70% w/v) in glacial acetic acid and fabricated into 3D porous scaffolds. Mechanical and material characterization of as printed scaffolds and the tubular conduits are done. In vitro accelerated degradation studies are also performed in order to determine the degradability of EHD-jetted PCL/PPy scaffolds. In vitro neural differentiation studies using hESC-derived neural crest stem cells (NCSCs) are also performed.

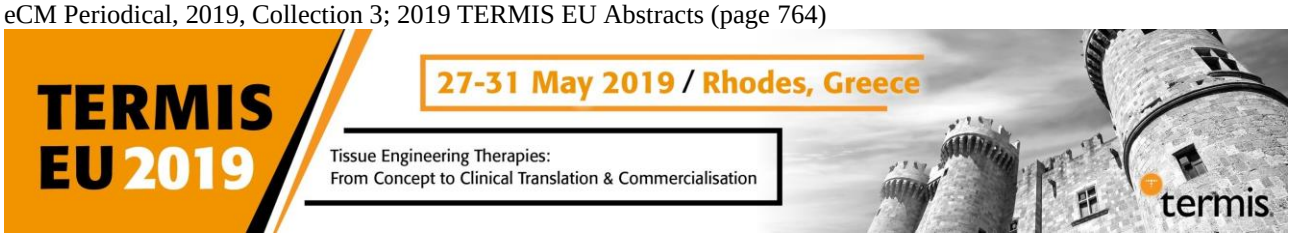
RESULTS & DISCUSSION: The mechanical properties of the scaffolds decrease with the addition of PPy-b-PCL. The yield strength of the NGC decreases from 12.95±1.26 MPa of that of pure PCL scaffolds to 5.02±1.68 MPa (PCL/PPy 0.5%) and 4.82±0.84 MPa (PCL/PPy 1%), which is closer to the properties of the native human peripheral nerve (~6.5 MPa). PCL/PPy 2% scaffolds are much weaker than the other scaffolds, which might be due to the viscosity changes due to higher concentration of PPy-b-PCL. Accelerated degradation studies showed that the highest PPy-b-PCL concentration scaffolds had a greater weight loss. In vitro neural differentiation studies using hESC-derived NCSCs showed that the PCL/PPy 1% scaffolds was the best in terms of neural differentiation, as quantified by RT-PCR studies and immunocytochemistry.

CONCLUSIONS: Collectively, these data clearly illustrate the beneficial effect of conductive PCL/PPy scaffolds in peripheral nerve injury repair.

ACKNOWLEDGEMENTS: Financial support was received from Agency for Science, Technology and Research (A*STAR) Singapore, Advanced Manufacturing and Engineering (AME) Individual Research Grant R265-000-630-305.

REFERENCES

[1] Vijayavenkataraman S et al. Mater. Des. 2019; 162:171-184
[2] Vijayavenkataraman S et al. Polymers 2018; 10(7):753



Hyaluronan-based 3D extracellular matrix model to study cancer progression

S. Amorim[1,2,3], D. Soares da Costa[1,2], I. Pashkuleva[1,2], C. Reis, R. L. Reis[1,2,3], R. A. Pires[1,2,3]
Presenting author: Sara Amorim, sara.amorim@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal [4]i3S, Universidade do Porto, Portugal; [5]IPATIMUP, Porto, Portugal [6]Department of Pathology and Oncology, Faculty of Medicine, Porto University, Portugal [7]Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal

INTRODUCTION: Hyaluronan (HA) molecular weight (Mw) present an important role in tumor invasiveness and metastasis [1, 2]. The reported trends are contradicting: in some cases, lower Mw species are associated with more invasive tumors [3], while other reports suggest that higher HA Mw are the culprit for the invasion. A possible reason for this contradiction can be due to the use of different models. Herein, we evaluated the impact of HA Mw in cancer invasiveness using core-shell hydrogels – a physiological relevant 3D model. We encapsulated MKN45 gastric cancer cells in the core of the hydrogel, composed by alginate and HA of different Mws, and evaluated the formation of cancer spheroids under a 3D environment.

METHODS: The core of the hydrogels (spheres) was made by a combination of alginate (Pronova VLVG, 20 mg/mL) and HA (of different Mws, i.e. 6.4, 752 and 1500 kDa, c=1mg/mL) and crosslinked with $CaCl_2$ (100mM). We encapsulated MKN45 cells (at $5x10^6$ cells/mL) during the processing of the core spheres, that were further embedded in an alginate disc (shell). Cell viability was accessed by live/dead staining using calcein (1.2 μL/mL) and propidium iodide (0.6 μL/min), respectively.

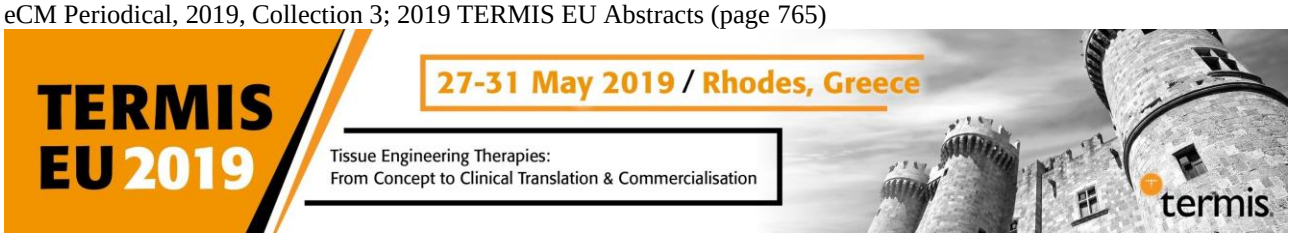
RESULTS & DISCUSSION: Cell viability analysis clearly shows a higher cell density in all mixed systems (combination of alginate and HA) as compared with the controls (only alginate). The increase of HA Mws induces a higher cell proliferation and formation of bigger cell clusters (spheroids).

CONCLUSIONS: We were able to correlate high Mw of HA with an increment on cancer cell viability and spheroid sizes (up to 150 μm for the HA of 1500kDa -measured in the Z axis). The presented data clearly illustrate the influence of HA Mw on tumor growth and in the formation of cancer spheroids.

ACKNOWLEDGEMENTS: We acknowledge the Portuguese FCT under the PhD Grant SFRH/BD/112075/2015. This work was supported by the EC, grant agreement numbers 692333–CHEM2NATURE, 668983–FORECAST and 739572-THE DISCOVERIES CTR.

REFERENCES

[1] S. Amorim et al. Sci. Rep. 2018; 8(1)
[2] T. Chanmee et al. Cancer Lett 2016; 375(1):20-30.
[3] L.Y. Bourguignon et al. Cytoskeleton 2011; 68(12):671-93.



Human platelet lysates-based hydrogels: A novel platform for tissue engineering applications

S. C. Santos[1], C. A. Custódio[1], J. F. Mano[1]
Presenting Author: Sara Santos, santossara@ua.pt
[1]Department of Chemistry, CICECO, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

INTRODUCTION: Platelet lysates (PL) are a human source of growth factors (GFs) and other bioactive proteins. Therefore, they have been explored for clinical and tissue engineering (TE) purposes [1,2]. Herein, we propose a novel human PL-based platform with tunable mechanical properties (PLMA) for human 3D cell culture.

METHODS: PLMA were prepared by mixing PL with methacrylic anhydride at different ratios. Two different degrees of modification were prepared (PLMA100 (low) and PLMA300 (high)) and fully characterized by NMR and Mass Spectrometry. Hydrogels were formed by irradiating with UV light a solution of PLMA containing 0.5% (w/v) of Irgacure 2959. Their structural and mechanical properties were assessed by SEM, compression tests and rheological studies. Release of proteins and GFs from these hydrogels was also evaluated. Biological assays were performed by encapsulating human adipose stem cells (hASCs) into PLMA hydrogels. Furthermore, PLMA sponges were formed after PLMA hydrogels freeze-drying and seeded with hASCs. hASCs were tested for cell adhesion, viability and proliferation.

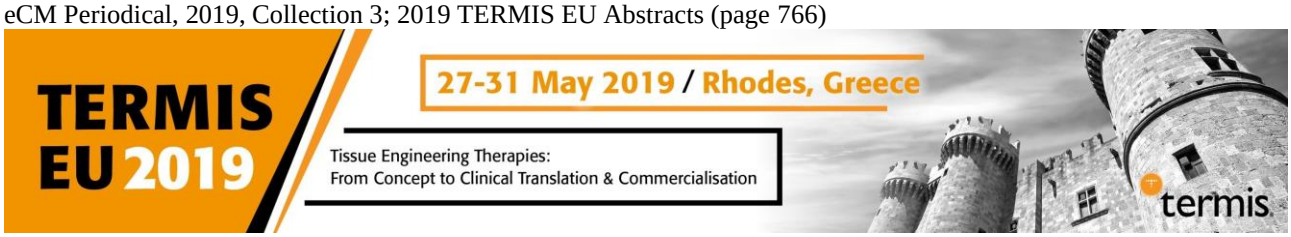
RESULTS & DISCUSSION: PLMA hydrogels here proposed are easy to handle and show tunable mechanical properties that can be easily adjustable by changing PL degree of modification or PLMA concentration. hASCs were encapsulated and cultured for 7 days. Results showed that PLMA hydrogels support cell adhesion, growth and invasion within the 3D matrix. Immunocytochemistry analysis of hASCs at 7 days of culture shows no evidence of differentiation of hASCs encapsulated in PLMA hydrogels. PLMA sponges were seeded with hASCs and cultured for 14 days. Overall, for both PLMA hydrogels and sponges, hASCs exhibited high cell viability, adhesion and proliferation showing their potential to be used as a humanized 3D cell culture platform. Further, PLMA hydrogels showed sustained release of proteins and GFs.

CONCLUSIONS: These results showed that PLMA hydrogels are a promising platform for TE applications. The material here developed could have an autologous origin, being adequate to produce customized robust matrices with no risk of cross reactivity, immune reaction or disease transmission.

ACKNOWLEDGEMENTS: Funding support from European Research Council grant agreement ERC-2017-PoC- 789760 for project MicroBone.

REFERENCES

[1] Santos SC et al, Tissue Eng. Part B: Reviews. 2018; 24(6):454-462
[2] Santos SC et al, Adv. Healthcare Mater. 2018;7, 1800849.



Liquid platelet-rich fibrin (i-PRF) enhances the vascularization of a non-crosslinked collagen matrix in vivo

S. Al-Maawi[1], A. Kubesch[1], R. Sader[1], CJ. Kirkpatrick[1], S. Ghanaati[1]
Presenting Author: Sarah Al-Maawi, Sarah.Al-Maawi@kgu.de
[1]Frankfurt Orofacial Regenerative Medicine (FORM) Lab, Department for Oral, Cranio-Maxillofacial and Facial Plastic Surgery, Medical Center of the Goethe University Frankfurt, Frankfurt am Main, Germany

INTRODUCTION: Vascularization is highly necessary for tissue regeneration [1]. Platelet-rich fibrin (PRF) is a blood-based concentrate system obtained from the centrifugation of peripheral blood without additional anticoagulants [2]. This system is clinically highly relevant and was developed to support regeneration in damaged tissues. In vitro studies proved that reducing the relative centrifugation force (RCF) during the preparation of PRF leads to significant enrichment of the matrices with platelets and leukocytes in addition to the significant increase of growth factor release [2]. The aim of the study was to investigate the influence of liquid PRF on the in vivo vascularization and integration of a non-crosslinked collagen matrix in vivo.

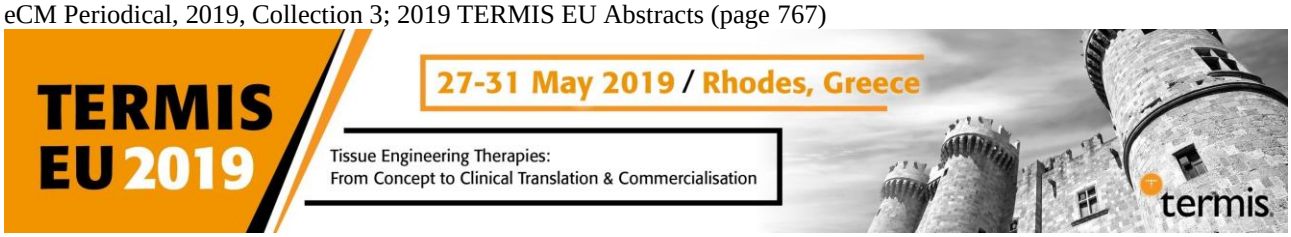
METHODS: Liquid PRF (i-PRF) was gained from human blood. The collected blood was immediately centrifuged at either highRCF (966 g) or low-RCF (60 g) and applied on the collagen matrix. The resultant biomaterial PRF construct was subcutaneously implanted in severe combined immunodeficiency (SCID) mice (n=4 per group). For the control group, the biomaterial was rinsed in phosphate-buffered saline (PBS) and subcutaneously implanted. After 10 days, the implantation areas were analyzed histologically. Immunohistochemical staining and histomorphometrical analysis was performed for the vessel density using CD-31 for mice and the distribution of human inflammatory cells, expressing vimentin, CD-3, CD-20, or CD-68 in the implantation bed.

RESULTS AND DISCUSSION: The vessel density within the biomaterial was significantly higher in the case of low-PRF compared to high-PRF (P<0,001) and control-group (P<0,0001). Vimentin expression as a pan marker for human cells was highly expressed in the group of low-PRF, whereas no cells were found in the group of high-PRF. Thereby, the expression of human cell markers was significantly higher in the group of PRF-low compared to PRF-high and control-group (CD-3 P< 0,001; CD-20 P<0,05 and CD-68 P<0,0001). Previous studies proved the angiogenesis effect of preculturing biomaterial using primary cells as a tool to enhance vascularization1. However, this concept is difficult to translate to clinical application.
The present findings highlight the use of PRF as an alternative to primary cells to enhance the biomaterial vascularization. Centrifugation using low-RCF led to significant increase of the cell-mediated vascularization in vivo. This concept is highly relevant for clinical application because of its simple preparation and high potential for translation to clinical application.

CONCLUSION: The use of i-PRF as an autologous source of inflammatory cells and growth factors provides pro-angiogenic stimuli and supports biomaterial vascularization as a clinically applicable tissue engineering concept.

REFERENCES

[1] Ghanaati et al 2011. Biomaterials.;32(32):8150-8160.
[2] Choukroun and Ghanaati (2017) Eur J trauma Emerg Surg



Autologous platelet-rich plasma (CuteCell™ PRP) boosts safely human in vitro fibroblast expansion

S. Berndt[1,2], A. Turzi[2], B. Pittet-Cuénod[1], A. Modarressi[1]
Presenting Author: Sarah Berndt: sarah.berndt@unige.ch
[1]Department of Plastic, Reconstructive and Aesthetic Surgery, Geneva University Hospitals and Faculty of Medicine, Geneva University, 4 Rue Gabrielle Perret-Gentil, 1211 Geneva 14, Switzerland
[2]RegenLab, En Budron B2, 1052 Le Mont-sur-Lausanne, Switzerland

INTRODUCTION: Nowadays autologous fibroblast application for skin repair presents an important clinical interest. In most cases, in vitro skin cell culture is mandatory. However, cell expansion using xenogeneic or allogenic culture media presents some disadvantages, such as the risk of infection transmission or slow cell expansion. In this study, we investigated an autologous culture system to expand human skin fibroblast cells in vitro with the patient's own platelet-rich-plasma (PRP).

METHODS: Human dermal fibroblasts were isolated from patients undergoing abdominoplasty and blood was collected to prepare fresh PRP using the CuteCellTM PRP medical device. Cultures were followed up to 7 days using a media supplemented with either fetal bovine serum (FBS) or PRP.

RESULTS & DISCUSSION: Fibroblasts cultured in medium supplemented with PRP showed dose-dependently significantly higher proliferation rates (up to 7 .7 times with 20% of PRP) and initiated a faster migration in the in vitro wound healing compared to FBS, while chromosomal stability was maintained. At high concentrations, PRP changed fibroblast morphology, inducing cytoskeleton rearrangement and an increase of alpha-SMA and vimentin expression.

CONCLUSIONS: Our findings show that autologous PRP is an efficient and cost-effective supplement for fibroblast culture and should be considered as a safe alternative to xenogeneic/allogenic blood derivatives for in vitro cell expansion.

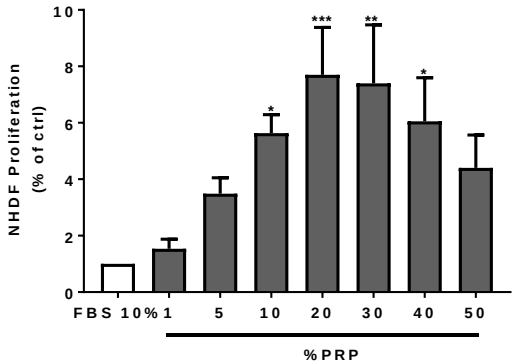
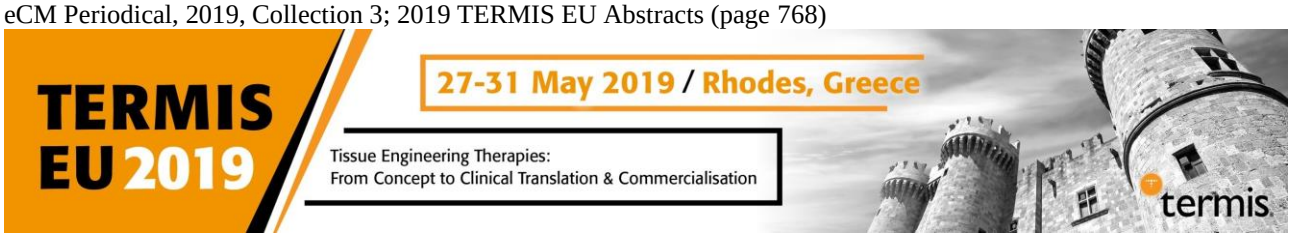


Figure 1: Assessment of PRP proliferative effect by flow cytometry using CellTrace Violet (vital dye). Proliferative effect of increasing PRP concentrations in comparison (1-50%) with FBS 10% (n=10 different patients) on NHDF for 7 days without medium change in a complete autologous system (cells and PRP from the same patient).



A therapeutic intraperitoneal reservoir (TherIP) and minimally invasive delivery system for regional, repeated intra-abdominal drug delivery

S.T. Robinson[1,2], E. Dolan[3], C. Maurerhofer[1], R. Levey[1], R. Beatty[1,2], G.P. Duffy[1,2,4]
Presenting Author: Scott Robinson, scott.robinson@nuigalway.ie
[1]Department of Anatomy, National University of Ireland Galway (NUIG), Galway, Ireland [2]Science Foundation Ireland (SFI) Advanced Materials and Bioengineering Research (AMBER), NUIG, Galway, Ireland [3]Department of Bioengineering, NUIG, Galway, Ireland [4]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), NUIG, Galway, Ireland

INTRODUCTION: Emerging technologies in the fields of regenerative medicine, cell therapy, gene therapy, and nanotechnology have broadened intraabdominal targets for multiple pathologies. However, maintaining a local therapeutic dose in deep tissue compartments while minimizing off-target effects remains a challenge. We previously developed a therapeutic reservoir for delivery to the heart [1]. The aim of this study was to develop a therapeutic intraperitoneal reservoir device (TherIP) and delivery system which enables targeted and repeated drug or cell delivery to the omentum in a large animal model through a minimally invasive procedure.

METHODS: The TherIP device was fabricated using porous thermoplastic polyurethane (TPU) sheets themoformed into interconnected reservoirs. An acute pig study was used to validate device deployment and delivery system design. Devices were targeted to the omentum of 35 kg landrace swine under general anesthesia (n=3), after which animals were euthanized to confirm device placement.

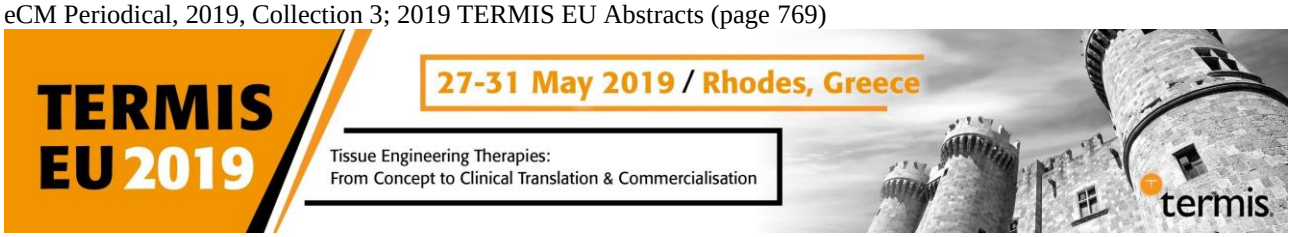
RESULTS & DISCUSSION: The intraperitoneal space was accessed via a percutaneous approach using an over the wire technique. A custom designed delivery system consisting of coaxial 3D printed sheaths was used to position the TherIP device over the omentum using ultrasound and fluoroscopic assistance. The reservoir system was deployed and then fixed with a cyanoacrylate adhesive. A radiopaque hydrogel was injected through an inlet line to simulate filling with therapeutic cargo. All animals tolerated the procedure in an acute setting, and post-mortem examination confirmed placement of the device.

CONCLUSIONS: We have developed a soft, flexible, reservoir system for local, repeated drug or cell delivery to intraabdominal targets. The device can be deployed, fixed, and filled through a minimally invasive, percutaneous procedure with minimal complications.

ACKNOWLEDGEMENTS: Funding for this project was provided in party by DRIVE (EU Horizon 2020 grant # 645991), EDGE (EU Horizon 2020 Marie Sklodowski-Curie grant # 713567) and the SFI research center AMBER under grant SFI/12/ RC/2278.

REFERENCES

[1] Whyte et al., Nature BME. 2018; 2(6):416-428



Deep insight into PEGylation of bioadhesive chitosan nanoparticles

U. Bozuyuk[1], N.O. Dogan[1], I.S. Gokulu[1], S. Kizilel[1]
Presenting Author: Seda Kizilel, skizilel@ku.edu.tr
[1]Koç University, Chemical and Biological Engineering, Sariyer, Istanbul, 34450, Turkey

INTRODUCTION: Chitosan nanoparticles (NPs) have great potential in nanomedicine; however, low solubility of chitosan severely limits their potential clinical translation [1]. Here, we present a systematic analysis to tune the size and zeta potential of NPs through both artificial neural network (ANN) model and experimental approaches to increase the solubility of chitosan NPs in physiological media. We use (i) PEG chain length, (ii) chitosan/PEG ratio, (iii) pH of solution as the three independent input variables. We utilize a novel, ultrafast and convenient method for the PEGylation of NPs through a photopolymerization along with ionic crosslinking. NPs with higher zeta potentials promoted adhesion of HEK293-T cells on NP-coated surfaces in cell culture medium, which was predicted through ANN. In summary, we present synthesis of PEGylated chitosan NPs through both ionic and covalent means, utilize ANN model as a tool to predict size and zeta potential which can capture cell adhesion behavior on surfaces prepared with these engineered NPs.

METHODS: HEK293-T cell line was used for cell culture experiments. Cells were seeded (10,000 cells/well) on 96-well plates and treated with altered concentrations of PEGylated chitosan NPs for 24 h and 48 h. Next, cells were incubated with shaking at 100 rpm using Cell Titer-Glo reagent and luminescence was measured using a plate reader (BioTek's Synergy H1). TNBS assay was used to characterize the degree of PEGylation.

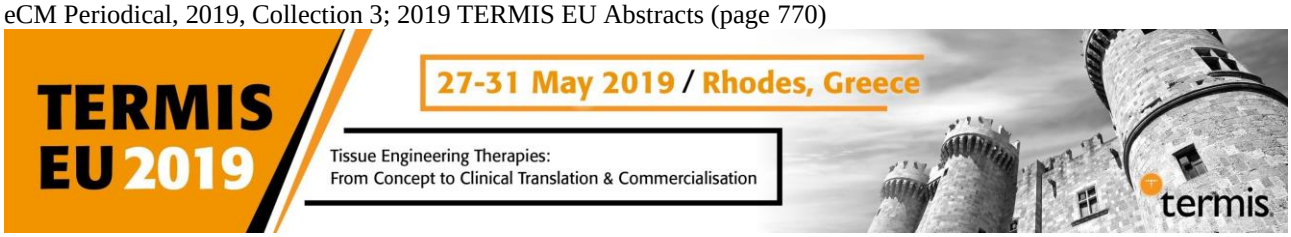
RESULTS & DISCUSSION: PEGylated chitosan was synthesized both through NHS-amine coupling chemistry and photopolymerization with TPP, PEG and methacrylamide chitosan (CSMA) with UV light. We synthesized chitosan NPs using 9 different types of PEGylated chitosan monomer through the reaction of positively charged chitosan and negatively charged TPP, formed polyelectrolyte complexes under rigorous stirring. We also used photopolymerization of CSMA ANNs could accurately estimate the size and zeta potential of different groups. We observed that 2 kDa PEG had the highest number of attached cells and uncoated sample had the lowest number of attached cells.

CONCLUSIONS: This study demonstrates fundamental aspects of PEGylated chitosan NPs and proves that NPs with the desired surface charge and size properties can be prepared in conjunction with the ANN model [2].

ACKNOWLEDGEMENTS: We acknowledge financial support by Scientific and Technological Research Council of Turkey (TUBITAK, project number: 116M995), COST Action-European Cooperation in Science and Technology–ENBA CA15216.

REFERENCES

[1] Bozuyuk U et al. ACS Nano 2018; 12(9):9617-25
[2] Bozuyuk U et al. ACS Applied Materials & Interfaces 2018; 10(40):33945-55



Fabrication of bilayer scaffolds composed of 3D printed SMP and bioactive composite to mimic the bone tissue at macro-nano level

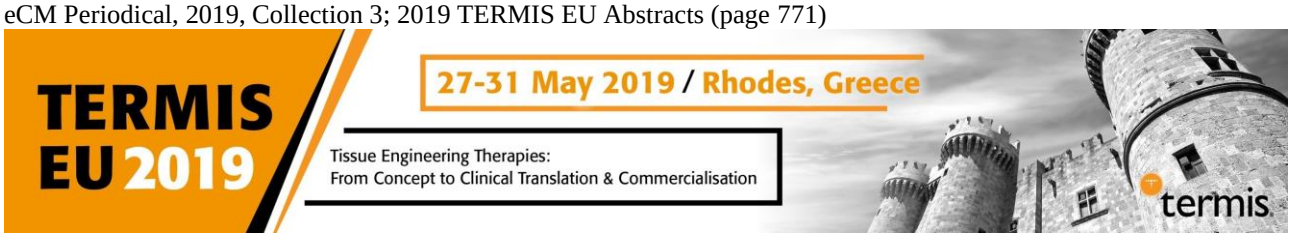
S. Tamburaci*[1] C. Ersanli[2], F. Tihminlioglu[1]
Presenting Author: Sedef Tamburaci, sedeftamburaci@gmail.com
[1]İzmir Institute of Technology, Department of Chemical Engineering, İzmir, Turkey [2]İzmir Institute of Technology, Department of Biotechnology and Bioengineering, İzmir, Turkey

INTRODUCTION: Recently, 3D printing technology has come into prominence in biomedical applications. Three-dimensional (3D) scaffold design plays an important role in tissue engineering to mimic the host tissue and defect morphology. Thus, shape-memory polymers (SMP)s are generally used for scaffold fabrication to obtain the desired morphology and structure. Among SMPs, thermoplastic polyurethane (TPU) is a biodegradable elastomer that is widely used for biomedical applications. However, TPU has low mechanical strength and biodegradability. These limitations can be improved blending with other polymers such as polylactic acid (PLA) which has good biocompatibility, high mechanical strength and biodegradability. In this study, it is aimed to fabricate PLA/TPU (1:1) blend to overcome the disadvantages of both polymers where PLA is used as hard segment and TPU is used as soft segment. However, these scaffolds generally do not provide the desired bioactivity and surface properties for tissue regeneration. Thus, biopolymer based porous layer was integrated in SMP scaffold. Highly porous and silica-based diatomite frustules were used as bioactive agent in chitosan-gelatin matrix. Hence, the main purpose of the study is to fabricate scaffolds for bone regeneration to mimic the composite structure of bone tissue at macro-nano levels. Diatomite particles were used to enhance bioactivity by inducing cell-material interaction at nano level and stimulating biomineralization on surface. Furthermore, SMP based bilayer scaffold can be fabricated in desired dimensions and easily replace bone defects by the help of its shape memory characteristic.

METHODS: Bilayered scaffolds were fabricated with two different methods and layers were combined. Outer layer TPU/PLA was fabricated with 3D printing and chitosan-gelatin porous layer was obtained with freeze-drying. Layers were integrated successfully. Shape memory effect of TPU/PLA blend as 3D layer was investigated. Scaffolds were characterized with SEM, Micro-Ct., swelling, mechanic tests. Open porosity of scaffolds were determined with liquid displacement method. In vitro bioactivity of scaffolds was evaluated with Saos-2 cell line and cell proliferation and osteogenic markers as ALP, osteocalcin and biomineralization were investigated. Cell attachment on each layer was observed with SEM analysis.

RESULTS & DISCUSSION: Fabricated bilayer scaffolds showed shape memory characteristic and desired bioactivity with diatomite incorporation which improved the cell-biomaterial interaction. Scaffolds provided desired porosity levels and pore size range for bone tissue formation. In vitro results indicated that Saos-2 cells proliferated on scaffolds at desired level and secreted osteogenic markers during incubation. Bioactive porous inner layer induced biomineralization of scaffold.

CONCLUSIONS: In conclusion, bioactive natural polymer and SMP based bilayered scaffolds showed potential for bone tissue regeneration. This scaffold design for the bone tissue regeneration can be used in osteochondral defects by taking advantage of its different mechanical properties and morphology.



Application of surface-selective laser sintering for tissue engineering and drug delivery

S.N. Churbanov[1,2], A.L. Fayzullin[1], N.V. Minaev[2], E.A. Grebenik[1], P.S. Timashev[1,2,3]
Presenting Author: Semyon N Churbanov, Churbanov.semyon@gmail.com
[1]Institute for Regenerative Medicine, Sechenov First Moscow State Medical University, 8-2 Trubetskaya st., Moscow, 119991, Russia [2]Institute of Photonic Technologies, Research Center "Crystallography and Photonics", Russian Academy of Sciences, 2 Pionerskaya s., Troitsk, Moscow, 142190, Russia [3]Department of Polymers and Composites, N.N. Semenov Institute of Chemical Physics, Kosygin st., Moscow, 119991, Russia

INTRODUCTION: The surface-selective laser sintering (SSLS) is a universal method which allows to create three-dimensional structures from polymer materials including FDA approved [1-2]. Herein, we assessed the potential of SSLS for creating scaffolds with prolonged drug release.

METHODS: The antifibrotic substance – pirfenidone was mixed with polylactide powder (PDL 04, PURAC) at the ratio of 600 μg/g, respectively. Next, the mixture was treated with $scCO_2$ (T=40 °C, P=140 MPa, τ=8 hours) in order to evenly impregnate the drug agent in the polymer volume. The resulting material was then ground to powder in a knife mill to obtain particles size less then 100 μm. Finally, the polylactide loaded with pirfenidone was used to design scaffolds with the dimensions 10×10×1 mm by SSLS method. Passive in vitro drug release was analyzed by the spectrophotometric method described in [3]. The obtained scaffolds were subcutaneously implanted into the ventral side of the rabbit ear cartilage plate (n=6). After 60 days of the implantation, the explants were collected for histochemical analysis.

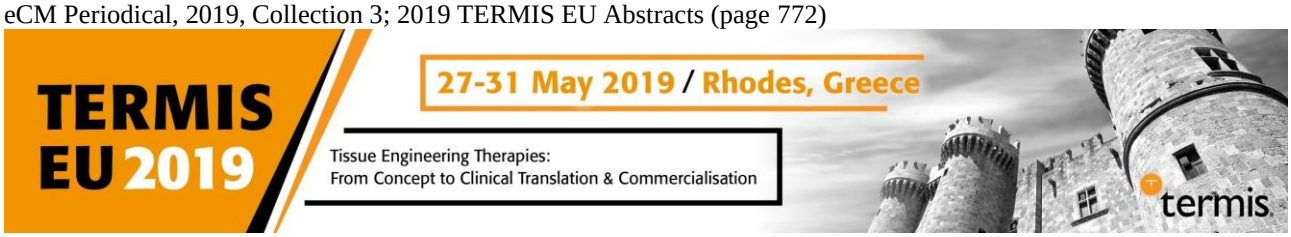
RESULTS & DISCUSSION: Using SSLS method, we fabricated PLA scaffolds impregnated with pirfenidone. The impregnation was achieved with the aid of $scCO_2$. Passive in vitro drug release study did not reveal burst release in the first few hours. It is known that implantation of polylactide matrices is complicated by the formation of a surrounding fibrous capsule. The encapsulation of the scaffold with pirfenidone inhibited the capsule formation. In details, after 60 days of the implantation, strong fibrosis was observed in the control scaffolds (without loaded drug), while the pirfenidone-loaded scaffolds showed no evidence of fibrosis.

CONCLUSIONS: The obtained data shown that the SSLS method can be used to form three-dimensional polymer structures loaded with drug. In the future, it is planned to create structures with loaded drugs that allow not only to prevent fibrosis, but also leveling all the negative reactions of the body during implantation.

ACKNOWLEDGEMENTS: Financial support was received from the Ministry of Science and Higher Education within the State assignment FSRC «Crystallography and Photonics» RAS in part of "Physico-chemical analysis", Russian Foundation for Basic Research № 18-32-20184 in part of "SSLS polymers samples" and Russian Scientific foundation №15-15-00132 in part of "biological tests".

REFERENCES

[1] Wagner, T., et al. International Polymer Processing 19.4 (2004): 395-401.
[2] Churbanov, S. N., et al. KnE Energy & Physics 3.2 (2018): 64-68.
[3] Trivedi, Ruchit, et al. Nanotechnology 23.50 (2012): 505101.



A newly developed defined medium for human keratinocyte culture and skin substitute production

S. Cortez Ghio[1,2], D. Larouche[1,2], A. Morissette[1,2], A. Garnier[1,3], L. Germain[1,2]
Presenting Author: Sergio Cortez Ghio, sergio.cortez-ghio.1@ulaval.ca
[1]Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, CRCHU de Québec – Université Laval, Canada and [2]Département de chirurgie, Faculté de Médecine [3]Département de génie chimique, Faculté de Sciences et de Génie, Université Laval, Canada

INTRODUCTION: Keratinocyte culture and self-assembled skin substitutes (SASS) have greatly improved the treatment of patients with severe burns. Currently, to properly culture human keratinocytes, fetal bovine serum (FBS) must be added to the medium. However, FBS exact biological composition is undefined and variations occur from batch-to-batch. FBS is also associated with a risk of xenogeneic pathogen transmission. For these reasons, discarding FBS usage in research and clinical contexts is desirable. Therefore, we aimed to develop a defined medium optimized for keratinocyte expansion and SASS production.

METHODS: Using a fractionated factorial design of experiment, we identified and optimized the concentration of a set of molecular factors capable of replacing FBS for keratinocyte culture, and produced our keratinocyte defined medium (DM). We compared the effects of this new medium to the serum-containing medium on keratinocyte cultures and SASS at the cellular and molecular levels.

RESULTS & DISCUSSION: Keratinocytes cultured in DM presented similar morphology and increased proliferative potential when compared to those cultured in FBS-containing medium. Transcriptomic profile analyses with RNA microarrays on 4 distinct cell populations cultured in either media revealed that only 0.16% of targets were differentially expressed. Most of the genes underexpressed in DM were associated to keratinocyte differentiation. SASS produced with either media were similar at the histological and mechanical levels. Additionally, SASS similarity was further confirmed with immunolabelling of various structure, adhesion, proliferation and differentiation biomarkers (Fig. 1). We also grafted SASS produced with either media onto athymic mice. For up to 6 months, the epidermis of SASS produced with DM regenerated, which confirms the presence of epithelial stem cells in the grafts.

CONCLUSIONS: In conclusion, this new defined medium is promising for keratinocyte culture and SASS production for research and clinical purposes.

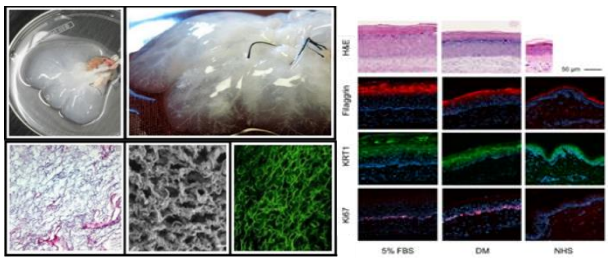
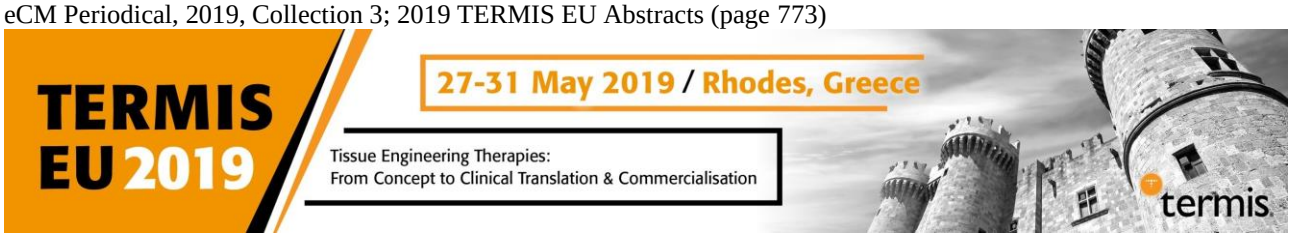


Figure 1: Histology and immunofluorescence imaging. Comparison of the structural integrity of SASS produced with either media and normal human skin

ACKNOWLEDGEMENTS: CIHR (MOP-12087 and FDN-14213), CRC, FPQGB, ThéCell network, FRQS, Fondation du CHU de Québec, LOEX, UL's Faculty of medicine.



Macromolecular crowding as a means to produce self-assembled scaffold-free tissue equivalents

S. Garnica-Galvez[1,4,5], I. Skoufos[1], A. Tzora[1], N. Diakakis[2], N. Prassinos[2], J. Dudhia[3], R. Smith[3], S. Korntner[4,5], D.I. Zeugolis[4,5]
Presenting Author: Sergio Garnica-Galvez, gsergio@vet.auth.gr
[1]Bioprocessing and biotechnology laboratory, University of Ioannina (Campus of Arta), Arta, Greece [2]School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece [3]Royal Veterinary College, London, United Kingdom [4]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), NUI Galway, Galway, Ireland, [5]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), NUI Galway, Ireland

INTRODUCTION: Musculoskeletal injuries are increasing in human and equine species. Cell sheets preserve the extracellular matrix (ECM) complexity, overcoming the problems derived from scaffolds [1]. However, the delayed in vitro ECM deposition is the biggest handicap for these therapies. Some polymers produced by several organisms in vivo appear associated to a great number of water molecules in solution, promoting a volume exclusion effect that enhances the ECM deposition in cultures. Also known as macromolecular crowding (MMC) phenomenon [2]. Herein, the MMC-related properties of a natural polymer (hyaluronic acid, HA), in comparison to Ficoll™ and carrageenan (CR) [3], were analysed.

METHODS: Equine adipose and bone marrow mesenchymal stem cells (MSCs) were seeded at 15.000 cells/cm^2 and WS-1 human dermal fibroblasts (hDFs) at 25.000 cells/cm^2. The media was supplemented with 100 μM L-ascorbic acid phosphate. Different concentrations of diverse HA molecular weights (MWs) were tested. SDS-PAGE, densitometry analysis and immunocytochemistry were conducted to detect ECM molecules deposition after 3, 5 and 7 days. Overall statistical significance was established at a p-value ≤ 0.05 by ANOVA one way analysis.

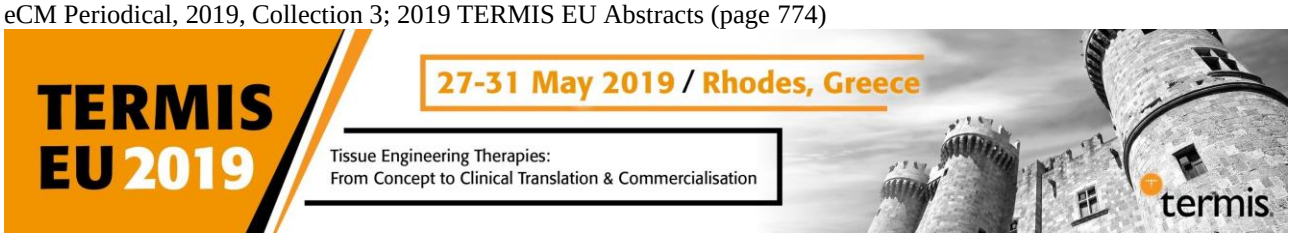
RESULTS & DISCUSSION: Densitomety analysis was performed to evaluate collagen type I α1 and α2 chains intensity for quantification. Some concentrations of the studied MWs corresponding to 60, 100, 500 and 1,000 KDa were significant in MSCs. However, HA-10 KDa was discarded from the study due to cell detachment. In contrast, PX did not promote collagen I deposition in WS-1 hDFs. However, HA induced collagen III deposition in WS-1 hDFs, being higher than Ficoll™ and CR. The highest collagen type I enhancer was CR (at 75 μg/ml) in all cell types. Equine MSCs were characterized by trilineage assays and FACS analysis; according to the current criteria for horse MSCs. CR did not alter the expression of the markers: CD29, CD44, CD105, CD90 and CD34.

CONCLUSIONS: HA has potential as MMC, enhancing collagen type I and type III deposition. Further studies will be needed to improve these PX properties.

ACKNOWLEDGEMENTS: This project has been funded by the European Union, Horizon 2020 Programme, under the Marie Skłodowska-Curie Actions, Innovative Training Networks, Grant Agreement 676338.

REFERENCES

[1] P. Y. Neo, et al. (2016). Connect Tissue Res. 57(6):428-442.
[2] C. Chen, et al. (2011). Adv Drug Deliv Rev. 30;63(4-5):277-90.
[3] D. Cigognini, et al. (2016). Sci Rep. 1;6:30746.



2-deoxy-D-ribose (2dDR) and 17β-Estradiol (E2) releasing functional scaffolds for stimulating angiogenesis in ex-ovo CAM assay

S. Dikici[1], N. Mangır[1, 2], M. Yar[3], F. Claeyssens[1], S. MacNeil[1]
Presenting Author: Serkan Dikici, sdikici1@sheffield.ac.uk
[1]Department of Materials Science and Engineering, Kroto Research Institute, University of Sheffield, UK [2]Royal Hallamshire Hospital, Department of Urology, UK [3]Interdisciplinary Research Centre in Biomedical Materials, COMSATS University, Pakistan

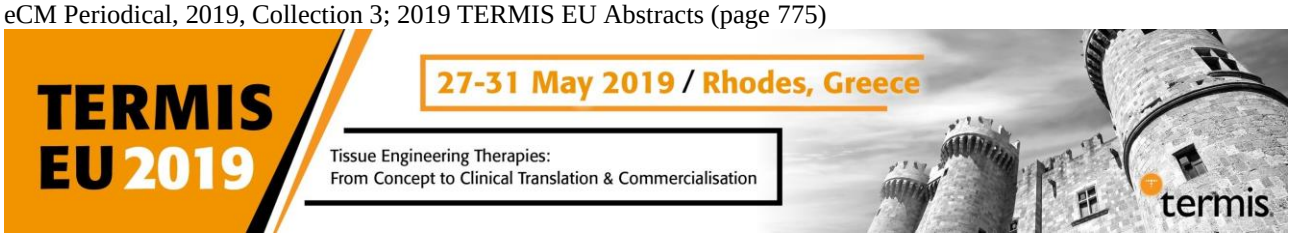
INTRODUCTION: Failure of tissue engineered (TE) constructs following implantation is one of the most challenging problems of tissue engineering. Thus, ensuring rapid vascularisation is considered as crucial for translation of TE constructs to the clinic. Functionalisation of the TE scaffolds with proangiogenic factors is a promising approach in order to overcome slow neovascularisation. Vascular endothelial growth factor (VEGF) is recognised as the gold standard for promoting angiogenesis but it is also unstable, expensive and the use of high doses of VEGF can result in leaky immature vessels in vivo. The aim of this study is to develop E2 and 2dDR releasing biodegradable scaffolds and assess their potency for promoting angiogenesis.

METHODS: We first assessed the angiogenic potency of 2dDR and E2 versus VEGF in an ex-ovo chick chorioallantoic membrane (CAM) assay. Both factors were then electrospun into PHBV fibres. Sustained releases of both agents were observed over 30 days from these scaffolds using a spectrophotometric method. The proangiogenic activities of the drug releasing scaffolds were assessed using ex-ovo CAM assay. Finally, a 20% solution of lens culinaris agglutinin (LCA) was injected into the circulation of CAM to visualize the microvasculature.

RESULTS & DISCUSSION: Both E2 and 2dDR were found approximately 80% as potent as VEGF in stimulating new blood vessels in CAM assay when applied directly onto CAM. Both factors were found to be effective at stimulating neovascularisation over 7 days when released from fibres. The structure of microvasculature of CAMs and the vascular areas showed that an endothelial cell hypertrophy together with smaller lacunae compared to PBS can be observed for VEGF, E2 and 2dDR groups

CONCLUSIONS: We conclude that both 2dDR and E2 provide attractive alternatives to VEGF for the functionalisation of TE scaffolds to promote angiogenesis. The gradual release of E2 and 2dDR from fibres stimulated the formation of new blood vessels in the ex-ovo CAM assay.

ACKNOWLEDGEMENTS: Financial support was received from Turkish Ministry of National Education.



Bone morphogenetic protein-9 and small-molecule phenamil synergistically induced osteogenic differentiation of intact human placenta membrane

S.S. Ebadi[1,2†], A. Ahmadi[1,2†], H. Niknejad[1]
Presenting Author: Seyed-Shayan Ebadi, ss.ebadi@sbmu.ac.ir
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [†]Co-first author

INTRODUCTION: Regeneration of bone with tissue engineering approaches is a promising way in order to reconstruct the bone defects without conflicting with side effects of bone grafts. Cell seeding, scaffold and signaling are three building blocks for regeneration of new bone. Intact human amniotic membrane, as a pre-formed sheet of stem cells, can be a proper candidate for osteogenesis which provide both seeding cells and scaffold together. [1]. Furthermore Bone morphogenetic protein-9 (BMP-9) and small molecule phenamil can induce osteogenic differentiation. [2, 3]. The objective of this study was to determine the synergistically effect of BMP-9 and phenamil on osteogenic differentiation of intact human amniotic membrane.

METHODS: In-situ differentiation of intact human amniotic membrane has been performed using four osteogenic media in four groups compared with control medium in 28 days. Basic osteogenic media was chosen as first group and the other three osteogenic media were made by addition of BMP-9, phenamil and BMP-9 alongside phenamil to basic osteogenic media in the second, third and fourth group, respectively. Alkaline phosphatase (ALP) activity, RUNX2 mRNA expression, Osteocalcin (OCN), Osteopenin (OPN), mineralization and calcium content were evaluated and compared among groups.

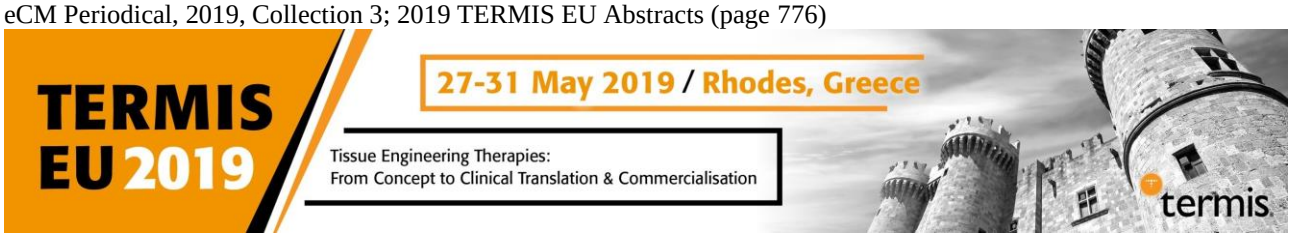
RESULTS & DISCUSSION: Addition of BMP-9 to basic osteogenic media had significant effect on mineralization and calcium content of amniotic epithelial cells. Furthermore, small-molecule phenamil changed the amount of RUNX2 mRNA expression significantly. Interestingly, addition of BMP-9 alongside phenamil to Basic osteogenic media had synergistic effect on osteocalcin, osteopnin and Alkaline phosphatase activity.

CONCLUSIONS: This study showed that utilization of BMP-9 alongside small-molecule phenamil can synergistically induce osteogenic differentiation of stem cells within intact human amniotic membrane which have resided in their natural environment.

ACKNOWLEDGMENTS: The authors would like to thank the personnel of Erfan hospital for assistance in this research. We also thank Ms Tayebi for her assistance during professional laboratory works. Research reported in this publication was supported by Research Grant Committee from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFRENCES

[1] Farhadihosseinabadi B et al. Artif Cells Nanomed Biotechnol. 2018: 1-10.
[2] Beederman M et al. J Biomed Sci Eng. 2013; 6(8A):32
[3] Lo K.V.H et al. J Tissue Eng Regen Med. 2014; 8(9):728-36



Design, construction and optimizing of a constant pressure bioreactor for whole liver decellularization

B.J. Nooshin[1,2†], Sh. Rahmani[1,3†], S.S. Shafiee[1,3], H. Niknejad[1]
Presenting Author: Seyyed Sepehr Shafiee, sepehr.shafiee@gmail.com
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Department of Tissue Engineering, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [3]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [†]Co-first author

INTRODUCTION: Design and construction an ideal decellurization bioreactor to obtain an intact liver scaffold can be of great help in regenerative medicine for the recovery of injured liver. The main reason of the damages to the scaffold during the decellurization process is mechanical stresses and prolonged exposure of the liver with decellularization agents. Therefore control of the pressure level and flow rate of solutions during decellurization process lead to the reducing mechanical damage to the scaffold structure, as well as effective penetration of the chemical agents into the depth of the tissue and clearing it in a shorter time.

METHODS: The built-in bioreactor has a seal main chamber with two inlet and an outlet valves, which provide a flow of cell removing solutions by using a constant pressure pump with a suitable flow rate. The Rat whole liver decellurization process was carried out with pressure of 50, 100 and 200 Psi. The structure and composition of decellularized organ were investigated using of SEM, staining with H&E, Van Gieson and Masson trichrome, amount of DNA content, IHC.

RESULTS & DISCUSSION: The results of the assessments revealed that the applying of constant pressure 100 Psi for whole liver decellurization compared to the other pressures resulted in minimal damage to the structure of the scaffold. The vascular network of the liver and its ECM structure, such as sinusoids, remained intact. Also, the amount and composition of ECM proteins such as collagen type 2, 3, 4, laminin and GAG`s are more similar to the normal liver.

CONCLUSIONS: The results of this study show that the optimization and utilize of constant pressure bioreactors plays an important role in the successful decellurization of whole liver. The applying of this method leads to obtaining of intact decellurized liver scaffolds by an appropriate combination in a shorter time.

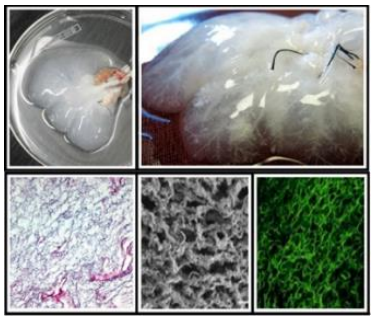
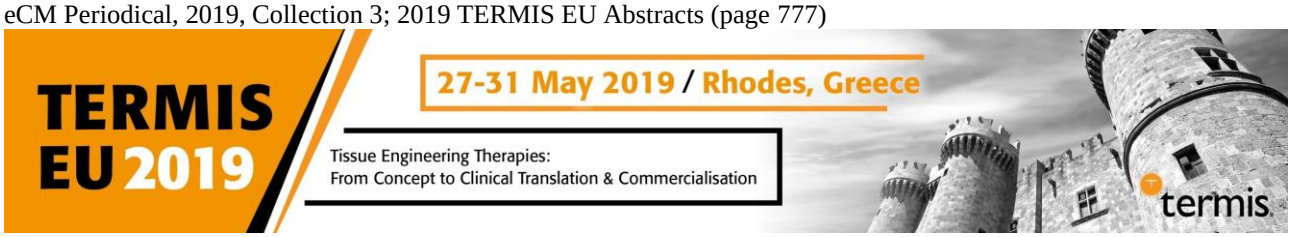


Figure1: A representative image of decellularized liver with constant pressure bioreactor

ACKNOWLEDGEMENTS: We would like to thank Ms. Tahereh Tayebi for her critical comments. Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Iran.



Bilayered, non-cross-linked collagen matrix for regeneration of facial defects after skin cancer removal: a new perspective for biomaterial-based tissue reconstruction

S. Ghanaati, S. Al-Maawi, P. Thönisson, A.M. Teiler, J. Lorenz, R. Sader
Presenting Author: Shahram Ghanaati, shahram.ghanaati@kgu.de
Frankfurt Orofacial Regenerative Medicine (FORM) Lab, Department for Oral, Cranio-Maxillofacial and Facial Plastic Surgery, Medical Center of the Goethe University Frankfurt, Frankfurt am Main, Germany

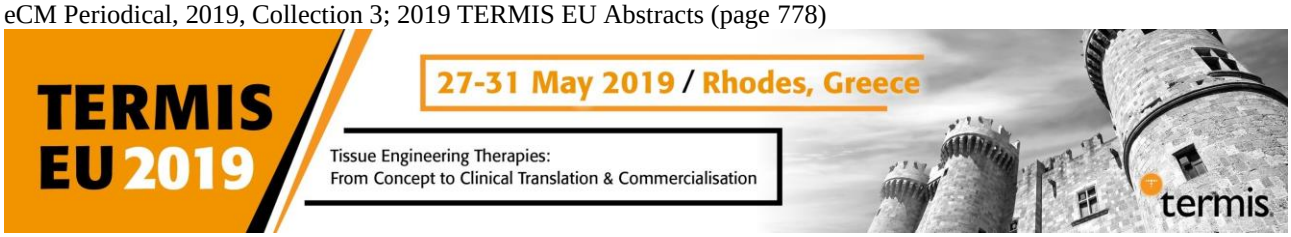
Introduction: Skin cancer resection results in a skin defect of different dimensions. In the reconstructive surgery skin transplants are considered as the golden standard. However, this intervention is accompanied by various disadvantages such as extended operation time, additional wound and pain. The aim of this case series was to evaluate the biomaterial-based skin regeneration using a three-dimensional collagen matrix of porcine origin.

Methods: The three-dimensional bilayered collagen matrix was originally developed for intraoral applications. In the course of a translational research series, the matrix was first evaluated during a preclinical study. The membrane was subcutaneously implanted in CD-1 mice for 60 days to evaluate the cellular reaction using histological and specific immunohistochemical methods to identify the inflammatory pattern. In a second step the matrix was evaluated in two independent clinical studies with different indications i.e. tooth recession coverage and vestibulopathy. These studies included histological evaluation of the treated region. Finally, the matrix was used for the regeneration of skin defects (up to 8 cm), which was resulted from skin cancer resection (basal cell carcinoma and malignant melanoma; n=30). The regeneration process was photo documented and analyzed using specific visual scores (three independent surgeons). Biopsied from 10 patients were evaluated histologically and the cases were followed for three years.

RESULTS & DISCUSSION: Preclinical: the matrix gets integrated into the implantation area and preserved its integrity over 60 days without any signs of a foreign body reaction. Clinical intraoral application: tooth resection coverage was achieved in all cases and vestibulopathy was successful without adverse events. In extraoral application Clinical extraoral application: defect regeneration was achieved in all cases and the original contour and texture of the skin was reconstructed to a large extent. Histological biopsies of the intra- and extraoral application shows the integration of the membrane as a scaffold to guide the newly formed tissue to regenerate the defect.

REFERENCES

[1] Ghanaati et al 2012 Biomed Mater. Feb;6:015010.
[2] Lorenz et al 2017 Clin Oral Investig. May;21:1103-11.
[2] Ghanaati et al 2017 J Cell Commun Signal. Mar;10:3-15



Development of PEG-fibrinogen hydrogel controlled release system of antisense nucleotide in the treatment of duchenne muscular dystrophy

S. Cohen[1], S. Testa[2], C. Fuoco[2], E. Fornetti[2], C. Gargioli[2], D. Seliktar[1]
Presenting Author: Shani Cohen, shani.c@campus.technion.ac.il
[1]Faculty of Biomedical Engineering, Technion, Israel Institute of Technology, Haifa, Israel
[2]Department Of Biology, Rome University Tor Vergata, Rome, Italy

INTRODUCTION: Duchenne muscular dystrophy (DMD) is caused by the almost complete absence of dystrophin protein due to out-of-frame mutations in the DMD gene. Antisense oligonucleotides (AONs) exon-skipping is one of the most promising therapeutic strategies for treating DMD, designed to skip a specific DMD exon, which produces a shortened transcript but a functional dystrophin protein. Here we designed a biomaterial strategy for encapsulation of therapeutic AONs in PEG-fibrinogen (PF) hydrogel-based microspheres as a controlled release system that can improve the pharmacokinetic properties of the AONs, increase their stability and greatly decrease the overall administered dosage.

METHODS: AONs loaded PF microspheres were formed using a dual photo-initiator, water-in-oil emulsion method. Prior to encapsulating, AONs were complexed with PEI, used as a transfection reagent. C2C12 mouse myoblasts were cultured and plated in 24 well plate. Cells were transfected with PF microspheres, each well contained 1 µg of 6-FAM labeled AONs and were incubated for 72 hours. The cellular uptake of AONs released from the microspheres was analyzed using confocal microscopy. For evaluation of dystrophin expression in mdx mice, isolated myotubes were cultured and dystrophin immunolabeling was performed after treatment of PF microspheres.

RESULTS & DISCUSSION: Figure 1 shows fluorescently 6-FAM labeled AONs loaded PF microspheres fabricated by the dual photo-initiator emulsion method, demonstrates spherical microspheres with encapsulation and homogenic distribution of the AONs within the PF hydrogel (i). In addition, penetration studies revealed that AONs polyplexes released from the microspheres and penetrated into C2C12 myoblasts after incubation of 72 hours (ii). For evaluation of dystrophin expression, mdx mouse isolated myoblasts treated with PF microspheres and immunostained with dystrophin antibody showed positive dystrophin expression (iii) compared to the untreated group.

CONCLUSIONS: PF hydrogel microspheres were able to release the therapeutic AONs, which demonstrate cellular uptake and successful restoration of dystrophin in vitro.

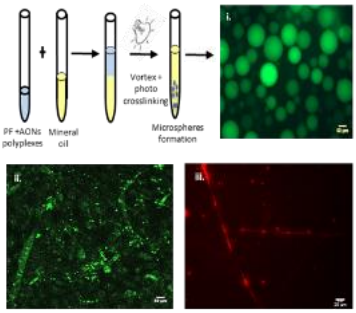
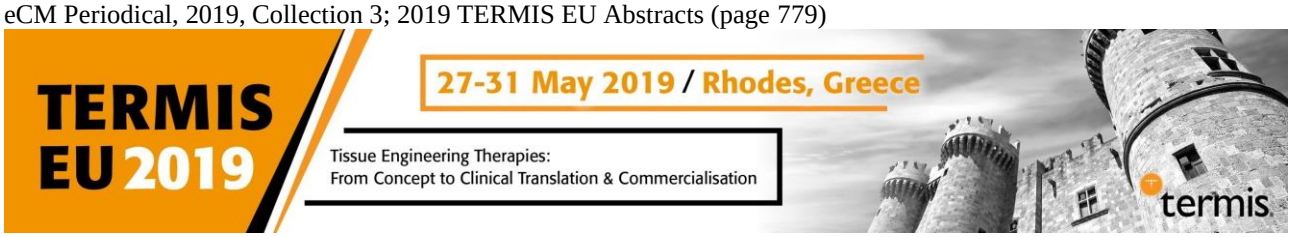


Figure 1: (i) Fluorescence microscopy of PF microspheres loaded with 6-FAM labeled AONs. (ii) AONs penetration study: transfection of 6-FAM labeled AONs into C2C12 myoblasts after 72 hours of incubation with PF microspheres encapsulating 1µg AONs per well. (iii) Dystrophin staining of isolated mdx derived myotubes after incubation of 72 hours with loaded PF spheres



Gelatin hydrogel with concentrated and refrigerated lyophilized human platelet lysate as full-thickness wound treatment

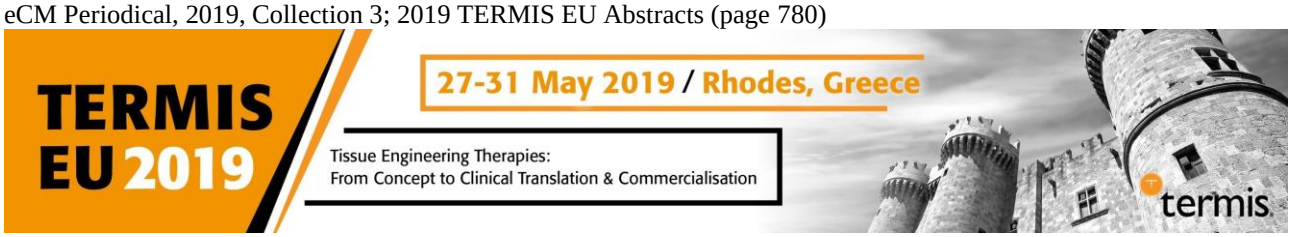
S. C. Notodihardjo[1], N. Morimoto[1], N. Kakudo[1], T. Mitsui[1], T. M. Le[1], Y. Tabata[2], K. Kusumoto[1]
Presenting Author: Sharon Claudia Notodihardjo, shar.claudia@gmail.com
[1]Department of Plastic and Reconstructive Surgery, Kansai Medical University, Osaka, Japan
[2]Laboratory of Biomaterials, Department of Regeneration Science and Engineering, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

Introduction: Shortly after wound occur, platelets play important roles such as releasing growth factors from its alpha granules to initiate the wound healing process. This mechanism has been adopted to in vitro condition by activating human platelet cell through freeze-thaw cycle to release the growth factors, then this cocktail is called as human platelet lysate (hPL). Efficacy of hPL has been widely used for cell culture medium and wound treatment in combination with bioscaffolds. In clinical fields, chronic wounds usually need months of treatment. An effective and efficient long-term preservation method of hPL is necessary to support its use in clinical setting. Some proteins are known stable for long-term preservation at 4°C after being lyophilized. Besides, we can use lyophilization process to adjust the concentration of hPL.

Methods: As biodegradation of hPL alone is short, we impregnated hPL into gelatin hydrogel (GH) before apply it as wound treatment. Full-thickness wound of male C57Bl6J/Jcl mice was used as the wound model. We considered 9 months are necessary as the period for long-term preservation. We compared cryopreservation of liquid form at -80°C and refrigeration of lyophilized form at 4°C. After being lyophilized, reconstitution with saline was adjusted to produce 1-fold, 2-fold and 3-fold concentrations of hPL. We use ELISA to measure quantity of growth factors in hPL. Histologic examinations using hematoxylin-eosin, Azan and anti-CD31 staining were performed on days 4, 7 and 14 to assess the wound healing process.

RESULTS & DISCUSSION: Concentration of PDGF-BB, VEGF, and TGF-β1 elevated in line with the concentration of hPL. From histologic examinations, neither the GH itself nor the simple administration of hPL accelerate the wound healing process. However, the combination of GH with 2-fold or 3-fold concentration of hPL accelerated the capillary and granulation tissue formation. In addition, the GH sheet impregnated with 3-fold concentrated hPL had the longest epithelium formation from the wound margin. There are no significant differences in the efficacy between preservation methods of hPL after 9 months.

CONCLUSIONS: Human platelet lysate in 2-fold or 3-fold concentration is effective to support wound healing in combination with gelatin hydrogel. Activities of the growth factors inside lyophilized human platelet lysate are maintained at 4°C for up to 9 months. This finding proposes a new long-term preservation method for human platelet lysate in clinical setting.



Opto-electrical fiber for real-time optical stimulation and electrochemical detection of dopamine exocytosis

S. Vasudevan[1], J. Kajtez[1], A-I. Bunea[1], N. B. Larsen[1], A. Gonzalez-Ramos[2], M. Kokaia[2], T. R. Moreno[2], A. Martínez-Serrano[3], A. Heiskanen[1], S. S. Keller[1], J. Emnéus[1]
Presenting Author: Shashank Vasudevan, shasv@nanotech.dtu.dk
[1]DTU Nanotech, Technical University of Denmark, K. Lyngby, Denmark [2]Wallenberg Neuroscience Center, Lund University, Lund, Sweden [3]Center of Molecular Biology Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain

INTRODUCTION: Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the midbrain leading to a dopamine deficit. Continuous delivery of dopamine has shown to reduce the risks associated with chronic motor complications. Here we describe a leaky opto-electrical carbon fiber as a potential neural implant for continuous supply and real-time modulation of dopamine in striatum using stem cells, optogenetics and electrochemistry.

METHODS: The polymer buffer layer on a commercial optical fiber was pyrolysed at 900 °C to obtain a carbon layer around the optical fiber. Pyrolysed carbon has proven to be biocompatible and to enhance the differentiation of human neural stem cells (hNSCs) into dopaminergic neurons. The carbon-coated optical fiber was made leaky using laser micro-ablation. Optogenetically modified hNSCs were cultured and differentiated in vitro on the fiber for 11 days (Figure 1a).

RESULTS & DISCUSSION: Figure 1b shows the initial amperometry results obtained first from potassium-induced depolarization followed by optical stimulation using blue light (460 nm). The peak heights are a direct measure of dopamine exocytosis from the cells. By comparing the current peaks, we see dopamine release from a large number of optogenetically modified dopaminergic neurons in the population. This dopamine can regulate physiological dopamine levels in the brain.

CONCLUSIONS: These initial results provide the first proof of concept for continuous real-time monitoring of dopamine release from stem cells cultured on the opto-electrical carbon fiber and its potential for use as PD therapy.

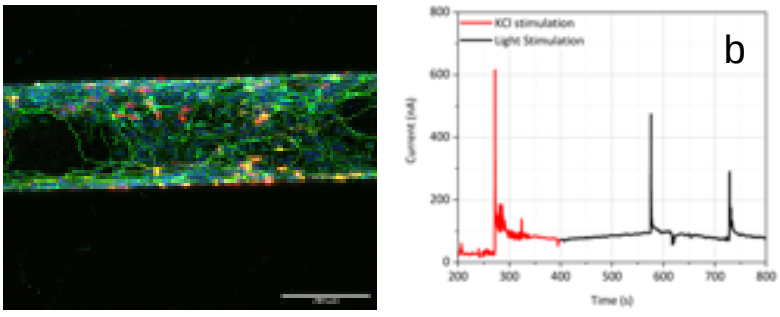
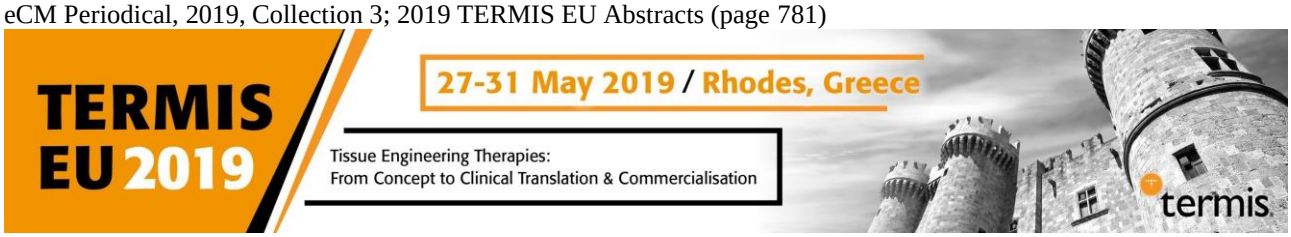


Figure 1: (a, left) Immunohistochemistry of opto-genetically modified hNSCs cultured on the fiber (Red: Tyrosine hydroxylase, Green: beta-tubulin, Blue: Nuclei). (b, right) Amperometry results showing chemical followed by optical stimulation.

ACKNOWLEDGEMENTS: This project has been funded by the European Union Horizon 2020 Programme (H2020-MSCA-ITN-2016) under the Marie Skłodowska-Curie Initial Training Network and Grant Agreement No.722779.



Inflammatory controllable hydrogel for improving tissue-engineering blood vessels formation

S. Wei, W. Chen, Y. Chen
Presenting Author: Shin-Yan Wei, tina84694@gmail.com
Department of Materials Science and Engineering, National Tsing-Hua University, Hsinchu 30013, Taiwan

INTRODUCTION: Recent studies have shown that host non-inflammatory myeloid cells mediate the blood perfusion of engraftment of bioengineered vascular networks[1,2,3], so developing a biomaterial to regulate host myeloid cells into biomaterials and control the interaction between biomaterial and non-inflammatory myeloid cells could control the vasculogenesis and angiogenesis. In this study, we developed the hydrogel with different crosslinkable bonds to control the interaction between biomaterial and non-inflammatory myeloid cells, and regulate stem cell-based perfused blood vessels formation.

METHODS: There are two different collagen hydrogels. One is "Acid collagen hydrogel" formed by self-assembly collagen fibrils, and the other one is "Collagen-Ph hydrogel" formed through covalently cross-linked collagen fibrils. 200 μL of a mixed solution of collagen-Ph, enzyme horseradish peroxidase (HRP), and hydrogen peroxide (H_2O_2) or acid collagen hydrogels with the same mechanical properties G'~50 Pa mixed with endothelial colony-forming cells (ECFCs), and mesenchymal stem cells (MSCs) (2 x 10^6 total; 40:60=ECFCs: MSCs) was subcutaneously injected into nude mice before gelation. At the indicated time point, each implant was excised, fixed, embedded, sectioned and immune-stained for various antibodies.

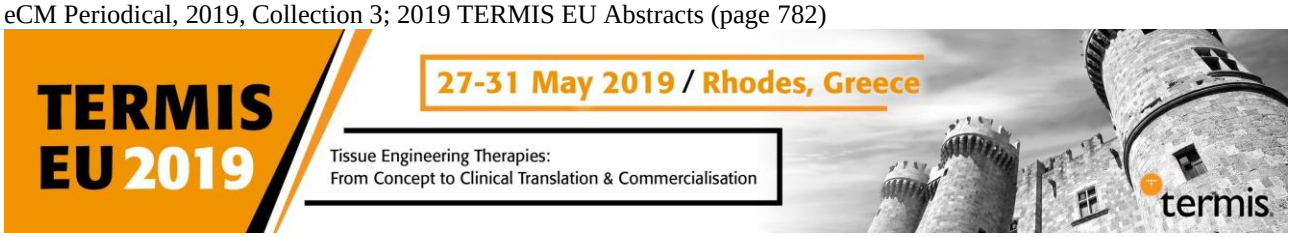
RESULTS & DISCUSSION: We compared the extent of vascular network formation in Acid collagen with Collagen-Ph. Acid collagen contained an extensive perfused blood vessels, and lumens expressed hCD31(human endothelial cells markers), confirming the human nature of the endothelium. By contrast, in the Collagen-Ph remained it organized as cellular cords on day7. To examine the role of myeloid cells in vasculogenesis, we further stained Ly6G (neutrophils markers), and CD45 (myeloid cells markers), the result show that the neutrophils-induced inflammatory time in Acid collagen was only two days, but in the Collagen-Ph it lasted four days. Collectively, these results confirmed that inflammatory time of $Ly6G^+$ neutrophils are effect on the ability implanted human stem cell to enable formation of vasculature in vivo.

CONCLUSIONS: We demonstrated that crosslinkable bonds of hydrogel play an important role in controlling the interaction between host myeloid cells, implanted stem cells (ECFC and MSCs) and hydrogel, to further modulate the bioengineering vascular network formation in vivo. Therefore, how the crosslinkable bond of hydrogel regulate the mechanism and interaction between inflammation and vasculogenesis was constructed and discussion.

ACKNOWLEDGMENTS: This work was supported by the Ministry of Science and Technology of Taiwan grant (MOST 106–2221-E-007–055, and MOST 107–2221–E-007–067-MY3)

REFERENCES

[1] Lin, R. Z. Nature Biomedical Engineering. 2017;**1**(6).
[2] Kwee, B.J. Annals of Biomedical Engineering. 2015;**43(3)**:628-640.
[3] Cattin, A.L.Cell 2015;**162(5)**:1127-1139.



Role for follistatin in bone tissue engineering?

S. Fahmy-Garcia[1,3], E. Farrell[2], J. Witte-Bouma[2], I. Robbesom-van den Berge[3], M. Suarez[4], D. Mumcuoglu[1,6], H. Walles[4], S.G.J.M. Kluijtmans[6], B.C.J. van der Eerden[3], G.J.V.M. van Osch[1,5], J.P.T.M. van Leeuwen[3], M. van Driel [3]
Presenting Author: Shorouk Fahmy-Garcia, s.fahmygarcia@erasmusmc.nl
[1]Department of Orthopedics, Erasmus MC.[2]Department of Oral and Maxillofacial Surgery, Erasmus MC [3]Department of Internal Medicine, Erasmus MC [4]Institute of Tissue Engineering and Regenerative Medicine, Julius-Maximillians University Würzburg, Germany [5]Department of Otorhinolaryngology, Head and Neck Surgery, Erasmus MC, Rotterdam, The Netherlands [6]Fujifilm Manufacturing Europe B.V, Tilburg, The Netherlands.

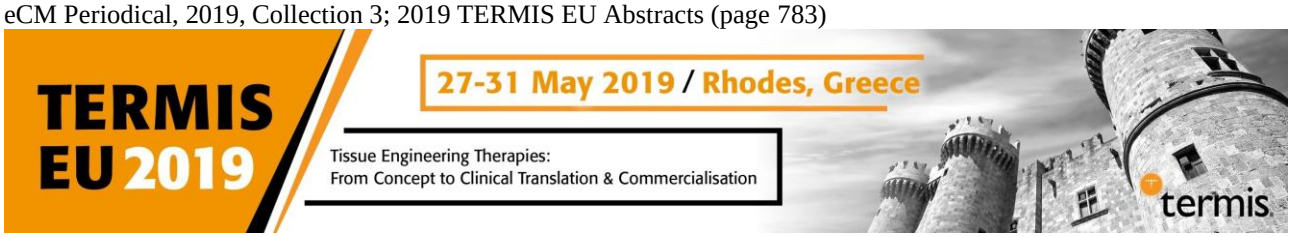
INTRODUCTION: Follistatin (FST) is a glycoprotein able to bind irreversibly to activin A, a protein that has been reported to inhibit bone formation. There are two FST variants (FST288 and FST315) with major differences in their ability to bind to the cell. This variation may influence their effect on bone repair. We investigated the effect of FST in critical processes for bone repair, such as cell recruitment, osteogenesis and vascularization, and ultimately its use for bone tissue engineering (TE). To do so, both FST variants were loaded in an in situ gelling formulation made by alginate and recombinant collagen-based peptide microspheres and implanted in a calvarial defect model.

METHODS: FST was added to the cell culture at a dose range (25-175 ng/ml) to study its effect on: MSC and EC migration; vascularization using tube-formation and spheroid-sprouting assays upon ECs; and MSC and pre-osteoblast osteogenic differentiation. The release profile of both FST variants from the alginate-based hydrogel was studied over 4 weeks. For in vivo studies FST315 and FST288 were loaded in the formulation and implanted in a calvarial defect model. Rats were scanned biweekly for 10 weeks. 4 Weeks postoperative, rats were subcutaneously injected with calcein. Samples were processed for histological evaluation.

RESULTS & DISCUSSION: FST stimulated MSC migration at all doses tested ($P<0.01$) and EC migration at 70 ng/ml ($P<0.05$). FST promoted tube-formation and sprouting at 28 ng/ml ($P<0.05$). FST did not enhance osteogenic differentiation of MSCs, but increased pre-osteoblast mineralization at all tested doses ($P<0.01$). Most of the loaded FST315 was released over 4 weeks, contrary to FST288, which was mostly retained in the formulation ($P=0.0003$). However, although a more homogenous mineralization progression was observed in the FST-treated samples, overall mineral density did not differ from the controls.

CONCLUSIONS: FST may be an early player in bone formation, having an angiogenic role and indirectly triggering ECM mineralization. Therefore, FST burst release could be necessary to trigger bone formation and its continuous release might not match with the pharmacokinetics of the protein. This is the first study that investigated the use of FST as a bone tissue engineering-based therapy, assessing protein´s effect both in vitro and in vivo. We have shown that FST stimulates, in vitro, essential processes for bone regeneration such as EC and MSC recruitment, formation and expansion of the vasculature and osteoblasts mineralization. Mechanistic studies are needed to clarify the biological activity of FST, and to further assess its potential and the most suitable FST carrier for bone tissue engineering applications.

ACKNOWLEDGEMENTS: Financial support was received from FP7/2007-2013/, grant agreement 607051.



Impact of polyvinylpyrrolidone on extracellular matrix deposition and cell proliferation of WS1 fibroblast

S. Rana[1,2], D.I. Zeugolis[1,2]
Presenting Author: Shubhasmin Rana, s.rana2@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway [2]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland

INTRODUCTION: Cell based therapies gained increasing interest for the treatment of musculoskeletal tissue and injuries or degeneration. Herein, we propose that functional regeneration of these tissues can be achieved by using native capacity of cells to create their own tissue-specific extracellular matrix (ECM) avoiding the use of biodegradable scaffolds [1]. Cell culture on polystyrene and similar surfaces represents an artificial environment with differs substantially from natural in vivo microenvironment of the respective cells. One important factor is the dilute media composition. Macromolecular crowding (MMC) has been reported to dramatically increase the ECM deposition in vitro. We propose the noncarbohydrate single crowder PVP as a promising candidate for tissue engineering applications [2].

METHODS: In this work four commercially available non carbohydrate polymers, a) polyvinylpyrrolidone (PVP) 40 kDa, PVP 55 kDa, PVP 360 kDa and PVP 1300 kDa were used as crowding agents. WS1 fibroblasts were expanded up to passage 4 in DMEM media, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. 25,000 cells/cm^2 were subsequently cultured for up to 7 days. Cell viability and metabolic activity and proliferation were accessed using Live/Dead, alamarBlue® and PicoGreen™ assays respectively. Extracellular matrix deposition was assessed with immunocytochemistry and sodium dodecyl sulfate polyacylamide gel electrophoresis (SDS-PAGE).

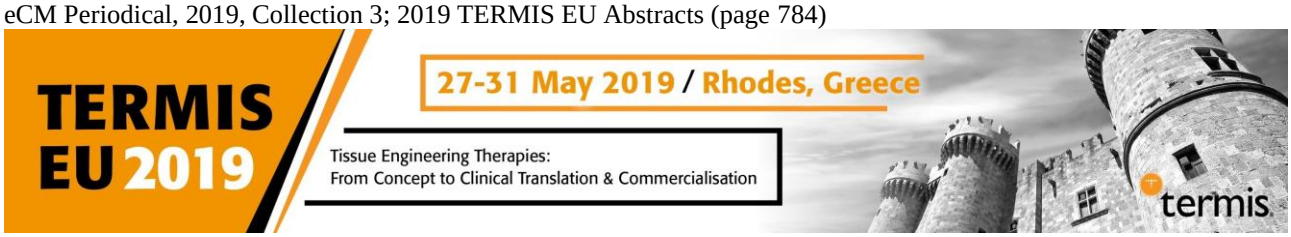
RESULTS & DISCUSSION: SDS-PAGE and densitometric analyses illustrated significant increase ($p < 0.001$) in collagen deposition in the presence of PVP grade crowder at all time points. Immunocytochemistry and relative fluorescence intensity analyses showed significant increase ($p < 0.001$) in collagen type I, deposition in the presence of PVP grade crowders. MMC did not affect cell proliferation and metabolic activity at all timepoint.

CONCLUSIONS: Mutually, these data clearly illustrate the positive effect of MMC in WS1 fibroblast cell culture.

ACKNOWLEDGEMENTS: The authors would like to acknowledge Hardiman Scholarship for financial support. SR and DZ have no conflict of interest.

REFERENCES

[1] Satyam A et al. Adv Mater. 2014; 26(19):3024-34
[2] Rashid, R., et al. Tissue Eng Part C Methods, 2014. **20**(12): p. 994-1002.



The co-application of freeze-dried amniotic membrane and lacto-n-neotetraose oligosaccharide as an inducer of type 2 immune response for full-thickness wound healing

B. Farhadihosseinabadi[1†], S. Shariatzadeh[2†], A. Jafari[2], T. Tayebi[2], B.J. Noushin[2], A. Manzari-Tavakoli[2], H. Niknejad[2] [†]Co-first author
Presenting Author: Siavash Shariatzadeh, siavash.shariatz@gmail.com
[1]Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

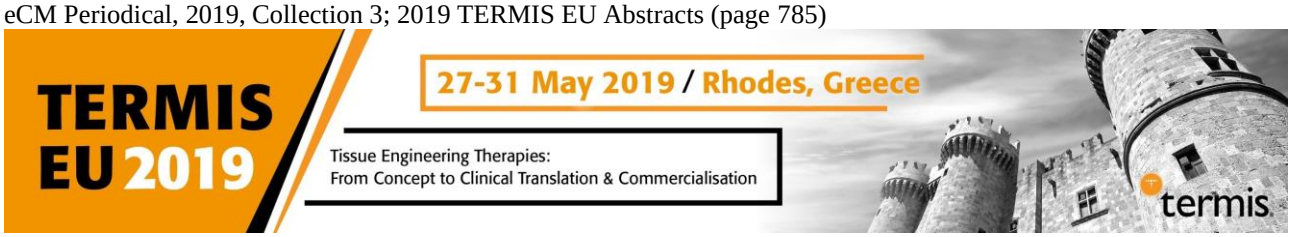
INTRODUCTION: The wound healing process is a complex mechanism in which many factors are involved. The various properties of the amniotic membrane (AM) such as antibacterial and anti-inflammatory effects, non-immunogenic characteristic, and the ability to promote re-epithelialization make it an excellent wound dressing. In this study, we aimed to assess the effect of co-application of freeze-dried amniotic membrane and Lacto-n-Neotetraose (an inducer of type 2 immune response) in the treatment of full-thickness wounds in the mice model.

METHODS: The placenta samples were obtained from the donors under the cesarean section. Then, the AM was separated from chorion and freeze-dried under vacuum condition using vacuum freeze-drying apparatuses. The full-thickness wound model was created by punch biopsy with a 6mm diameter on the dorsal region of mice. Experiments were performed in 3 groups including G1 (wound with no wound dressing), G2 (wound covered by FDAM) and G3 (wound covered by AM/ LNnT (200 µg)) at four postulated times including 3, 7, 14, and 21 days post-surgery. The wound closure rate was compared in the three groups at the mentioned times. After sacrificing the mice, the wound samples were collected for histological and molecular analysis including H&E and Masson's trichrome stain and measuring the gene expression rate of Collagen I, III, VEGF-a, IL-4, IL10 and IL-13 using real-time PCR.

RESULTS & DISCUSSION: The wound closure rate in the G3 group was significantly higher than the G1 group at day 3 and G1 and G2 groups at day 7. H&E staining revealed that the epithelialization rate in the G3 group was better than the other groups at day 3. The epithelial layer was completely formed in G3 group at the day 7 while this condition was not observed in the G1 and G2 groups at the mentioned time. Also, the hair follicle was higher in the G3 group at days 14 and 21 in compared with G1 and G2 group. Masson's trichrome staining showed a better collagen deposition in G3 group than the two other groups which was in line with data obtained from the molecular analysis where the gene expression rate of col I and III was higher in the G3 group. An increase in gene expression rate of IL-4, IL10 and IL-13 showed that the use of LNnT could induce the type 2 immunity in G3 group.

CONCLUSIONS: Our results showed that the simultaneous use of LNnT with AM shows more positive outcomes than using amniotic membrane alone in wound healing.

ACKNOWLEDGEMENTS: The authors would like to thank the Biotechnology Department of Shahid Beheshti University of Medical Sciences for their assistance in this research. Research reported in this publication was supported by Elite Researcher Grant Committee from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.



Design of a new bioactive matrix to guide ligament regeneration

S. Chiera, W. Bonani, A. Motta, C. Migliaresi
Presenting Author: Silvia Chiera, silvia.chiera.1@unitn.it
BIOtech Research Center and Department of Industrial Engineering, University of Trento, Trento, Italy

INTRODUCTION: Ligament disorders and injuries are among the most common health problem influencing the adult population. To overcome problems related to autografts and allografts for ACL (anterior cruciate ligament) repair, tissue engineering (TE) could be a valid alternative strategy using scaffolds able to recruit cells while exerting the necessary biomechanical functions. An ideal scaffold for ACL TE (as well as for all other tissues) has to combine appropriate physical, mechanical and biological properties [1]. We present here the design and fabrication of a multicomponent scaffold with silk fibers in a porous silk sponge having the role of recruiting cells and host the regenerative pathway, whose mechanical properties can be adjusted to ACL biomechanics.

METHODS: Mechanical properties of dry and wet silk fibers bundles with different twisting levels were assessed with monoaxial tensile tests, to define type and number of filament bundles needed for the mechanical matching with the natural ACL. ACL model scaffolds were prepared by embedding the selected fibers in a fibroin gel that underwent to directional freeze-drying in order to induce the formation of vertical porous pathways [2]. Scaffolds were seeded with AdMSC p3 cells at 30,000 cells/cm^2 and cultured in static and perfusion chamber conditions. Cell adhesion, proliferation and gene expression tests were performed at different time points (1-3-7-14-28 days) and results were evaluated with biochemical assays, confocal microscopy and qPCR.

RESULTS & DISCUSSION: At increasing twisting levels, the stiffness of the investigated silk fiber bundles increased. Stiffness and load at break of the selected silk bundles are compared in table 1 with those of an ACL. The results indicate that about 30 bundles would be required to fabricate a scaffold with stiffness and strength comparable to those of a natural ACL. Biological tests showed that cells, seeded on the surface, are able to penetrate inside the porous fibroin sponge and also in between the fibers of the bundle, assuming an elongated orientation that is typical of native ACL.

CONCLUSIONS: We demonstrated that using silk twisted fibres, it is possible to fabricate model ligament prostheses with mechanical properties comparable with the natural ACL. Moreover, the use as a matrix of a properly morphological designed fibroin sponge with vertical pores could allow cell penetration and orientation.

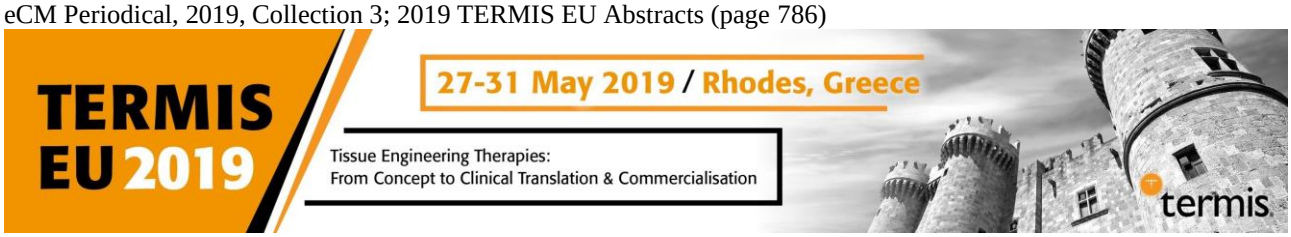
Condition:	Dry	Wet	Natural ACL [3]
Stiffness N/mm	37 ± 1	9 ± 1	242 ± 28
Load at break N	102 ± 3	78 ± 2	2160 ± 157

Table 1: values obtained from the mechanical tensile test of silk fibroin fiber bundles considering N>=7 and assuming ACL length as 30 mm.

ACKNOWLEDGEMENTS: University of Trento financial support.

REFERENCES

[1] Butler DL et al. Tissue Eng Part B Rev. 2009; 15(4):477-84
[2] Font Tellado S et al Tissue Eng Part A. 2017; 23(15-16):859-872



Tissue engineering experimental experience in Costa Rica: PCL/Dental LT resin scaffolds for skeletal muscle and bone tissue engineering

A. Ulloa-Fernández[1], S. Castro-Piedra[1], L. Rojas-Rojas[1,2], W. Vargas-Segura[3], T. Guillén-Girón[2]
Presenting Author: Silvia E Castro-Piedra, scastro@tec.ac.cr
[1]Centro de Investigación en Biotecnología, Instituto Tecnológico de Costa Rica [2]Centro de Investigación en Materiales, Instituto Tecnológico de Costa Rica [3]Laboratorio de Irradiación Gamma, Instituto Tecnológico de Costa Rica, Costa Rica

INTRODUCTION: Skeletal muscle and bone tissue engineering have great potential to be used to improve repair or to replace damaged tissue [1]. Also, tissue engineering allows to fabricate study models for myopathies and other tissue related diseases. Herein, we have fabricated two polymeric scaffolds that mimic the in vivo tissue and were used to evaluate muscle and bone cell growth. The scaffolds would be probed in a bioreactor with mechanical stimulation

METHODS: We fabricated poly-Ɛ-caprolactone (PCL) microfilaments for muscle tissue and dental LT resin from FormLabs ® was used for bone scaffolds, through stereolithography (SLA), and sterilized by 25 kGy gamma radiation. To fabricate bone scaffolds we take microCT images to model and imitate the structure of trabecular bone. C2C12 and Saos-2 cells lines were seeded at 50 000 cells/ cm2, for each 1 mm scaffold height. We evaluate mechanical properties of materials, cell adhesion and viability of muscle and bone cells, for 3, 7, 11 days.

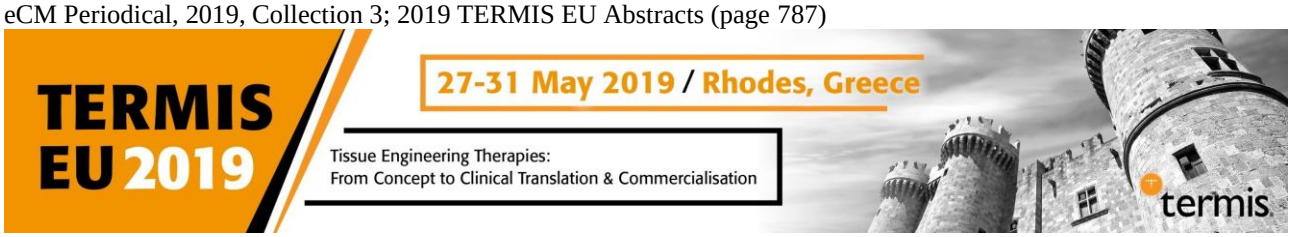
RESULTS & DISCUSSION: Mechanical characteristics were obtained for the polymeric scaffolds, Young modulus was 347.25 ± 30.10 MPa for bone-like structure and 80.02 ± 23.14 MPa for microfilaments. Also, we have found that cells can adhere and grow in both scaffolds, however, cells do not penetrate deep in bone scaffolds, therefore, we need to improve cell seeding. Nevertheless, we note some cell growth on scaffolds edge.

CONCLUSIONS: Scaffolds that simulate muscle tissue and trabecular bone were fabricated. Cells adhere in both biomaterials, however, for bone scaffolds it's necessary to improve seeding conditions to enhance cell adhesion, growing and distribution into the scaffold. This work represents the beginning at TEC to combine cells with biomaterials to improve tissue regeneration for Costa Rican population.

ACKNOWLEDGEMENTS: Finantial support: Vicerrectoría de Investigación, Instituto Tecnológico de Costa Rica. Project No. 15101013 María Laura Espinoza, Jessica Mora and Ian Cordero for collaboration as project assistants.

REFERENCES

[1] Porcionato A et al. Int.J.Mol.Sci. 2018; 19(4117):1-79.



Hypertrophy associated regulation of RUNX3 and MEF2C during chondrogenesis of human mesenchymal progenitor cells

S. Dreher[1], V. Dexheimer[1], V. Tonnier[1], J. Fischer[1], T. Walker[2], S. Diederichs[1], W. Richter[1]
Presenting Author: Simon Dreher, simon.dreher@med.uni-heidelberg.de
[1]Research Centre for Experimental Orthopaedics, Orthopaedic University Hospital Heidelberg, Heidelberg, Germany [2]Department of Orthopaedic and Trauma Surgery, Orthopaedic University Hospital Heidelberg, Heidelberg, Germany

INTRODUCTION: Bone marrow-derived mesenchymal progenitor cells (MPC) are attractive for cartilage-defect regeneration. Unfortunately, MPC unlike articular chondrocytes (AC), undergo hypertrophy during chondrogenic (re-)differentiation and produce transient cartilage in vitro and bone in vivo [1]. During mouse embryonic development, balancing of transcription factors SOX9, RUNX2, RUNX3 and MEF2C is associated with chondrocyte hypertrophy [2]. Surprisingly little is known on regulation of these key fate-determining factors during MPC chondrogenesis versus AC redifferentiation. Aim was to uncover regulation of SOX9, RUNX2, RUNX3 and MEF2C during MPC and AC chondrogenic (re-)differentiation and to identify signaling pathways driving possible misregulation in MPC.

METHODS: Human MPC and human AC were isolated after informed consent, expanded and subjected to chondrogenic conditions for up to 6 weeks. qRT-PCR, Western-Blotting, ALP activity assay and histology were performed weekly during (re-)differentiation and after withdrawal of TGF-ß, treatment with antagonists or intermittent PTHrP application.

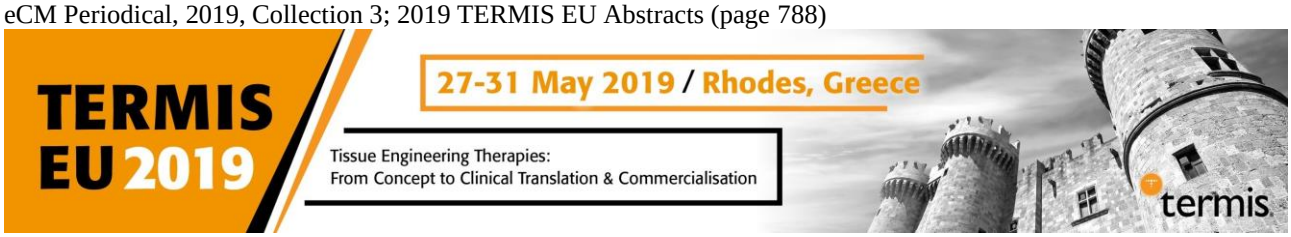
RESULTS & DISCUSSION: AC had higher SOX9-levels but remained negative for RUNX2 and RUNX3 protein throughout redifferentiation. During MPC chondrogenesis, RUNX2 protein was undetectable while RUNX3 and MEF2C mRNA and protein levels were significantly upregulated together with hypertrophic markers. Reduction of TGF-ß and BMP-activity but not WNT- or HH-signaling reduced MPC chondrogenesis. Only PTHrP-pulse and IWP-2 treatment specifically reduced hypertrophy. Hypertrophic RUNX3 was driven by TGF-ß and BMP-signaling while MEF2C induction involved BMP- and HH-signaling.

CONCLUSIONS: RUNX2, considered as master regulator of endochondral development, apparently did not drive hypertrophy of human MPC. Reduction of RUNX3 and MEF2C transcription factors to AC levels directly or via manipulation of relevant pathways may be promising for guiding MPC differentiation towards an articular chondrocyte phenotype.

ACKNOWLEDGEMENTS: We thank patients for donation of tissue samples.

REFERENCES

[1] Pelttari K et al. Arthritis Rheum 2006; 54: 3254-3266.
[2] Lefebvre V et al. Curr Top Dev Biol 2010; 90: 291-317.



Tissue-derived ECM hydrogels for peripheral nerve repair

S.C. Kellaway[1,2], J.B. Phillips[1], L.J. White[2]
Presenting Author: Simon Kellaway, simon.kellaway.16@ucl.ac.uk
[1]Centre for Nerve Engineering (CNE), University College London (UCL), London, UK [2]Division of Regenerative Medicine and Cellular Therapies (RMCT), University of Nottingham, Nottingham, United Kingdom

INTRODUCTION: Engineered neural tissue (EngNT) made using aligned cellular collagen promotes axonal regeneration across critical sized defects [1]. We present alternative hydrogel materials that are suitable for use in EngNT and may improve its regenerative capacity.

METHODS: Decellularization protocols for a range of tissues were adapted [2-3]. dsDNA and glycosaminoglycan (GAG) contents were quantified, and hydrogels were formed. Mechanical properties were characterized using a rheometer (Anton Paar, Hertford, UK). Viability of Schwann cells (SC) incorporated within gels was assessed using the Cell Titer-Glo assay (Promega, Southampton, UK). SC were seeded at 0.5, 1, 2 and 4 x 10^6 cells/ml to produce contraction profiles and cellular alignment was visualized via immunofluorescence microscopy.

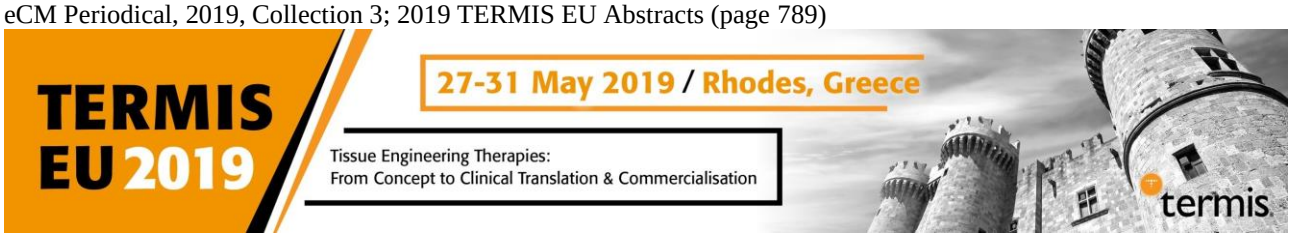
RESULTS & DISCUSSION: Bone extracellular matrix (ECM, B-ECM) possessed the lowest dsDNA content (27.4 ± 5.3 ng/mg) whilst small intestinal submucosa ECM (SIS-ECM) had the highest (424.4 ± 4.0 ng/mg). Similarly, B-ECM had the lowest GAG content at 1.9 ± 0.8 µg/mg, compared to the highest of 5.6 ± 0.9 µg/mg for SIS-ECM. The B-ECM gel displayed the fastest gelation and exhibited a sigmoidal gelation profile. The liver ECM (L-ECM) gel was the stiffest at 453 ± 9 Pa, whilst the urinary bladder ECM (UBM-ECM) was the softest at 61 ± 8 Pa. SC viability was significantly higher for 6 mg/ml compared to 4 mg/ml gels and was highest for SIS-ECM and lowest for B-ECM. Contraction was dependent on both gel and cell concentrations; higher cell concentrations and lower gel concentrations produced the most contraction. Adequate SC alignment was seen in 4 and 6 mg/ml B-ECM and SIS-ECM gels however, no alignment was observed in L-ECM or UBM-ECM gels.

CONCLUSIONS: Biochemical and mechanical properties affect SC viability. B-ECM and SIS-ECM are further suitable for use in EngNT. Future work will involve co-cultures of neurons to assess axonal regeneration.

ACKNOWLEDGEMENTS: EPSRC grant EP/L01646X

REFERENCES

[1] Georgiou M et al. Biomaterials 2014; 34(30): 7335-7343.
[2] Sawkins M et al. Acta Biomaterialia 2013; 9(8): 7865-7873
[3] Crapo P et al. Biomaterials. 2011;32(12): 3233-3243



Development of an injectable thermoresponsive polymer clay nanocomposite for the intervertebral disc

S.W. Partridge[1,2], J. Snuggs, C.L. Le Maitre[2], C. Sammon[1]
Presenting Author: Simon William Partridge, S.Partridge@shu.ac.uk
[1]Materials Engineering Research Institute [2]Biomolecular Sciences Research Centre, Sheffield Hallam University, S1 1WB, United Kingdom

INTRODUCTION: Injectable hydrogels via minimally invasive surgery offer benefits to the healthcare system reducing the risk of infection, scar formation and cost of treatment. Degradation of the intervertebral disc (IVD) currently has no preventative treatment. The use of an injectable hydrogel material could support IVD mechanical properties and promote tissue regeneration. We present a hydrogel material based on Laponite® associated poly(N-isopropylacrylamide) co poly(dimethylacrylamide). Previous research from our group has already demonstrated in vivo and in vitro efficacy for cell differentiation, migration and tissue integration for both nucleus pulposus (NPgel) and bone tissues (Bgel) [1-3].

METHODS: Hydrogels were synthesised in accordance with previous research [1-3]. Physical properties of different hydrogel formulations were assessed using rheometry, SEM, DSC and swelling for 7 days in PBS at 37^{o}C. The NPgel formulation has also been characterized using dynamic uniaxial unconfined compression (DMA) and degradation was assessed in water, PBS and DMEM (10% FCS) over >48 weeks at 37^{o}C. Gravimetric, ATR-FTIR and ICP-MS data from the degradation study have been analysed using PCA, PLS and multivariate statistical analysis degradation from the data sets.

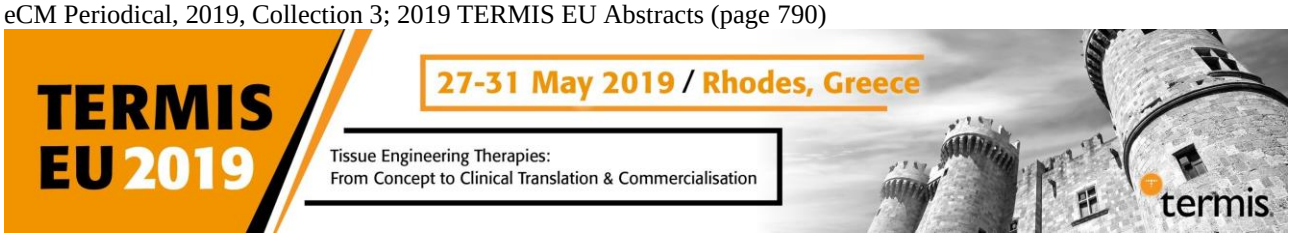
RESULTS & DISCUSSION: Using a box-Behnken experimental design, we were able to produce hydrogels with ranging shear moduli G* (600-2800 Pa), gelation temperatures (35-38^{o}C) and swelling characteristics (-10% to -40%). DMA of the NPgel formulation demonstrated complex modulus E* of > ~1800 Pa when first gelled; this increased to > ~5000 Pa following 24 hours in PBS. The degradation study showed distinct changes in the NPgel material behaviour at 37^{o}C in salt-based media (PBS and DMEM) compared with water. FTIR of dried NPgel from the degradation study revealed some subtle alterations over time.

CONCLUSIONS: We have demonstrated the ability to customise the hydrogel physical properties to best suit the needs for the IVD. The shrinkage of NPgel in salt-based media was likely the cause of the increased mechanical properties observed in the DMA (Fig 1). The real-time degradation study is still ongoing; however initial results show subtle changes to the material in physiological media over time (Fig 1).

ACKNOWLEDGEMENTS: We would like to thank Arthritis Research UK grant number 21497 for supporting this research.

REFERENCES

[1] Thorpe AA et al. Eur. Cells Mater. 2016b; 32:1–23.
[2] Thorpe AA et al. Acta Biomater. 2017;54: 212–226.
[3] Thorpe AA et al. Oncotarget. 2018; 9:18277–18295.



Engineering an environment for the study of fibrosis: A 3D human muscle model with endothelium-specificity and endomysium

S. Bersini[1], M. Gilardi[1,2], G.S. Ugolini[3], V. Sansoni[1] G. Talò[1], S. Perego[1], S. Zanotti[4], P. Ostano[5], M. Mora[4], M. Soncini[3], M. Vanoni[2], G. Lombardi[1], M. Moretti[1,6,7]
Presenting Author: Simone Bersini, sbersini@salk.edu
[1]IRCCS Istituto Ortopedico Galeazzi, Milano, Italy [2]University of Milano Bicocca, Milano, Italy [3]Politecnico di Milano, Milano, Italy [4]Fondazione IRCCS Istituto Neurologico C. Besta, Milano, Italy [5]Fondazione Edo ed Elvo Tempia, Biella, Italy [6]Ente Ospedaliero Cantonale, Lugano, Switzerland [7]Fondazione Cardiocentro Ticino, Lugano, Switzerland

INTRODUCTION: The integration of vascular structures and mesenchymal compartments into in vitro cultured tissues is critical to study complex cell interactions [1]. Several works highlighted that muscle disorders including dystrophies show an abnormal cross-talk between muscle fibers, endothelium and fibroblasts, emphasizing how targeting different cell types would be critical to effectively tackle this condition. Despite the incidence of dystrophies and their consequences (e.g. fibrosis), in vitro systems are still far from recapitulating the complexity of skeletal muscle and its degeneration. Based on a previous model [2], we here developed a mesoscale system of the human muscle environment to study the onset of fibrosis.

METHODS: The mesoscale system enabled casting of three non-planar muscle fibers (600 µm diameter, 600 µm interfiber distance) and subsequent addition of fibrin hydrogels embedding endothelial cells (ECs) and fibroblasts derived from healthy or Duchenne (DMD) donors [3]. TGF-β1 treatment was used as positive control to induce a fibrotic environment.

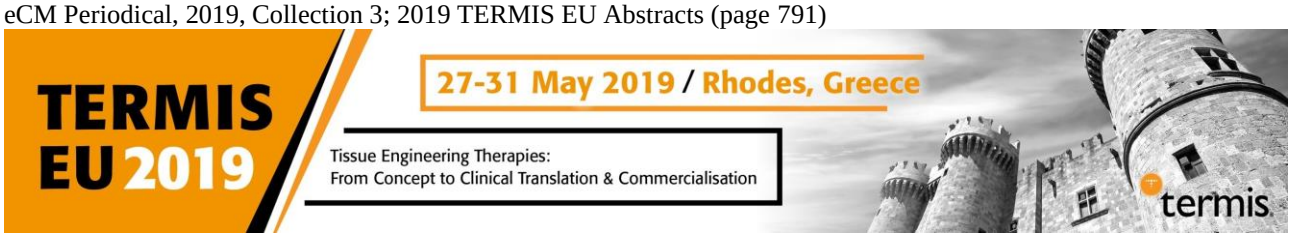
RESULTS & DISCUSSION: We characterized the model in terms of vasculature topological parameters, muscle fiber architecture and expression of endothelial and muscle markers. We demonstrated the induction of muscle-specific endothelial features and the self-organization of an endomysium around muscle fibers. We employed the model to mimic the fibrotic environment of muscle dystrophies, which is characterized by abnormal matrix deposition. The expression of collagen I and fibronectin in tissues containing DMD fibroblasts was higher compared to control and TGF-β1-treated samples ($p<0.001$). DMD fibroblasts also showed higher expression of α-smooth muscle actin compared to control fibroblasts ($p<0.001$), which is an additional indication of differentiation towards pathological myofibroblasts.

CONCLUSIONS: Musculoskeletal tissue has a distinctive 3D architecture which is fundamental to promote intercellular crosstalk and maintain tissue functionality. Herein, we exploited our 3D mesoscale model to show that the inclusion of DMD derived fibroblasts is sufficient to increase the expression of fibrosis-associated proteins, highlighting differences that are overlooked by traditional 2D assays.

ACKNOWLEDGEMENTS: Italian Ministry of Health and U.S. Office of the Assistant Secretary of Defense for Health Affairs, Breast Cancer Research Program Award W81XWH-15-1-0092.

REFERENCES

[1] Bersini S et al. Biotechnol Adv. 2016;34, 1113
[2] Bersini S et al. Biomaterials. 2016; 76, 157
[3] Bersini S et al. Cell Reports. 2018; 25, 3858



Tissue engineering scaffolding: Computer aided in-vitro degradation model

S. Russo[1], M. Prieto Fraga[2], M. Arruebo[2], M.A. Pérez[1], J.M. García-Aznar[1]
Presenting Author: Simone Russo, simonerusso@unizar.es
[1]Multiscale in Mechanical and Biological Engineering (M2BE), Aragon Institute of Engineering Research (I3A), University of Zaragoza, Spain [2]Department of Chemical Engineering, Institute of Nanoscience of Aragon (INA), University of Zaragoza, Spain

INTRODUCTION: Tissue Engineering (TE) development is strictly related to changes in materials fabrication technology. Biomaterials design and their properties play a critical role in the success of designed scaffolds for TE. A better understanding and control of the biodegradation process may help in the development of successful TE strategies. Moreover, computational modelling offers an efficient framework to predict and understand the behaviour of biomaterials. This work's aim is to develop a combined in-vitro/in-silico method to quantify artificial scaffold's degradation.

METHODS: 3D-printed PolyCaproLactone scaffolds were used for the in-vitro degradation tests. To evaluate the influence of perfusion in the degradation process, a customized bioreactor has been developed (Figure 1). Scaffold degradation was evaluated as weight ratio loss. An in-silico approach was also developed to provide a predictive model of degradation. Polyester hydrolysis was modelled through physiochemical rate equations. The hydrolysis kinetic coefficient is a thermodynamic quantity associated with the probability of molecular scission and can depend on different parameters: pH and temperature were considered constant, at 7.4 and 37°C, respectively.

RESULTS & DISCUSSION: Each polymeric molecule is randomly broken at a given ester group. Thus, the number of carboxylic end groups will increase with degradation time, whilst the number of ester groups decrease. The variation of ester bonds molar concentration was used to estimate the molecular weight variation. It seems that perfused scaffolds degrade faster than static ones, Figure 2. This may be due to the shear stress increasing the probability of bond scissions. So, the reaction rate coefficient depends on the shear, whilst it was considered constant for the static condition.

CONCLUSIONS: Driving the polymers degradation is crucial for TE to let the new-born extracellular matrix gradually replace the polymeric artificial structure. However, tests for scaffolds degradation in physiological conditions require very long experimental times. This combined model permits to predict long term behaviour starting from data retrieved from experiments run at short term.

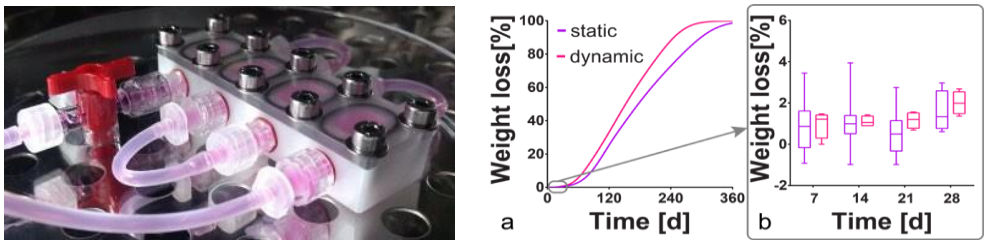
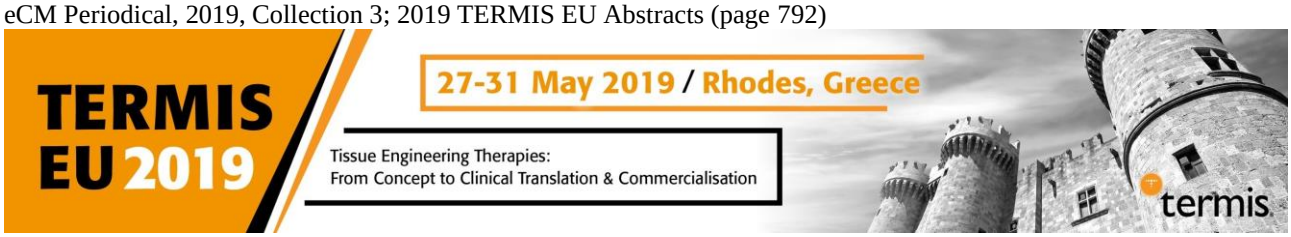


Figure 1: Customised bioreactor's chamber (left). Normalised weight loss a) in-silico, b) in- vitro (min to max data displayed) (right).

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 722535 and the Spanish Ministry of Economy and Competitiveness (DPI 2014-53401-C2-1-R).



The angiogenic potential of dental pulp stem cell - loaded hydrogels in an ex vivo bovine tooth model

S. Zhang[1], D.F. Duarte Campos [2], M. Esteves-Oliveira[3], S. Jockenhoevel[1], C. Apel[1]
Presenting Author: Siyuan Zhang, zhang@ame.rwth-aachen.de
[1]Department of Biohybrid & Medical Textiles, AME- Applied Medical Engineering, RWTH Aachen University, Germany [2]Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Germany [3]Department of Operative dentistry, Periodontology and Preventive Dentistry, Medical Faculty, RWTH Aachen University Hospital, Germany

INTRODUCTION: Dental pulp tissue plays a major role to maintain the biological and physiological vitality of the tooth. It is the only vascularized tissue encapsulated in highly mineralized tissue including dentin and enamel. [1-2] Here we introduce an ex vivo bovine tooth culture model to assess the angiogenic potential of different cell-seeded hydrogels.

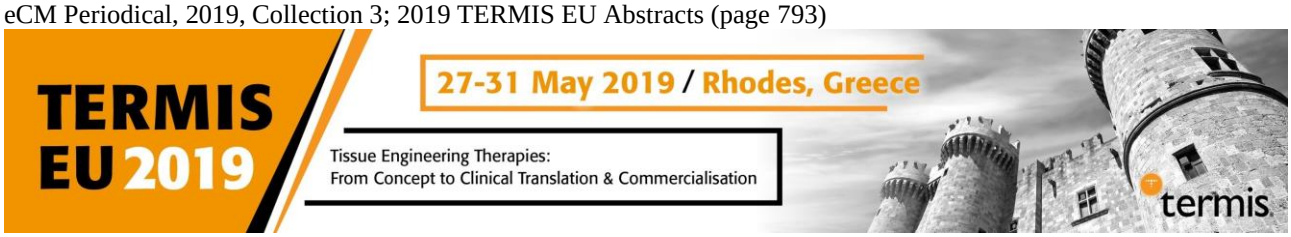
METHODS: Bovine teeth were prepared to the same length and canal diameter, and were fixed in 24-well plate by agarose gels. Type I collagen (COL), fibrin gel (FIB) and agarose collagen blends (AG-COL) were optimized at the concentration 0.2% COL, 0.5% FIB and 0.5%AG- 0.2% COL. Human dental pulp stem cells (DPSC) and human primary umbilical vein endothelial cells (HUVEC) were co-cultured at the concentration of 3×10^6 respectively in each hydrogel. Cell-loaded hydrogels were cast directly on to the well plates and into bovine teeth canals. The bovine tooth was static in the well plate during the culture period and only allowed medium to get through from the top of the canal mimicking the natural environment of human dental pulp. After 14 days, the hydrogels were evaluated by two-photon laser scanning microscopy (TLSM). The vascularized structures were visualized using anti-CD31 immunostaining and quantified.

RESULTS & DISCUSSION: TLSM three-dimensional (3D) images showed the formation of vascular-like networks in 0.2% COL, 0.5% FIB and 0.5%AG- 0.2% COL cultured in well plates and in bovine teeth. The lumen structures were detected in the side view of 3D images in all conditions. In the bovine tooth-supported culture model, the root canal limited the contacting area of medium and hydrogel, and the tubular formation was shown only in a certain depth (around 200 µm). The tubular length in bovine tooth model and in culture well was measured and compared. The statistical results did not reveal any significant difference ($P > 0.05$).

CONCLUSIONS: The results demonstrate that the bovine tooth model allows angiogenesis and can be further developed to assess the regenerative potential of different cell-loaded hydrogels ex vivo mimicking the natural dental environment.

REFERENCES

[1] Huang G. Regen Med. 2009; 4(5): 697-707.
[2] Dissanayaka WL et al. J Endod. 2017; 43(9): S102-S106.



Design and characterization of synthetic biodegradable films for soft tissue engineering

S. Ribeiro[1,2], E. M. Fernandes[4,5], M. E. Gomes[4,5], R. L. Reis[4,5], Y. Bayon[1], D. I. Zeugolis[2,3]
Presenting Author: Sofia Ribeiro, sofia.ribeiro@medtronic.com
1Medtronic, Sofradim Production, Trevoux, France [2]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL) National University of Ireland Galway (NUI Galway), Galway, Ireland [3]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM) National University of Ireland Galway (NUI Galway), Ireland [4]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [5]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

INTRODUCTION: To repair soft tissue, it is vital to ensure that the biomaterial is able to mimic the complex elasticity of the native tissue. Substrate stiffness has a huge influence on cell physiology and behavior [1,2]. The present study presents a set of polymeric films as initial support matrix of cells.

METHODS: Six (6) resorbable polyesters made from different combination of monomers, such as lactic acid, glycolic acid, trimethylene carbonate, dioxanone & β-caprolactone, were selected for their physico-chemical intrinsic properties. Even though the selected polymers have similar chemistry they show different mechanical and degradation properties.
The films were manufactured by compressing moulding using a thermal presser. The mechanical properties of the films were assessed by tensile test, dynamic mechanical analysis (DMA) and atomic force microscopy (AFM) in dry and wet conditions. In vitro assays were performed using human bone marrow mesenchymal stem cells (SC) to assess the proliferation and differentiation potential of the biodegradable films. It was also performed single cell analysis to assess the substrate influence in stem cell adhesion.

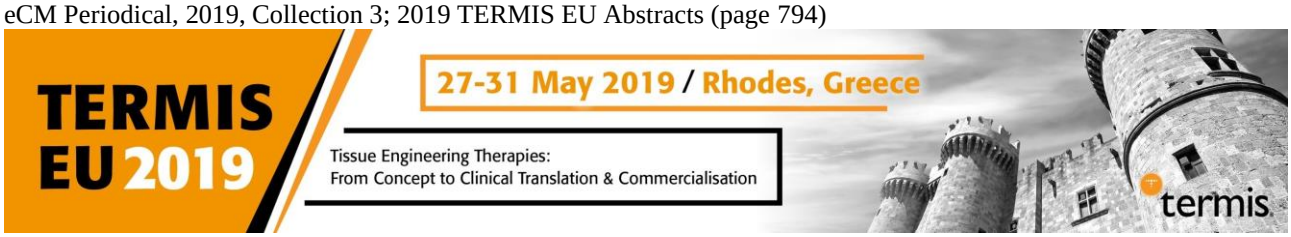
RESULTS & DISCUSSION: The mechanical properties of the successfully processed films were analyzed regarding at their macro level by DMA at 37°C in a phosphate buffer bath, with storage moduli ranging from 0.1 up to 2.6 GPa. Similar ranking of polyester films was confirmed by AFM.
Biological assays with the stem cells showed good cell adhesion, proliferation & viability in all conditions. Stem cells were able to differentiate into adipogenic, osteogenic, chondrogenic and tenogenic lineages. But cell morphology and cluster formation were very different from one to another polymer. The focal adhesion pattern has been analyzed as well.

CONCLUSIONS: The selection of polymers gives us good options to cover a large range of tissue repair indications, from their mechanical features. The choice of the right polyester should be driven not only by their mechanical properties and degradation profile, but also by the nature of the tissue to be repaired and the bio-instructive properties of the polyesters

ACKNOWLEDGEMENTS: This work received funding from H2020-MSCA-ITN-2015 - Tendon Therapy Train - Project N° 676338. It has also been supported from Science Foundation Ireland and the European Regional Development Fund (Grant Agreement Number: 13/RC/2073).

REFERENCES

[1] Chen Q et al. Prog. Polym. Sci. 2013; 38 :584-671
[2] McMurray R et al. Nat. Mat. 2011; 10 :637-644



Multipurpose liquid support matrixes-enabled 3D printing of engineered hydrogel constructs

L.R. Sousa, S.G. Patrício[1], L.S. Pires[1,2], J.L. Luís[1,2], T.R. Correia[1], V.M. Gaspar[1], J.M. Oliveira[1,2], J.F. Mano[1]
Presenting Author: Sónia Patrício, sgpatricio@ua.pt
[1]Aveiro Institute of Materials (CICECO), University of Aveiro, Portugal [2]School of Design, Management and Production Technologies Northern Aveiro (ESAN), University of Aveiro, Oliveira de Azeméis, Portugal

INTRODUCTION: A crucial key milestone for the 3D organ bioprinting is the ability to create specific anatomical features, such as complex shape in the 3D space, vasculature and functional inner structures that cannot be achieved from the standard layer-by-layer planar fabrication [1]. Herein, in a proof-of-principle experiment, we demonstrate the versatility of xanthan gum-based medium to generate **i)** freeform 3D complex hollow structures and **ii)** perfusable channels, by acting as a sacrificial or permanent support material, respectively.

METHODS: The 3D model of the arterial tree was designed with computer-aided design (CAD) software (SolidWorks) and printed by using the pneumatic dispensing bioplotter (Envisiontec, Germany). The hydrogel ink was a solution of 3.5% (w/v) sodium alginate. Xanthan gum (XG) was obtained from food industry. Methacrylate-modified XG was obtained via epoxide ring-opening reaction of glycidyl methacrylate [2]. For alginate ionic crosslinking, 50 mM of $CaCl_2$ was added to both support baths exposure.

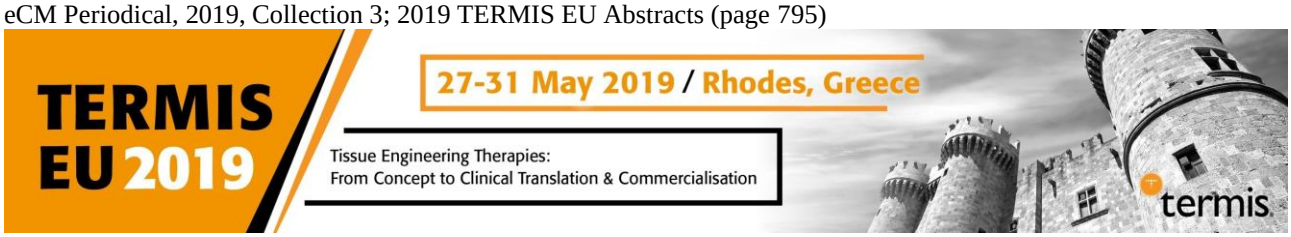
RESULTS & DISCUSSION: XG medium has enabled to generate a self-supported arterial tree construct with high resolution. Also, the well embedded arterial structure in the XG support bath allowed the formation of three asymmetric branched hollow lumens without collapsing or deforming, during the printing process. After removing from the XG bath, the arterial tree construct still maintained its structural integrity, confirming the alginate printed layers were well fused together. The 3D printing of a spiral alginate filament within a XG support material containing methacrylated moieties allowed the further photocrosslink of the support material forming a hydrogel. The subsequent alginate liquefying process, left a well-defined open channel with the printed pattern through the XG-based hydrogel.

CONCLUSIONS: Xanthan gum provides a support material platform for 3D bioprinting technology of functionally complex biological constructs, opening new prospects for biofabrication technologies in tissue engineering and regenerative medicine.

ACKNOWLEDGEMENTS: Financial support was received from the European Research Council grant agreement through project ATLAS (ERC-2014-ADG-669858).

REFERENCES

[1] Moroni L et al. Nat. Rev. Mater. 2018; 3:21-37.
[2] Reis AV et al. J. Org. Chem. 2009; 74(10); 3750-57.



Development of anisotropic fibrous bundle-like PCL structure for muscle tissue regeneration

S.J. Chae[1], J.U. Lee[1], D. Yoon[2], G.H. Kim[1]
Presenting Author: Soo Jung Chae, sjchae65@skku.edumailto:diana.gaspar@nuigalway.ie
[1] Department of Biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea [2] Burn Institute, Hangang Sacred Heart Hospital, Colleage of Medicine, Hallym University, Seoul, South Korea

INTRODUCTION: Basic concept of tissue engineering is fabrication of biological substitution to overcome the limited self-healing ability of human body. Especially, mimicking the native extracellular matrix (ECM) has been an issue in tissue engineering. The ECM of skeletal muscle is well known for its anisotropic pattern and micro fibrous structure. To mimic these biophysical cues, many fabrication methods have been used, such as freeze-drying, electrospinning, 3D printing, etc. In this study, we fabricated 3D structure composed of fibrous PCL bundle using 3D printing technology and a blended sacrificial material.

METHODS: To fabricate a fibrous Poly(□-caprolactone) (PCL, M_W=45,000 g/mol) bundle, PCL and pluronic F-127 (PF127) were mixed and printed. The printed structures were immersed in the 3rd distilled water to dissolve the PF-127. Conventional printed PCL structure was used as control group. Porcine collagen type I and EDC/NHS solutions were used for collagen coating process for both groups. Scanning electron microscopy (SEM) was used for capturing surface morphology. The myoblasts (C2C12 cells) were seeded onto the scaffolds, and cell bioactivity such as cell proliferation and differentiation were evaluated.

RESULTS & DISCUSSION: In this study, we were able to fabricate micro-fibrous PCL bundle structure by mixing PCL with PF127. Structure composed of micro-scale PCL fibers shows anisotropic aligned morphology compared to conventional PCL structure. Fibrous-bundle PCL group shows enhanced cell proliferation and myotube formation, compared to PCL group.

CONCLUSIONS: Fabricated fibrous structure showed enhanced proliferation of myoblasts, cell alignment, and myotube formation compared to conventional PCL structure. Based on the results, we conclude that fibrous-bundle PCL could be a new strategy for muscle tissue regeneration.

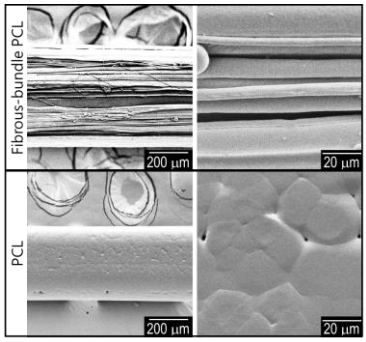
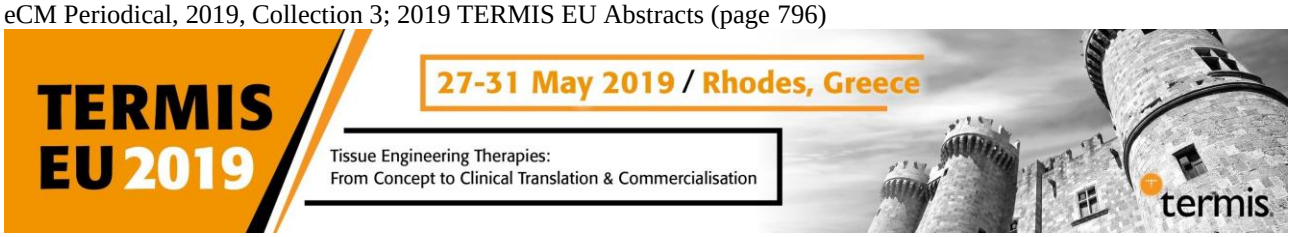


Figure 1: SEM images showing surface morphology of fibrous-bundle PCL and PCL.

ACKNOWLEDGEMENTS: National Research Foundation of Korea (NRF) Grant funded by the Ministry of Science and ICT for Bio-inspired Innovation Technology Development Project (NRF-2018M3C1B7021997) and the National Research Foundation of Korea grant funded by the Ministry of Education, Science, and Technology (MEST) (NRF-2018R1A2B2005263).



Mechanical regulation of the corneal epithelium

S. Masterton[1], M. Ahearne[2]
Presenting Author: Sophia Masterton, masterts@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin
[2]Department of Mechanical and Manufacturing Engineering, Trinity College Dublin, Ireland

INTRODUCTION: Mechanical stress on cells is known to affect their activity [1], however, little is known about its effect on the corneal epithelium. This study examines how material stiffness regulates epithelial cell behaviour using a human telomerase immortalised corneal epithelial cell line (hTCEpi) with the aim of determining optimal conditions for generating new tissue

METHODS: Polydimethylsiloxane (PDMS) substrates with an elastic modulus ranging from 12kPa to 2240kPa were fabricated. Proliferation marker phosphorylated extracellular regulated kinase (pERK) was examined using western blot and gene expression of cytokeratin 3 (CK3) and cytokeratin 14 (CK14) was quantified at day 7. Immunocytochemistry analysis of intermediate filament protein vimentin was examined at day 3.

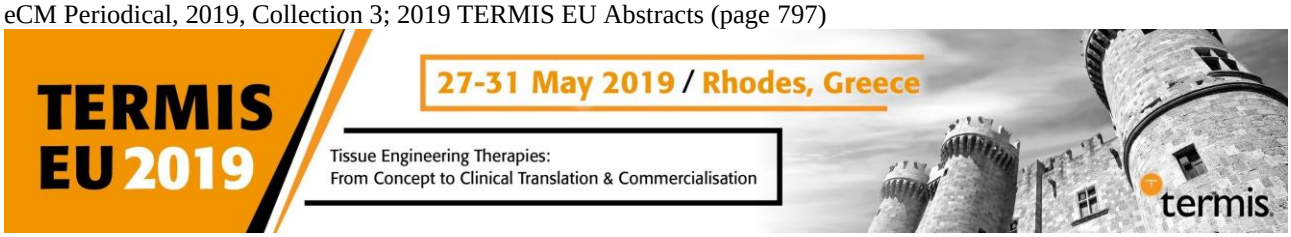
RESULTS & DISCUSSION: The medium stiffness group displayed the most significant proliferation compared to all other groups. The stiff group expressed CK3 at a significantly higher rate than other groups. CK14 was significantly increased compared to the control in the medium-soft and soft groups (Fig 1 D). Vimentin expression was lowest in the control and soft groups.

CONCLUSIONS: Substrate stiffness has a significant role to play in corneal epithelial cell proliferation, gene expression and intermediate filament formation. CK14 is a corneal stem cell marker [2] and its expression was increased with softer substrates. CK3 is a mature corneal epithelial marker [2] and its expression was significantly decreased in softer substrates. Cells on the softest samples also had less proliferation and intermediate filament formation. This indicates more of a stem cell phenotype with lower proliferative potential for cells grown on softer substrates and a more mature, higher proliferative phenotype for cells grown on stiffer substrates. Results may be used to design a biomaterial for corneal epithelial tissue regeneration.

ACKNOWLEDGEMENTS: Research supported by the Eurpoean Research Council Starting Grant (Project Number 637460)

REFERENCES

[1] DuFort, C.C., et al. (2011). Nature Reviews Molecular Cell Biology, 12:308-319.
[2] Merjava, S., et al. (2011). Histol Histopathol 26(3): 323-331.



Elastin like recombinamers molecularly programed with a sequential three-stage gelation process as novel bioinks for 3D bioprinting

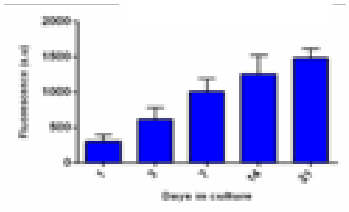
S. Salinas-Fernández[1], M. Santos[1], M. Alonso[1], J. C. Rodríguez-Cabello[1]
Presenting Author: Soraya Salinas-Fernández, ssalinas@bioforge.uva.es
[1]BIOFORGE Lab, University of Valladolid-CIBER-BBN, Spain

INTRODUCTION: A novel sequentially gelating smart bioink based on elastin like recombinamers (ELRs) has been developed whose thermoresponsive behavior is exploited for the deposition of well controlled fibers under a heated platform.

METHODS: An ELR bioink showing synergistically combination of thermal responsiveness (hydrophobic aggregation), Leu-Zipper domains, for fast setting and silk-like sequences for late stabilization is tested and compared with control recombinamers showing partial functionallity. Printability as a function of circularity and scaffold stability in excess of aqueous medium is measured, as well as rheological experiments. In vitro tests are also performed using HFF-1 cell line to prove the Biocompatibility of the bioink.

RESULTS & DISCUSSION: Best ink concentration has been optimised (Figure 1). Printed structures exposed to aqueous media show long term stability in comparison to the disaggregation of the Zipper ELR over time and the inability of the Silk sequence to induce a fast gelation into the Silk ELR. Zipper-Silk ELR bioink shows a Newtonian behaviour at low concentrations with very low viscosity, allowing the formation of complex structures by using small-diameter needles while keeping the shear forces constant (Figure 1).In vitro tests further proved adherence and proliferation of cells, supporting the potential of the presented material (Figure 2).

CONCLUSIONS: Results of this work accentuates the great applicability and novelty of printed biomimetic ELRs structures based on a molecularly programme of gelation setting and stabilization. Applications such as micro-organ, or tissue printing are in the scope of future investigations.



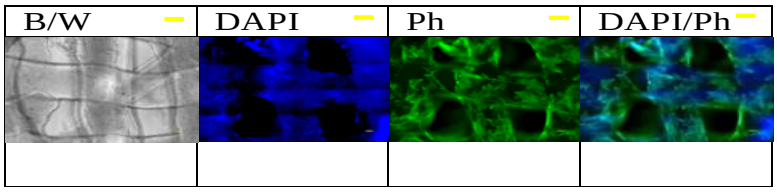
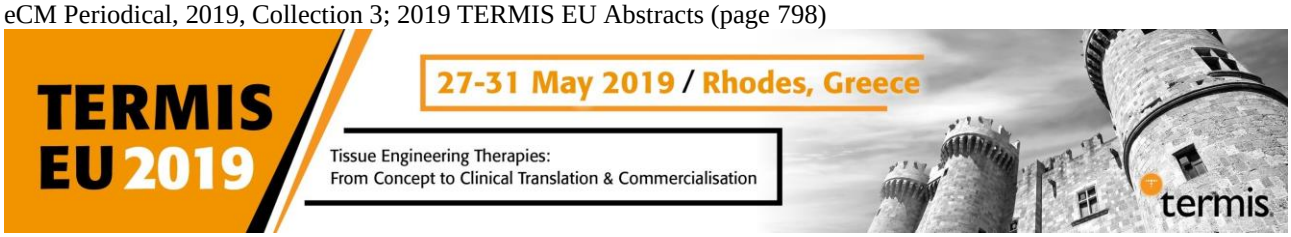


Figure 1: Pr calculated value of the printed structures of the ZELR; SELR, and Zipper-Silk for 120, 150, 180, 200, 250 and 300 mg/mL in PBS 1X (left). Viscosity of the ELR bioink upon increasing of a determined shear rate for different concentrations in PBS 1X (right). **Figure 2:** AlamarBlue® reduction measurement over HFF-1 cell seeded printed scaffolds (left). Cell staining of a 21 days cell seeded printed scaffold. Scaffold in black and white, cell nuclei in blue (DAPI) and actin filaments in green (Phalloidin). 300 µm scale bars (right).

ACKNOWLEDGEMENTS: The authors are grateful for the funding from the European Commission (NMP-2014-646075), MINECO of the Spanish Government (PCIN-2015-010, MAT2016-78903-R), Junta de Castilla y León (VA317P18) and Centro en Red de Medicina Regenerativa y Terapia Celular de Castilla y León.



Developmental biology inspired 3D bioprinted bone construct

S. Ghosh[1,2], S. Chawla[1], A. Bandopadhay[3]
Presenting Author: Sourabh Ghosh, sghosh08@textile.iitd.ac.in
[1]Regenerative Engineering Laboratory, Indian Institute of Technology, New Delhi, India [2]HST, ETH Zurich [3]Biological Science and Bioengineering Department, Indian Institute of Technology, New Delhi, India

INTRODUCTION: The worldwide prevalence of bone disorders and conditions such as bone defects either from trauma or congenital disorders is exponentially increasing. Until now, approaches for bone tissue engineering involve seeding mesoderm derived mesenchymal cells on scaffolds followed by their osteogenic differentiation. Although this could mimic the intramembraneous route for bone formation, which is a unique feature of ectoderm derived mesenchymal stem cells; however efficacy of such constructs is yet to be demonstrated in load bearing long bones.

METHODS: 3D bioprinted constructs with mesenchymal stem cells were cultured in (1) Osteogenic differentiation media containing an additional 1 nM T3 along with 10 nM Dexamethasone, 50 mg/mL ascorbic acid, 10 mM β-glycerophosphate, penicillin, streptomycin, 10% FBS for 21 days or (2) first in chondrogenic differentiation media comprising DMEM, Ham's F12 media, 50 µg/mL ascorbic acid, 10 µg/mL insulin, 10 µg/mL transferrin, and 100 nM hydrocortisone for 21 days, followed by Osteogenic differentiation media +T3 for 14 days.

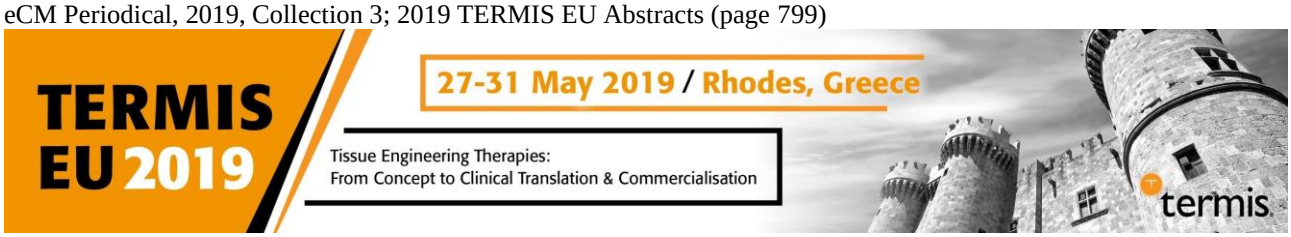
RESULTS & DISCUSSION: This study explored the 3D bioprinting strategy recapitulating the path of embryonic bone formation which are formed from a pre-figured cartilage template during embryonic stage. 3D bioprinting of silk-gelatin laden with bone marrow stem cells leads to the chondrogenic differentiation of mesenchymal stem cells followed by osteogenic differentiation thus mimicking the conditions in vivo that takes place during embryonic stage. This finding was supported by the temporal changes in gene expression corresponding to early and end stage osteogenic differentiation of the mesenchymal stem cells in the 3D bioprinted construct, closely mimicking the conditions in vivo. Interestingly, upon addition of T3 and following the endochondral ossification route could trigger the parathyroid hormone (PTH), IHH, and Wnt/β-catenin pathways thus resulting in gene and protein expression similar to one that takes place in vivo. Presently, we are following stable incorporation of trans-genes to precisely control differentiation pathways.

CONCLUSIONS: Innovative bioprinting strategy could replicate (1) chondrogenic condensation and hypertrophic cartilaginous template development, (2) involvement of IHH signaling indicative of the development of bony collar by perichondral ossification, (3) involvement of Wnt/β-catenin signaling, (4) involvement of PTH signaling, and (5) synthesis and deposition of bone-specific mineral. Hence this replication of developmental biology within in vitro set up could ultimately lead to better and stable bone formation akin to the one observed in vivo.

ACKNOWLEDGEMENTS: Financial support was received from Department of Biotechnology, India Govt.

REFERENCES

[1] Chawla S et al, ACS Biomaterials Science and Engineering, 2018, 4(10), 3545-3560



Tailor-made biointerfaces on oxidic substrates via polymer brushes

S. Helfert[1,3], J. Hellmeier[2,3], E. Sevcik[2,3], R. Liska[1,3], S. Baudis[1,3]
Presenting Author: Stefan Helfert, stefan.helfert@tuwien.ac.at
[1]Institute of Applied Synthetic Chemistry - Polymer Chemistry and Technology, Technische Universität Wien, Vienna, Austria [2]Institute of Applied Physics – Biophysics, Technische Universität Wien, Vienna, Austria [3]Austrian Cluster for Tissue Regeneration, Vienna, Austria

NTRODUCTION: The concept of building an interface between substrate surfaces and biomolecules became very attractive for applications in the biomedical field. These interfaces can be used for manufacturing of biosensing platforms, cell culturing [1] and tissue engineering [2]. Specific surface engineering via synthetic polymer brushes can introduce a chemical environment that can promote cell adhesion, proliferation, viability and enhanced extracellular matrix-secretion functions [3].

METHODS: Flat silicon oxide substrates (e.g. glass, silicon wafer) were activated and amino-functionalized via chemical vapor deposition (CVD) of alkoxysilanes. Reversible addition-fragmentation chain transfer (RAFT) polymerization was applied, to generate polymer brush interfaces on these substrates. An activated ester RAFT reagent was bound to the amino-functionalized surface and in a thermal- or light induced grafting-from polymerization, acrylamide polymer brushes were generated. Aminolysis reactions were used to generate thiol end groups onto the brushes and these were further coupled to maleimide (Mal) nitriloacetic acid (NTA) linkers for His-Tag coupling reactions. The substrates were analyzed by water contact angle (WCA), ellipsometry and total internal reflection fluorescence microscopy (TIRFM).

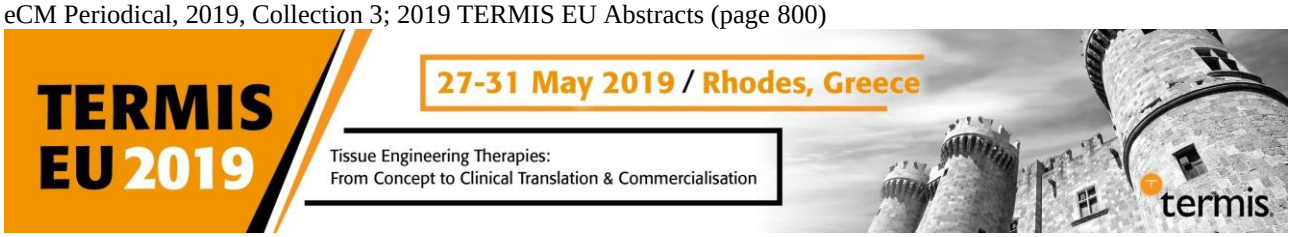
RESULTS & DISCUSSION: After CVD amino-functionalization, the generation of poly-N-acryloylmorpholine (NAM) polymer brushes on flat silicon oxide substrates was accomplished via thermal and light induced RAFT polymerization. WCA (wetting behavior) and ellipsometric layer thickness analysis confirmed the presence of a polymer brush layer of approx. 20 nm. End group modifi-cation by the use of aminolysis was confirmed by ellipsometry and resulting thiol groups were labeled with Alexa 647-Mal to confirm their presence in TIRFM. Thiol end groups were linked to a Mal-NTA spacer and coupled to an Alexa 555 labeled bovine serum albumin (BSA) molecules and the bioconjugation was monitored via TIRFM.

CONCLUSIONS: Based on the collected data, it was possible to create a poly-NAM polymer brush biointerface with BSA protein bioconjugated chain ends.

ACKNOWLEDGEMENTS: Special thanks for funding to the doctoral school of BioInterface, TU Wien

REFERENCES

[1] Krishnamoorthy M. et al. Chemical Reviews 2014, 114 (21), 10976-11026.
[2] Cole, M. A. et al. Biomaterials 2009, 30 (9), 1827-1850.
[3] Ma, Z. et al. Colloids and Surfaces B: Biointerfaces 2007, 60 (2), 137-157.



Meniscus tissue engineering through 3D printing

S. Scheurer[1,2], R. Schipani[1], DJ. Kelly[1,3,4], CJ Moran[1,2,4]
Presenting author: Stefan Scheurer, scheures@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin [2]Orthopaedics and Sports Medicine, School of Medicine, Trinity College Dublin [3]Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of, Surgeons in Ireland and Trinity College Dublin, Dublin 2, Ireland [4]Sports Surgery Clinic and Trinity College Dublin, Dublin, Ireland

INTRODUCTION: The menisci are semilunar tissues in the knee responsible for load distribution and joint stability. Menisci can fulfill this function due to the unique structure and composition of their extracellular matrix (ECM), which results in anisotropic mechanical properties that exhibit significant tension-compression non-linearity [1,2]. 3D printing allows tuning of the compressive and tensile parameters of scaffolds to replicate the structure of complex biological tissues. The objective of this study was to use 3D printing to fabricate PCL scaffolds which replicate aspects of the internal architecture of menisci in order to mimic the mechanical properties of the native tissue.

METHODS: A 3D Discovery printer (RegenHU, Switzerland) was used to produce polycaprolactone (PCL, Capa, 40kDa) scaffolds. Constructs with spacing between the fibres of 0.25, 1 or 2 mm, with aligned or offset fibre patterns were printed and imaged. Compressive and tensile testing was conducted with a Zwick/Roell material testing machine.

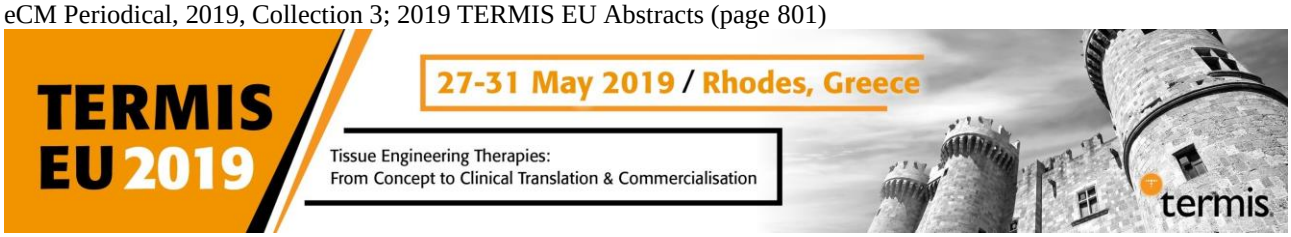
RESULTS & DISCUSSION: The spacing and printing pattern dramatically influenced the compressive and tensile modulus of the scaffolds. A fibre spacing of 0.25 mm and 2 mm resulted in tensile moduli approaching that observed in the circumferential and radial direction of native meniscus [3]. In compression only an offset pattern resulted in a modulus near the native range, independent of the fibre spacing. The pattern therefore affected the compressive modulus without affecting the tensile modulus.

CONCLUSIONS: Using a scaffold architecture of 2 mm spacing and an offset print pattern it is possible to print scaffolds mimicking the compressive and tensile modulus (in radial direction) of the native meniscus. Decreasing the fibre spacing was necessary to match the tensile stiffness in the circumferential direction. Future studies will seek to combine such PCL frames with bioinks to produce functional meniscal implants.

ACKNOWLEDGEMENTS: Funding by SFI 12/IA/1554 and by ERC 258463 and 647004.

REFERENCES

[1] Makris E., Biomaterials 2011; 32:7411-7431
[2] Huang C., J of Biomechanics 2005; 38:799-809
[3] Tissakht M., J of Biomech 1995; 28:411-422



Delayed reconstruction of peripheral nerves by mean of chitosan conduit filled with muscle fibers

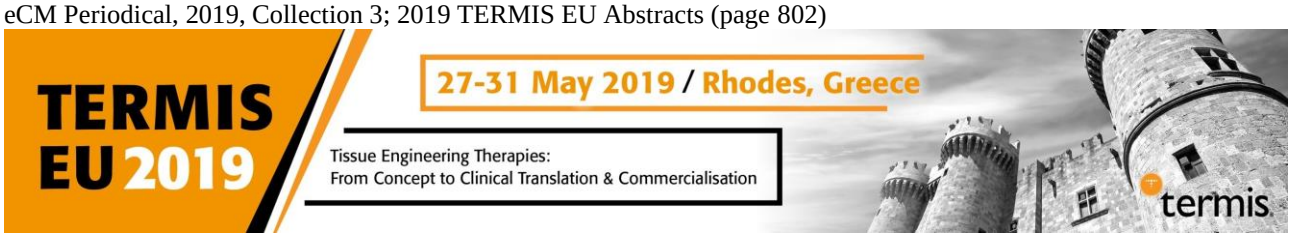
S. Raimondo
Presenting Author: Stefania Raimondo, stefania.raimondo@unito.it
Department of Clinical and Biological Sciences, and Cavalieri Ottolenghi Neuroscience Institute, University of Turin, Regione Gonzole 10, 10043, Orbassano, Italy

Nerve fiber regeneration and complete functional recovery after peripheral nerve injury do not always occur and can be influenced by many factors, including time interval that elapses before performing surgical repair. The poor outcome occurring after a long delay can be due to loss of neuron ability to regenerate, loss of Schwann cell ability to support regeneration and the consequent ineffective support for nerve regeneration. One of the most important factors regulating Schwann cell action is Neuregulin1 (NRG1), which is highly up-regulated following acute nerve injury, while it is strongly down-regulated during chronic nerve degeneration. Taken together these data suggest that providing a source of soluble NRG1 might be a good strategy to improve the outcome after delayed nerve repair. In the present study, to provide a three-dimensional environment and trophic factors supporting Schwann cell activity and axon re-growth, we combined the use of an effective conduit (a chitosan made tube) [1] with a promising intraluminal structure (fresh longitudinal skeletal muscle fibers). Fresh skeletal muscle produces and releases high levels of soluble isoforms of NRG1 and for this reason we chose it to enrich hollow chitosan tubes. In particular in this study the efficacy of this enriched tubular device was evaluated as a support of nerve regeneration after a delayed surgical nerve reconstruction (secondary repair). After 3 months of denervation, the median nerve was repaired with autologous nerve grafts (as control) or chitosan tubes filled with a longitudinal piece of pectoralis major muscle (muscle-in-tube). Regenerating nerve samples were harvested 4 months after nerve reconstruction for morphological and stereological analysis. Results showed that the chitosan conduit enriched with muscle fibers is as effective as the autograft, the "gold standard" technique for nerve reconstruction, in promoting nerve regeneration [2].

ACKNOWLEDGEMENTS: Financial support was received from The European Community's Seventh Framework Programme (FP7-HEALTH-2011) under grant agreement nº 278612 (BIOHYBRID).

REFERENCES

[1] Meyer C et al. Biomaterials. 2016; 76:33-51.
[2] Crosio A et al. Neural Regen Res. 2019;14:1079-1084.



Comparing scaffold and scaffold-free extracellular-matrix rich tissue substitutes for tendon enthesis regeneration

S. Korntner[1,2], A. De Pieri[1,2,3], Z. Wu[1,2], E. Pugliese[1,2], D.I. Zeugolis[1,2]
Presenting Author: Stefanie Korntner, stefanie.korntner@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland [2]Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland [3]Proxy Biomedical Ltd., Coilleach, Spiddal, Galway, Ireland

INTRODUCTION: Macromolecular crowding (MMC) was shown to significantly increase extracellular matrix (ECM) deposition and to promote chondrogenic differentiation of stem cells. Herein, we assess the influence of MMC on chondrogenic differentiation of human adipose derived mesenchymal stem cells (hADSCs) in different 3D culture systems in order to fabricate ECM-rich chondrogenic tissue substitutes for tendon enthesis repair.

METHODS: hADSCs were seeded on Collagen type I and type II sponges, on thermoresponsive electrospun fibres, in order to obtain viable cell sheets, and in pellet culture. Carrageenan (50 µg/ml) was used as MMC agent. Histologic and gene expression analysis were conducted after 7, 14 and 21 days of chondrogenic induction.

RESULTS & DISCUSSION: MMC induced increased ECM deposition and therefore thicker cell sheets could be harvested compared to controls. Alcian blue staining showed increased deposition of sulphated glycosaminoglycans (sGAGs) in collagen type II sponges after 21 days in the presence of carrageenan (Figure 1). In a scaffold-free approach, ADSCs showed the highest chondrogenic differentiation potential compared to all other culture systems after 21 days and viable multilayer cell sheets could be harvested (Figure 1).

CONCLUSIONS: Collectively, these data indicates a beneficial effect of MMC on chondrogenesis in different 3D culture systems with chondrogenic cell sheets as a promising candidate for transplantation into tendon enthesis defects. The fabrication of tissue equivalents can be achieved within very short culture times, preventing phenotypic drift of stem cells.

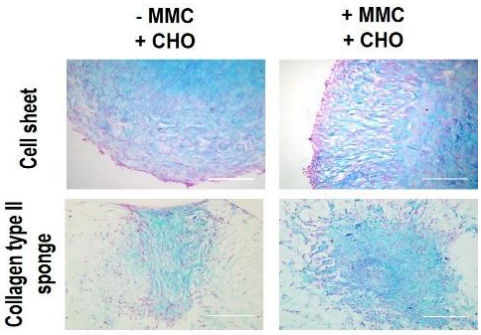
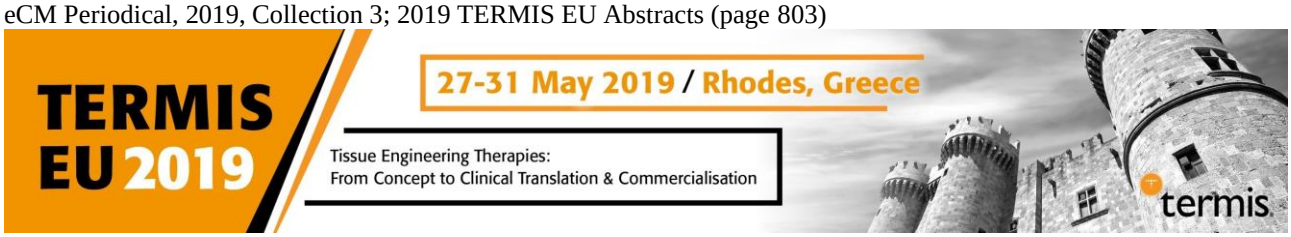


Figure 1: Alcian blue/ Fast red staining of hADSCs-constructs after 21 days of chondrogenic induction. MMC: macromolecular crowding; CHO: chondrogenic induction; Scale bars: 100µm

ACKNOWLEDGEMENTS: European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713690; Science Foundation Ireland (SFI) / European Regional Development Fund (Grant Number 13/RC/2073)



Therapeutic effect of neural progenitor cells expanded in the 3D nano-engineered Nichoid substrate in a Parkinson's disease preclinical model

S. Carelli[1,2], T. Giallongo[1], F. Rey[1], T. Zandrini[3], G. Cerullo[3], R. Osellame[3], A.M. Di Giulio[1,2], M.T. Raimondi[4]
Presenting Author: Stephana Carelli, stephana.carelli@unimi.it
[1]Department of Health Sciences, University of Milano, Italy [2]Pediatric Clinical Research Center Fondazione "Romeo ed Enrica Invernizzi", University of Milan, Italy [3]Istituto di Fotonica e Nanotecnologie and Department of Physics, Politecnico di Milano, Italy [4]Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy

INTRODUCTION: The use of biomaterials allows to generate active biophysical signals for directing stem cell fate through 3D microscaffolds, such as the one named "Nichoid" [1]. The aim of this study was to investigate: i) the proliferation, differentiation and stemness properties of neural precursor cells (NPCs) [2, 3] following their cultivation in the Nichoid substrate; ii) the therapeutic effect and safety in vivo of NPCs cultivated in the Nichoid in preclinical experimental model of Parkinson's Disease (PD).

METHODS: Nichoids were fabricated by 2PP onto circular glass coverslips using a home-made SZ2080 photoresist. NPCs were grown inside the Nichoid for 7 days ($1x10^4$ cells/cm^2), counted and characterized with immunofluorescence, western blot, and Real Time PCR analysis. NPCs were transplanted in a murine experimental model of PD after a 7 days' growth inside the Nichoid. Parkinsonism was induced by the intraperitoneal administration of MPTP in C57/bl mice (Fig.1) [3].

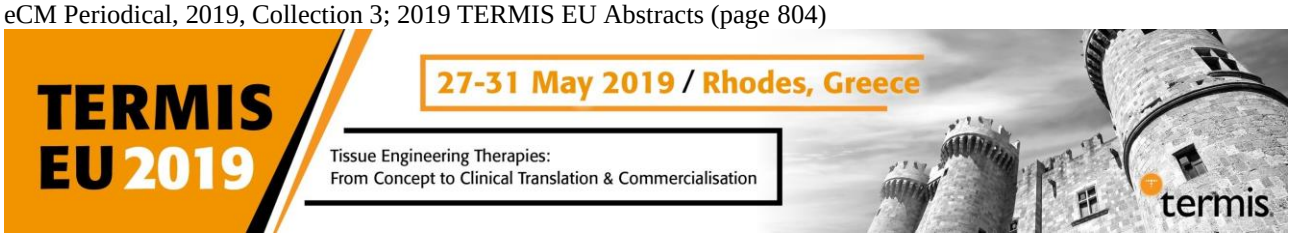
RESULTS & DISCUSSION: NPCs grown inside the Nichoid create a 3D carpet expanding inside the scaffold. 7 days after plating, cells grown inside the Nichoid show a significantly higher cell viability and proliferation than in normal floating culture conditions. Furthermore, after being re-plated in floating conditions for 7 more days, the cells formed smaller but more abundant neurospheres respect to control. The replated cells, analyzed by immunofluorescence, Real Time-PCR and Western blot, demonstrate an increase in stemness markers. The therapeutic potential and safety of Nichoid-grown NPCs was evaluated by their intrastriatal infusion ($7x10^4$ cells) in the brain of PD affected mice. Behavioral performances were evaluated with two different tests showing that Nichoid-grown NPCs promoted the recovery of PD symptoms better than NPCs maintained in normal floating conditions.

CONCLUSIONS: Stem cells demonstrated an increase in stemness potential when grown inside the Nichoid, showing great promise and strong application in the field of regenerative medicine applied to neurodegenerative disease.

ACKNOWLEDGEMENTS: Financial support was received from "Neurogel-en-Marche" Foundation (France) to SC; Fondazione "Romeo and Enrica Invernizzi" to AMDG. ERC grant NICHOID, G.A. 646990 and NICHOIDS, G.A. 754467.

REFERENCES

[1] Raimondi et al. Acta Biomater. 2013;9(1):4579-84.
[2] Carelli et al. Cell Transplant. 2015;24(4):703-19.
[3] Carelli et al. Neuropharmacol. 2017;119:76-90.



Radio-metal cross-linking of alginate hydrogels for non-invasive imaging

P.S. Patrick[1], J.C. Bear[2], H.E. Fitzke[1], M. Zaw-Thin[1], I.P. Parkin[2], M.F. Lythgoe[1], T.L. Kalber[1], and D.J. Stuckey[1]
Presenting Author: P. Stephen Patrick, peter.patrick@ucl.ac.uk
[1]Centre for Advanced Biomedical Imaging; Division of Medicine, University College London
[2]Materials Chemistry Centre, Department of Chemistry, University College London, UK

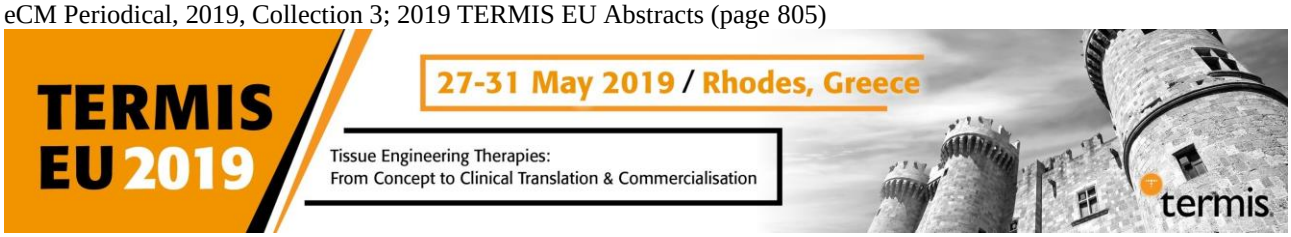
INTRODUCTION: The similarity of alginate hydrogels to soft tissue often renders them undetectable with biomedical imaging. In vivo material behaviour is thus poorly understood, posing a challenge to preclinical development and clinical translation. To address this, we demonstrate a new labelling technique for tracking alginate hydrogels using translational, quantitative in vivo imaging techniques SPECT (single photon emission computed tomography) and PET (positron emission tomography).

METHODS: Alginate's ability to bind metal cations including Ca^{2+} suggested to us that the radiometals $^{111}In^{3+}$ and $^{89}Zr^{4+}$ might act as cross-linkers to enable rapid radiolabeling (1a). In and Zr crosslinked alginate hydrogels were synthesized and probed using X-ray Photo-electron Spectroscopy (XPS), and FTIR (Fourier Transform Infrared) to determine metal-alginate binding. Rheometry and scanning electron microscopy probed the effect on visco-elasticity and porosity or hydrogels crosslinked with Ca^{2+} with/without $InCl_3$ or $ZrCl_4$ equivalent to 1 GBq/mL. Radiolabelling stability was measured using dialysis. ^{111}In-alginate was imaged with SPECT in a model of cardiac tissue engineering.

RESULTS & DISCUSSION: Co-ordination of In^{3+} and Zr^{4+} with carboxyl and hydroxyl groups on alginate sugar subunits was demonstrated by FTIR and XPS analysis. Porosity and viscoelasticity of Ca^{2+}-crosslinked hydrogels was unchanged at concentrations of In and Zr relevant for clinical imaging with PET and SPECT. Alginate hydrogels were synthesized in sheets, beads, and microbeads using ^{89}Zr and ^{111}In and imaged with PET and SPECT (1b). In vivo tracking of radiolabelled alginate hydrogels was evaluated in various clinically-relevant applications including cardiac tissue engineering. Co-localisation of viable luciferase-expressing MSCs transplanted in 2% w/v ^{111}In-alginate to the cardiac wall was shown over 9 days with *in vivo* and *ex vivo* bioluminescence imaging, SPECT-CT, and autoradiography, allowing determination of injection success and retention period.

CONCLUSIONS: Alginate polymers co-ordinate metal cations enabling rapid radiolabeling with $^{89}Zr^{4+}$ and $^{111}In^{3+}$ for PET and SPECT imaging. In a cardiac tissue engineering model, cell location and retention period, and injection success or failure could be determined non- invasively. This ability to obtain information on whole-body biodistribution dynamics of alginate hydrogels will facilitate translation of novel therapeutic formulations for drug delivery, cell encapsulation and tissue engineering.

ACKNOWLEDGEMENTS: MRC grant MR/R026416/1



Antibiotic loaded poly(ε-caprolactone) microspheres functionalized with poly(aspartic acid) as bone targeting delivery system to treat infection

S. Rotman[1,2], M. Wang[3], S. Yang[3], D.W. Grijpma[2], R.G. Richards[1], F.T. Moriarty[1], D. Eglin[1], T.T. Tang[3], O. Guillaume[1]
Presenting Author: Stijn Rotman, stijn.rotman@aofoundation.org
[1]AO Research Institute Davos, Davos, Switzerland [2]Department of Biomaterials Science and Technology, Faculty of Science and Technology, Technical Medical Centre, University of Twente, Enschede, The Netherlands [3]Shanghai Key Laboratory of Orthopedic Implants, Department of Orthopedic Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

INTRODUCTION: The recurrence rate of chronic osteomyelitis in adults is close to 30% [1]. Bacteria are known to migrate deeper into bone tissue through canaliculi and evade common systemic- and local-antibiotic therapies [2]. By fabricating antibiotic loaded poly(ϵ-caprolactone) (PCL) microspheres conjugated with the bone-chelator poly(aspartic acid) (PAA) we aim to prolong the microsphere residency near the site of infection, increasing bactericidal potential.

METHODS: Hydrophobic Gentamicin-dioctyl sulfosuccinate (Gen-AOT) loaded PCL microspheres were made by oil/water emulsion methods. In vitro antimicrobial properties were tested by zone of inhibition (ZOI) in a serial plate transfer test with S. aureus. In vivo antimicrobial efficacy of 1 mg of microspheres was tested in a femoral defect in rats (n =5), infected with $2 \cdot 10^6$ colony forming units (CFU) of bioluminescent Xen-29 a week prior to treatment. In a 2nd study, the PCL microspheres underwent conjugation with PAA by carbodiimide chemistry. Interaction with bone mineral was assessed in the same model as above. IR780 iodide loaded PCL or PCL-PAA microspheres (1 mg) were injected in the bone defect and traced using an in vivo imaging system (IVIS Lumina III, Perkin Elmer).

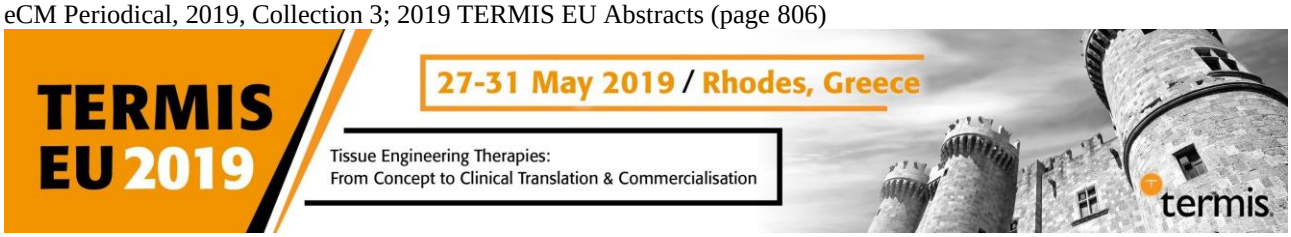
RESULTS & DISCUSSION: ZOI of Gen-AOT loaded PCL was measurable for 5 days, while a ZOI for bactericidal collagen-sheets was visible for 3 days. The Gen-AOT loaded PCL microspheres caused an 81% reduction in CFU compared to untreated control. In vivo, a brighter signal was measured for PCL-PAA compared to PCL microspheres, validating the hypothesis that PAA-grafted PCL resides longer in bone as control PCL.

CONCLUSIONS: In the presented animal model, a monotreatment of 1 mg PCL microspheres caused an 81% CFU reduction. PCL-PAA microspheres enhance bone affinity by chelation of the PAA to bone mineral at the femoral defect. Further work is required to optimize the bone-targeted drug delivery system to bone.

ACKNOWLEDGEMENTS: Project funding was received from AO Trauma.

REFERENCES

[1] Tice AD et al. Am J Med. 2003; 114(9):723-728
[2] Brady A et al. FEMS Immunol. Med. Microbiol. 2008; 52(1):13-22



Oral epithelial-on-a-chip reveals a FOXO1 mechanism regulated in epithelial diabetic barrier

S. Alimperti[1,2], K. Keim[1,2,3], P. Salipante[1], S. Hudson[1], D. Graves[4]
Presenting Author: S. Alimperti, styliani.alimperti@nist.gov
[1]American Dental Association Foundation, Volpe Research Center, USA [2]National Institute of Standard and Technology, Gaithersburg, USA [3]Department of Microbiology, Texas Tech University, USA, [4]Dental Medicine, University of Pennsylvania, USA

INTRODUCTION: Diabetes is a chronic, incurable disorder characterized by lack of resistance to insulin leading to an excess of sugar in the blood controlling the oral epithelium homeostasis [1,2]. Alterations or disruptions of the epithelium integrity can ultimately result in chronic inflammation, and tooth loss.

METHODS: We engineered a 3D disease-like organ on a chip system to deconvolute the dynamic contribution of epithelium in diabetic periodontitis. The in-vitro disease epithelium model is a microfluidic platform that is comprised of 3D cylindrical channel (diameter 160 μm) embedded with collagen I. Human oral keratinocytes (HOKs) were seeded and allowed to adhere through the collagen wall.

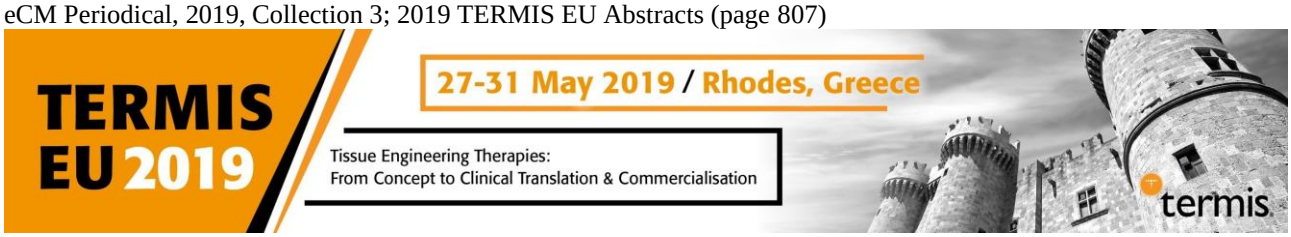
RESULTS & DISCUSSION: The epi-oral tube was exposed in 11 nmol/ml of glucose to mimic diabetic epithelium. In addition, we introduced a pro-inflammatory stimulus into the chip, named lipopolysaccharides (LPS) (100 ng/ml) for 1hr. The diffusion of fluorescent dextran (70 kDa Texas Red, Thermo Fisher) (12.5 μg/mL) was imaged in real time with a confocal microscope (LSM 800, Carl Zeiss). The diffusive permeability coefficient (Pd) was calculated by measuring the flux of dextran into the collagen gel and fitting the resulting diffusion profiles to a dynamic mass conservation equation as described previously [3]. The Pd was increased dramatically in LPS treated diabetic conditions compared to non-diabetic ones. Finally, LPS treatment increases FOXO1 translocation in diabetic HOKs.

CONCLUSIONS: Collectively, the 3D epi-oral model substantial provides insights on periodontitis area by developing sophisticated approaches, based on the tissue-on-a-chip technology, avoiding the limitations of the 2D standard cell culture and enabling us to approach FOXO1 mechanisms in a more detailed manner.

ACKNOWLEDGEMENTS: Financial support was received from ADAF (9720056).

REFERENCES

[1] Masset, A., et al. Ann Anat, 2006. 188(6):529-33.
[2] Mealey, B.L. J Am Dent Assoc, 2006. 137 26S-31S.
[3] Alimperti et al. PNAS. 2017 114(33):8758-8763.



Patient-specific spheroid-on-chip for cancer treatment: a combinatory drug treatment

S. Sankar[1], A. Bhatt[1], S. Raavi[1], C. S. Sharma[2], S. N. Rath[1]
Presenting author: Subha Narayan Rath, subharath@iith.ac.in
[1]Regenerative Medicine and Stem Cell Laboratory (RMS), Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Telangana, India [2]Creative & Advanced Research Based On Nanomaterials (CARBON) Laboratory, Department of Chemical Engineering, Indian Institute of Technology Hyderabad, Telangana, India

INTRODUCTION: Screening tumor biopsies from cancer patients and estimation of the drug combinations to be administered has been a challenge[1], [2]. Hence, we have developed a microfluidic platform with strategically placed U-shaped wells for screening the combinatory effect of drugs on tumor spheroids. The system enables to study the individual effect of 3 drugs (Paclitaxel(P), Vinorelbine(V) and Etoposide(E)) and the combination (P+V, P+E, V+E, P+V+E) of drugs on tumor spheroids (A549 cell line).

METHOD: The platform allows self-formation of tumor spheroids on passing the cancer cells through the inlet port. We have compared the effect of drugs on the spheroids in chip with the effect of drugs on spheroids in static culture in a 96-well plate. We performed live/dead assay, MTT assay and picogreen assay to determine the cell viability in the tumor spheroids in presence of drugs. Cryo-sections of the drug treated spheroids were stained with hematoxylin and eosin to study the necrosis/apoptosis of cells within the 3D spheroid.

RESULTS & DISCUSSION: The combined effect of all three drugs (Paclitaxel- 10 nM, Vinorelbine- 10nM, Etoposide- 25nM) showed an IC50 at 72 hours which was much lower than the effect of drugs individually or as pairs. The design of the chip is shown in Figure 1.

CONCLUSIONS: A rapid, cost-effective and patient specific microfluidic system has been developed for identifying the exact combination and concentration of drugs required for individual treatment. This would be an invaluable step for developing a personalized healthcare set-up for cancer diagnostics and treatment.

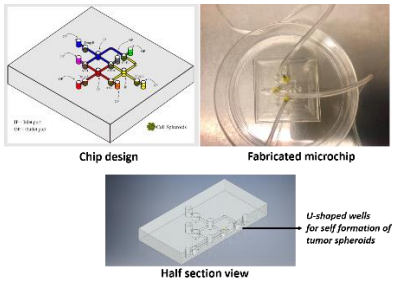
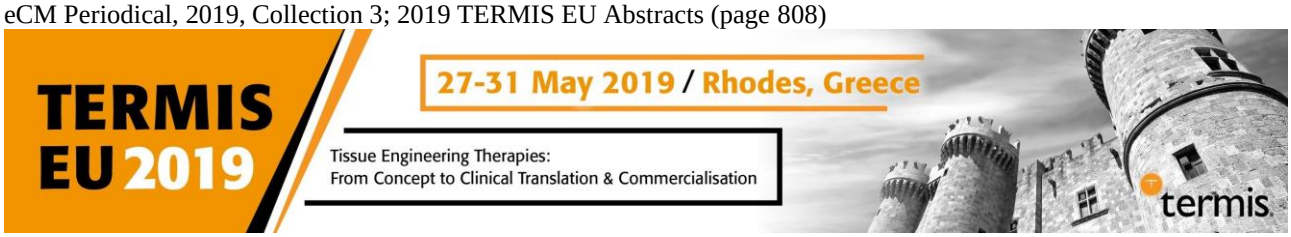


Figure 1: Image shows the chip design and the image of the actual chip

ACKNOWLEDGEMENTS: This work was funded by the Nano Mission project, Department of Science and Technology.

REFERENCES

[1] J. Zhou, J. Su, X. Fu, L. Zheng, and Z. Yin, Exp Hematol Oncol, vol. 6, Aug. 2017.
[2] Y. Chen, D. Gao, H. Liu, S. Lin, and Y. Jiang, Anal. Chim. Acta, vol. 898, pp. 85–92, Oct. 2015.



Triggering endogenous cardiac regeneration via human amniotic stem cell paracrine effects

C. Balbi[1,2], K. Lodder[3], A. Costa[1], S. Moimas[4], F. Moccia[5], F. Campagnoli[1], V. Rosti[6], T. van Herwaarden[3], F. Santini[7], L. Barile[2], M. Giacca[4], A. Palmeri[8], P. De Biasio[8], M.J. Goumans[3], A.M. Smits[3], S. Bollini[1]

Presenting Author: Sveva Bollini, sveva.bollini@unige.it

[1]Regenerative Medicine Lab., Dept. of Experimental Medicine, University of Genova, Genova, Italy [2]Molecular and Cell Cardiology Lab., CardioCentro Ticino, Lugano, Switzerland [3]Dept. of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands [4]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy [5]Dept. of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy [6]Myelofibrosis Study Centre, IRCCS Ospedale Policlinico San Matteo, Pavia, Italy [7]Cardiac Surgery Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy [8]Dept. of Obstetrics and Gynecology, IRCCS Ospedale Policlinico San Martino, Genova, Italy

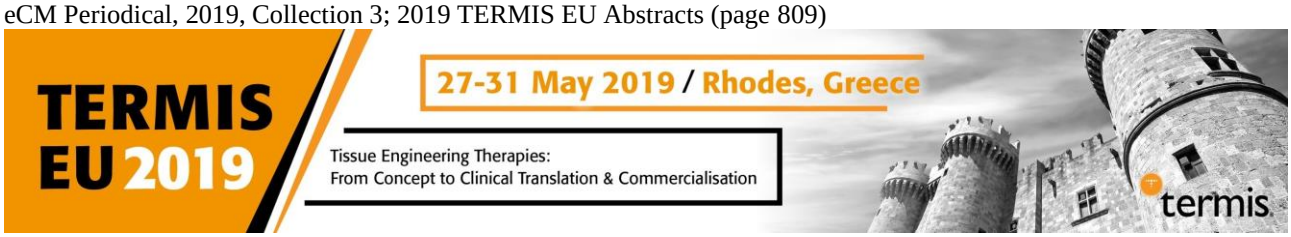
INTRODUCTION: Following injury, cardiovascular disease may arise from inefficient cardioprotection, defective repair and lack of myocardial renewal. We reported that human amniotic fluid stem cells (hAFS) are cardioprotective on rodent ischemic myocardium and on cardiac cells exposed to cardiotoxicity. Here we analyze the hAFS secretome potential to: i) enhance cardiac repair and ii) trigger endogenous regenerative mechanisms.

METHODS: c-KIT+ hAFS were isolated from leftover samples of II trimester amniotic fluid for prenatal screening and stimulated under 1% O_2 to enrich their conditioned medium (hAFS-CM) with paracrine factors. Paracrine anti-apoptotic, angiogenic, and proliferative effects were evaluated on rodent neonatal cardiomyocytes (NVCM), human endothelial colony forming cells (hECFC) and human epicardial progenitors (hEPDC). A preclinical mouse model of myocardial infarction (MI) was treated with a single intra-myocardial injection of: total hAFS-CM; conditioned medium depleted of extracellular vesicles (hAFS-DM), and hAFS-extracellular vesicles (hAFS-EV).

RESULTS & DISCUSSION: hAFS-CM improved survival of NVCM undergoing oxidative and hypoxic damage; it induced Ca^{2+}-dependent angiogenesis in hECFC and triggered hEPDC and NVCM proliferation. In contrast to hAFS-DM, hAFS-CM enriched with EV counteracted scarring, supported cardiac function and triggered cardiomyocyte cell cycle progression ($p<0.05$). hAFS-CM also induced reactivation of endogenous EPDC, similarly to hAFS-EV ($p>0.05$). Although no EPDC cardiovascular differentiation was observed, our data suggest paracrine contribution to local angiogenesis. Transfer of hAFS-EV microRNA into myocardial tissue following MI might explain the increase in resident cardiomyocyte cell-cycle progression

CONCLUSIONS: From our data the secretome soluble component (hAFS-CM and hAFS-DM) is more likely to drive therapeutic angiogenesis, while hAFS-EV improved cardiac function and triggered EPDC reactivation and cardiomyocyte cell cycle re-entry. Thus, the hAFS secretome fractions may be relevant for cardiac regenerative medicine.

ACKNOWLEDGEMENTS: Financial support was received from Programma “Rita Levi Montalcini” by Italian Ministry of Research and Education (S. Bollini) and by a Leiden University Medical Center fellowship (A.M. Smits).



Two-step cross-linkable, injectable and customized serum hydrogel

S. Mao[1], C. Chen[1], Y. Chen[2]
Presenting Author: Syuan-Yu Mao, s106012504s@gapp.nthu.edu.tw
[1]Department of Biomedical Engineering and Environmental Sciences, National Tsing-Hua University, Hsinchu 30013, Taiwan, R. O. C [2]Department of Materials Science and Engineering, National Tsing-Hua University, Hsinchu 30013, Taiwan

INTRODUCTION: Clinically anti-adhesive therapies are mostly based on hyaluronic acid-based membranes, but it is expensive, incomplete coverage, and uncontrollable mechanical property and gelation time for different patient. Since each patient's the severe degree of tissue adhesion is different, it is necessary to prepare an anti-adhesive hydrogel that can meet various needs, such as high mechanical strength, flexible, low immune response and durable anti-adhesion.

METHODS: Serum from the rabbit blood, and serum-based conjugate are modified with tyramine or 2-Aminoethl methacrylate hydrochloride (AEMA). 80 ul of albumin-Ph conjugated, 50 ul of albumin-AEMA conjugated and 10 % of photoinitiator were mixed to form 5 % albumin-based hydrogels. The 1st step of chemical crosslinking is carried out by adding 10 µl HRP and 10 µl H2O2. The 2nd step of photocrosslinked is formed by irradiation with UV.

RESULTS & DISCUSSION: In this study, we developed an adjustable, biocompatible, customized and two-stage cross-linked serum hydrogel through different chemical modification with functional groups. The serum hydrogel shows controllable gelation time, mechanical properties, proteolytic crosslinking. A mouse model of sidewall defect-bowel abrasion was employed, and a significant reduction of post-operative peritoneal adhesion has been found (fig1). It is easy to use and potentially promising for adhesion prevention and drug release for clinical therapy.

CONCLUSIONS: By extracting autologous blood, we have customized a two-stage anti-adhesive material that can release the drug slowly, and can adjust the properties of the material according to the needs of the patient.

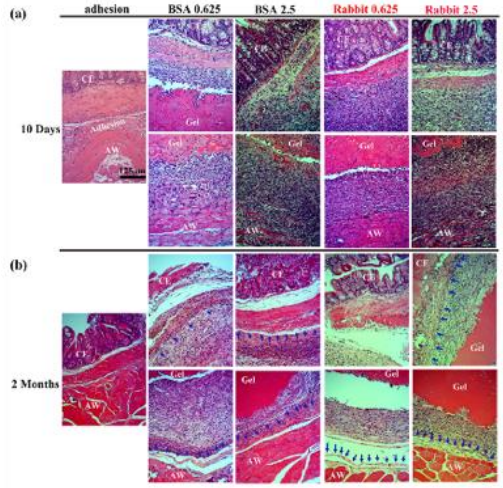
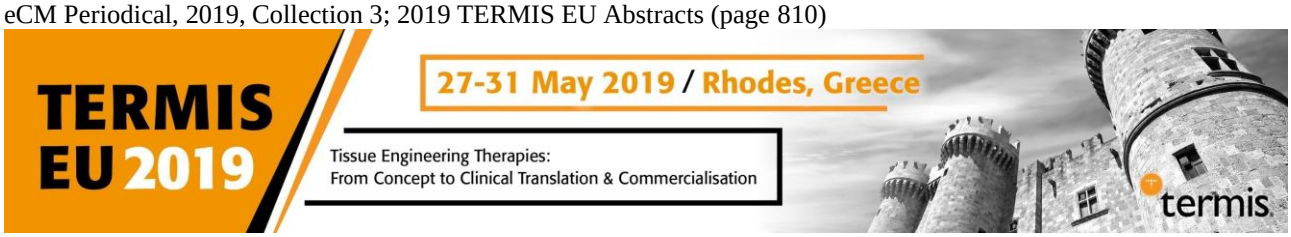


Figure 1: The hematoxylin and eosin staining of tissue sections from peritoneal model of mice

ACKNOWLEDGEMENTS: This work was supported by the Ministry of Science and Technology of Taiwan grant (MOST 106–2221-E-007–055, and MOST 107–2221–E-007–067)



Biofabrication of corneal endothelial grafts through culturing the human corneal endothelial cells on nanochitosan-polycaprolactone membrane

T. Tayebi[1], A. Baradaran-Rafii[2], A. Zafari[3,4], B. Farhadihosseinabadi[4], H. Niknejad[4]
Presenting author: Tahereh Tayebi, Tayebi_t@sbmu.ac.ir
[1]Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Ophthalmic Research Center, Labbafi-nejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran [3]Student research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [4]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

INTRODUCTION: The main reason for sever visual impairment following an endothelial trauma is the limited proliferative capacity of human corneal endothelial cells (HCECs). To date, corneal transplantation has been the only therapeutic choice for corneal failures, yet the global donor shortage makes limitation for this therapy (1). **Tissue engineering strategies can be promising** to compensate for this limitation and **production of HCEC grafts (2).** The purpose of this study was to produce a transplantable corneal endothelial cell grafts by culturing the human corneal endothelial cells on a membrane made of Nano-chitosan (NCh)-polycaprolactone (PCL) as a transparent carrier.

METHODS: Chitosan nanoparticles were prepared using ionic-gelation method. NCh solution were added to PCL solution to achieve the final ratio of 3:1 (3). Biomechanical features of the membrane were examined after its preparation. HCECs were isolated and cultured on the NCh-PCL membrane. The culture medium contained 5µm Y-27632 and 5 µm Cycloastragenol (CAG) for induction of cells proliferation. The cells phenotype was evaluated by immunostaining against ZO-1, Na^+/K^+ ATPase. Proliferative activity was examined using MTT assay and related genes expression was analyzed.

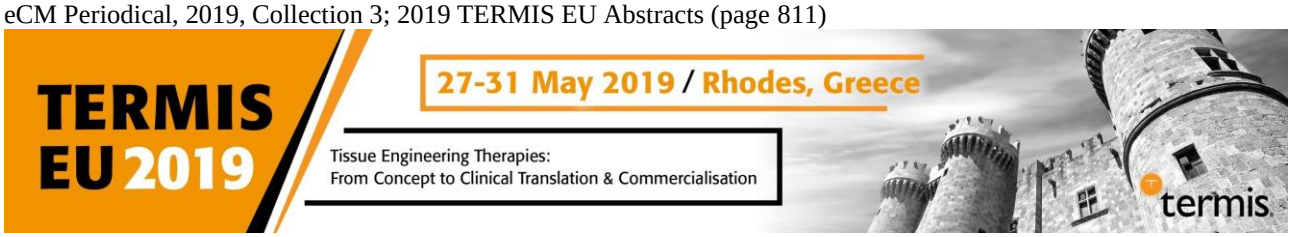
RESULTS & DISCUSSION: Replacing of the chitosan with nanochitosan in the NCh-PCL membranes was found to be appropriate scaffold, for its desired biomechanical and safety characteristics as well as improving cell behaviors such as cell adhesion and expansion. Immunostaining against ZO-1, Na+/K+ ATPase and actin filaments showed that treatment with Y-27632 and Cycloastragenol not only promoted the cells adhesion and proliferative activity of the endothelial cells but also maintained the cells shape and phenotype while the irregular cell shapes was obtained in the control cultured HCECs. Investigation of gene expression showed that expression of ZO-1, Na^+/K^+ ATPase, and HTERT remarkably affected by culturing HCECs on NCh-PCL membrane and treating with Y-27632 and CAG with compared to control.

CONCLUSIONS: NCh-PCL membrane has favored effects on cultured HCECs behaviors such as cell adhesion and proliferation, so, it can be used as a suitable carrier for corneal endothelial cells transplantation. Y-27632 and CAG have synergistic effect on the HCECs phenotype.

ACKNOWLEDGEMENTS: The authors would like to thank the operation room personnel of Shahid Labbafinezhad hospital for their assistance in this research.

REFERENCES

[1] Tan DT et al. The Lancet. 2012;379(9827):1749-61.
[2] Zhu Y-T et al. Current ophthalmology reports. 2015;3(3):207-17.
[3] Wang T-J et al. Molecular vision. 2012;18:255.



High-performance iPS-derived hepatocytes by fabricating 3D culture

T. Akama, F. Tao, N. Kojima
Presenting Author: Takeshi Akama, akama@yokohama-cu.ac.jp
Department of Life and Environmental System Science, Graduate School of Nanobioscience, Yokohama City University, Japan

INTRODUCTION: For drug screening and for toxicity assays, human hepatocytes are indispensable even combined with or without animal models because metabolic characters in hepatocytes are different between human and other animals' derivation. Therefore, primary human hepatocytes are widely accepted although they cost with metabolic variations among lots, which are hard to maintain for long time in vitro. Differentiation of hepatocytes from iPS cells has a possibility to solve those issues, however, produced hepatocytes have quite less metabolic activity. Meanwhile 3D culture is applicable for the purpose of increasing cellular functions. We have a unique technique to fabricate 3D by using high dose of methylcellulose (MC) [1]. By combining our 3D fabrication with long term culture, we aimed to increase hepatic function in iPS-derived cells.

METHODS: iPS-derived hepatocytes supplied from Reprocell were tested cell aggregation approached by drop culture, V-bottom multiwell, and our unique methylcellulose method. Methylcellulose was dissolved in ReproHepato culture medium to be 3%, then injected 1 µl suspended 2k cells, followed by releasing obtained spheroids with cellulase and transferring into ultra-low attachment surface culture wells. Progression of cell aggregation was observed over time. After culture, RNA was harvested to evaluate hepatic function by measuring CYP3A4 gene expression.

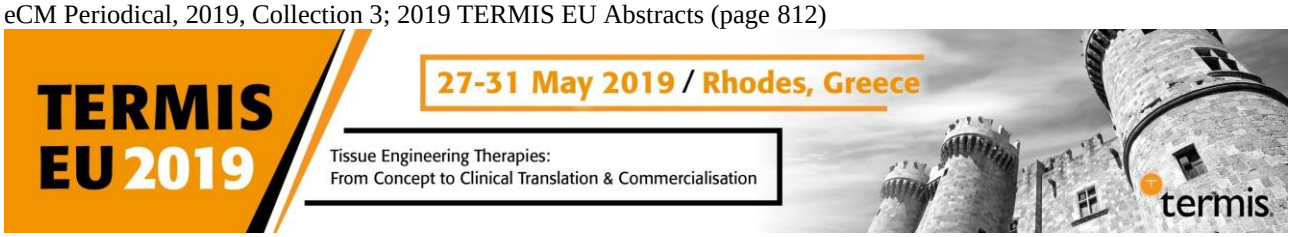
RESULTS & DISCUSSION: Among cell aggregation methods, drop culture could not aggregate to the end, 48 hours later. V-bottom wells gradually fabricated cell spheroids over 24 hours. Meanwhile, 3% MC medium quickly aggregated cells within 30 minutes after injecting cell suspension, then firm spheroids were fabricated even after releasing from MC medium. Fabricated spheroids were cultured for 6 and 14 days together with monolayer 2D culture control. Then harvested RNA was analyzed gene expression, demonstrated 3D culture did not have remarkable different expression with 2D control after 6 days culture. However, after 14 days culture, CYP3A4 gene was induced drastically. The trend of gene induction was shown by all of multiple cell lots with upregulation range between 30 to 300 times.

CONCLUSIONS: Taken together, we succeeded in establishing more functional hepatocytes without gene modifications or inducible drug treatments. Our method will lead to more physiological liver function assays in vitro not depending on human liver primary.

ACKNOWLEDGEMENTS: This research was supported by Japan Agency for Medical Research and Development (AMED).

REFERENCES

[1] Kojima N., et al.: Biomaterials, 33, 4508 (2012).



Engineering prevascularized tissue engineered constructs by harnessing the leaf vasculature on natural-origin multilayered membranes

T. Pesqueira[1], J. Borges[1], J.F. Mano[1]
Presenting Author: Tamagno Pesqueira, tamagno@ua.pt
[1]Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

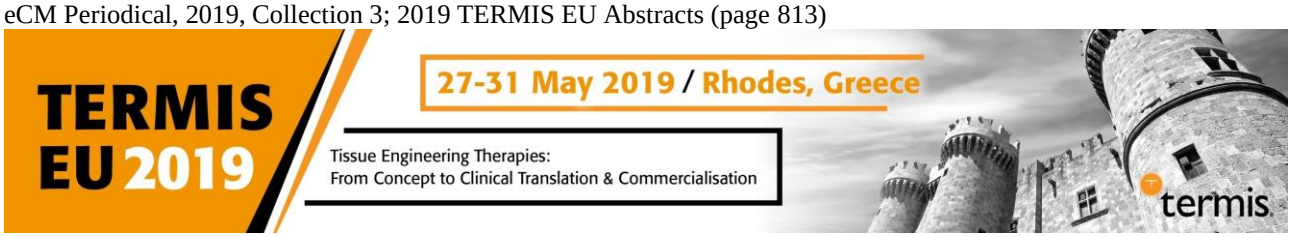
INTRODUCTION: Despite significant research efforts to date, the lack of vascularization in tissue engineered constructs remains one of the major challenges in tissue engineering and regenerative medicine (TERM) strategies. The inefficiency of nutrient and oxygen delivery within highly intricate structures hinders their scale-up and translation into clinical setups. Plant leaves' microvascular network greatly resembles the human circulatory system representing a promising platform to create pre-vascularized tissues. Layer-by-Layer (LbL) is a simple technology for the build-up of highly ordered and organized nanosized-based multilayered structures on top of any substrate by resorting to a plethora of building blocks exhibiting complementary intermolecular interactions. This versatility allows the development of prevascularized platforms which present great relevance in TERM strategies and own great potential for clinical translation.

METHODS: Innate leaf vasculature was replicated in a PDMS mold, later used as substrate for LbL assembly of chitosan (Sigma-Aldrich) and alginate (Sigma-Aldrich) biopolymers into free-standing multilayered membranes. Fibronectin (20 μg/mL, Sigma-Aldrich) was immobilized on their surface via EDC/NHS coupling chemistry. Human umbilical vein endothelial cells (HUVECs) were cultured with endothelial cell culture medium (M199, Sigma-Aldrich) and seeded at 25,000 cells/cm^2 on top of the membranes up to 21 days. At specific time-points, cell viability, DNA quantification, and nuclei and cytoskeleton fluorescent staining were performed. Non-vascularized membranes were used as control.

RESULTS & DISCUSSION: HUVECs remained viable and proliferated over time when cultured on both control and vascularized membranes. However, the cell migration towards the vascular network pattern and its density was significantly enhanced on the vascular network, as observed by fluorescence and brightfield microscopy.

CONCLUSIONS: HUVECs not only migrated towards the vascular network but also along the network leading to the development of de novo blood vessels. The cell migration was assessed by fluorescence microscopy and confocal laser scanning microscopy (CLSM). The cells were stained with non-toxic cell membrane tracers for real-time monitoring. The evaluation of microcapillary tube formation was performed by flow cytometry (e.g. CD31, CD144, VEGF). CLSM was also used to assess protein expression (e.g. collagen type I, CD31, vWF). Microscopy techniques enabled the visualization of the vascular network pattern and assessment of endothelial cell lumen and tube formation. Collectively, we foresee the development of synthetic blood vessels with promising clinical applicability as patches and cell sheets or as a model for cell co-culture and cell crosstalk studies.

ACKNOWLEDGEMENTS: The authors acknowledge the financial support by the Portuguese Foundation for Science and Technology (FCT) in the scope of the Marine Biotechnology ERA-NET project "BLUETEETH" (ERA-MBT/0002/2015) and postdoctoral grant (SFRH/BPD/103604/2014) awarded to J. Borges.



Design and implementation of a 3D PEG-fibrinogen hydrogel system for high-throughput diagnostics and cancer cell growth characterization

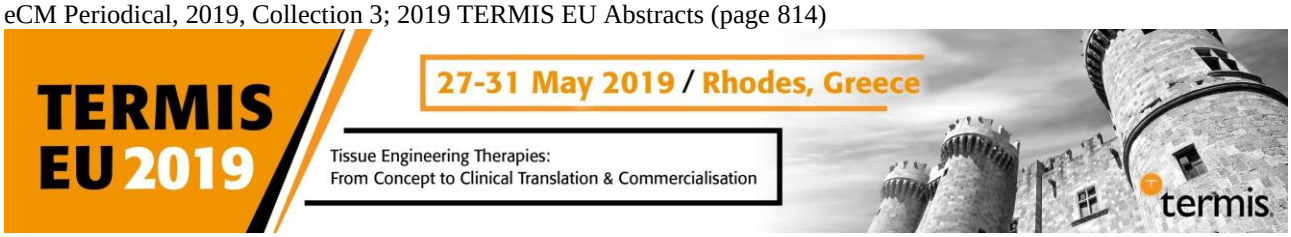
T. Birman[1], T. Nguyen[1], D. Seliktar[1]
Presenting Author: Tamara Birman, btamara@campus.technion.ac.il
[1]Department of Biomedical Engineering, Technion Israel Institute of Technology, Israel

INTRODUCTION: The main goal of our study is to establish a high-throughput growth model system that will be used as an in-vitro drug testing, diagnostics, and cell characterization tool. First, an optimal PEG-Fibrinogen (PF) hydrogel composition for cancer cells outgrowth from cell-line based tumors as well as from human biopsies was established. Our current efforts are focused on validating the established conditions and implementing them in a design of a unique high-throughput microfluidic device.

METHODS: "Artificial tumors" were made by encapsulating a co-culture of the malignant MDA-MB-231 cells and fibroblasts in PF precursor hydrogels, followed by UV-light crosslinking [Fig.1A]. Next, to promote cancer cell outgrowth from human biopsies, the hydrogel composition was altered in various strategies, including the alteration of matrix stiffness and fibrinogen concentration, as well as added fibronectin, Laminin-511, or fibroblast cells into the hydrogel matrix. Finally, a PDMS microfluidic device was designed [Fig.1B] to implement the hydrogel system for high-throughput production of hydrogel μspheres.

RESULTS & DISCUSSION: Preferential cancer cell outgrowth was validated using cells that express fluorescent proteins, eliminating any unspecific migration of fibroblast or EMT/MAT effects that can alter future drug screening results. Next, explants from different types of human primary tumors were retrieved from liver metastases and were encapsulated using the conditions proven successful for the artificial tumors. Various strategies were employed to enhance the cancer cell outgrowth from the biopsies. Among them were alterations of matrix stiffness, incorporation of Fibronectin or Laminin-511. Whereas most conditions failed to promote cancer cell invasion, namely a higher matrix stiffness together with a lower Fibrinogen concentration did lead to cancer cell invasion. Finally, we designed a high-throughput microfluidic device to enable us to produce many highly homogeneous tumor hydrogel μspheres without or with MDA-MB-231 cancer cells.

CONCLUSIONS: The objective of our study is to engineer a high-throughput device that will enable us to produce highly homogeneous tumor hydrogel μspheres from dissociated cells isolated from relatively small tumor biopsy samples. Consequently, we will be able to examine the motility and outgrowth parameters of the cells invading the surrounding hydrogel milieu. Moreover, the device could be implemented as an in-vitro drug testing and diagnostics tool.



Innovative bone substitute to prevent device-associated infections

T. Padrão [1,2,3], C. C. Coelho[1,2,4,5], P. Costa[6], P. A. Quadros[5], S. R. Sousa[1,2,3], F. J. Monteiro[1,2,4]
Presenting Author: Tatiana Padrão, tatiana.padrao@i3s.up.pt
[1]Instituto de Investigação e Inovação em Saúde, U.Porto, Portugal [2]Instituto de Engenharia Biomédica, U.Porto, Portugal [3]Instituto Superior de Engenharia, Politécnico do Porto, Portugal [4]Faculdade de Engenharia, U.Porto, Portugal [5]Fluidinova, S.A., Maia, Portugal [6]UCIBIO, REQUIMTE, Laboratório de Tecnologia Farmacêutica, Departamento de Ciências do Medicamento, Faculdade de Farmácia U.Porto, Portugal

INTRODUCTION: Among the most challenging problems in bone surgery are device-associated infections. This type of infections causes major morbidity, an enormous financial burden to the healthcare systems and a lot of suffering to the patients [1]. Therefore, it is mandatory to develop bone substitutes with antibacterial properties to prevent them. In addition, for the commercial success of these products, several features must be taken into account such as shelf-life and handling properties [2, 3]. For this purpose, a granular bone substitute was produced, combining the biological properties of hydroxyapatite (HAp) together with the antibacterial activity conferred by magnesium oxide (MgO).

METHODS: The granules were produced as described [3] using two sintering temperatures and its effect on morphology, phase composition, surface charge and mechanical strength were evaluated. In addition, their antibacterial effect was studied towards S. epidermidis, one of the major pathogens associated to implanted medical device infections.

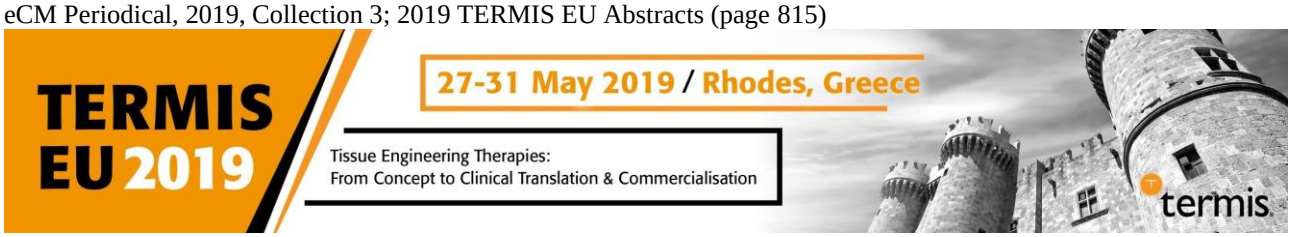
RESULTS & DISCUSSION: XRD results showed that the inclusion of MgO did not induce changes in calcium phosphate phases. However, there was a change in the zeta potential of these materials. SEM images revealed a different surface topography in terms of HAp grain size. The results of compression strength showed that higher sintering temperature also resulted in granules with increased mechanical strength, as expected. These differences also influenced the antibacterial activity, as granules sintered at higher temperature presented a decreased antibacterial effect.

CONCLUSIONS: Both HAp/MgO granules sintered at two temperatures were able to significantly reduce S. epidermidis growth, in comparison with pure HAp materials.

ACKNOWLEDGEMENTS: This work was financed by FEDER funds through the COMPETE 2020–POCI, Portugal 2020, Portuguese funds through FCT/MCTES in the framework of the project "Institute for Research and Innovation in Health Sciences (POCI- 01-0145-FEDER-007274) by projects Biotherapies (NORTE-01-0145FEDER-000012) and NoMic2Bone (NORTE-01-0247-FEDER-017905). The provision of HAp by FLUIDINOVA, S.A. (Portugal) is acknowledged.

REFERENCES

[1] Percival SL et al., J Med microbiol. 2015; 64(4):323-34.
[2] Bohner M., Materials Today. 2010; 13:24-30.
[3] C. Coelho et al., Mat Sci Eng C. 2019; 97:529–538.



Making it softer: Using plasticisers to modify mechanical properties of microfabricated electrospun corneal membranes

T.E. Paterson [1,2], D.V. Navarrete [1,2], F. Claeyssens [2], S. MacNeil [2], I. Ortega [1]
Presenting Author: Thomas Paterson, t.paterson@sheffield.ac.uk
[1]School of Clinical Dentistry, University of Sheffield, Sheffield, UK [2]Kroto Research Institute, Department of Materials Science and Engineering, University Sheffield, Sheffield, UK

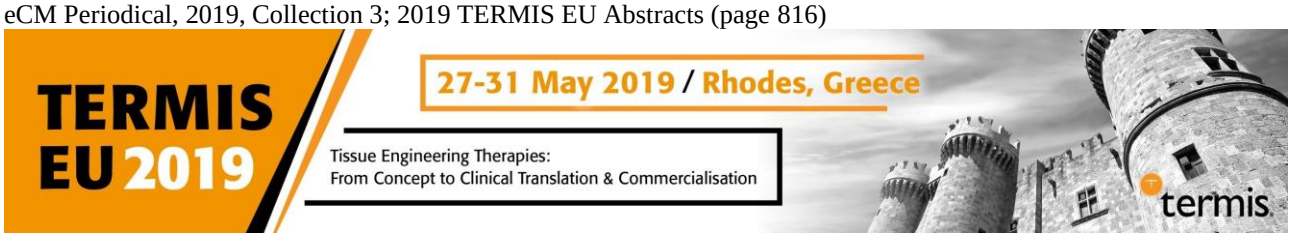
INTRODUCTION: Corneal blindness is the third most common cause of blindness worldwide, disproportionately impacting populations in the global south. Corneal disease generally involves damage to the limbal area which results in cells invading and occluding the normally clear cornea and leads to reduced vision or blindness. A successful surgical technique, called SLET (Simple Limbal Epithelial Transplantation), uses an explant limbus of healthy cornea stem cells which are held in place using human amniotic membrane. This technique relies on the availability of tissue banks for amniotic membrane, which are far less common in the global south and has the risk of disease transition and problematic supply chain. Our group has previously produced synthetic electrospun membranes in collaboration with India to be used as artificial replacements for the amniotic membrane. Previous work in India has resulted in human trials using these membranes but surgeons found the membranes were too stiff and brittle to handle easily. The aim of this project is to produce membranes with a lower Young's modulus than previous membranes while keeping all other properties as similar as possible.

METHODS: Plasticisers PCL, PEG400 and glycerol were added to PLGA 50/50 for electrospinning into membranes. Resulting membranes underwent mechanical testing to determine their Young's modules and DLC to determine their glass transition temperature. Membranes were stored at -20°C for 6 weeks and then re-tested to determine any impact from storage. Accelerated degradation studies were carried out to ensure membranes would degrade within 8 weeks once implanted. Cell viability with human cornea cells and porcine cornea models was investigated to test membrane toxicity using MTT and PrestoBlue metabolic assays. Confocal microscopy was used to image cell morphology on the membranes using DAPI and FITC phalloidin.

RESULTS & DISCUSSION: The addition of plasticisers altered the mechanical properties of the membranes, reducing the Youngs modulus and the glass transition temperature. The morphology of the fibres was also impacted by the plasticisers. Degradation tests showed that scaffolds degraded within the 8 week window required for the operation parameters. No significant toxicity was observed with the addition of the plasticisers.

CONCLUSIONS: Membranes for cornea SLET have been manufactured with a lower Young's modulus while maintaining the previous desired properties. Plasticisers have long been used to influence the mechanical properties of plastics and their use in low dosages has had little impact on the other properties of the membranes. This project will allow the production of membranes which are more suitable for human use and will potential remove the requirement for amniotic membrane in SLET surgery.

ACKNOWLEDGEMENTS: Financial support was received from the University of Sheffield QR GCRF Capacity and Capability Funds Sustainable Partnership Award.



Prevascularization of collagen-glycosaminoglycan scaffolds: Stromal vascular fraction versus adipose tissue-derived microvascular fragments

T. Später[1], F.S. Frueh[2], R.M. Nickels[1], M.D. Menger[1], M.W. Laschke[1]
Presenting Author: Thomas Später, thomas.spaeter@uks.eu
[1]Institute for Clinical & Experimental Surgery, Saarland University, 66421 Homburg/Saar, Germany
[2]Division of Plastic Surgery and Hand Surgery, University Hospital Zürich, University of Zürich, 8091 Zürich, Switzerland

INTRODUCTION: The seeding of scaffolds with the stromal vascular fraction (SVF) of adipose tissue is a common prevascularization strategy. We herein demonstrate benefits of using the novel approach of scaffold prevascularization with adipose tissue-derived microvascular fragments (ad-MVF).

METHODS: SVF single cells and ad-MVF were enzymatically isolated from epididymal fat pads of green fluorescent protein $(GFP)^+$ donor mice to assess their viability, activity and cellular composition using fluorescence microscopy and flow cytometry. Additionally, collagen-glycosaminoglycan matrices (Integra®) were seeded with identical amounts of the isolates and implanted into full-thickness skin defects within dorsal skinfold chambers of GFP^- recipient mice for intravital fluorescent microscopic, histological and immunohistochemical analysis of scaffold vascularization and incorporation throughout an observation period of 2 weeks.

RESULTS & DISCUSSION: While both isolates contained a comparable fraction of endothelial cells, perivascular cells, adipocytes and stem cells, ad-MVF exhibited a significantly higher viability. After in vivo implantation, the vascularization of ad-MVF-seeded scaffolds was improved when compared to SVF-seeded ones, as indicated by a significantly higher functional microvessel density. This was associated with an enhanced cellular infiltration, collagen content and density of $CD31^+/GFP^+$ microvessels particularly in the center of the implants. In contrast, non-seeded matrices exhibited a rather poor vascularization, incorporation and epithelialization over time.

CONCLUSIONS: The present study demonstrates that ad-MVF are highly potent vascularization units that markedly accelerate and improve scaffold vascularization when compared to the SVF.

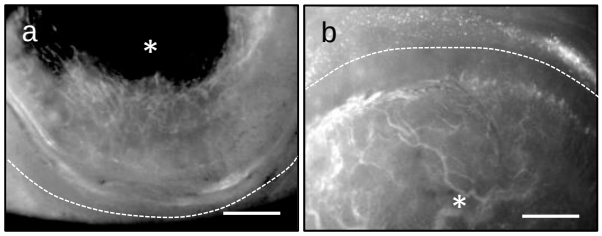
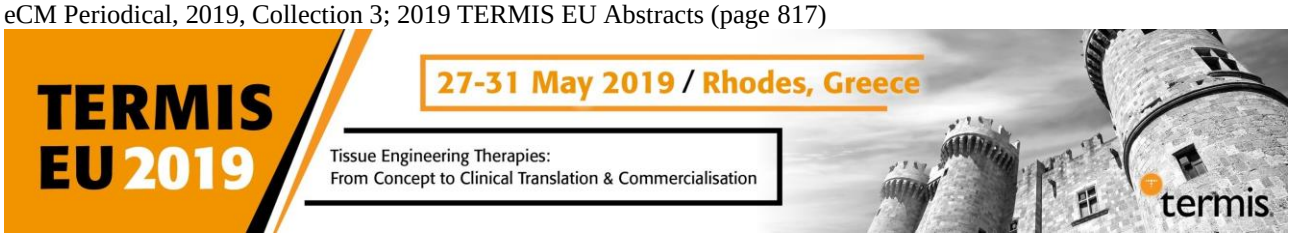


Figure 1: Intravital fluorescence microscopy of implanted, SVF-seeded (a) and ad-MVF-seeded (b) scaffolds on day 14 after implantation into dorsal skinfold chambers pf C57BL/6 recipient mice (dotted lines = scaffold borders; asterisks = center zones; scale bars = 500 µm). Note that newly formed blood vessels did not reach the center zones of SVF-seeded scaffolds whereas ad-MVF induced a strong vascularization throughout the entire scaffolds.

ACKNOWLEDGEMENTS: We are grateful for the excellent technical assistance of Janine Becker, Caroline Bickelmann and Sandra Hans.



Monocyte behaviour under perfusion conditions for development of granuloma on-a-chip

M. Nikolic[1,2], T. Sustersic[1,2,3], C.B. Muller[4], Y.S. Zhang[5], N. E. Vrana[4], N. Filipovic[1,2,3]
Presenting Author: Tijana Sustersic, tsustersic@risk-technologies.com
[1]Faculty of Engineering, University of Kragujevac (FINK), Kragujevac, Serbia [2]Steinbeis Advanced Risk Technologies Institute doo Kragujevac (SARTIK), Kragujevac, Serbia [3]Bioengineering Research and Development Center (BioIRC), Kragujevac, Serbia [4]PROTIP SAS, Strasbourg, France [5]Brigham and Women's Hospital, Cambridge, MA, USA

INTRODUCTION: Multiscale modelling has gained importance in simulating different levels of organs [1]. Defining influence of biomaterial presence to cell growth and cell proliferation is a topic of wide research, as well as immune response to biomaterials [2], in order to define level of biomaterial compatibility with innate immune cells and to see if the biomaterial presence induces adverse immune reactions. We aim to develop a granuloma-on-a-chip system for risk assessment of new biomaterials by i) finding the right cytokine conditions for granuloma induction ii) simulating the behavior of monocytes for optimal physiological attachment to biomaterial surfaces.

METHODS: Simulations of bioreactor on macro scale would mean examining behavior of the fluid flow, monocytes distribution, attachment etc. [3]. We first analyzed fluid flow within the reactor with monocyte cells. Geometry of the bioreactor was based on the dimensions obtained from literature. Prescribed boundary conditions included inlet velocity (4mm/s) and zero pressure at the outlet. Monocytes were introduced with the fluid at the inlet. Model was analyzed using two commercial software - ANSYS and COMSOL, as well as open solver PAK, developed at the University of Kragujevac and BioIRC. Navier-Stokes equation together with the continuity equation were used to model fluid inside bioreactor, and movement of monocytes was modelled as mass transport through the fluid. Trajectories of the monocytes were obtained based on calculated drag force.

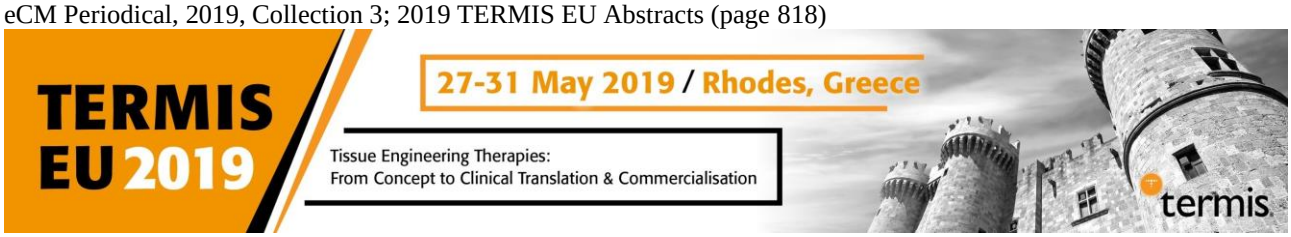
RESULTS & DISCUSSION: Presented model of bioreactor was used for simulation of tracking the monocyte cells inside the bioreactor. Obtained results from different solvers – ANSYS, COMSOL and PAK, show good agreement with each other and with experimentally obtained results.

CONCLUSIONS: The presented model of fluid flow through bioreactor and monocyte trajectories sets good basis for further analysis of monocyte behavior. Future models will include equations for monocyte deposition within the bioreactor, which would simulate inflammatory process.

ACKNOWLEDGEMENTS: This study was funded by the European Project H2020 PANBioRA 760921 and grants from the Serbian Ministry of Education, Science and Technological Development III41007 and OI174028.

REFERENCES

[1] Karabasov S et al. Phil Trans R Soc A 2014; 372 (2021), 20130390
[2] Moriarty TF, Zaat SA, Busscher, HJ (Eds.). Springer Science & Business Media 2012
[3] Williams KA et al. Biotechnol Prog. 2002; 18(5): 951-963.



Pore size-dependent polarization of human macrophages towards the M2-type on melt-electrowritten scaffolds

T. Tylek[1], C. Blum[1], T. Schilling[1], K. Schlegelmilch[1], Andrei Hrynevich[1], P. Dalton[1], J. Groll[1]
Presenting Author: Tina Tylek, tina.tylek@fmz.uni-wuerzburg.de
[1]Department of Functional Materials in Medicine and Dentistry, University Hospital Wuerzburg, Germany

INTRODUCTION: The cell-mediated immune response to biomaterials is an important research field in the development of new materials for medical applications. Especially macrophages play a significant role in the defense against foreign bodies and the formation of fibrous capsules. To prevent such an undesired pro-inflammatory reaction, e.g. upon implantation of a biomaterial, different approaches can be used. In our study, we used the novel technology of melt-electrowriting (MEW) to produce 3-dimensional polycaprolactone (PCL) scaffolds with a porous topography, which influence the spontaneous in vitro-polarization of human macrophages into the anti-inflammatory, regenerative M2-type.

METHODS: Human monocyte-derived macrophages were cultivated for seven days on PCL scaffolds with square-shaped pores of 40, 50, 60, 80 and 100 µm in side length. A 2-dimensional (2D) PCL film was used as a control. To detect their spontaneous polarization, cell culture medium without differentiation factors was used. After seven days, the differentiation was analyzed on gene expression, cytokine release and phagocytic activity.

RESULTS & DISCUSSION: Significant ($p < 0.05$) upregulation of M2 markers (e.g. CD163) and downregulation of M1 markers (e.g. IL-1b) at the gene expression level were observed for macrophages cultivated on scaffolds with the smallest pore size (40 µm) compared to scaffolds with bigger pore sizes and to the 2D-PCL control. Furthermore, the release of the anti-inflammatory cytokine IL-10 was significantly higher for macrophages cultivated on scaffolds with a pore size of 40 µm. Additionally, the investigation of the phagocytic activity showed a pore size-dependent effect: With increasing pore size of the scaffolds, the macrophages increased the uptake of 2 µm latex beads.

CONCLUSIONS: We have proven that especially macrophages cultivated on scaffolds with 40 µm pore size upregulate typical anti-inflammatory macrophage markers without the need for additional stimulating chemicals. Hence, the production of MEW-derived 3D PCL scaffolds with this pore size and the resulting topography might provide a promising tool for medical applications to avoid the undesired pro-inflammatory immune response and to support tissue regeneration.

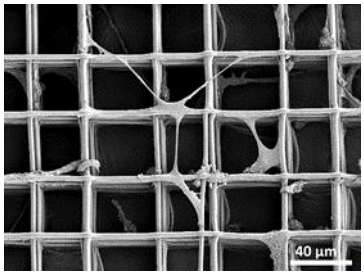
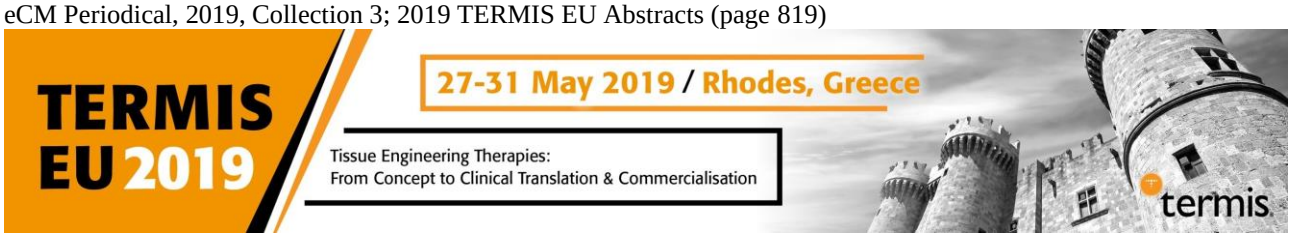


Figure 1: Macrophages cultivated on MEW-derived PCL Scaffolds with a pore size of 40 µm.

ACKNOWLEDGEMENTS: We thank the European Research Council (ERC, consolidator grant Design2Heal, contract no. 617989) for funding this work.



A sound-induced technology for multiscale organization of perfusable micro-vessels networks

D. Pellicciotta, R.G. Richards, D. Eglin, M. Alini, T. Serra
Presenting Author: Tiziano Serra, tiziano.serra@aofoundation.org
AO Research Institute Davos, Switzerland

INTRODUCTION: Cell patterns are important for studying morphogenesis, unravelling biophysical mechanisms, and in the development of novel tissue engineering approaches. Surface acoustic wave (SAW) technologies, based on Faraday wave principle, enable the generation of spatially orchestrated particulate systems (cells, spheroids, inorganic aggregates). Patterns shape can be tuned on demand by varying a set of parameters, such as sound frequency, amplitude, chamber shape. Here we propose the use of a SAW-based technology, named 3D sound induced morphogenesis (3D-SIM), which makes possible the generation of precise and reproducible patterns of particulates systems such as cells, spheroids, bioactive particles[1]. We will use 3D-SIM to create precise and reproducible microvascular networks formed by interconnected and perfusable vessels.

METHODS: Primary human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs) were used to create complex 3D cell structures. Also, patterns composed by calcium phosphate particles (three different sizes: 32-75 µm, 125-250 µm and 250-500 µm) were tested. As hydrogels: i) gelatin methacryloyl (GelMA, 5%w/v) / Irgacure 2959 solution in PBS and ii) fibrin gel (fibrinogen-thrombin, SIGMA) were used. Finite element analysis (FEA), particle dynamics simulation, was conducted to properly select cell pattern shapes. Morphological analysis via optical and confocal microscope has been carried out. Spheroids formed by HUVECs and hMSCs are patterned in few seconds within an extracellular matrix-like hydrogel. Then, HUVECs sprouting from the patterned spheroids and self-organization into micro-vessels will do the rest.

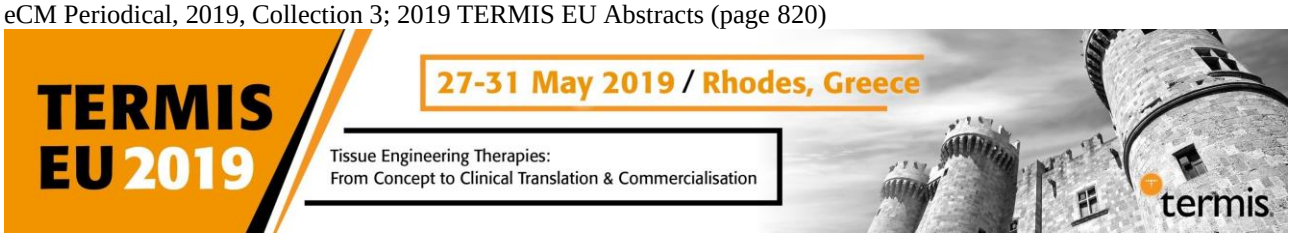
RESULTS & DISCUSSION: We show that acoustic waves can move cells/particulates systems dispersed in a fluid over an area of 28 cm^2 in less than 15s. The process is applicable to a wide range of off-the-shelf gelling biomaterial matrices. Layers composed by several combinations of hydrogel and cells/bioactive particles were generated and employed as matrices. Spheroids patterns morphology confirmed FEA investigation. 3D constructs were created by staking layers of patterned cells embedded in hydrogel matrices. Biological evaluation confirmed that 3D-SIM is a mild fabrication process which does not affect cell viability.

CONCLUSIONS: We demonstrate that 3D-SIM is an affordable and user-friendly technology to create 3D cell models in a time-effective manner, with sufficient spatial complexity, retaining cell viability. Hierarchically shaped vessels with a multiscale organization (meso-micro scale), obtained via 3D-SIM, can be integrated into fluidic chip where perfusion can be performed in a reproducible manner with a controlled flow rate.

ACKNOWLEDGEMENTS: The authors would like to thank BRIDGE programme (SNSF-Innosuisse) for providing financial support to this project (SNSF grant number: 20B1-1_178259).

REFERENCES

[1] Serra T et al. Surface Acoustic Wave (SAW) 3D Printing Method - Nr. 01058/17



Spatiotemporal biomaterial functionalization via competitive supramolecular complexation of avidin and biotin analogs

T. Kamperman[1], M. Koerselman[1], C. Kelder[1], J. Hendriks[1], J.F. Crispim[1], X. de Peuter[1], P.J. Dijkstra[1], M. Karperien[1], J. Leijten[1]
Presenting Author: Tom Kamperman, t.kamperman@utwente.nl
[1]Department of Developmental BioEngineering, University of Twente, The Netherlands

INTRODUCTION: Native tissues are characterized by a dynamic nature. Recapitulating such dynamicity in engineered tissues requires spatiotemporal control over their biochemical composition. Here, we pioneered supramolecular desthiobiotin/avidin complexation to enable dynamic functionalization of biomaterials. Desthiobiotin is a non-sulfur containing analog of biotin that also interacts with avidin, but with substantially lower binding affinity than biotin [1]. We hypothesized that combining biotinylated hydrogel with desthiobiotinylated bioactive molecules would enable the spatiotemporal functionalization of biomaterials via in situ desthiobiotin/biotin displacement, which is a novel, facile, and fully cytocompatible material modification strategy.

METHODS: Biotinylated dextran-based (Dex-TA) hydrogel constructs were functionalized with 1µM neutravidin (i.e., avidin analog), 1µM desthiobiotin-FITC (D-FITC) and 1µM biotin-atto565 (B-atto565), and subsequently analyzed using fluorescence recovery after photobleaching (FRAP) and confocal microscopy. Surface plasmon resonance imaging (SPRi) and reporter cells were used to analyze the reversible presentation and bioactivity of desthiobiotinylated BMP7 antibodies and BMP7 growth factors.

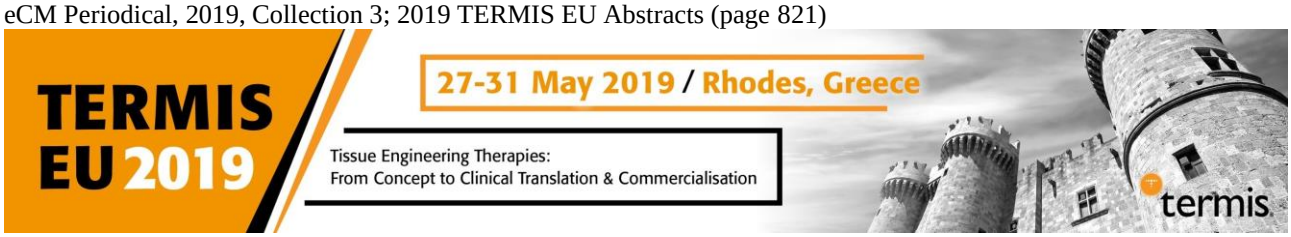
RESULTS & DISCUSSION: The reversible and sequential modification of the hydrogel constructs was demonstrated by displacing D-FITC with B-atto565. By tuning the concentration and incubation time of B-atto565, we reproducibly controlled its penetration depth into the hydrogel. An injection-molded bone-shaped 3D construct was spatially modified by controlling the thickness of the biotin-displaced layer. Moreover, competitive supramolecular complexation enabled the temporal presentation of desthiobiotinylated BMP7 antibodies and growth factors, as confirmed using SPRi and reporter cells.

CONCLUSIONS: We have successfully developed and characterized a novel spatiotemporal biomaterial modification strategy based on competitive supramolecular complexation.

ACKNOWLEDGEMENTS: The authors acknowledge funding from Dutch Arthritis Foundation (#12-2-411, #LLP-25), NWO VENI (#14328), and ERC Starting grant (#759425).

REFERENCES

[1] Hirsch JD et al. Anal Bio. 2002;308(2):343-57



Quantitative characterization of adhesion and cytomechanics of living cells on biomaterials and tissues

T. Neumann[1], T. Müller[1], D. Stamov[1], H. Haschke[1], T. Jähnke[1]
Presenting Author: Dr. Torsten Mueller, To.Mueller@bruker.com
[1]JPK BioAFM Business, Bruker Nano GmbH, Colditzstr. 34-36, 12099 Berlin, Germany

INTRODUCTION: The versatility and refinement of biomaterials and tissues engineered for regenerative medicine is growing, as is the need to characterize their properties and host interactions. The topography and mechanical properties of biomaterials are crucial parameters that influence cell adhesion/motility, morphology and mechanics as well as the fate of stem and progenitor cells [1, 2, 3].

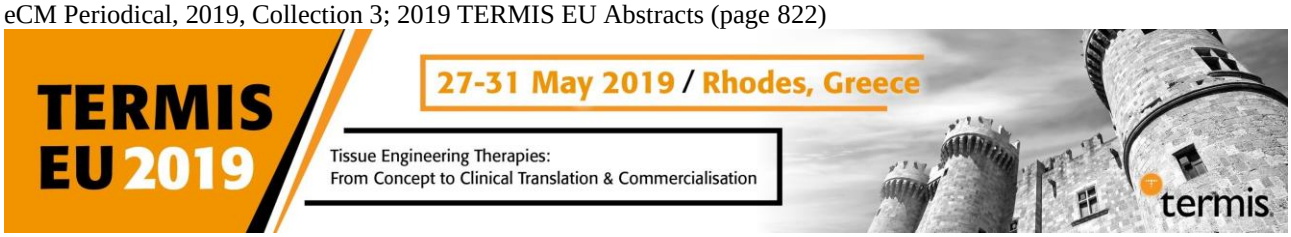
METHODS: Atomic force microscopy (AFM) is a powerful tool which allows the comprehensive study of all these properties and interactions with nanometer scale resolution under controlled environmental conditions. The inherent drawbacks of traditional AFM imaging modes for fast imaging or for challenging samples like living cells has been impressively overcome by the novel NanoWizard® ULTRA Speed AFM, which not only enables high-speed studies of time-resolved dynamics associated with cellular processes, it's latest scanner technologies and compact design also allow full integration of AFM into advanced commercially available light microscopy techniques. Thus, fast AFM imaging of several frames per second can be seamlessly combined with methods such as epi-fluorescence, confocal, TIRF, STED microscopy, and many more.

RESULTS & DISCUSSION: Using AFM, mechanical properties like the Young's modulus of biomaterials, tissues or cells can be determined. Furthermore, the nanostructure of biomaterials like aligned collagen matrices and cell alignment on such structures have been resolved [3]. Using Single Cell Force Spectroscopy (SCFS), cell-substrate or cell-cell/tissue interactions can be measured down to single protein unbinding. The nano-mechanical analysis of cells is increasingly gaining in importance in different fields in cell biology like cancer research and developmental biology.

CONCLUSIONS: We will present how the latest advances in the ULTRA Speed AFM are being applied to study a wide-range of biological specimen, from individual biomolecules to collagen type I fibrillogenesis to mammalian cells and tissues.

REFERENCES

[1] Elter et al., Eur Biophys J 2011: 40: 317-327.
[2] Engler et al., Cell 2006: 126:677-689
[3] Cisneros et al., Small 2007: 3:956-63



Interaction between contusion velocity and depth influences astrocyte reactivity in an in vitro model of traumatic spinal cord injury

T.K. Nguyen[1], J.L. Tipper[2], J.B. Phillips[3], R.M. Hall[4], J.C.F. Kwok[5, 6]
Presenting Author: Trang Kim Nguyen, mntkn@leeds.ac.uk
[1]Institute of Medical and Biological Engineering, University of Leeds, UK [2]School of Biomedical Engineering, University Technology Sydney, AU [3]UCL Centre for Nerve Engineering, University College London, UK [4]Institute of Thermofluids, University of Leeds, UK [5]School of Biomedical Sciences, University of Leeds, UK [6]Institute for Clinical and Experimental Medicine, Prague, CZ

INTRODUCTION: Traumatic injuries to the spinal cord lead to a microenvironment where regeneration is poor. Replicating the biomechanics of injury has been shown to be important in understanding the heterogeneity of pathophysiological events [1]. The aim of this work was to investigate the interactions between injury biomechanics and astrocyte responses in a 3D hydrogel model.

METHODS: P2-3 primary Wistar rat astrocyte-seeded collagen gels were prepared at $1x10^5$ cells $p.ml^{-1}$ of gel [2]. A contusion regime was implemented after 24 hours: contusion (100 or 1000 $mm.s^{-1}$) to 25, 50, or 75% gel height, 100 ms dwell, and return to 0%. Astrocyte reactivity was measured over a 14-day period using glial fibrillary acidic protein (GFAP) immunostaining.

RESULTS & DISCUSSION: An increase in both displacement depth and velocity resulted in higher loads and thus energy applied to the gel (Table 1). Quantification of the volume of GFAP expression suggests increasing reactivity with increasing displacement, at 100 $mm.s^{-1}$. Interestingly, at 1000 $mm.s^{-1}$ a similar level of reactivity was observed across all displacement depths over time.

CONCLUSIONS: Results here suggest an interaction between displacement and velocity, which is in agreement with Lam et al who used a rat SCI model [3]. Between 100 and 1000 $mm.s^{-1}$ lies a threshold where, below this, injury depth defines astrocyte reactivity. This work has provided the basis for the development of a distraction model. An aligned astrocyte-DRG co-culture model has been developed to investigate the effects of tensile forces on cell behaviour. Increasing the biological complexity and generating an organised cellular architecture aims to bring the model closer to that of the human spinal cord.

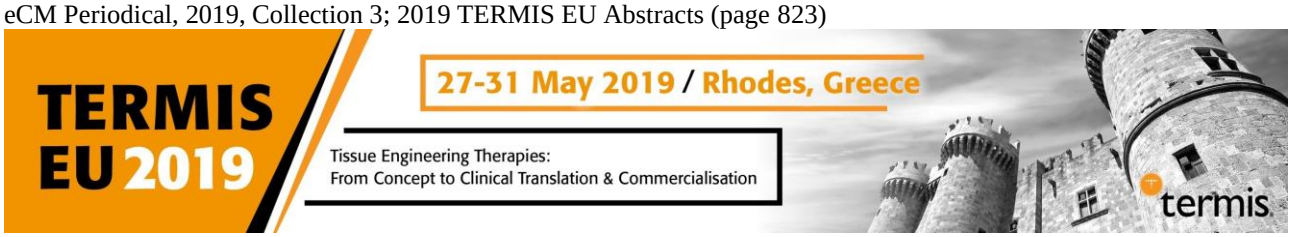
	100 $mm.s^{-1}$	**1000 $mm.s^{-1}$**
Displacement (%)	**Work Done (mJ)**	**Work Done (mJ)**
25	0.150 ± 0.01	0.954 ± 0.10
50	0.506 ± 0.07	1.836 ± 0.07
75	0.945 ± 0.10	3.709 ± 0.26

Table 1: Work done for simulated contusion injury. Mean ± SEM (n=12 per condition).

ACKNOWLEDGEMENTS: EPSRC CDT in TERM. Grant number EP/L014823/1.

REFERENCES

[1] Mattuci S et al. Clin Biomech. 2018. 18: 30262.
[2] East E et al. J Tissue Eng Regen Med. 2009. 3(8): 634-46.
[3] Lam CJ et al. J Neurotrauma. 2014. 31(24):1985-97.



Quantification of collagen fibre structure in osteogenesis imperfecta using second harmonic generation imaging on polycaprolactone scaffold

T. Cebe[1], A de Leeuw[1], M. Balasubramanian[2], G. Reilly[1]
Presenting Author: Tugba Cebe tcebe1@sheffield.ac.uk
[1]Department of Materials Science and Engineering and INSIGNEO Institute for in silico Medicine, University of Sheffield, Sheffield, UK[2]Academic Unit of Child Health, Department of Oncology & Metabolism, University of Sheffield, Sheffield, UK

INTRODUCTION: Osteogenesis imperfecta (OI) is a heritable disorder of bone matrix formation, usually caused by mutations in the type I collagen genes, COL1A1 and COL1A2, which leads to increased bone fragility, and deformity [1]. It is characterized by poor bone quality, mass, and strength. In this project, we aimed to create a 3D model of extracellular matrix production using electrospun polycaprolactone (PCL) scaffolds [2] to investigate OI effects on in vitro collagen production in order to better understand how type 1 collagen mutations alter early collagen formation processes.

METHODS: PCL pellets were dissolved in dichloromethane, non-aligned and aligned fiber scaffolds were fabricated by electrospinning. Primary fibroblasts and controls were collected from human donors under informed consent from Sheffield's Children Hospital. For this project, cell-secreted collagen was analyzed using a laser scanning confocal microscope fitted with a Ti: sapphire multiphoton laser. Samples were illuminated and second harmonic generation (SHG) signals were detected.

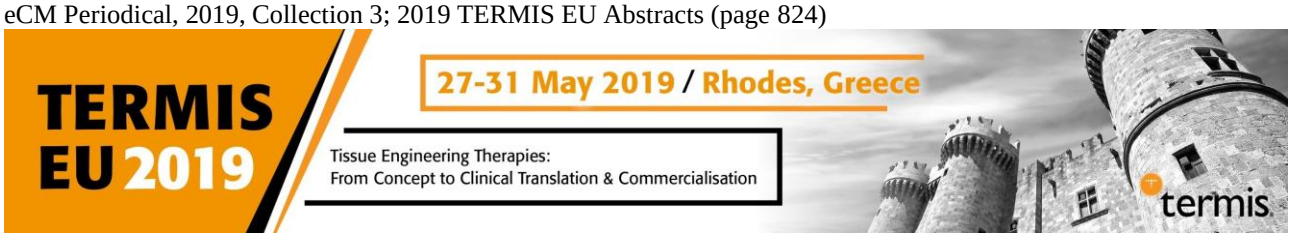
RESULTS & DISCUSSION: SHG is a powerful imaging tool capable of elucidating collagen structure. SHG has been utilized to assess collagen deposited by fibroblasts in 2D and 3D [3]. As expected, collagen secreted by fibroblasts from healthy donors aligned in the direction of the electrospun PCL fibres of the substrate scaffold (3D). Notably, collagen secreted by OI fibroblasts cultured on aligned fibres produced very low SHG signals suggesting decreased collagen deposition or presence of immature procollagen. Conversely, OI fibroblasts cultured on both non-aligned fibres and tissue culture plastic (2D) produced stronger SHG signals.

CONCLUSIONS: Our results demonstrate that fibrous scaffolds traditionally used for tissue engineering can be used to create in vitro human cell-based, patient-specific models of matrix deposition in 3D. This will be a powerful tool to better understand the mechanisms behind diseases of extracellular matrix production such as OI.

ACKNOWLEDGEMENTS: Marie Skłodowska - Curie Actions: H2020 – MSCA – RISE - 2017 (777926) TC is funded by Republic of Turkey Ministry of National Education.

REFERENCES

[1] Lindert, U et al. J. Biol. Chem (2015): jbc-M115.
[2] Agarwal S et al. Polymer 49.26 (2008): 5603-5621.
[3] Delaine-Smith, R et al. PloS one 9.2 (2014): e89761



Differentiation of pluripotent stem cells on biomaterials immobilized ECM and ECM-derived peptides

T.C. Sung[1], H.F. Li[1], C.H. Liu[1], A. Higuichi[1,2]
Presenting Author: Tzu-Cheng Sung, stc321@yahoo.com.tw
[1]Department of Chemical and Materials Engineering, National Central University(NCU), Zhongli, Taiwan [2]Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama, Japan

INTRODUCTION: Stem cells are an attractive prospect for regenerative medicine and tissue engineering. However, stem cell are difficult to differentiate into specific cell types. Thus, reliable methods to differentiate stem cells into desired cell types are necessary to be developed. Here, we developed nanosegment-grafted biomaterials having different elasticity. [1] We also developed optimal differentiation media to induce the differentiation of hES cells into cardiomyocytes.

METHODS: We developed several biomaterials having different elasticity for hPSCs differentiation into cardiomyocytes. We prepared (1) ECM (extracellular matrix)-coated dishes, (2) PVA-IA (polyvinylalcohol-co-itaconic acid) hydrogel dishes having different elasticity that are grafted with several ECMs [2], and (3) PVA-IA hydrogel dishes having different elasticity that are grafted with cell-adhesion oligopeptide (oligovitronectin). We also investigated the differentiation efficiency using different induction medium: (a) Commercial cardiomyocyte induction medium, (b) RPMI 1640 medium supplemented with B27 and bovine serum albumin (BSA), (c) CDM3 medium (xeno-free culture medium) developed by literature [3] and in this study.

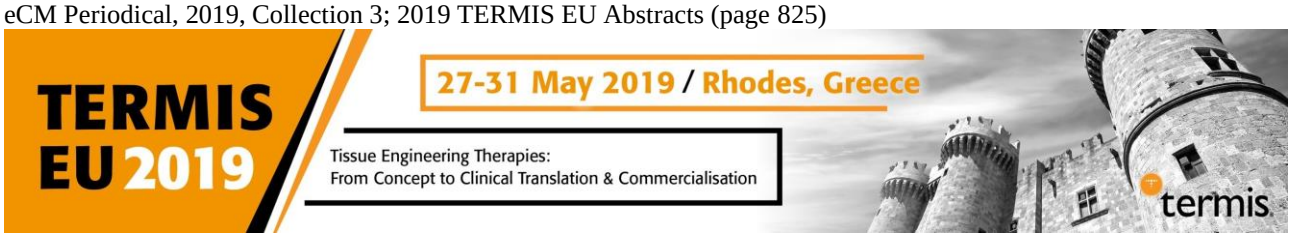
RESULTS & DISCUSSION: On day 0, we replaced the expansion medium into cardiomyocytes differentiation medium containing the GSK3β inhibitor. On days 1-2, we observed that 30%~40% of the cells in the medium shown in medium and 50~60% of the cells in the medium were died and detached from the surface. However, the center of the colony of living cells were getting thicker and became compact. These cells were differentiated into cardiomyocytes on days 5-6. On day 8-10, we successively observed the contracting colonies on each biomaterial surface.

CONCLUSIONS: We successfully screened the optimal elasticity of bio-materials, preferable nanosegments immobilized on the biomaterials as well as the cell culture medium for the optimal differentiation of hPSCs into cardiomyocytes. This system will be used for developing for cell sorting system, which will be a great benefit to its clinical application in regenerative medicine.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Science and Technology (MOST 107-2119-M-008-002).

REFERENCES

[1] A. Higuchi, et al. Biomaterials, 2016;76, 76.
[2] T.C. Sung, A. Higuchi, et al. J. Vis. Exp., 2018;132, e57314.
[3] P. W Burridge, et al. Nat. Methods, 2014; 11, 855.



3D printed Sr^{2+} and Mg^{2+} doped calcium deficient apatite scaffolds as an angiogenic custom bone graft

U. K. Roopavath[1], S. N. Rath[1]
Presenting Author: U. K. Roopavath, bm15resch11005@iith.ac.in
[1]Regenerative medicine & Stem cell Laboratory (RMS), Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Hyderabad, India

INTRODUCTION: 3D printing or additive manufacturing is employed to manufacture complex anatomical shapes and patient specific tissue grafts based on CT/MRI scans. Strontium promotes osteoblasts proliferation and inhibits osteoclasts. Magnesium Induces nitric oxide production in endothelial cells and promotes angiogenesis.

METHODS: Sr^{2+} and Mg^{2+} doped calcium deficient apatite (CDA) powders were synthesized by wet chemical precipitation method. Ceramic ink was formulated and scaffolds were 3D printed using extrusion 3D printer. The 3D printed green bodies were tested for invitro biocompatibility, osteogenic differentiation and angiogenetic potential.

RESULTS & DISCUSSION: SEM micro graphs (Fig 1A & B) of 3D printed structures show complete removal of binder used to formulate the ceramic ink and tightly packed and intact external morphology without any cracks. The hUMSCs seeded on top of the 3D printed structures show good proliferation and biocompatibility after 2 days of culture as evident from live/dead staining (Fig 1(C, D & E)). Alamar blue dye reduction assay confirm significant increase in metabolic activity after seven days of culture. ALP staining indicates increased osteo differentiation with increased Sr^{2+} and Mg^{2+} ions concentration. In vitro tube formation assay performed using media extracts of Sr and Mg doped CDA shows increased sprouting, tube length and diameter formed by Endothelial cells on Matrigel matrix after 6 hours when compared to CDA.

CONCLUSIONS: Co doping these two elemental ions in mechanically stable CDA show a synergistic effect on angiogenesis and osteogenesis simultaneously and can be a suitable candidate for bone tissue engineering. As the ink formulated using the prepared CDA powders is effortlessly 3D printable with complex internal architecture this material can be employed to fabricate patient-specific bone defects.

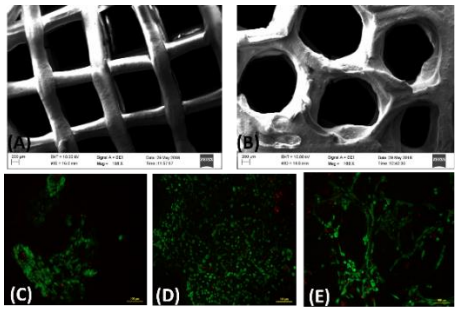
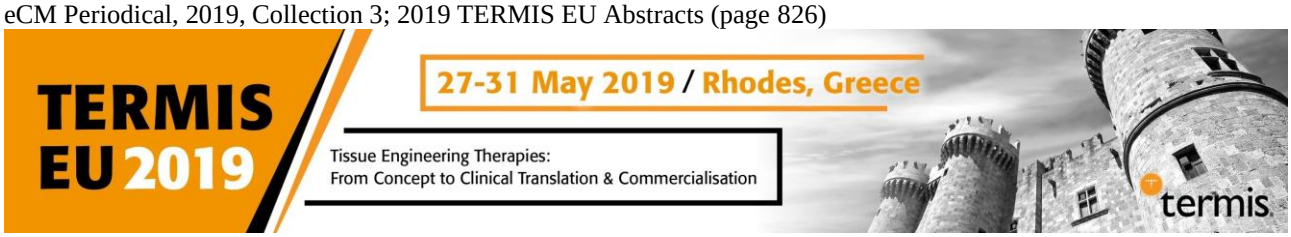


Figure 1: SEM images of Sr^{2+} and Mg^{2+} doped CDA linier and hexagonal infill (A, B). Live dead images of CDA (C), 0.03 M Sr^{2+} and Mg^{2+} doped CDA (B) and 0.06 M Sr^{2+} and Mg^{2+} doped CDA (C) after 72 hours.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Human Resource Development (MHRD), Government of India.



A novel pro-angiogenic fibrin-alginate technology for repair and regeneration of multiple tissues

V. Sharma[1], S. Brown[1], N. Kohli[1], L. Hook[2], E. García-Gareta[1]
Presenting Author: Vaibhav Sharma, sharmav@raft.ac.uk
[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood, UK[2]Smart Matrix Ltd, Leopold Muller Building, Mount Vernon Hospital, Northwood, UK

We describe a novel patented technology developed in our laboratory based on a fibrin-alginate mesh that is pro-angiogenic and shows excellent cell attachment and infiltration properties. The first product developed using this technology is a dermal replacement scaffold called Smart Matrix®. Advantageously, this fibrin-alginate technology can be combined with synthetic polymers, either inert (i.e. silicones) or bioactive (i.e. polycaprolactone, PCL) in various shapes (sheets, 3D structures), or osteogenic components for repair and regeneration of various tissues. Extensive in vitro and in vivo analysis has shown that Smart Matrix® allows a rapid initial infiltration of cells and blood vessels [1]. The product has gone through detailed structural characterization using techniques like SEM, histological analysis, laser scanning confocal microscopy and AFM. The results have suggested that the material is highly porous, and the interconnected pores are gradient in nature. The cellular behavior towards the biomaterial has been confirmed through numerous cell viability assays, cytotoxicity assays and angiogenic assays including the very popular chick chorioallantoic membrane (CAM) assay. To further expand the applications of the technology, a novel two-component dermal scaffold for the treatment of pressure sores was designed using a polydimethylsiloxane (Sil) backing membrane to make the dermal scaffold more robust [2]. Moreover, the fibrin-alginate mesh was combined with PCL structures for the treatment of non-union fractures: the pro-angiogenic properties of the PCL/fibrin-alginate composite scaffold were increased compared to PCL alone as shown by the CAM assay. PCL/fibrin-alginate scaffolds are currently being tested in a rat model. Moreover, the fibrin-alginate mesh has been modified by introducing an osteogenic element into the mesh to be used as a bone void filler. We present here a pro-angiogenic fibrin-alginate technology for repair and regeneration of soft and hard tissues. The first product of this technology is currently undergoing clinical trials. Future development will see its combination with 3D printing for development of custom-made implants.

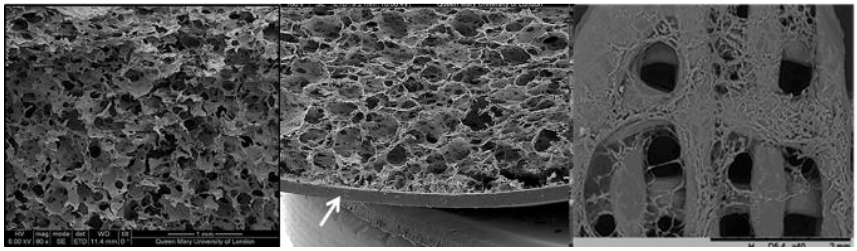
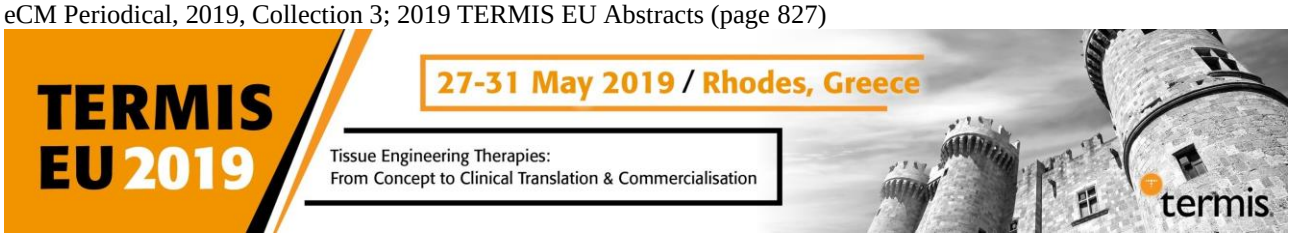


Figure 1: Left to right; SEM image of Smart Matrix®, fibrin-alginate/Sil composite and PCL/fibrin-alginate composite scaffold

ACKNOWLEDGEMENTS: This work was supported by the Restoration of Appearance and Function Trust (RAFT, UK, registered charity number 299811) and Smart Matrix Ltd.

REFERENCES

[1] García-Gareta et al. Bioresearch Open Access 2(6), 412, 2013.
[2] Sharma et al. Macromolecular Bioscience 1700185, 2017.



Role of dexamethasone and (+)-ZK 216348 during chondrogenic fate in bone marrow stem cells

V. Basoli [1], E. Della Bella [1], M. Alini [1], M.J. Stoddart [1]
[1]Musculoskeletal Regeneration, AO Research Institute Davos, Davos Platz, Switzerland

INTRODUCTION: The chondrogenic commitment in vitro requires the use of glucocorticoids (GCs)[1]; however, the exact role of them in this process is still not clear. Here, we investigate how glucocorticoids play a role during chondrogenesis in the presence or absence of dexamethasone (DEX) or (+)-ZK 216348[2], a novel synthetic glucocorticoid agonist that modulates NF-κB pathway.

METHODS: We induced chondrogenesis in hBMSCs (n= 6, obtained with full ethic consent) using 10 ng of TGFβ for the 2 hours in the presence or absence of DEX (100nM) and (+)-ZK 216348, then for 7, 14 and 21 day respectively. The gene expression analysis by RT^2 array was performed after 2h and respective days for the interesting genes. GAG and Histology using Safranin-O and IHF for Col 2 were analyzed after 21 Days.

RESULTS & DISCUSSION: GC treatment by (+)-ZK 216348 downregulated gene expression of cartilage matrix components Collagen Type 2, aggrecan, and collagen type X and decreased cartilage matrix-sulfated proteoglycans and GAG production. Interestingly, chondrogenic commitment in the absence of conventional DEX used at 100 nM showed an increase of SOX9 expression in the early moment of differentiation compared to hBMSC pellets in the presence of DEX.

CONCLUSIONS: The use of GC treatment during chondrogenic commitment strongly influences stem cell fate. The use of (+)-ZK 216348 counteracts differentiation, potentially by controlling NF-κB translocation and interfering with gene expression activation of genes involved in chondrogenesis. Our results suggest that the transactivation pathway plays a role in chondrogenic differentiation. This provides new insights for understanding the molecular mechanisms behind MSC fate in vitro.

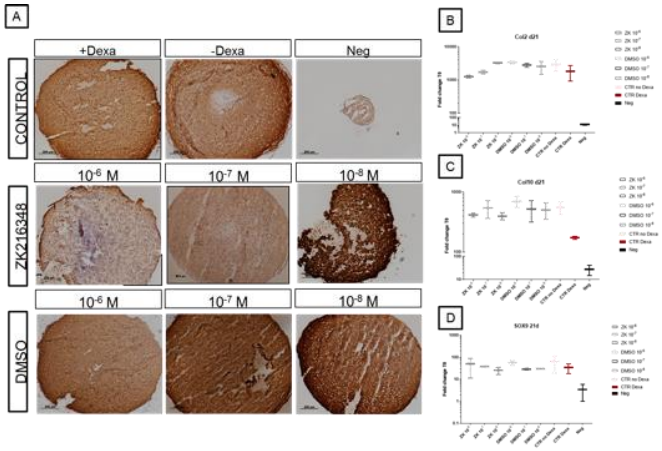
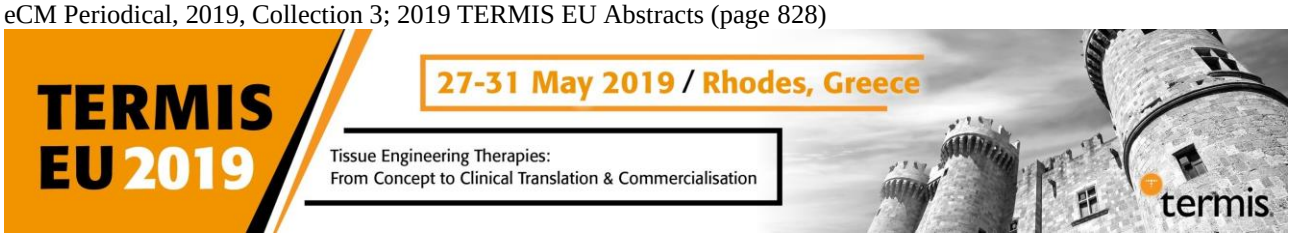


Figure 1: (A) IHC for Col2 on pellets at day 21, (B-D) relative mRNA quantification of COL2, COL10 and SOX9 at day 21.

ACKNOWLEDGEMENTS: Financial support was received from AO foundation.

REFERENCES

[1] Johnstone et al. Experimental cell research. 1998;238(1):265-72.
[2] Schäcke H et al. Proceedings of the National Academy of Sciences. 2004;101(1):227-32



Characterization of stem cell differentiation in a 3D Nichoid scaffold through label-free multimodal microscopy

V. Parodi[1], T. Zandrini[1,3], R. Osellame[3], D. Polli[2], G. Cerullo[2], M.T. Raimondi[1]
Presenting Author: Valentina Parodi, valentina.parodi@polimi.it
[1]Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, 20133 Milano, Italy [2]Department of Physics, Politecnico di Milano, 20133 Milano, Italy [3]Istituto di Fotonica e Nanotecnologie (IFN)-CNR, 20133 Milano, Italy

INTRODUCTION: 3D live bioimaging of in-vitro cell culture requires invasive staining which often affects the physiology of the investigated process and may result toxic in the long run. To avoid these effects, we used label-free multimodal microscopy, to observe mesenchymal stem cells (MSC) cultured inside a biomimetic 3D microscaffold called Nichoid, capable of maintaining cells stemness through architectural cues.

METHODS: Nichoid scaffolds were fabricated by multi-foci direct laser writing. Parallelization was achieved by adding a spatial light modulator to the laser fabrication setup. MSC at a density of 20,000 cells/cm^2 were seeded on the scaffolds and cultured for 3 weeks with adipogenic induction medium. Cells were observed directly in the scaffolds with coherent Raman microscopy and TPEF microscopy combined in the same setup, at different time points during culture. Images were elaborated with Matlab and Fiji-ImageJ.

RESULTS & DISCUSSION: Parallelized multi-foci fabrication of Nichoids allowed to greatly reduce the fabrication time of these micro structured substrates, overcoming the main limitation of this fabrication technique by increasing the number of samples available for cell culture in a short time. MSCs expansion and differentiation in the 3D environment showed lipid vesicle formation and their distribution through Nichoid pores. Coherent Raman microscopy allowed to clearly reconstruct fat droplets inside the microstructure in their whole volume, excluding any other signal. This technique allowed to filter out the autofluorescent signal of the Nichoid scaffold, that can be acquired by two-photon acquisition.

CONCLUSIONS: Our experiments suggest that multimodal label-free microscopy is a promising tool for observation of the evolution of cell culture in-vitro in a 3D environment.

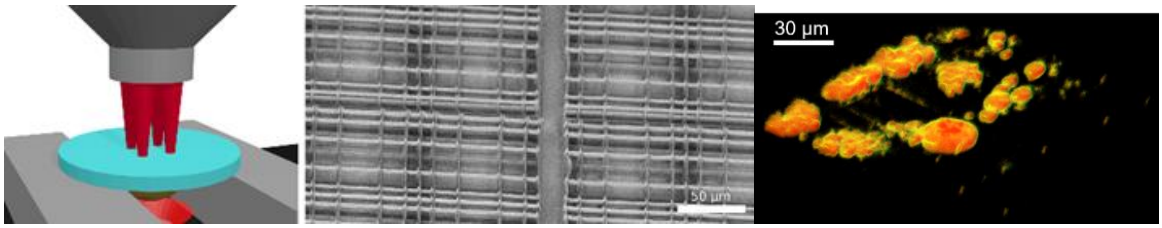
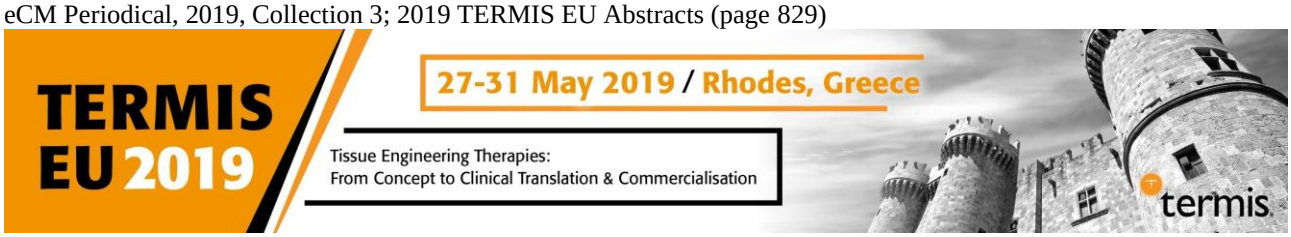


Figure 1: (left) Scheme of multi-foci laser direct writing. (middle) SEM of the 3D Nichoid microscaffold fabricated using this technique. (right) 3D rendering of a coherent Raman image (CARS). Lipid vesicles showed an intense signal (2850 cm^{-1}) and they are reconstructed from Z-stacks of MSCs cultured inside the Nichoid scaffold

ACKNOWLEDGEMENTS: This work received founding from the ERC grants NICHOIDS (GA 754467) and VIBRA (GA 648615).



Tendon biomimetic scaffolds influence amniotic epithelial stem cells genotype and phenotype towards the tenogenic lineage

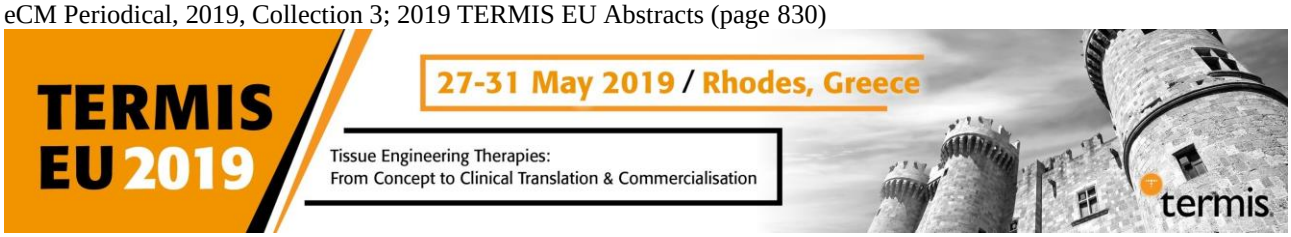
V. Russo[1], M. El Khatib[1], A. Mauro[1], P. Berardinelli[1], M.R. Citeroni[1], O. Di Giacinto[1], M. Turriani[1], M. Ancora[2], M. Schnabelrauch[3], R. Wyrwa[3], J. Weisser[3], B. Barboni[1]
Presenting Author: Valentina Russo, vrusso@unite.it
[1]Faculty of Bioscience and Agro-Food and Environmental Technology, Italy [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy [3]INNOVENT, Department of Biomaterials, Germany

INTRODUCTION: Tendon injuries have prompted researchers to find alternative solutions in the field of tissue engineering. Two aspects are crucial and must be taken into consideration to accelerate tendon regeneration: the design of a tendon biomimetic scaffold and the choice of the stem cell source. In this context, amniotic epithelial stem cells (AECs) represent an attractive cell source due to their high tenogenic attitude. Since it has been already demonstrated that PLGA is biocompatible for AECs; in this study we fabricated with this biopolymer a tendon biomimetic scaffold, with highly aligned fibers, and explored its biomechanical properties and tenogenic potential on AECs.

METHODS: Highly aligned and random, used as control, PLGA electrospun scaffolds (PLGA-HA and PLGA-R, respectively) were fabricated using the electrospinning technique. Their ultrastructure (SEM) and their mechanical properties were evaluated. Moreover, ovine AECs were seeded onto each scaffold type (0.5cm×1cm), and then cultured. Subsequently, we compared their viability (Calcein AM and propidium iodide), morphology and proliferation index (48h culture), as well as gene and protein expression for epithelial mesenchymal transition (EMT) (24h, 48h, and 8 days culture). Finally, it was also investigated the effect of fetal tendon co-culture [2] on teno-differentiation of AECs seeded onto both scaffold types (14 and 28 days of culture).

RESULTS & DISCUSSION: Scaffolds' mechanical properties showed that the alignment of fibers resulted in a higher tensile stress and strain compared to the PLGA-R scaffolds ($p<0.05$). AECs were viable on all type of PLGA scaffolds, whereas AECs proliferation was significantly higher on PLGA-R rather than the PLGA-HA scaffolds. Interestingly, cells on PLGA-HA, acquired an elongated morphology, spindle-like tenocyte, just after 24h culture, whereas AECs on PLGA-R retained their cobblestone typical morphology. Morphological changes were also genotypically and phenotypically confirmed by the upregulation of EMT-related genes (Vimentin and Snail mRNAs) ($p<0.05$), and α-SMA (mesenchymal marker) protein expression, in parallel to Cyto-8 (epithelial marker) downregulation of AECs seeded on PLGA-HA respect to PLGA-R engineered scaffolds ($p<0.05$). AECs cultivated on PLGA-HA expressed collagen type I (COL1), the main protein of a tendon, in their cytoplasm during the first 24h of culture. This result was also confirmed by analyzing the mature tendon specific markers COL1 and TNMD gene expressions that were significantly higher in PLGA-HA bio-hybrid scaffolds compared to those in PLGA-R ($p<0.05$). After 28 days' culture, COL1 and TNMD mRNAs reached their highest values compared to PLGA-R ($p<0.05$), and COLI protein became extracellular. Tenogenic AECs differentiation was even accelerated when PLGA-HA bio-hybrid scaffolds were co-cultured with fetal tendon explants. Indeed, cells were able to express COL1 and TNMD mRNAs at their highest levels and COLI already at day 14 on PLGA-HA scaffolds.

CONCLUSIONS: PLGA-HA electrospun scaffolds bio-mimic tendon biomechanics and structure. Indeed, these features are able to induce an early EMT and promote AECs teno-differentiative potential that is even accelerated when co-cultured with fetal tendon explants. In conclusion, PLGA-HA electrospun scaffolds have great teno-inductivity and could serve as tendon construct.



SilkBridge: A novel biomimetic and biocompatible silk-based nerve conduit

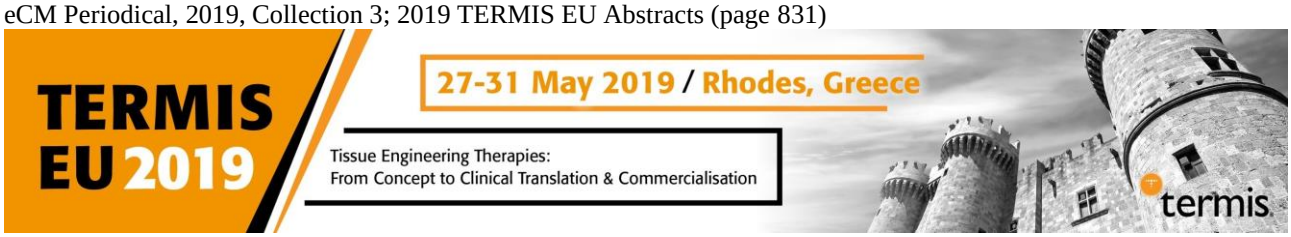
V. Vincoli[1], L. Muratori[2,3], G.A. Bassani[1], G. Ronchi[2,3], M. Biagiotti[1], A. Alessandrino[1], G. Freddi[1], S. Geuna[2,3]
Presenting Author: Valentina Vincoli, valentina@silkbiomaterials.com
[1]Silk Biomaterials Srl, Lomazzo (Co) Italy [2]Department of Clinical and Biological Sciences, University of Torino, Italy [3]Neuroscience Institute Cavalieri Ottolenghi, University of Torino, Italy

INTRODUCTION: In this study, silk fibroin was used to manufacture a nerve conduit (SilkBridge) characterized by a novel 3D architecture. Results of morphological, physical, and mechanical characterization of the SilkBridge are presented. In vitro cell interaction studies were performed using glial RT4-D6P2T and neuronal NSC34 cell lines. In vivo pilot trials for the repair of rat median nerve are also reported.

METHODS: The SilkBridge was manufactured according to a patented technology (WO 2016/067189 A1) and fully characterized. In vitro cell tests were performed using RT4-D6P2T, a schwannoma cell line and Mouse Motor Neuron NSC-34 cell line. After 2, 4, and 6 days (RT4-D6P2T cells) and after 5 days (NSC-34 cells) of culture cells were analyzed to evaluate adhesion, proliferation, and differentiation. In vivo nerve regeneration assays were performed on 4 adult females Wistar rats. A 12 mm long gap in the median nerve was repaired with SilkBridge. After 2 weeks post-operative, regenerated nerves analyzed by light and confocal microscopy, Masson's trichrome staining, immunohistochemistry, high resolution light and electron microscopy.

RESULTS & DISCUSSION: The 3 cm long SilkBridge with an inner diameter of 1.6 mm and a wall thickness 0.52 mm consists of two ES (inner and outer) and one TEX (middle) layers perfectly integrated at the structural and functional level, able to respond as a single body to mechanical stresses, without showing slipping or separation between adjacent layers, thus avoiding the emergence of biomechanical mismatch at the implantation site. The manufacturing technology conferred high compression strength (up to190 N/m at 60% strain under submersed state at 37°C), higher than commercial collagen-based nerve conduits, thus meeting requirements for clinical application, being able to withstand reported physiological and pathological compressive stresses. In vitro direct contact assays of glial RT4-D6P2T and neuronal NSC-34 cell lines with SilkBridge revealed that the material is capable to sustain cell proliferation; glial cells increased their density and organized themselves in a glial-like morphology. Neuronal NSC-34 cell line showed a natural tendency to differentiation, with a more pronounced neuritic elongation respect to the control conditions. In vivo tests at two weeks from surgery showed that several cell types colonized the lumen of SilkBridge. Cells and vessels were also visible between the different layers of the conduit wall. Moreover, the electron microscopy analysis allowed to reveal the presence of few regenerated myelinated fibers with a thin myelin sheath at proximal level.

CONCLUSIONS: SilkBridge shows an optimized balance between biomechanical and biological properties. Both in vitro and in vivo results demonstrated that SilkBridge is a valid and biomimetic substrate for cells to grow on. In vitro, nervous cells are able to differentiate and start the fundamental cellular regenerative activities, while in vivo studies showed a perfect cellular colonization of the conduit and the progressive growth of the regenerating nerve fibers. Long-term in vivo studies are in progress.



Hyaluronic acid hydrogels with prolonged antibacterial activity

V. Gribova[1,2], N.E. Vrana[3], P. Lavalle[1,2]
Presenting Author: Varvara Gribova, varvara.gribova@inserm.fr
[1]INSERM U1121, Biomaterials and Bioengineering, Strasbourg, France [2]Faculté de Chirurgie Dentaire, Université de Strasbourg, France [3]PROTiP Medical, Strasbourg, France

INTRODUCTION: Implantation of biomedical devices is often followed by excessive immune response to the implant, as well as by bacterial, yeast and fungal infections. Inflammation and infection may seriously affect implant functionalities and even lead to their failure. To address these issues, our group is developing antimicrobial and anti-inflammatory coatings made of polypeptides and polysaccharides [1-3]. Recently, we developed hyaluronic acid (HA) hydrogels that can be loaded with polyarginine (PAR), a homopolypeptide, and provide a long lasting antibacterial effect. Such hydrogels can be deposited onto wound dressings and mesh prosthesis to prevent infections, thus improving tissue regeneration and/or implant integration.

METHODS: To fabricate HA hydrogels, HA solution was cross-linked with BDDE (1,4-butanediol diglycidyl ether). After cross-linking, the hydrogels were cut into 4 mm diameter pieces and loaded with 0.05 to 1 mg/mL PAR. For antibacterial assays, Staphylococcus aureus suspension was added to the wells of a 24-well plate containing HA hydrogels loaded with PAR. Antibacterial effect of HA-PAR hydrogels was quantified by measuring $OD_{620\ nm}$ after 24h of bacterial culture. After each measurement, fresh bacterial suspension was added.

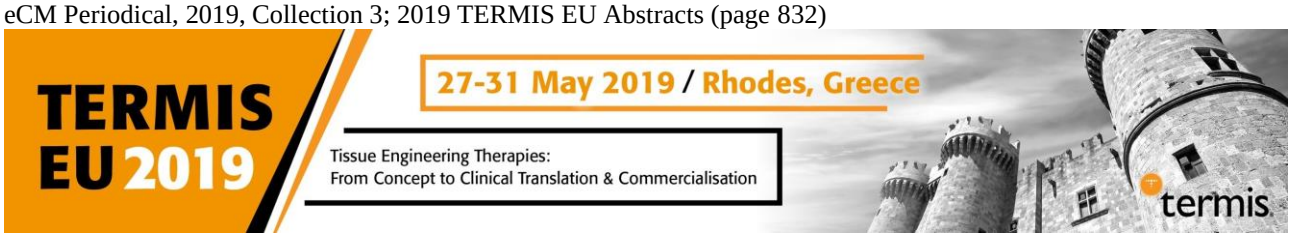
RESULTS & DISCUSSION: Loading of HA hydrogels with PAR was first characterized by confocal microscopy (data not shown). Then, antibacterial activity of HA-PAR hydrogels was studied. Hydrogels loaded with higher PAR concentrations (0.5 and 1 mg/mL) maintained their antibacterial activity for 8 days. Interestingly, the antibacterial activity depended on PAR length, PAR with 30 arginine residues (PAR30) being the most efficient.

CONCLUSIONS: These data illustrate the ability of HA-PAR hydrogels to ensure prolonged antibacterial activity.

ACKNOWLEDGEMENTS: Financial support was received from the European Union's Horizon 2020 research and innovation program under grant agreement No 760921.

REFERENCES

[1] Özçelik H et al. Adv Healthc Mater. 2015;4:2026–2036
[2] Mutschler A et al. Chem Mater. 2016;28(23):8700–8709
[3] Knopf-Marques H et al. ACS Omega. 2017;2(3):918–929.



Adaptation of 3D printing bioinks to mimic the mechanical properties of embryonic tissue for the purpose of vascularization

V.D. Trikalitis[1], F. Stein[1], J. Perea-Paizal[1], N. Salehi-Nik[1], Jeroen Rouwkema[1]
Presenting Author: Vasileios Trikalitis, v.trikalitis@utwente.nl
[1]Department of Biomechanical Engineering, Faculty of Engineering Technology, University of Twente, The Netherlands

INTRODUCTION: In order for engineered tissue grafts and eventually organs to successfully integrate in a clinical setting, a functional vascular network is imperative. Current artificial vascular networks are insufficient in scale, poorly controlled regarding their sprouting length and direction and current biofabrication methods fail so far to mimic the dynamic process which occurs in nature[1]. Recently, it has been observed that embryonic tissue acts as a jammed suspension which can be locally unjammed by cellular forces. Embryonic vascularization occurs within this mechanical environment. In this work in order to mimic the embryonic environment, we created granular bioinks of different material compositions, which can be locally unjammed by HUVEC/SMC cells for the purpose of 3D printing microvascular structures.

METHODS: For the patterning phase of the granular inks we tested blended Agarose-Collagen conjugate particles with a diameter of 25μm. For the preparation the alginate granular ink, in-Air microfluidics technology were produced with the In-Air Microfluidics technology, and subsequently coated with 5% w/v collagen solution to allow DCAM cell adhesion onto the particles. 1:1 Human umbilical vascular endothelial cells (HUVEC) / Smooth Muscle Cells (SMC) Spheroids, functioning as the 3D tissues were used to dope randomly the granular ink in different ratios to find the maximum concentration of cells/ml that can be printed. SMC and HUVECs cell high concentration pellet was also mixed with the granular ink particles in order to find the maximum concentration of cells that can be loaded in the interstitial space of the granular ink. Optical and confocal microscopy was employed in order to characterize the samples.

CONCLUSIONS: The ability to 3D print and pattern microvascular constructs through granule size and composition modification and which offers an embryo-like mechanical environment, establishes that granular inks are a potent alternative to their shear thinning hydrogel counterparts.

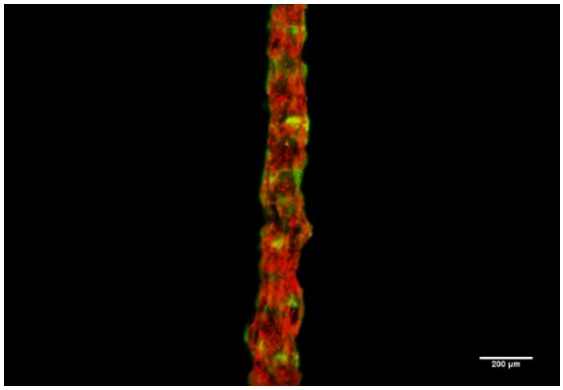
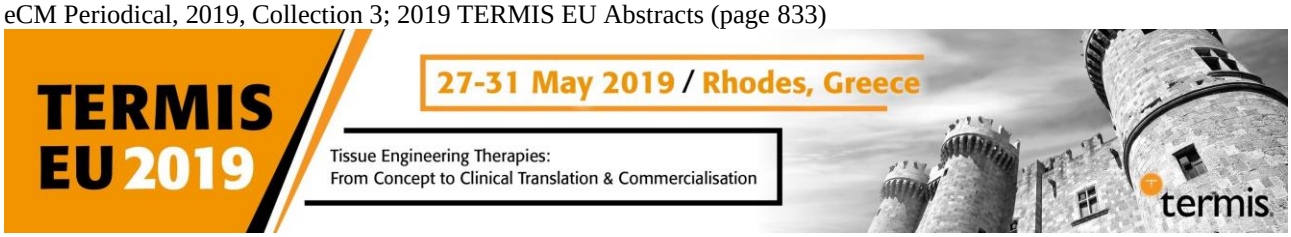


Figure 1: 3D printed granular agarose ink(red) doped with FITC dextran labelled 75μm 0.25% alginate microparticles(green) as proof of concept of a 3D printable doped granular ink.

ACKNOWLEDGEMENTS: The alginate particles were provided from IamFluidics B.V. This work is supported by an ERC Consolidator Grant under grant agreement no 724469.



Inverse electron-demand Diels-Alder HA-based hydrogel improves retinal explant imaging

V. Delplace,[1] A. Ortin-Martinez,[2] P. Nickerson,[2] A.E.G. Baker,[1] V. Wallace,[2] M.S. Shoichet[1]
Presenting Author: Vianney Delplace, vianney.delplace@gmail.com
[1]Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, ON, M5S 3E5, Canada [2]Donald K. Johnson Eye Institute, Krembil Research Institute University Health Network, Toronto, ON M5T 2S8, Canada

INTRODUCTION: Biohydrogel design is a booming field of research. Over the last 10 years, many new crosslinking strategies have been proposed to allow 3D cell culture, cell/drug delivery or bioprinting, holding the promise of versatile and user-friendly platforms to come for use by biologists and clinicians. [1-2] Yet, most of the existing systems (i) require external stimuli or catalysts/activating agents (e.g., photochemistry), (ii) are not entirely biorthogonal or compatible with physiological conditions (e.g., hydrazone, disulfide), or (iii) have inherent limitations such as limited stability or slow gelation rate (e.g., Diels-Alder). Thus, a hydrogel that would be fully tunable, fast-gelling, biocompatible and, yet, easy to synthesize and use, remains to be designed.

In this context, we hypothesized that a novel hyaluronan (HA)-based hydrogel that uses the inverse Electron-Demand Diels-Alder (iEDDA) «click » reaction as a crosslinking mechanism, would meet all the design criteria. As part of our strategy, we hypothesized that crosslinking two HA components together, as opposed to using a synthetic crosslinker such as polyethylene glycol, would greatly reduce the gelation time and offer new possibilities.

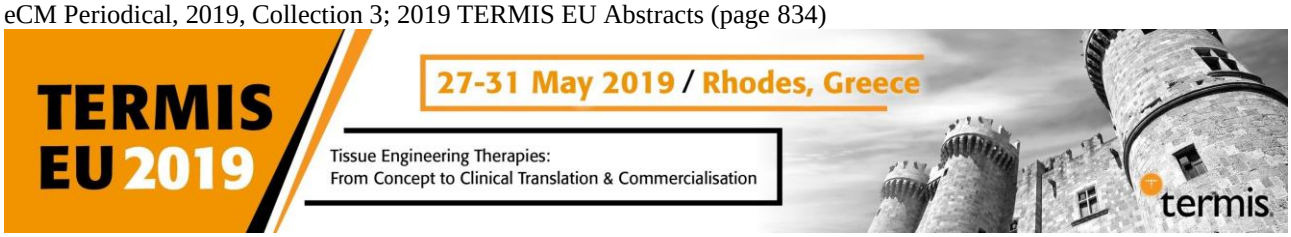
METHODS: The components of this new class of HA-based hydrogels were easily synthesized in single-step reactions from commercially available compounds. Optimizing the HA MW, component ratio and polymer content, we successfully designed fast-gelling hydrogels that form under physiological conditions. Interestingly, the optimized hydrogels were non-swelling, transparent and enzymatically biodegradable within days.

RESULTS & DISCUSSION: To demonstrate their versatility, the newly designed hydrogels were tested as matrices for 3D cell culture and explant imaging in the context of retinal degeneration investigation. Using two retinal cell types (αRPE-19 cells and $Nrl^{-/-}$ photoreceptor cells), iEDDA hydrogels were shown to be cytocompatible. The evaluation of 3D cell distribution confirmed the ease of mixing, and showed that optimal encapsulation requires gelation time < 10 min. The HA-based hydrogels were then tested for 2-photon retinal explant imaging. Compared to gold standard agarose thermogels, our new hydrogels best preserved retinal tissues, and prolonged remarkably the fluorescence of GFP-labeled explanted retinas, allowing 2-photon imaging over days vs hours. Finally, subcutaneous injections of iEDDA hydrogels were performed, confirming proper gelation in vivo and, thus, their possible use as drug/cell delivery vehicles. While we explored their potential in the context of innovative ophthalmological investigation, iEDDA HA-based hydrogels constitute a novel hydrogel platform for in vitro and in vivo applications.

ACKNOWLEDGEMENTS: CFREF through Medicine by Design (M.S.S., V.W.), and by CIHR and NSERC (M.S.S.).

REFERENCES

[1] Highley CB et al. Curr Opin Biotechnol. 2016, 40:35–40
[2] Freudenberg U et al. Adv Mater. 2016, 28, 8861–8891



Controlling the release from supported vesicle layers on LbL films

V. Domínguez-Arca[1], R. R. Costa[2,3], P. Taboada[4], R. L. Reis[2,3,5], G. Prieto[1], I. Pashkuleva[2,3]
Presenting Author: Vicente Domínguez-Arca vicente.domarc@gmail.com
[1]Grupo de Biofísica e Interfases, Universidade de Santiago de Compostela, Spain [2]3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal [3]ICVS/3B's, PT Government Associated Laboratory, Braga/Guimarães, Portugal [4]Grupo de Física de Coloides y Polímeros, Universidade de Santiago de Compostela, Spain, [5]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

INTRODUCTION: Liposomes are among the most used delivery vesicles. However, their adsorption on solid surfaces usually results in their spontaneous reorganization and formation of a supported lipid bilayer [1,2]. This reorganization compromises the compartmentalization and change the uptake and release of bioactive agents. We hypothesize that soft layer-by-layer (LbL) supports would minimize colloidal instability of liposomes upon adsorption and promote supported vesicular layers (SVL).

METHODS: Gold-coated quartz crystals were modified with 3 bilayers of cationic poly-L-lysine (PLL) and anionic hyaluronic acid (HA). A top layer of cationic liposomes loaded with Nile Red (d=120 nm, ζ-potential=10 mV) was added, followed by 2 HA/PLL bilayers. The assembly was monitored by quartz crystal microbalance with dissipation (QCM-D). The encapsulation efficiency (EE) and release to NaCl 0.15 M were quantified by fluorescence spectroscopy.

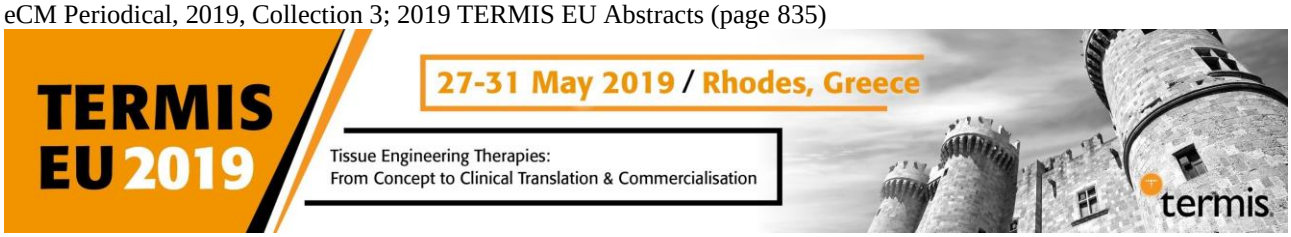
RESULTS & DISCUSSION: Our QCM-D data demonstrated that indeed a phospholipid bilayer is formed upon adsorption of the liposomes on bare gold crystals. When the deposition was carried out on soft LbL substrates instead, we observed adsorption of intact spherical liposomes. We determined an average EE of 5.4 ng/cm^2 for these constructs and 12% release after 1 h. Further coating of the constructed SVL with HA/PLL bilayers did not compromise their stability and increased the diffusion barrier: only 4% (three fold less) of Nile Red were released from the SVL coated with 2 HA/PLL bilayers. This result showcases that the release can be controlled by additional top layers but also the protective efficiency of this coating as compared to free liposomes in aqueous suspension (~29%).

CONCLUSIONS: SVLs show promise for compartmentalized 2D films where small amounts of biomolecules are encapsulated for diagnostic and therapy. Liposomes will allow expanding these systems further to bioactive hydrophilic species.

ACKNOWLEDGEMENTS: Portuguese FCT (PTDC/BTM-MAT/28327/2017, IF/00032/2013, SFRH/BPD/95446/2013), FSE and POCH, EU H2020 ELASTISLET (NMP-2014-646075), Spanish AEI, ERDF (project MAT2016-80266-R).

REFERENCES

[1] Kumar A. et al. Journal of Colloid and Interface Science 2016; 473:152-61
[2] Gillissen J. J. J. et al. Langmuir 2018; 34(1):503-111



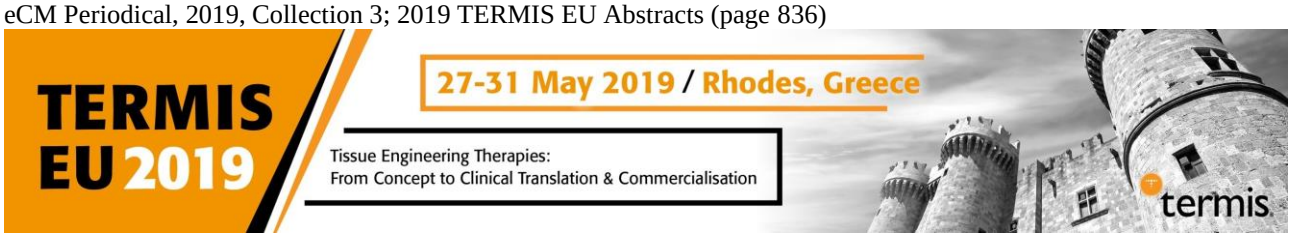
Automation of cellular and tissue therapy manufacturing

V. Ronfard[1,2,3], C. Holenstein[3], L. Barnes[3], Y. Bayon[4]
Presenting Author: Vincent Ronfard, vincent.ronfard@cutiss.swiss
[1]UNTHSC College System of Pharmacy, Fort Worth Texas USA [2]Cutiss AG, Zurich Switzerland [3] HairClone Manchester UK [4] Medtronic, Trevoux France

There is an uneven increasing worldwide shortage of transplantable tissues (in the US, approximately 100,000 individuals are waiting for a kidney transplant) (1) for the treatment of cancer and chronic disease conditions. And this trend is strengthened by the population aging, worldwide. The use of cellular or tissue engineering products is one solution to cover these unmet medical needs. They started to be developed since the late 70's, but still marginally used in the clinics, essentially due to high selling prices and the lack of reimbursement in most cases. Cellular or tissue engineering therapies are designated as ATMPs (Advanced Therapy Medicinal Products) in Europe or Biologic/RMATs (Regenerative Medicine Advanced Therapies) in the USA. They can be divided into two main groups according to the cell origin: (autologous) or (allogeneic). These two groups are differentiated by their mechanism of action, manufacturing processes and, business models. Autologous cell-based therapies are usually produced at small-scale (scale-down), in dedicated suites, with centralized or localized manufacturing facilities at point-of-care. Allogeneic therapies have the potential to be produced at large scale (scale-up) in large facilities where they can be stored and shipped to the users at the time of clinical needs for patients. While allogeneic manufacturing allows cost reduction due to large volume, both groups require numerous human resources which represent the most part of the cost of goods (COGs) for manufacturing. This prevents widespread clinical adoption of these therapies. The development of automatization is then seen as a relevant option for both reducing COGs and improving the quality of cells with higher batch to batch reproducibility. Automation should deliver three major outcomes: 1- Improving safety by using disposable closed system with fewer or no manual operations, 2- Improving cell culture efficiency by reducing manufacturing time while optimizing the quality of amplified cells, 3- Lowering cost by reducing culture time and human resources. Automation should play a key role in the development of cellular and tissue therapies by creating standardized processes that can be replicated everywhere, without neglecting the quality of the product and aligning with regulatory guidelines of ATMPs and, RMATs. And it is even seen as a cornerstone to guarantee the economic viability of tissue engineered and cell therapies to be delivered in a more cost-effective way. This is mandatory for reimbursement which should lead to larger market adoption.

REFERENCES

[1] https://www.eurekalert.org/pub_releases/2018-10/ason-dtu102218.php (American Society of nephrology, October 2018)



Microfluidic intestine-on-chip model induces stroma remodeling enhancing epithelial cell differentiation

V. De Gregorio[1], B. Corrado[2], S.Sibilio[1], G. Imparato[1]*, F. Urciuolo[3], P.A. Netti[1, 3, 4]
Presenting Author: Vincenza De Gregorio, vincenza.degregorio@iit.it
[1]Center for Advanced Biomaterials for HealthCare@CRIB, Istituto Italiano di Tecnologia, Naples, Italy [2]National Research Council, Institute for Microelectronics and Microsystems, Naples, Italy [3]Interdisciplinary Research Centre on Biomaterials (CRIB), University of Naples Federico II, Italy [4]Department of Chemical Materials and Industrial Production (DICMAPI) University of Naples Federico II, Italy

INTRODUCTION: There is a great interest in the development of in vitro alternatives to animal testing by using tissue-on-chip technologies [1]. Here, we developed an Intestine-on-a-chip (In-OC) platform used to increase the epithelial differentiation on a cell-instructive stromal equivalent fabricated by using a bottom-up approach technique [2, 3].

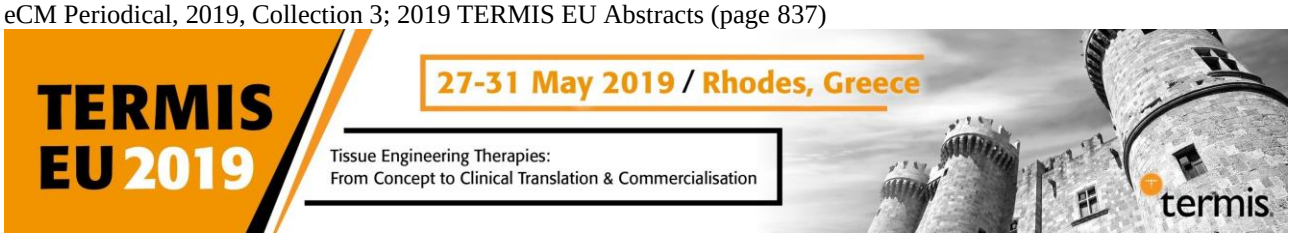
METHODS: Porous gelatin microscaffolds were used to culture human intestinal subepithelial myofibroblasts to make intestine-microtissues that were further used as building-blocks to construct a 3D stromal intestine equivalent (3D-ISE) [2]. To obtain the complete model, human colon-carcinoma cells (Caco-2) were layered and induced to differentiate on 3D-ISE into a properly designed chip. In-OCs were obtained by using replica molding of PDMS; a polycarbonate membrane (0.4µm pore) separated the lower layer consisting in a single chamber from the upper chamber on which is placed the 3D-ISE. Immuno-histotypical, ultra-structural analyses and Trans-epithelial electrical resistance (TEER) measurements were performed to assess the differentiation markers expression and to evaluate the epithelial barrier integrity. The auto-produced collagen signal was also examined by using multiphoton microscopy combined with second harmonic generation imaging.

RESULTS & DISCUSSION: The H/E staining of 3D-human intestine-equivalent showed that the villous-like thickness increased in In-OC samples respect to the static culture. The epithelial differentiation markers were analyzed by confocal (villin) and ultrastructural (SEM) analyses. TEER induction revealed the enhancement of the barrier properties during the dynamic culture confirmed by ZO-1 intensity enhancement. SHG signal indicated an increase in collagen remodeling in In-OC respect to the static conditions.

CONCLUSIONS: Taken together these data indicated that In-OC allows to obtain a better full thickness equivalent human tissues in a shorter time, that can be employed in the future as an effective alternative to animal model for drug testing.

REFERENCES

[1] Olson H et al. Regul Toxicol Pharmacol, 2000; 32,56–67
[2] De Gregorio V et al. Biotechnol Bioeng, 2018,115(4):1062-1075
[3] De Gregorio V et al. Acta Biomater, 2018, 81:43-59



3D heterotypic breast cancer model based on silk fibroin matrices

V. Brancato[1,2], B. Kundu[1,2], J. M. Oliveira [1,2, 3]; V. M. Correlo [1,2, 3]; R. L. Reis[1,2, 3]; S. C. Kundu[1,2]
Presenting Author: Virginia Brancato, virginia.brancato@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Drug development is a high-costly process and may last 7-10 years with no guarantees for the final drug approval and efficacy particularly for cancer treatment. There is an urgent need to develop the new tools to be able to copycat the tumor microenvironment and more realistic drug-screening platform to predict the drug efficacy and toxicity. Biomaterials support the growth of cancer cells together with the other cellular and acellular players, which offer different options to recapitulate the tumor micro-environment [1, 2]. In this work, we develop a 3D in vitro cancer model based on naturally-derived silk protein fibroin biomaterial. Fibroin shows good biocompatibility, suitable mechanical properties, and tunable biodegradability. Silk fibroin-derived freeze-dried scaffolds are produced in order to recapitulate faithfully the tumor microenvironment.

METHODS: 2 % silk fibroin solution is used to fabricate the scaffolds. The breast cancer cells (MCF-7) and normal mammary fibroblast are seeded or embedded on the scaffolds. The cell proliferation is monitored by means of Alamar blue assay at different time points for 14 days. 3D breast cancer model morphology is observed by confocal microscopy. The gene expression modulation concerning the extracellular matrix markers is evaluated by mean of qRT-PCR. Further, 3D bioengineered breast cancer models are treated with doxorubicin. The cell growth inhibition is assessed at 24, 48 and 72h post-treatments.

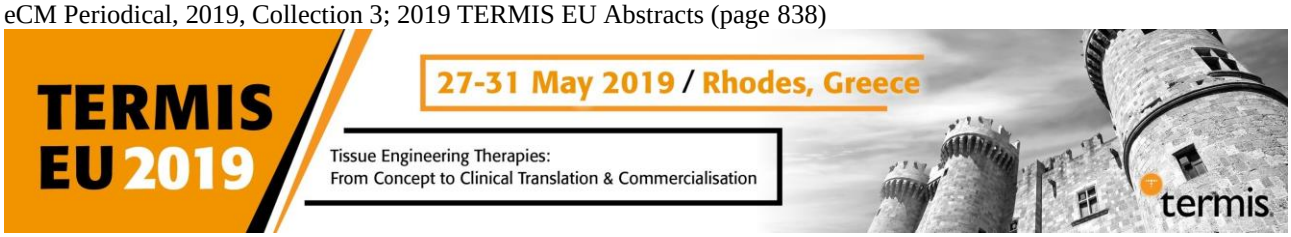
RESULTS & DISCUSSION: Silk fibroin scaffolds support the proliferation of the cancer cells and fibroblasts. The growth of cells is enhanced when cancer cells and fibroblasts are seeded together ($p<0.001$). Histological staining shows 3D cell organization. MMP-1, MMP-2, MMP-3, Col-1 and fibronectin expressions are upregulated in co-culture ($p<0.001$). Doxorubicin treatment reduces the proliferation of cells proliferation. However, higher doxorubicin concentration is needed to kill the cancer cells when they are in co-culture with fibroblasts.

CONCLUSIONS: The platforms designed may represent promising model for understanding the crosstalk between cancer cells, fibroblasts and extracellular matrix. The developed 3D in vitro tumor tissue model is expected to be an appropriate platform for drug-screening.

ACKNOWLEDGEMENTS: Financial support was received from the European Union Framework Programme for Research and Innovation Horizon 2020 under ERA-Chair grant agreement nº 668983 — FoReCaST and the FCT project BREAST-IT (IF/028168/2017)

REFERENCES

[1] Caballero, D. et al. Biomat. 2017;149:98–115.
[2] Rodrigues, T. et al. Pharmacol Ther. 2018;184:201–211.



In vivo survival of de novo bone generated by chondrogenically primed paediatric MSCs in the presence of an allogeneic humanised immune system

V. Palomares Cabeza[1,2,3], N. Fahy[1], J. Witte-Bouma[1], C. H. Kiernan[1], E. Wolvius[1], P. A. J. Brama[2], M. J. Hoogduijn[3], E. Farrell[1]
Presenting author: Virginia Palomares Cabeza, v.palomarescabeza@erasmusmc.nl
[1]Department of Oral and Maxillofacial Surgery, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands [2]School of Veterinary Medicine, University College Dublin, Dublin, Ireland [3]Department of Internal Medicine, Erasmus MC, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands

INTRODUCTION: Chondrogenically primed Mesenchymal Stem Cells (MSCs) have been shown to form bone in vivo via the endochondral ossification pathway [1]. Nevertheless, the outcome is highly influenced by donors' age and consequently MSC differentiation capacities. Here, we study the potential of chondrogenically primed paediatric MSCs (pMSCs) for bone formation in an immune allogeneic setting within a humanised mouse model.

METHODS: 21 day chondrogenically primed pMSCs were subcutaneously implanted in IL2Rγ-/-RAG2-/- immunodeficient mice for 8 weeks. After that time, $5x10^6$ allogeneic human peripheral blood mononuclear cells (PBMCs) were intraperitoneally injected for 4 weeks. Bone formation was quantified by uCT imaging for up to 12 weeks. Upon retrieval, bone structure was assessed in the constructs by immunohistochemistry (IHC). Human engraftment was determined in blood and spleen by FACS. Human immune cell infiltration in the constructs was analysed by IHC and PCR.

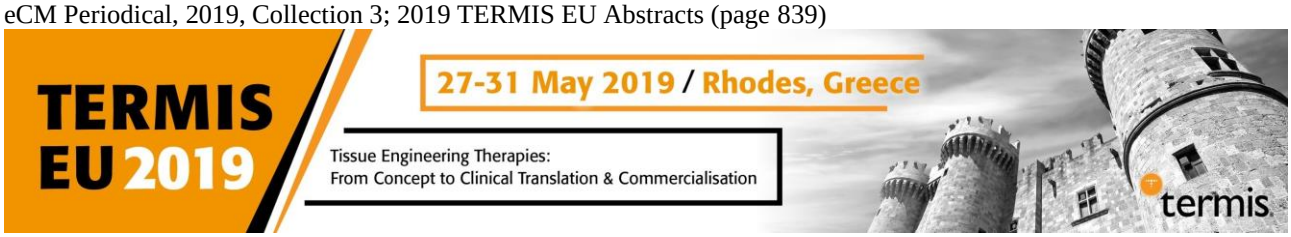
RESULTS & DISCUSSION: uCT scans showed bone formation at 8 weeks upon implantation until the endpoint of the experiment (12 weeks). FACS results revealed successful human PBMC engraftment in the humanised animals. $CD45^+$ human cells were detectable 4 weeks after humanisation in blood and spleens, and no significant differences were observed in the $CD45^+$ percentages between the humanised animals regardless of the presence of the bone constructs (2.6±0.7 versus 4.4±1.4 in blood, and 31.7±4.9 vs 36.9±3.9 in spleens). Upon retrieval of the constructs, H & E staining revealed a bone like structure. Human immune T cell infiltration in the humanised mice was identified by CD3 human IHC. In these animals, expression of CD4 and CD8 T cells was detected by PCR in the constructs, as well as CD8 cytotoxicity associated markers PRF-1 and GZMB.

CONCLUSIONS: Here, we show that chondrogenically primed pMSCs are able to form bone in vivo that is maintained in a successfully engrafted allogeneic humanised animal model. No evidence of a destructive immune response was detected in blood nor spleens. Nevertheless, human allogeneic T cells infiltrated in the bone structure of the allografts upon 4 weeks of humanisation. All together, these results illustrate the potential of pMSCs for bone formation via the endochondral ossification process in the presence of a donor mismatched immune system. However, further investigation into possible cytotoxic T cell local responses towards the allografts is required.

ACKNOWLEDGEMENTS: Financial support to carry this project was received from the AO foundation (**AOCMF-15-27F)**

REFERENCES

[1] Farrell et al. BMC Musculoskeletal Disorders 2011;12(31)



Engineering pseudo-3D and 3D osteosarcoma in vitro disease models in ECM-mimicking microenvironments

M. Monteiro[1], V.M. Gaspar[1], L.P. Ferreira[1], J.F. Mano[1]
Presenting Author: Vitor Gaspar, vm.gaspar@ua.pt
[1]Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

INTRODUCTION: The development of 3D in vitro cancer models that mimic the microenvironment of human tumors is a promising approach to accelerate the discovery of novel anti-tumor therapies [1,2]. Herein, we engineered 3D scaffold-based in vitro osteosarcoma cancer models by using GelMA and Matrigel™ ECM-mimicking hydrogel microenvironments and compared the physio-pathological features of cell-laden hydrogels (Pseudo-3D culture) with those of 3D spheroid-laden scaffolds in order to evaluate which platform could better mimic solid tumors and the biological cues of the in vivo tumor microenvironment.

METHODS: Osteosarcoma 3D cancer models were established by using the MG-63 (ATCC® CRL-1427™) cell line. Cancer cells were seeded within GelMA 10 % (w/v) and Matrigel™ hydrogels. At the same time, MG-63 3D spheroids were formed by the force-floating technique in ultra-low adhesion 96-well plates for 7 days and were then encapsulated in GelMA or Matrigel™ hydrogels. Cells and spheroids morphology, as well as necrotic core formation were evaluated in both in vitro models at 7, 14 and 21 days.

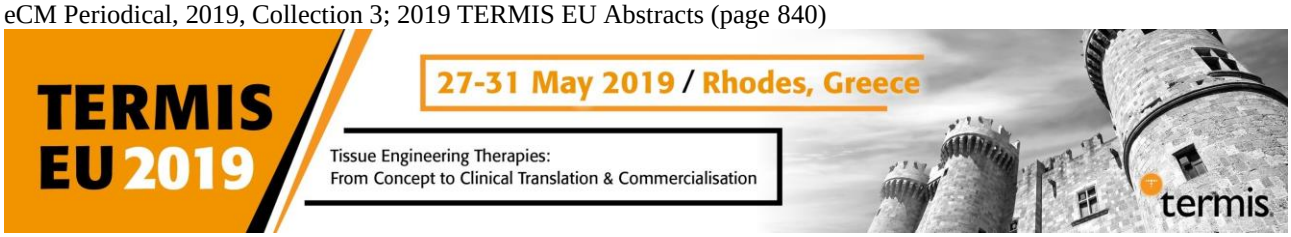
RESULTS & DISCUSSION: 3D tumor spheroids cultured in ECM-mimicking hydrogels maintained their spherical spheroidal morphology acquired during force-floating aggregation and exhibited necrotic core formation. On the other hand, the pseudo-3D culture did not exhibit the typically necrotic core and the single macrostructure of a solid tumor mass.

CONCLUSIONS: Overall, the pre-formed 3D spheroids laden in ECM-mimetic hydrogels recapitulate better solid tumor hallmarks and represent a more promising model for drug screening at later disease stages.

ACKNOWLEDGEMENTS: Financial support was received from ERC for project ATLAS (ERC-2014-ADG669858), for project Microbone (PoC-2017-7897602017), and from FCT as a Post-Doc grant (SFRH/BPD/119983/2016, Vítor Gaspar).

REFERENCES

[1] Ferreira, L.P. et al, Acta Biomater. 2018; 75:11-35.
[2] Ferreira, L.P. et al, Biomaterials. 2018; 185:155-173.



Gellan gum-based biomimetic hydrogels to regenerate the skeletal muscle

O. Alheib[1,2,3], L.P. da Silva[1,2], R.L. Reis[1,2,3], V.M. Correlo[1,2,3]
Presenting Author: Vitor M Correlo, vitorcorrelo@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: The progress of active methodology to heal a large-scale muscular defect is still a topic of noteworthy concern, and a possible alternative consists of an injectable dosage form to be directly inserted into muscle. Gellan Gum (GG) is commonly used hydrogel owing to its appropriate jellifying temperature (37ºC) [1]. However, its cell adhesive properties can be enhanced by adding sulfonyl functional group which in turn allow the binding of a desired cell adhesive peptide important for cell encapsulation traits. Herein, we propose GG-based hydrogels with tailorable physical-chemical and mechanical features, biofunctionalized with muscle-derived laminin peptide sequences specifically selected to mimic the muscle extracellular matrix and promote skeletal muscle cell binding.

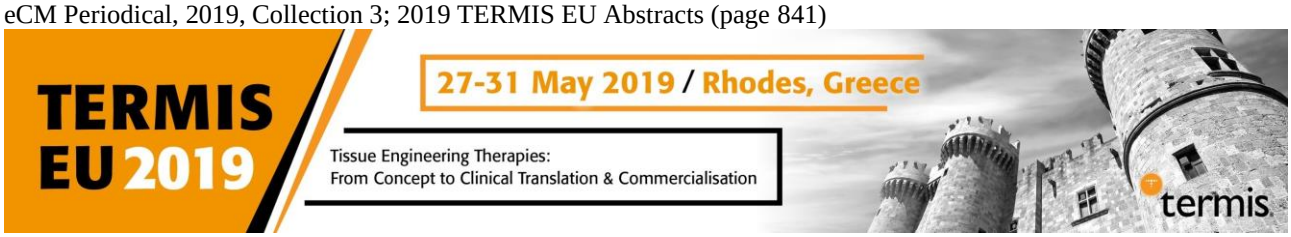
METHODS: GG polymer was chemically functionalized with divinyl sulfone (DVS) groups then reacted with three specific peptides (CIKVAVS (V), KNRLTIELEVRTC (T), RKRLQVQLSIRTC (Q)) by a one-step reaction. The efficiency of the reaction was determined by ^{1}H NMR and µ-BCA assay. Different hydrogel formulations were prepared by mixing GG-DVS-Peptide and GG at different amounts and characterized in terms of injectability, mechanical properties, swelling and molecules diffusion. Murine skeletal muscle cells (c2c12) were encapsulated within hydrogels, and cell viability, adhesion, spreading and differentiation was evaluated after 1, 7, 14 and 21 days, by phalloidin, calcein/PI staining and immunocytochemistry for Myosin Heavy Chain (MYH).

RESULTS & DISCUSSION: Biofunctionalization of GGDVS with X, Y and Z peptides was confirmed by ^{1}H NMR and µBCA yielding a conjugation efficiency of V=47.34%, T=31.57% and Q=92.65%, respectively. Hydrogel's properties varied with the formulation; all hydrogel formulations were injectable, allowed the diffusion of molecules up to 250 kDa, presented a deswelling ratio up to 15% and an elastic modulus ranging from 10 to 100 kPa. Cell death (<50%) was detected after 1 day of encapsulation but the majority of the cells were alive after 7 days. Since day 1, cells started spreading most remarkably in hydrogels with higher polymer amount and depicting higher mechanical properties. In these hydrogel formulations, cells proliferated and elongated showing MHC expression at day 14.

CONCLUSIONS: GG-based hydrogels showed tailorable physical-chemical and mechanical properties, as well as cell-binding properties that improved skeletal muscle cell performance.

REFERENCES

[1] Oliveira JT. J Biomed Mater Res A 2010; 93, 852.



Cartilage derived extracellular matrix incorporated silk fibroin hybrid scaffolds for endochondral ossification mediated bone tissue regeneration

V. Jeyakumar[1], N. Amraish[2], E. Niculescu-Morsza[1], C. Bauer[1], D. Pahr[2], S. Nehrer[1]
Presenting Author: Vivek Jeyakumar, vivek.jeyakumar@donau-uni.ac.at
[1]Center for Regenerative medicine, Danube University Krems, Krems, Austria [2]Department of Anatomy and Biomechanics, Karl Landsteiner University of Health Sciences, Krems, Austria

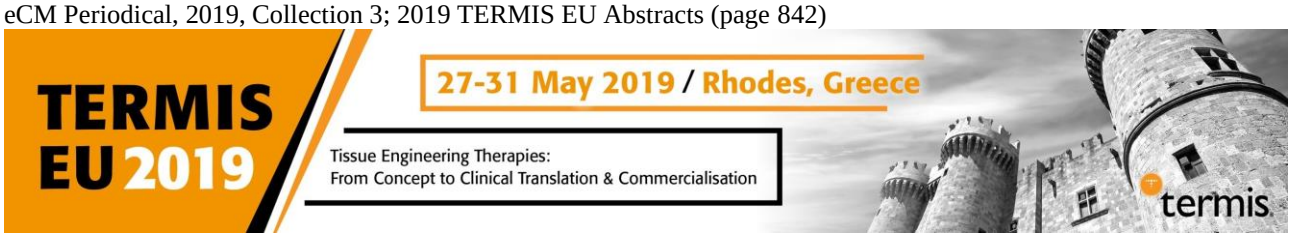
INTRODUCTION: Hypertrophic chondrocytes mediate the enduring pathway for endochondral bone formation. However, challenges in tissue-engineered bone constructs include functionality under the mechanical environment inside in vivo conditions. We aim to fabricate a mechanically stable Silk fibroin (SF) carrier incorporating decellularized cartilage derived extracellular matrix (CD-ECM) and hypertrophic chondrocytes as a model of endochondral ossification and characterize superior bone-like tissue formation non-destructively in the case of in vitro studies to find the optimal time point when the constructs should be implanted.

METHODS: Human bone marrow stem cells (hBMSC's) were seeded onto CD-ECM/SF or SF constructs and primed for 2-week chondrogenesis following a further 8-week hypertrophy or 4-week hypertrophy + 2-week osteogenesis in differentiation medium. Biochemical assays, SEM/EDX, RT-qPCR, Biomechanical tests followed by non-destructive μCT scanning were used as methods at 4 and 8 weeks to determine hypertrophy mediated ossification.

RESULTS & DISCUSSION: Calcium deposition biochemically determined increased significantly from 4-8 weeks in both SF and CD-ECM/SF constructs ($p < 0.05$) and retention of sGAG's were observed in CD-ECM/SF constructs. SEM/EDX revealed calcium and phosphate crystal localization by hBMSC's under all conditions. Compressive modulus reached a maximum of 70 KPa in 8-week hypertrophic induction group than 4-week hypertrophy+2-week osteogenesis group ($p<0.05$). μCT scanning at 8 weeks indicated a cloud of denser minerals in groups only induced for 8-week hypertrophic induction. Gene expression by RT-qPCR revealed that hBMSC's expressed hypertrophic markers VEGF, COL10, RUNX2 but absence of early hypertrophic marker ChM1 and presence of later hypertrophic marker TSBS1. Further induction of osteogenesis indicated presence of osteogenic markers ALPL, IBSP, OSX under all conditions.

CONCLUSIONS: Our data indicates a new method to prime hBMSC'S into late hypertrophic stage in vitro in mechanically stable constructs for endochondral ossification mediated bone regeneration.

ACKNOWLEDGEMENTS: LSC16-024 from NÖ Forschungs- und Bildungsges.m.b.H (NFB) and the provinical government of Lower Austria.



Does in vitro mechanical stimulation mimic in vivo loading of the Achilles tendon in mice? Pilot study

V. Fleischhacker[1,3], S. Minkwitz[1], F. Klatte-Schulz[1], M. Rummler[2], A. Seliger[1], B. M. Willie[2], B. Wildemann[1,3]
Presenting Author: Viviane Fleischhacker, viviane.fleischhacker@med.uni-jena.de
[1]Berlin-Brandenburg Center for Regenerative Therapies, Julius Wolff Institute, Charité-Universitätsmedizin Berlin, Germany [2]Department of Pediatric Surgery, McGill University, Shriners Hospital for Children-Canada, Montreal, Canada [3]Experimental Trauma Surgery, Universitätsklinikum Jena, Jena, Germany

INTRODUCTION: Tendons require mechanical loading to maintain the homeostasis and function [1]. This study aimed to investigate if mechanical stimulation of tenocytes in 2-dimensional (2D) monoculture is able to mimic loading of the Achilles tendon in an in vivo mouse model.

METHODS: The left tibiae of female BALB/c mice (10 weeks) (n=14) were subjected to axial compression (10N, 216 cycles/day at 4 Hz) for 3 weeks. Right tibiae served as non-loaded control. Tenocytes were isolated from Achilles tendons of female BL6J mice (26 weeks) and mechanically loaded (216 cycles, 4% stretch and 4 Hz) for various periods. Histology (n=8) was performed for in vivo loaded tendons. Gene expression was analyzed with qPCR (in vivo and in vitro) (n=6).

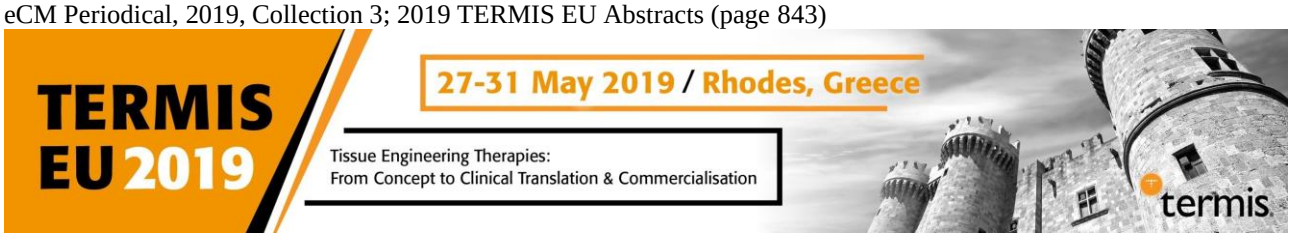
RESULTS & DISCUSSION: Histology: no differences in cell morphology or collagen fiber network in the in vivo loaded tendons. The expression of Scleraxis (Scx) and Tenomodulin (Tnmd) showed no significant changes between in vivo and in vitro stimulation (Fig. 1B). Significant changes in expression were found for Collagen type I (Col1), Col3 and Metalloproteinase 2 (MMP2) (Fig. 1C).

CONCLUSIONS: This study showed that gene expression of tendon markers was similar, whereas significant changes in the extracellular matrix compartment were detected between in vivo and in vitro loading. The differences might be explained by the 2D in vitro set-up [2]. In this study young and adult mice of different genetic strains were compared. It is known that age can affect gene expression in bone cells in response to mechanical loading [3], which cannot be excluded for tendon. Moreover, the loading regimes differ in duration of loading, which might also influenced the outcomes. However, this first pilot study is important for the development of an in vitro stimulation set-up of tenocytes that mimic in vivo characteristics.

ACKNOWLEDGEMENTS: The project was funded by the Berlin-Brandenburg Center for Regenerative Therapies (BCRT; Bundesministerium für Bildung und Forschung (BMBF) grant 1315848A).

REFERENCES

[1] Galloway M. et al. J. Bone Joint Surg. 2013; 95(17): 1620–1628
[2] Zhang J. et al. PLos One 2013; 8(8):e71740
[3] Holguin et al. JBMR 2016 31(12):2215-2226.



Tissue engineered constructs for the treatment of spinal cord injury. The results of in vivo study on nonhuman primates

V.P. Baklaushev[1], V.G. Bogush[2], O.V. Durov[1], V.A. Kalsin[1], M.A. Konoplyannikov[1], J-E. Ahlfors[3]
Presenting Author: Vladimir Baklaushev, serpoff@gmail.com
[1]Federal Research and Clinical Center of Specialized Medical Care and Medical Technologies FMBA of Russia Moscow, Russia [2]Scientific Center of Russian Federation "Research Institute for Genetics and Selection of Industrial Microorganisms", Moscow, Russia [3]New World Laboratories Inc. Laval, Canada

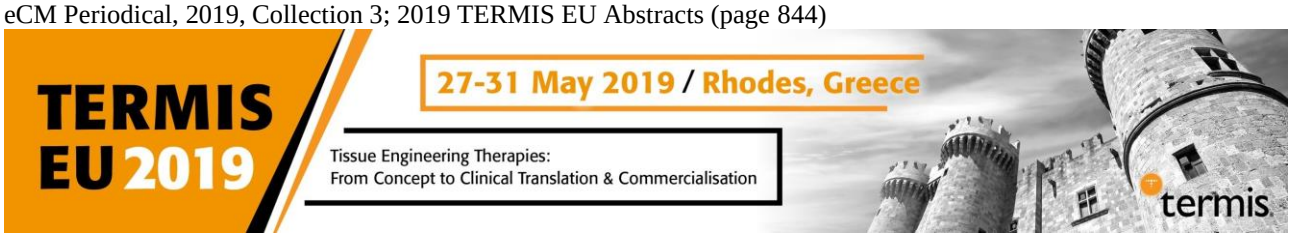
INTRODUCTION: One of the most promising experimental approaches for spinal cord regeneration is transplantation of neural stem cells-containing biomimetic hydrogel with the ability to adjust the axon's directionality.

METHODS: In vitro study was conducted using directly reprogrammed neural precursor cells (drNPCs) obtained as described earlier (J.-E. Ahlfors et al., 2012, 2018), that were cultured in PRP-based hydrogel. Anisotropically structured complex scaffold has been prepared by electospinning using mixture of recombinant rS1/9 spidroin and PCL. The in vivo experiments was conducted on 12 male rhesus macaques (2 healthy and 10 with control complete spinal cord modeled as described earlier (VP Baklaushev et. al, 2018).

RESULTS & DISCUSSION: We have designed a novel two-component matrix (SPRPix) for the encapsulation of drNPCs. The combination of PRP and spidroin promoted drNPC proliferation with the formation of neural tissue organoids and dramatically activated neurogenesis. Differentiation of drNPCs generated large numbers of βIII-tubulin and MAP2 positive neurons as well as some GFAP-positive astrocytes, which likely had a neuronal supporting function. Interestingly the SPRPix microfibrils appeared to provide strong guidance cues as the differentiating neurons oriented their processes parallel to them. Implantation of the SPRPix matrix containing human drNPCs into the brain and spinal cord of healthy Rhesus macaque monkeys showed good biocompatibility: no astroglial and microglial reaction was present around the implanted construct. Importantly, the human drNPCs survived for the 3 month study period and differentiated into MAP2 positive neurons. Monkeys with SCI after implantation of drNPCs demonstrated the recovery of paralyzed hindlimb as well as recovery of SSEP and MEP of injured pathways.

CONCLUSIONS: Tissue engineered constructs based on SPRPix exhibits important attributes that warrant further examination in spinal cord injury treatment.

ACKNOWLEDGEMENTS: The study was financially supported by the Russian Science Foundation, Grant No. 16-15-10432, and New World Laboratories Inc.



Magnetic levitational bioassembly in space

V. Parfenov[1], Y. Khesuani[1], S. Petrov[1], E. Koudan[1], E. Nezhurina[1], F. Pereira[1], A. Krokhmal[1], A. Gryadunova[1], E. Bulanova[1], M. Vasiliev[2], K. Brakke[3], S. Belousov[4], T. Grigoriev[4], E. Osidak[4], P. Karalkin[1], O. Petrov[2], V. Mironov[1]
Presenting Author: Vladislav Parfenov, vapar@mail.ru
[1]Laboratory for Biotechnological Research "3D Bioprinting Solutions", Moscow, Russia [2]Joint Institute for High Temperatures, Russian Academy of Sciences, Moscow, Russia [3]Susquehanna University, Selinsgrove, Pennsylvania 17870, USA [4]National Research Center «Kurchatov Institute», Moscow, Russia

INTRODUCTION: Tissue engineering promises to solve acute clinical problem – a shortage of human tissues and organs for transplantation. Traditional tissue engineering is based on using synthetic or natural scaffolds as temporal and removable supports or templetes [1]. Magnetic levitational bioassembly of 3D tissue constructs represents a novel rapidly emerging scaffold-free and label-free approach and alternative conceptual advance in tissue engineering [2,3].

METHODS: The magnetic levitational bioassembly of 3D tissue construct from tissue spheroid consists of a directional levitational assembly under the action of magnetic forces of the construct from tissue spheroids, followed by fusion of tissue spheroids collected at a specific location.

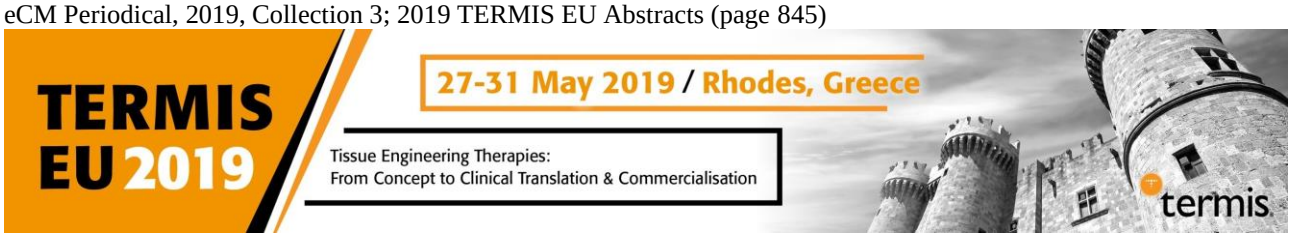
RESULTS & DISCUSSION: New magnetic bioassembler have been designed, developed and certified for life space research. Tissue spheroids (thyrospheres and chondrospheres) and magnetic bioprinter were delivered on the «Soyuz MS-11» ship to the Russian segment of the international space station in a temperature-sensitive non-adhesive hydrogel. From tissue spheroids were fabricated in a magnetic printer by 6 constructs from thyrospheres and chondrospheres. The constructs fused within 24 hours for the thyrospheres and 48 hours for the chondrospheres in a magnet field at of 37°C. Then, the fused constructs were removed from the magnetic printer and fixed with formalin. Finally, the cuvettes with the constructs were sent to Earth where histology and histochemistry of the constructs were performed.

CONCLUSIONS: Scaffold-free and label-free magnetic levitational bioassembly of 3D tissue constructs from tissue spheroids (chondrospheres) have been performed first time at the condition of microgravity.

ACKNOWLEDGEMENTS: The project was implemented with the assistance of the «Roscosmos» State Corporation, Russia.

REFERENCES

[1] Parfenov V et al. Biofabrication. 2018; 10(3):034104
[2] Tasoglu S et al. Adv Healthc Mater. 2015; 4(10):1469-76
[3] Bulanova E et al. Biofabrication. 2017; 9 034105



Establishment of patient-specific cancer cell lines from colon cancer tissues by membrane filtration method via nylon mesh filter and PLGA-silk screen membranes

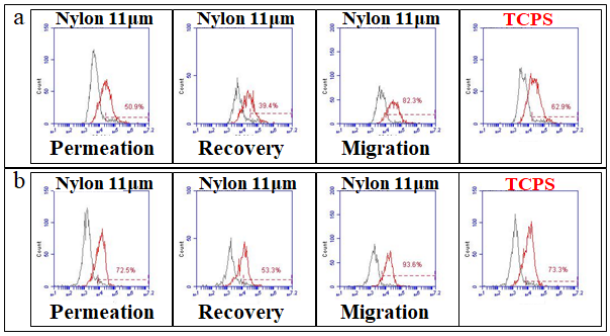
W.L. Huang[1], T.Y. Chen[1], A. Higuchi[1,2]
Presenting Author: Wei Lun Huang, allen840528@gmail.com
[1]Department of Chemical and Materials Engineering, National Central University, Taoyuan, Taiwan
[2]Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama, Japan

INTRODUCTION: Cancer stem cells (CSCs) typically comprise 1%–5% of the total tumor cell population. This cell population is considered to be primarily responsible for tumor initiation, growth, and metastasis. Therefore, we develop membrane filtration and migration method to target and purify rare primary CSCs based on their high migration mobility characteristics compared with other tissue cells. Further, establish the primary colon cancer cell line

METHODS: Nylon mesh filter with pore size of 11 μm and 20 μm and PLGA-silk screen membrane with different PLGA (Sigma-Aldrich) concentration 3%, 5%, and 10% are the membrane we used in the experiment. Subsequently, primary human colon cancer cells and colon cancer cell line will pass through the membrane and then be divided into three fractions. Cells without any treatment were used as control in this experiment. Cancer stem cells marker, CD44 and CD133 will be analyzed by flow cytometry. And the malignancy index will evaluate by soft agarose colony forming assay.

RESULTS & DISCUSSION: Flow cytometry analysis illustrated that colon cancer stem cell surface markers CD44 and CD133 expression in the cells from migration method were higher than the cells from permeation and recovery solutions (Fig. 1). Soft agarose colony forming assay revealed the cells from migration method were able to form bigger size of the colonies compared to the cells from permeation and recovery parts (Fig. 2).

CONCLUSIONS: The data showed that the membrane filtration method can be used to target and purify cancer stem cells. It would be a great benefit for establishing patient-specific cancer cell line.



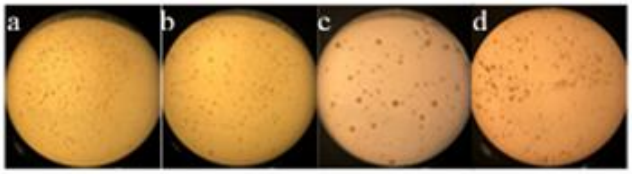
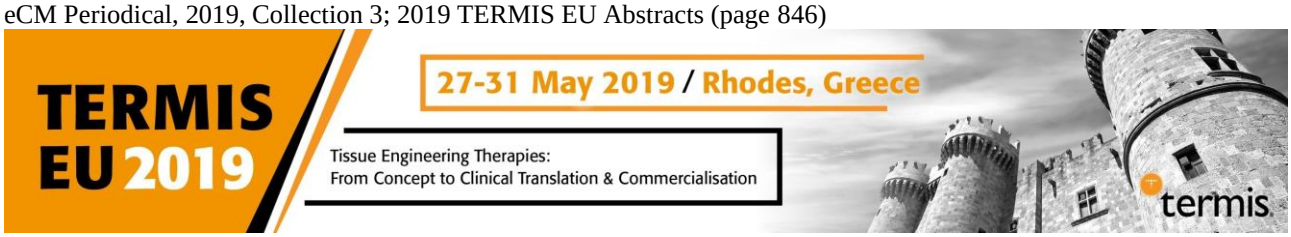


Figure 1: (left) Flow cytometry analysis. The cells from three fraction and non-treated cells were evaluated by colon cancer stem cell surface marker (a) CD44 and (b) CD133 expression. **Figure 2:** (right) Soft agarose colony forming assay. The cells were seeded on soft agarose coating dishes. After 2-4 weeks, colony formation was counted for data analysis: (a) cells in permeation part, (b) cells in recovery part, (c) cells in migration part used Nylon 11μm membranes and (d) non-treated cells.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Science and Technology (MOST 107-2119-M-008-002).



Comparison and evaluation of tissue-engineered 3D tumor model based on different resources of biomaterials

W. Li[1], Xiangqin Li[1], T. Liu[1], K. Song[1*]
Presenting Author: Wenfang Li, linan0072@163.com
[1]State Key Laboratory of Fine Chemicals, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian 116024, China

INTRODUCTION: Tissue-engineered tumor models can better mimic in vivo tumor microenvironment and provide a more representative platform for in vitro studies and drug discovery [1]. Herein, we developed tissue-engineered breast cancer models based on three different types of materials, and further evaluated cell viability and the responsiveness to chemotherapeutic agent.

METHODS: Three derived scaffolds (decellularized lung scaffold, chitosan/gelatin scaffold, and poly-L-lactic acid scaffold) were prepared. A total of 10^5 cells were seeded on both sides of each scaffold in 24-well plates. Dead/Live staining was conducted to assess cell viability in three scaffolds. SEM and H&E were conducted to evaluate cell growth and distribution. Ki67 immunofluorescence (IF) was determined to evaluate cell proliferation. The cell response to anti-cancer drug 5-fluorouridine (5-FU) was examined in 2D and 3D culture using Dead/Live staining and CCK-8 assay.

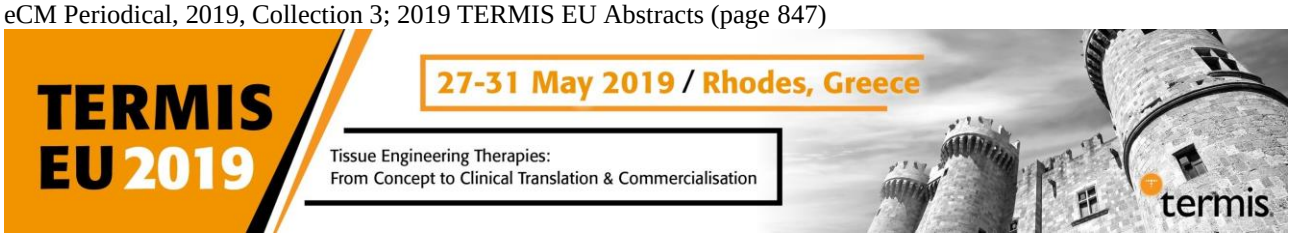
RESULTS & DISCUSSION: Dead/Live staining indicated good biocompatibility of the three scaffolds. SEM showed cells formed contracted tumor spheroids in three scaffolds which is significant difference compared to 2D culture. H&E assay showed cell well distributed in the skeleton and aperture of three scaffolds. IF staining showed a high expression of Ki67 in MCF-7 cells, suggesting favorable proliferative activity of cells in scaffolds. The 3D structure of tumor model was visualized by the positive Ki67, F-actin, and DAPI staining, which well depicted protein expression and cell distribution with clear architecture of three scaffolds. Cells grown in 3D exhibit stronger drug resistance compared to their 2D counterparts, no significant difference was identified in three scaffolds.

CONCLUSIONS: Overall, these 3D culture systems provide a reliable and cost-effective platform to study tumor biology and drug discovery.

ACKNOWLEDGEMENTS: Financial support was received from the National Natural Science Foundation of China (31670978/21676041), and the Natural Science Foundation of Liaoning (20180510028).

REFERENCES

[1] Rodenhizer D et al. Adv. Healthc. Mater. 2018; 7(8): 1701174.



Controlled delivery of IL-1Ra to enhance long bone healing

W. Lackington[1], M. Hildebrand[1], M. Alini[1], S. Zeiter[1], M. Stoddart and K. Thompson[1]
Presenting Author: William Lackington, william.lackington@aofoundation.org
[1]AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland

INTRODUCTION: Despite the intrinsic healing capacity of bone and modern orthopedic fixation technologies, large defects do not always heal successfully resulting in fracture non-union. Modulating the immune response to fractures might enable innovative solutions to enhance the repair of large bone defects. Administration of interleukin-1 receptor antagonist (IL-1Ra) can enhance bone healing in mouse calvarium [1]; however, its therapeutic efficacy in long bone defects, which heal by endochondral ossification, and its optimal course of administration have yet to be identified. Thus, the overall objective of this study is to investigate the efficacy of IL-1Ra to enhance long bone healing.

METHODS: To determine the optimal mechanical fixation to promote endochondral ossification in an osteotomy model, 2 mm femoral osteotomies were carried out in Fischer 344 rats and fixed with either a thin 1.25 mm PEEK plate, a thicker 2.00 mm PEEK plate, or an external fixator, while histology was carried out on samples collected from animals euthanized 10 days post-surgery to check for callus formation. 4-point bending and torsional tests were also carried out on the individual fixators to determine their relative stiffness. To assess endogenous IL-1β and IL-1Ra levels up to 14 days post-osteotomy, a second round of osteotomies was carried out using the optimal plate and cytokine levels were assessed 3, 7, and 14-days post-surgery using ELISA. To assess the healing capacity of IL-1Ra, a thermoresponsive hyaluronic acid-based scaffold (T-HyA) is currently under development for the controlled delivery IL-1Ra.

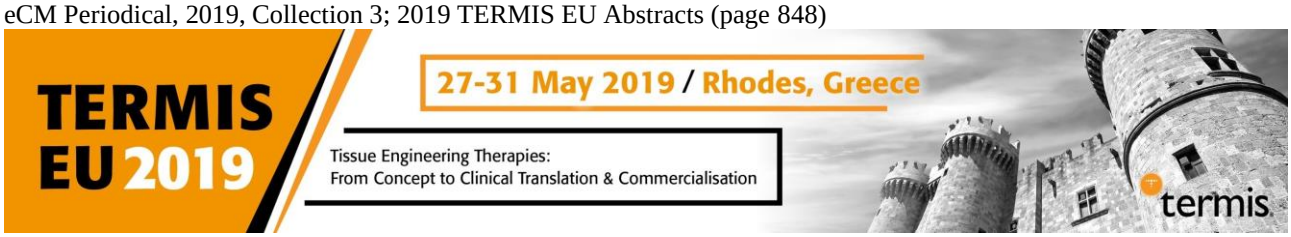
RESULTS & DISCUSSION: Fixation of a 2 mm femoral osteotomy using a 1.25 mm PEEK plate led to significantly higher levels of systemic IL-1β and greater callus formation observed 10 days post-surgery compared to when fixed using a 2.00 mm PEEK plate, or an external fixator. Bending and torsional testing of the individual plates shows that the 1.25 mm plate is less stiff compared to the thicker 2.00 mm plate and the external fixator. Local IL-1β concentration was seen to decrease 7 days post-surgery, while local IL-1Ra concentration drastically increases afterwards. Release kinetics of IL-1Ra from T-HyA show that after a small burst release, >80% of the loaded protein is retained and released only after enzymatic digestion of T-HyA, while the bioactivity of the retained IL-1Ra was confirmed using HEK-Blue-IL-1β cells.

CONCLUSIONS: A long bone defect model has been optimized to mechanically induce healing via endochondral ossification while a thermoresponsive natural polymer-based scaffold has been developed for the controlled delivery of IL-1Ra. Future work will assess its efficacy to enhance healing

ACKNOWLEDGEMENTS: AO Foundation

REFERENCES

[1] Nat Commun. 2016 Mar 22;7:11051



Nanotopography and substrate stiffness enhances mesenchymal stem cell chondrogenesis for articular cartilage regeneration

W. Yingnan[1,2], V. Deslin[1], X. Ren[1,2], C.S. Lee[3], F.L. Yap[3], Y. Zheng[1], L.E. Hin[1,2]
Presenting Author: Wu Yingnan, doswuy@nus.edu.sg
[1]NUS Tissue Engineering Program, Life science Institute, National University of Singapore
[2]Department of Orthopeadic Surgery, YLL School of medicine, National University of Singapore
[3]Institute of Materials Research and Engineering, Agency for Science, Technology and Research, Singapore

INTRODUCTION: Adult articular cartilage mechanical functionality is dependent on the unique zonal organization of its tissue. The phenotype of cartilage derived from MSCs has been reported to be influenced by the microenvironmental biophysical cues, such as the surface topography and substrate stiffness [1]. In this study, the application of these pre- differentiated cells for cartilage repair was explored with the in vitro optimization

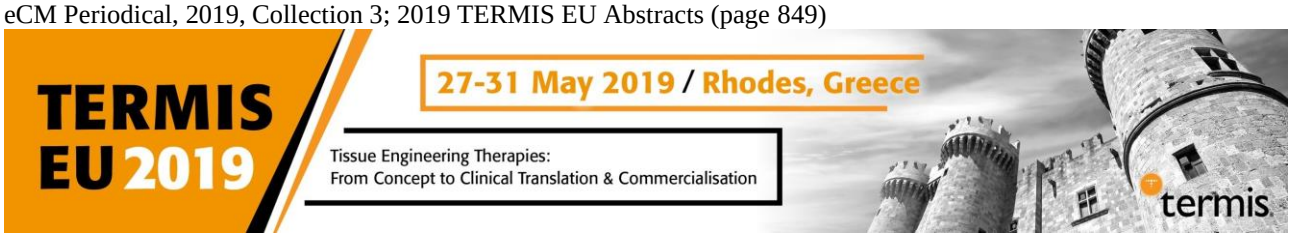
METHODS: Specific nano-topographic patterns on polymeric substrate were generated by by nano-thermal imprinting on the PCL surface. Human bone marrow MSCs seeded on these surfaces were subjected to chondrogenic differentiation and the phenotypic outcome of the differentiated cells was analysis. The ability of these pre-differentiated MSCs on different nano-topographic surfaces to form zonal cartilage was verified in in vitro 3D hydrogel culture and applied in rabbit osteochondro defect model.

RESULTS & DISCUSSION: Nano-topographical patterns triggered MSC morphology and cytoskeletal structure changes, and cellular aggregation resulting in specific chondrogenic differentiation outcomes. MSC chondrogenesis on nano-pillar topography facilitated robust hyaline-like cartilage formation, while MSCs on nano-grill topography were induced to form fibro/superficial zone cartilage-like tissue. the nano-topography pre-differentiated cells possessed phenotypic memory, forming phenotypically distinct cartilage in subsequent 3D hydrogel culture. Lastly, implantation of the bilayered hydrogel construct of superficial zone-like chondro-progenitors and middle/deep zone-like chondro-progenitors resulted in regeneration of phenotypically better cartilage tissue with higher mechanical function.

CONCLUSIONS: Our results demonstrate the potential of nano-topographic cues in guiding the differentiation of MSCs to chondrocytes of a specific phenotype. Implantation of these chondroctyes in a bilayered hydrogel construct yielded cartilage with more normal architecture and mechanical function. Our approach provides a potential translatable strategy for improved articular cartilage regeneration using MSCs.

REFERENCES

[1] Wu Y, Yang Z and Lee EH, etc al Nanomedicine: NB, 2014 Oct;10(7):1507-16



Physiomimicking tumour microenvironment for phenotypic screening facilitated via 3D in vitro models

X. Wan[1], M. Hill[2], D. Ebner[1]
Presenting Author: Xiao Wan, xiao.wan@ndm.ox.ac.uk
[1]Target Discovery Institute, University of Oxford [2]Department of Oncology, University of Oxford, UK

INTRODUCTION: The dismal success rate of preclinical drug candidates [1] and even surprisingly lower preclinical 5% success rate of cancer drug candidates compounds reaching the clinic [2] is at least in part due to the very complex nature of cancer pathophysiology where a complex mix of factors can lead to resistance to small molecule drugs in human patients. These can include extracellular matrix and other supporting cells such as endothelial cells and immune cells, which affect cell polarity, growth characteristics, and gene expression [3]. The two-dimensional (2D) reductionist approach where cells are incubated in a mono-layer on hard plastic microtiter plates is not physiologically relevant, then must in vivo rodent models be employed to validate the potentially positive hits emerging from a large primary screening campaign. Well developed and applied Three Dimensional (3D) in vitro models could be employed to bridge the gap between 2D in vitro primary screening and in vivo rodent models, by incorporating key features of in vivo tumour environment, such as extracellular matrix, co-culture, tension and perfusion, to replace several hundred rodents in the validation cascade.

METHODS: Human neural progenitor cells from middle brain (ReN VM, Merck Millipore, UK) were expanded as instructed by the supplier (Merck Millipore), and then seeded at 3000, 2000, 1000, 500 and 250 cells/well in 96-well plates. ReN VM were differentiated via growth factor deprivation and were imaged for a desired period. Radiotherapy was mimicked via gamma-radiation at 2Gy.

RESULTS & DISCUSSION: As shown in Figure 1, after 14 days of differentiation, neuron early differentiation marker (Tuj1, red) started to be expressed among the cells expressing neural stem cell marker SOX2 (green). Post-radiation effects include impaired tissue growth, and lower pluripotency and neuron differentiation marker expression were detected by immunofluorescence staining.

CONCLUSIONS: Combined with the benefit of greatly reducing the issues associated with in vivo rodent models, including overall numbers of animals, cost, ethics, and potential animal welfare burden, we fell that well developed and applied Three Dimensional (3D) in vitro models will be very compelling to provide a crucial tool to evaluate combinatorial therapies, optimal drug concentrations and treatment dosages.

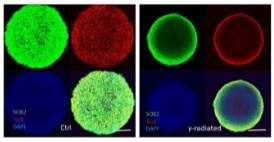
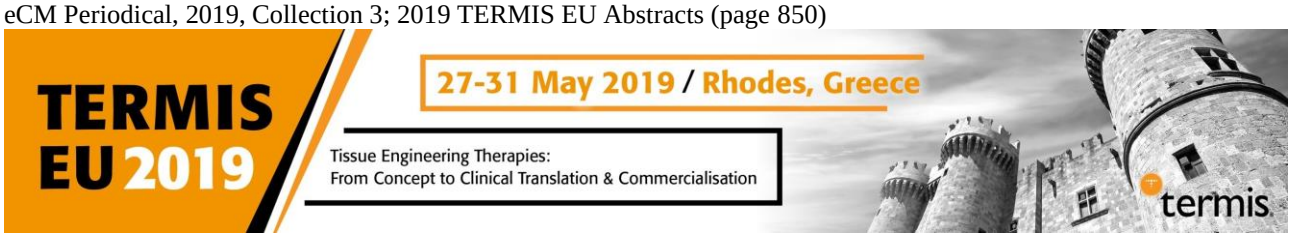


Figure 1: Neural progenitor cell-originated 3D spheroids. After 14 days of differentiation, neuron early differentiation marker (Tuj1, red) started to be expressed among the cells expressing neural stem cell marker SOX2 (green).

ACKNOWLEDGEMENTS: National Centre for the Replacement, Refinement, and Reduction of Animals in Research (Grant: NC/P002374/1).



In situ photopatterning of 3D osteocyte microenvironments

X.H. Qin[1], R. Müller[1]
Presenting Author: Xiao-Hua Qin, E-mail: qinx@ethz.ch
[1]Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

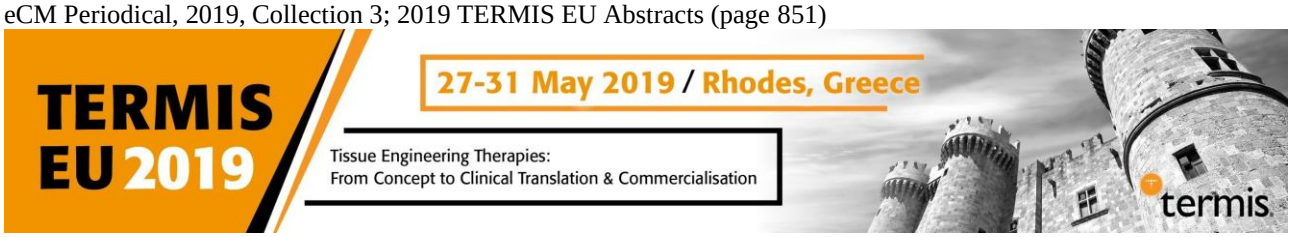
INTRODUCTION: Osteocytes are mechanosensory cells in the bone matrix with long dendritic processes within the lacuno-canalicular network (LCN). So far, it remains challenging to construct a functional 3D osteocyte network in vitro that resemble the features of a LCN system. This limitation hampers a deeper understanding of bone biology and the development of functional bone in vitro models for regenerative medicine. Here we build microengineered bone models by interfacing high-resolution photopatterning with 3D cell culture technology. By scanning a laser beam through a 3D cell-laden matrix, canaliculi-like microchannels can be generated as physical cues to guide dendrite formation through two-photon-induced photoablation (2PiP).

METHODS: A series of protein gels with different stiffness (ca. 0.5-10 kPa) were prepared by UV-curing of gelatin methacryloyl (GelMA). Single IDG-SW3 mouse osteocytes were photoencapsulated in GelMA gels (soft-5%, middle-7.5%, stiff-10%) at a final concentration of 1 M/mL and then cultured in differentiating media. Cell viability and morphology were assessed via live-dead and phalloidin-DAPI staining at day 1, 7 and 14. GM6001 was added as a broad-spectrum matrix metalloproteinase inhibitor to assess the effect of proteolysis on dendrite formation. Arbitrary micrometer-scale guidance channels were created inside 3D cell-laden hydrogels via 2PiP on a Nanoscribe GT 3D microprinter.

RESULTS & DISCUSSION: When embedded in 3D, osteocytes remained highly viable (>90%), demonstrating cell-compatibility of the photo-encapsulation process. Confocal live-cell tracking showed that the early formation of dendrites during 3D culture was strongly influenced by matrix stiffness: after 12h cells were able to form up to ca. 50-100 µm long processes in a soft matrix whereas cells remained round in a stiff matrix. Addition of GM6001 (10 µM) fully blocked dendrite formation, demonstrating the importance of proteolysis and matrix degradation in dendrite formation. Biomimetic microchannels were engineered inside a 3D matrix through 2PiP to foster dendrite formation. It was found that the threshold for 2PiP is greatly influenced by the amount of residual photoinitiator and processing parameters such as laser power and scanning speed. By positioning the focal spot in close proximity to live cells, user-dictated microchannels were generated at high spatial resolution. After 12h, guided formation of dendrites was observed.

CONCLUSIONS: In summary, we have developed a new method to create microengineered bone systems by using the 2PiP technique. This approach holds the potential to enable user-defined construction of 3D osteocyte networks as functional in vitro models for tissue engineering, disease modeling and drug screening.

ACKNOWLEDGEMENTS: This work was supported by an ETH Zurich Career Seed Grant (NO. SEED-21 18-2).



Functionalised chondroitin sulfate based injectable hydrogel system for cartilage regeneration

X. Li, W. Wang,
Presenting Author: Xiaolin Li, xiaolin.li1@ucdconnect.ie
Charles Institute of Dermatology, School of Medicine, University College Dublin (UCD), Dublin 4, Ireland

INTRODUCTION: The application of stem cell-laden hydrogel systems has drawn increasing attention in cartilage repair and regeneration. However, slow fabrication, fast degradation and low stem cell retention rate have limited the clinical application of hydrogel systems. Herein, we developed an injectable hydrogel system based on functionalized chondroitin sulfate (CS), a cartilage specific extra cellular matrix (ECM) material, to generate a stem cell-laden structure for cartilage tissue regeneration.

METHODS: Functionalised CS were synthesised and crosslinked with hyperbranched poly (ethylene glycol) multi-acrylate macromer (HB-PEG) with a series concentration of both components to form hydrogels. Rheology test were conducted at 37 ºC to determine the gelation time and mechanical strength of the hydrogels. Hydrogel structure were observed under scanning electron microscopy (SEM) and in vitro hydrogel degradation were monitored for 28 days. Rat adipose derived stem cells (ADSCs) were encapsulated in hydrogels at a concentration of 1.0×10^7/mL. AlamarBlue® assay were applied at 1, 3, 7 days post-encapsulation and LIVE/DEAD® assays were used to determine the cell viability. Hydrogels with ADSCs were cultured in chondrogenesis medium for 28 days. Subsequently, RT-qPCR and histochemistry were conducted after 14 and 28 days to monitor chondrogenesis process.

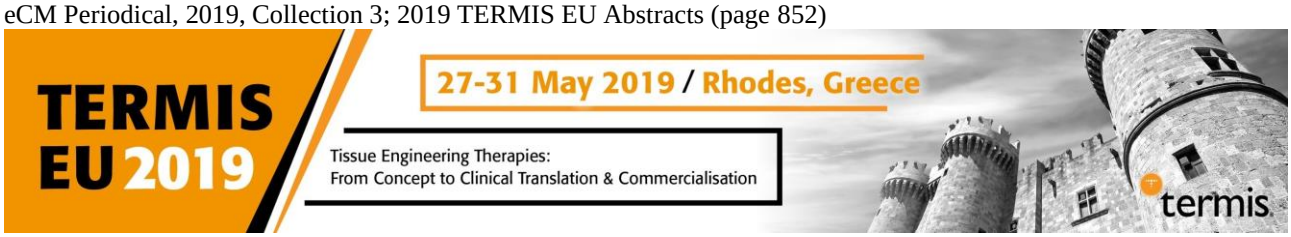
RESULTS & DISCUSSION: Rheological analysis showed that the hydrogels formed in situ within 5 min and achieved a comparable mechanical strength as natural cartilage tissue. The hydrogels present an extra low degradation rate over a time scale of at least 28 days. ADSCs encapsulated in the regular porous scaffolds showed good cell viability over a period of 7 days. The enhanced chondrogenic differentiation were determined indicated by increased cartilage specific gene expression and ECM production. Significant increase ($p < 0.001$) of Sox9 at 14-days and Col 2a, ACAN 28-days post-encapsulation were recorded. The decrease expression of IL-1β exhibited no inflammation effect were induced by the hydrogel systems. Histochemistry intensity analyses demonstrated increased production of collagen type II at both 14- and 28-days post-encapsulation.

CONCLUSIONS: As indicated by the results, the functionalized CS based hydrogels serve as a stem cell delivery system that rapidly restores metabolism of encapsulated stem cells and promotes chondrogenesis. This tissue engineered injectable scaffolds hold promise for clinical translation in cartilage tissue regeneration.

ACKNOWLEDGEMENTS: Financial support was received from Irish Research Council, (EBP/2017/487).

REFERENCES

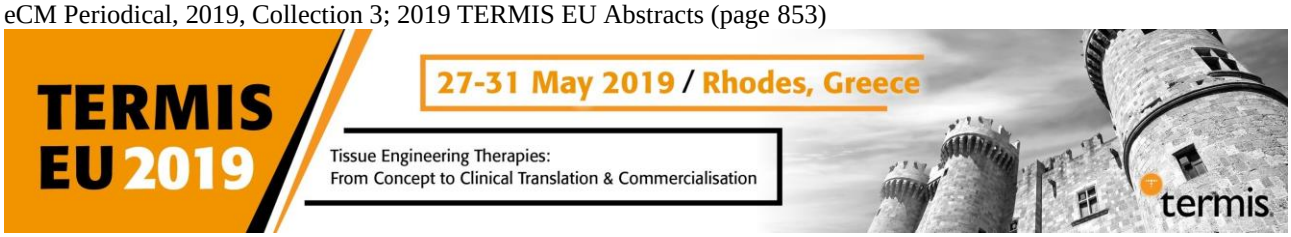
[1] Liu, M et al. Bone Res. 2017; 5 (January), 17014.
[2] Makris, E. A et al. Nat. Rev. Rheumatol. 2014, 11 (1), 21–34.
[3] Huang, B. J et al. Biomaterials 2016, 98, 1–22.



Human reconstructed epidermis and vascularized bioprinted skin tissues as tissue-in-a-dish models for drug screening

X. Liu[1], M.J. Song[1,2], S. Michael[1], M. Ferrer[1]
Presenting Author: Xue (Lucia) Liu, lucia.liu@nih.gov
[1]National Center for Advancing Translational Sciences/NIH, USA [2]National Eye Institute/NIH Bethesda, MD, USA

In vitro cell assays and animal models are extensively used in early stage drug development for efficacy, risk and toxicity tests. However, these in vitro and in vivo models lack human physiologically relevance and therefore have limited predictive value of drug responses in humans. Three-dimensional (3D) tissue models are currently being developed as an alternative, more predictive assays for high throughput screening (HTS) for drug discovery. In this study, we have developed a novel bio-printed vascularized fullthickness skin equivalent (VFS) and a reconstructed human epidermis (RHE) in a multi-well plate format for the purpose of drug testing. The morphology and functionality of the skin tissue equivalents were confirmed with H&E staining, immunohistological characterization, and transepithelial electrical resistance (TEER) measurements. In the dermis of the VFS, confocal images of intact tissues stained with collagen IV and CD31 showed the 3D microvascular network and basement membrane of microvessels. Late epidermal differentiation markers illustrated that the epidermal layer was fully differentiated in both VFS and RHE tissues. Interleukin (IL)-4 treatment was used to induce atopic dermatitis (AD) like in the skin equivalents constructed. These AD models were able to reproduce several clinical hallmarks of AD including (i) spongiosis and hyperplasia; (ii) early and terminal expression of differentiation proteins; (iii) increases in levels of pro-inflammatory cytokines. Janus Kinase (JAK) inhibitors have been reported to be efficacious for the treatment of AD. The AD RHE was used to screen known JAK inhibitors and other compounds being tested for the treatment of AD in a 96-transwell format. Six effective JAK inhibitors from the primary screening were further tested in the AD VFS tissue model. The assay system for the AD VFS model includes tight junction barrier value, quantification of inflammatory cytokines, pro-angiogenic factors measurements and structural morphology changes in the microvasculature. In our study, we first provide an innovative and reproducible bio-printing biotechnology to create a vascularized human skin equivalent. Second, this construct combined with RHE is used in screening and developing pre-clinical drugs for skin disease. Third, endpoint readouts are quantifiable, robust, AD relevant, and can be scaled up to allow HTS for compound discovery. Thus, the skin equivalents developed in this study offers an in vitro approach for understanding pathological mechanism, efficacy of action and toxicity of dermal drugs.



Icariin protects annulus fibrosus cells in a rat model of intervertebral disc degeneration by MAPK signaling inhibition

X. Wang[1], M. Li[1], Z. Bian[1], C. Hou[1], L. Cao[2] and L. Zhu[1]
Presenting Author: Xuepeng Wang, wangxuepeng22@163.com
[1]Department of Orthopedics Surgery, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou 310006, Zhejiang, People's Republic of China
[2]Department of Orthopedics Surgery, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, People's Republic of China

INTRODUCTION: Icariin is the primary active ingredient of the herb Epimedium pubescens, a traditional Chinese medicine, which has been proved to be an alternative approach to treat bone-related diseases [1-3]. In the present study, the potential efficacy of icariin in protecting intervertebral disc was investigated using annulus fibrosus (AF) cells isolated from a rat model of intervertebral disc degeneration (IVDD).

METHODS: In total, 24 male 8-week-old Sprague-Dawley rats were used to develop a lumbar IVDD model. The differential expression of extracellular matrix in normal and degenerated rat AF tissues was observed using immunohistochemical and TUNEL staining. Isolated AF cells were treated with 0, 10−8 or 10−6 mol/l icariin as the IVDD group and the low- and high-concentration groups, respectively. The MTT assay was performed for cell viability, real-time polymerase chain reaction and immunocytochemistry for catabolic marker gene and protein expression and western blot for protein expression of mitogen-activated protein kinase (MAPK) signaling.

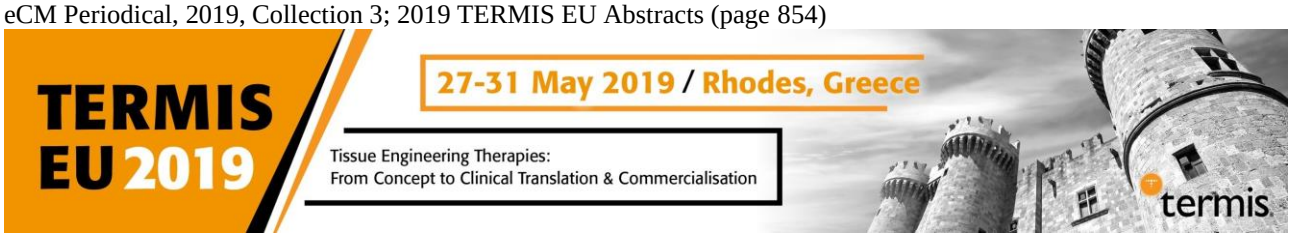
RESULTS & DISCUSSION: Degenerated AF tissues from the IVDD model at 8 weeks displayed increased collagen X and matrix metalloproteinase (MMP)-13 expression and cell apoptosis. Icariin treatment of isolated AF cells reversed the reduced proliferation induced by IVDD and significantly decreased catabolic marker gene and protein expression, including collagen X, MMP-13 and MMP-3. MAPK signaling was involved with the progress of IVDD in vitro. Furthermore, inhibition of MAPK phosphorylation, and thus activation, by icariin treatment reduced ERK, JNK and p38 protein expression in AF cells.

CONCLUSIONS: Our results suggest that icariin prevented intervertebral disc matrix degradation in vitro, and that acting as an MAPK inhibitor, it may be used as a potential therapy for the treatment of disc degeneration.

ACKNOWLEDGEMENTS: This work was supported by a grant awarded by the Zhejiang Provincial Natural Science Foundation (No. LY16H060009) and the Wu Jieping Medical Foundation (No.320.6750.16102 and 320.6750.16103).

REFERENCES

[1] Jia C, et al. Int J Clin Exp Pathol 7:9104-9109, 2014.
[2] Qin S, et al.Int J Clin Exp Med 8:7125-7133,2015.
[3] Zhang W, et al. Indian J Exp Biol 51:313-321, 2013.



A biomimetic cartilage gradient hybrid scaffolds for functional tissue engineering of cartilage

X. Hu[1], X. Li[1], T. Liu[1], K. Song[1*]
Presenting Author: Xueyan Hu, huxueyan@mail.dlut.edu.cn
[1]Dalian University of Technology, State Key Laboratory of Fine Chemicals, Dalian R&D Center for Stem Cell and Tissue Engineering, Liaoning Dalian 116024, China

INTRODUCTION: According to the recent World Population Survey, there are approximately 1.66 million cases of articular cartilage damage worldwide each year and are increasing year by year. Tissue engineering is a means of repairing the integration of cells, biological materials and growth factors. The performance of tissue engineering materials largely determines the characteristics of the composite scaffold, so choosing the right materials and cells is especially critical. And the ideal scaffold should have a physiological layered.

METHODS: The control mixture was mixed according to Cs, GP, and Gel in a volume ratio of 3:1:2, 6:1:3.5, 9:1:5, 12:1:6.5, 15:1:8, 18:1:9.5. According to the principle of the connector, the mixture of the Cs/GP/Gel biomimetic gradient scaffolds was prepared by gradually diluting the concentration of the single layer mixture which is 0.15 mol/L. Rat bone marrow stem cells were seeded at 20,000 cells/cm2 on Cs/GP/Gel biomimetic gradient scaffolds. SEM, water absorption, porosity, degradation rate, biocompatibility were conducted.

RESULTS & DISCUSSION: Cs/GP/Gel gradient hybrid scaffolds were successfully prepared in this study by controlling the concentration of monolayer solution, and the real physiological structure of natural cartilage was simulated. The scaffold had suitable water absorption (584.24±3.79 ~ 677.47±1.70%), porosity (86.34±5.10 ~ 95.20±2.86%) and degradation rate (86.09±2.46 ~ 92.48±3.86%). Staining showed that bone marrow mesenchymal stem cells had the ability to differentiate into bone and cartilage. BMSCs adhered and stretched well on this scaffold, which further confirmed its good biocompatibility.

CONCLUSIONS: The biomimetic gradient scaffold designed and prepared in this study provides an important basis for the development of new gradient composite biomedical materials and osteochondral repair.

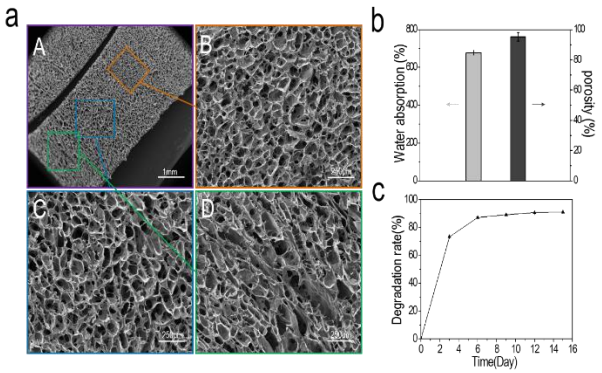
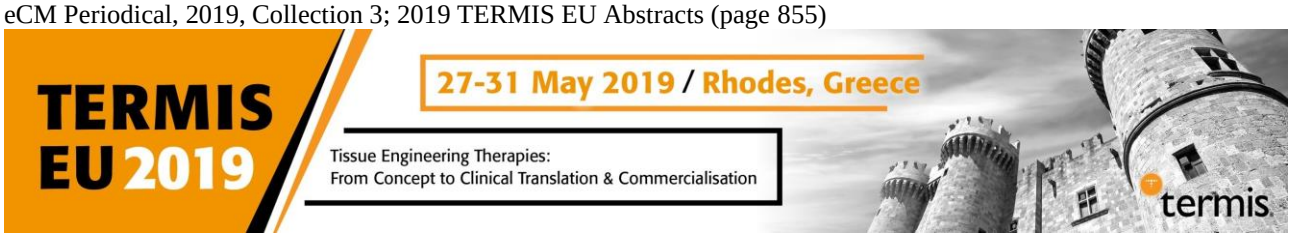


Figure 1: Physical properties of Cs/GP/Gel biomimetic cartilage gradient scaffolds.

ACKNOWLEDGEMENTS: This work was supported by the National Natural Science Foundation of China (31670978/21676041), and the Natural Science Foundation of Liaoning (20180510028).



Secretome of cartilage tissue engineered from chondrocytes or bone marrow-derived mesenchymal stem cells induce angiogenesis

Y. Nossin[1], C. Gorgun[4,5], R. Tasso[4,5], J.L.M Koevoet[1], E. Farrell[2], G. J.V.M. van Osch[1,3]
Presenting Author: Yannick Nossin, y.nossin@erasmusmc.nl
[1]Dept. Otorhinolaryngology [2]Dept. Oral and Maxillofacial Surgery [3]Dept Orthopaedics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands [4]Dept Experimental Medicine, University of Genova, Italy [5]Ospedale San Martino, IRCCS per l'Oncologia, Genova, Italy

INTRODUCTION: Cartilage can be tissue engineered from chondrocytes or from bone marrow derived mesenchymal stem cells (bMSC). Chondrocyte derived cartilage remains stable upon subcutaneous implantation whereas cartilage derived from bMSC transitions into bone via a process known as endochondral ossification (EO) [1]. The onset of EO is marked by the invasion by blood vessels in the cartilage [2]. We hypothesise that this process is induced by soluble factors secreted by hypertrophic chondrocytes. Here we compare the secretome of stable and transient cartilage for their effect on angiogenesis.

METHODS: Conditioned medium (CM) was derived from culture models for transient and stable cartilage [3]. As a model for transient cartilage we used 3D pellets of bone marrow derived mesenchymal stem cells (MSC) and as model for stable cartilage we used expanded primary articular chondrocytes. Three donors of each were differentiated over 21 days. [3] Pellets were characterized by histology and qPCR. The CM was used in different in vitro and in vivo/ex vivo assays: human endothelial cell migration and proliferation assays as well as a chick chorioallantoic membrane (CAM) assay and mouse metatarsal assay.

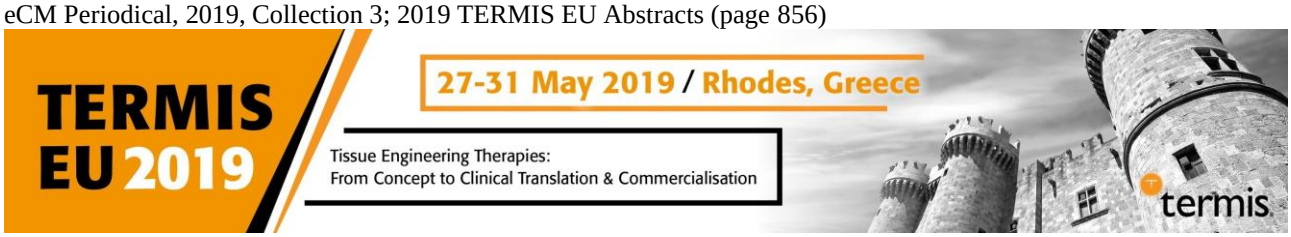
RESULTS & DISCUSSION: At the time of CM harvest chondrocytes and bMSC from all donors had deposited a matrix that stained positive for chondrocyte markers. Their gene expression mirrors this, whereas only the bMSCs expressed COL10A1 and ALPL. Both chondrocyte and MSC CM induced HUVEC migration, albeit the effect of MSC CM was significantly higher. Both CM stimulated proliferation of HUVECs and pro-angiogenic effects in both in vitro assay.

CONCLUSIONS: Secreted factors from engineered cartilage from both cell sources showed increased pro-angiogenic behavior in in vitro and in vivo angiogenesis assays. The migration assay reflected results of the metatarsal assay the most. Although both cell sources stimulated angiogenesis differently it is unclear if this can explain the difference in vessel ingrowth seen after in vivo implantation in mice. A physical rather than chemical barrier might be the explanation for this behaviour.

ACKNOWLEDGEMENTS: This project is funded by EU's H2020 research and innovation programme under MSCA No 721432

REFERENCES

[1] Hellingman, C. A., et al. Cell Transplant, 2011. **20**(6), 925–940.
[2] Gerber, H.P., et al. Nat Med, 1999. **5**(6): p. 623-8.
[3] Johnstone, B., et al. Exp Cell Res, 1998 **238**(1), 265–272.



Mussel protein nanoparticle-mediated photo-responsive system for cancer-specific photothermal-chemotherapy

Y. Jeong[1], Y. K. Jo[2], H. J. Cha[1]
Presenting Author: Yeonsu Jeong, skyyyhu@gmail.com
[1]Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, Korea [2]Department of Chemical and Biomolecular Engineering, University of Pennsylvania, USA

INTRODUCTION: Photothermal-chemotherapy is a promising synergistic cancer therapy due to high selectivity, and remote controllability [1] However, most of near-infrared (NIR) photothermal agent (PTAs) have challenges in the clinical translation due to their poor biodegradation and long-term toxicity [2]. Therefore, organism-based PTAs would be highly beneficial for clinical applications with excellent biocompatibility. Here, we proposed a photo-responsive system based on ascidian-inspired mussel adhesive protein (MAP) nanoparticles (NPs) for synergistic photothermal-chemotherapy.

METHODS: The catechol-metal complex-containing MAP NPs, and ammonium bicarbonate (ABC) and doxorubuicin (DOX) co-loaded MAP NPs (MAP@ABC/DOX NPs) were fabricated by electrospraying. The real-time temperature of MAP NPs was detected by using a thermometer during exposure to an 808 nm laser. Next, human breast cancer cells MCF7 were incubated with MAP@ABC/DOX NPs and irradiated with laser for 2, 5, and 10 min respectively. After 24 h of incubation, the cell viability was evaluated by Cell Counting Kit-8 assay.

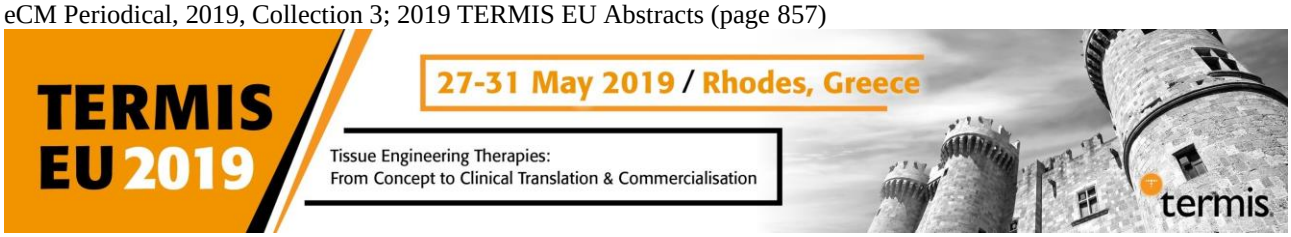
RESULTS & DISCUSSION: The MAP NPs successfully generated heat, elevating temperature of 60 ℃ upon laser irradiation for 10 min. In addition, MAP@ABC/DOX NPs efficiently released DOX upon laser irradiation through photothermal-induced generation of microbubbles from ABC. Next, the cytotoxic effects were investigated against MCF7 cells. The MAP NPs and MAP@ABC NPs showed negligible toxicity on the cells without laser exposure. Upon laser irradiation, the MAP NPs exhibited photocytotoxicity in an irradiation time-dependent manner and about 20 % of MCF7 cells remained alive after irradiation for 10 min. Compared to the photothermal therapy alone, less than 2% of MCF7 cells remained alive, demonstrating the synergistic efficacy of photothermal-chemotherapy.

CONCLUSIONS: The marine organism-based protein NPs had potential for clinical application of cancer-specific combined therapy with biocompatibility and high photocytotoxic effects.

ACKNOWLEDGEMENTS: Financial support was provided by the Marine Biomaterials Research Center grand from Marine Biotechnology Program of the Korea Institute of Marine Science & Technology Promotion funded by the Ministry of Oceans and Fisheries, Korea.

REFERENCES

[1] Chen M C et al. ACS Nano 2016; 10(1):93-101 [2] Dong Z et al. Theranostics 2016; 6(7):1031-1042



Designing materials to control stem cell-based blood vessel network formation for vascular tissue engineering

Y.C. Chen
Presenting Author: Ying-Chieh Chen, yisschen@mx.nthu.edu.tw
Department of Materials Science and Engineering, National Tsing-Hua University, Hsinchu 30013, Taiwan, R. O. C.

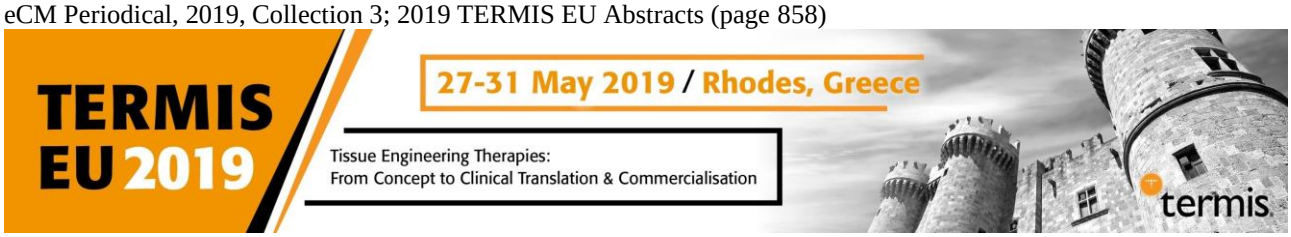
INTRODUCTION: Timely tissue vascularization and integration of engineered tissues into a patient plays an important role in the successful translation of engineered tissues into clinically relevant therapies. To decrease the time needed to vascularize an engineered adipose tissue, suitable local microenvironments provided by hydrogels to support cell-based functional vascular network formation have been investigated. In this study, we compare the capability of engineering vascular network in vivo in different types of collagen and gelatin hydrogels, with controllable physical and chemical crosslinking. The relationships between vascular network formation and hydrogel properties for the hydrogels are discussed.

METHODS: Using the same biomolecule in solution, collagen and gelatin hydrogels with various crosslinking methods can be obtained. Human endothelial colony-forming cells (ECFCs) and mesenchymal stem cells (MSCs) were resuspended in pre-polymer solution and the mixture was implanted or injected into 6-week-old male nu/nu mice.

RESULTS & DISCUSSION: In order to demonstrate how and why physicochemical properties of collagen hydrogels regulate the extent of the ECFC-MSC based vascular network formation (Fig1a), the perfused functional lumen density and size, weight of engineered vascularized constructs, and percentage of human cells within the lumen were plotted against the hydrogel properties (G', concentration of RGD and MMP, swelling, diffusion, mesh size, mesh density and in vivo bio-degradation) and host immune response (murine leukocytes surrounding and inside the hydrogel constructs) of the self-assembled collagen hydrogels (physical, Fig1a), enzymatically cross-linked collagen-Ph hydrogels (chemical, Fig1a), and collagen hydrogels (i.e. no matter which crosslinking methods used, Fig1b). In physically cross-linked collagen hydrogels, the lumen density is proportional to the diffusion rate and number of murine leukocytes inside gels (data not shown). These results revealed that the extent of vascular network formation is highly dependent on the diffusion of gels and the number of infiltrating host leukocytes observed inside gels. On the other hand, in collagen-Ph hydrogels (data not shown), a higher diffusion and biodegradation rate of collagen-Ph hydrogels resulted in more infiltrating murine leukocytes, illustrating that these constructs have higher microvessel density and smaller lumens. Regardless of which cross-linking methods (Fig.1b) were used to polymerize collagen, the lumen density inside collagen gels is proportional to the diffusion property of gels and the degree of infiltration by murine leukocytes. Moreover, the weight and diameter of engineered collagen tissue constructs are highly proportional to the concentration of collagen instead of the crosslinking methods and stiffness.

CONCLUSIONS: These results show manipulating the polymerized methods of a hydrogel could not only modulate vascular network formation, but also regenerate adipose tissue in vivo.

ACKNOWLEDGEMENTS: This work was supported by the Ministry of Science and Technology of Taiwan grant (MOST 106–2221-E-007–055, and MOST 107–2221–E-007–067)



Stearic acid assisted poly(1,3-trimethylene carbonate) deposited on boiled-AZ31 for biodegradable bone implants application

Y. Li[1], S. Zhao[2], H. Wei[2], Y. Zhang[1]
Presenting Author: Yixuan Li, lyxnju@163.com
[1] State Key Laboratory of Pharmaceutical Biotechnology, Department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, PR China [2]Department of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing University, PR China

INTRODUCTION: The fast corrosion rate and complex biological activities of Mg challenge its orthopedic application [1-3]. Here, we designed a composite coating $Mg(OH)_2$/SA/PTMC to improve corrosion resistance.

METHODS: There were three steps to prepare the composite coating. Hydrothermal method, immersion, and evaporation were used in this study. SEM, XRD, XPS, water contact angle and adhesion test were investigated to reveal the quality of the composite coating. Electrochemical corrosion test and immersion test in PBS were performed to evaluate degradation of materials in vitro. Rat bone mesenchymal stem cells (rBMSCs) cultured in sample extracts and surfaces were used to access cytocompatibility. Furthermore, samples were implanted into rat femur bone cavity, micro-CT was a technique to analysis degradation of materials in vivo and new bone formation around implants.

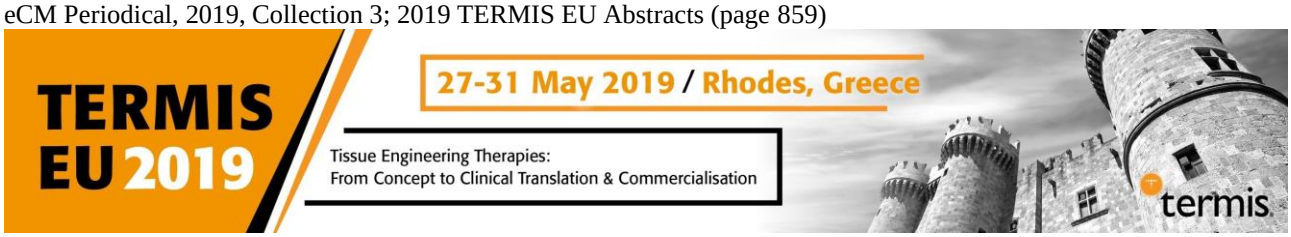
RESULTS & DISCUSSION: The thickness of PTMC layer and $Mg(OH)_2$&SA layer was 12.48 ± 0.70 μm and 10.58 ± 1.20 μm, respectively. With the linkage effect of SA, PTMC layer was adhered strongly onto $Mg(OH)_2$ layer without gaps. The electrochemical corrosion and 30-day immersion test results demonstrated high improvement of anti-corrosion properties in AZ31-OH&SA@PTMC compared with bare AZ31. Cell results suggested that AZ31-OH&SA@PTMC possessed better cell viability, adhesion and survival than bare substrates, indicating the composite coating had an excellent cell biocompatibility. Less volume loss and more new bone formation implied AZ31-OH&SA@PTMC could not only protect substrate from corrosion but also enhance bone formation in vivo.

CONCLUSIONS: A de novo designed sandwiched coating $Mg(OH)_2$/SA/PTMC was developed to modify Mg based biodegradable implants, paves a promising way for the development of fully biodegradable orthopedic implants.

ACKNOWLEDGEMENTS: Financial support was received from NSFC(81772402, 81802152), Key Program of NSFC (81730067), Excellent Young Scholars NSFC (81622033), Jiangsu Provincial Key Medical Center Foundation, Jiangsu Provincial Medical Outstanding Talent Foundation, Jiangsu Provincial Medical Youth Talent Foundation and Jiangsu Provincial Key Medical Talent Foundation (BK20160633), the Scientific Research Foundation of Graduate School of Nanjing University (2016CL12)

REFERENCES

[1] Witte F et al. Biomaterials. 2005;26(17): 3557-3563
[2] Xu W et al. Nat Mater. 2015; 14(12):1229-1235
[3] Zhang Y et al. Nat Med. 2016;22(10):1160-1169



Towards the production of a new animal model of tissue engineered vascular graft (TEVG): The production of CAM sheets from large animal skin fibroblasts

Y. Torres[1], N. Da Silva[1], N. Chellaoui[1], A. Grémare[1], R. Siadous[1], N. L'Heureux[1]
Presenting Author: Yoann Torres, yoann_t@yahoo.fr
[1]Inserm U1026, Université de Bordeaux – Bioingénierie Tissulaire, Bordeaux, France

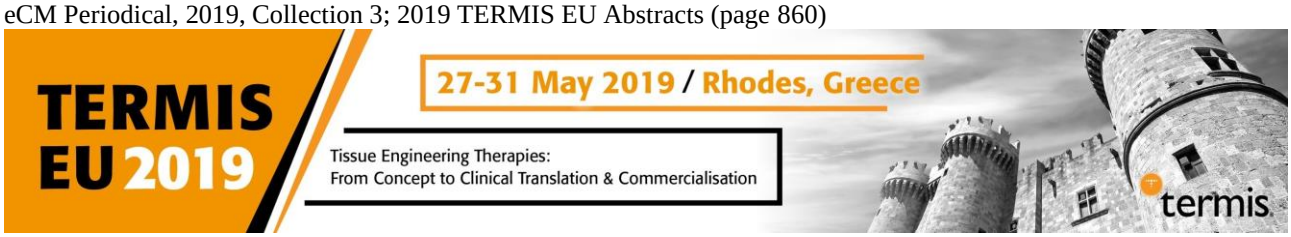
INTRODUCTION: Synthetic vascular grafts perform poorly in small diameter applications (<6 mm) with high risks of thrombosis and intimal hyperplasia. Our goal is to develop an entirely biological tissue-engineered vascular graft (TEVG) using Cell-Assembled extracellular Matrix (CAM). Large sheets of CAM were produced by cells in vitro, and then cut into yarns to be woven into TEVGs. The in vivo evaluation of a human CAM-based TEVG remains limited to immunodeficient or immunosuppressed animals, which are too small, too expensive, ethically challenging and/or too unstable for long-term studies. Consequently, a large animal TEVG is essential to evaluate the potential of the new approach in a clinically relevant setting (size and allogeneic). The goal of this study is the first step towards the production of a TEVG: the production of CAM sheets for a large animal model.

METHODS: Ovine, porcine, canine and human skin fibroblasts were cultured for two months in 6-well plates in DMEM/F-12 (1:1) medium supplemented with 20% of two selected serums (Biowest and Hyclone) alone or in combinations, antibiotics and 0.5 mM Na L-ascorbate. Perforation strength was measured with a spherical probe (95 mm diameter) at a rate of 20 mm/min. Large CAM sheets (10 x 18 cm) were cut using a custom device with blades that can cut ribbons with a uniform width between 1 and 20 mm. Yarn mechanical properties were studied with tensile tests (Shimadzu AGS-X 10kN) performed at a rate of 1% L_0/s until break detection.

RESULTS & DISCUSSION: Serum alone greatly influenced the production of CAM sheets in a clearly species-dependent manner. Ovine emerged as the most promising species to produce CAM, although ovine sheets strength were almost four times weaker than that of human sheets (respectively 117±14 gf, n=5 and 424±76 gf, n=5). Ovine cells showed higher proliferation than human cells and produced less collagen. Also, serum concentration did not significantly change ovine sheet strength while it had a direct effect on human sheets. The addition of Insulin-Transferrin-Selenium to the culture medium slightly increased the ovine sheet strength (respectively 170±8 gf, n=5 and 161±26 gf, n=9, $p>0.05$). Larger ovine sheets, in T225 flasks, were produced and cut into yarns. Ovine yarns were weaker than their human counterparts when produced with the same width of sheet. An increase of the yarn width increased the strength at break of ovine yarns. Also, when yarns were twisted, their strength at break were increased.

CONCLUSIONS: An immediate solution to move forward in the production of TEVGs, would be to cut wider ovine yarns to obtain a similar strength than that of human yarns. This study demonstrated the important species-to-species variability in cell behavior, which becomes very apparent in long-term and complex culture systems.

ACKNOWLEDGEMENTS: Financial support was received from Initiative d'Excellence U. bordeaux (IdEx) (Senior Chair 2016-0389); Agence Nationale de la Recherche (ANR) (R16060 GG HumTex); ERC Advanced Grant (Human Textiles #785908)



Wound healing of adipose stem cell-derived extracellular matrix sheet on full thickness skin defect in diabetic rat model

Y.J. Lee[1], J.W. Rhie[2]
Presenting Author: Yoon Jae Lee, yoonjae1116@gmail.com
[1]Department of Plastic and Reconstructive Surgery, Yeouido St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Korea [2]Department of Plastic and Reconstructive Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Korea

INTRODUCTION: Impaired diabetic wound healing is associated with a dermal extracellular matrix (ECM) protein profile favoring proteolysis [1,2,3]. The ECM is an intricate network of various combinations of elastin, collagens, laminin, fibronectin, and proteoglycans that plays a key role in stimulating cell proliferation and differentiation [1]. Treatment of diabetic wounds with extracellular matrix sheet dressing material may be a promising source to improve wound healing.

METHODS: Using in vitro models of ECM sheet derived from human adipose tissue and in vivo diabetic murine wound healing models, we tested the hypothesis that ECM sheets enhance wound healing by decreasing proteolysis, hence enhanced re-epithelization rate and denser granulation tissue.

RESULTS & DISCUSSION: Microscopic analysis indicated that the re-epithelization rate of the wound was higher in the ECM sheet group compared to that in the control group ($p < 0.001$). Moreover, a denser and more organized collagen tissue was formed in the ECM sheet group compared to that in the control group($p < 0.001$). The ECM sheet also showed the highest microvessel density compared to the control group.

CONCLUSIONS: These findings suggest a potential mechanism through which ECM sheet dressing enhance diabetic wound healing by improving collagen alignment and higher re-epithelization rate. We suggest that a bioactive ECM sheet dressing derived from human adipose can provide therapeutic proteins for wound healing in diabetic wound.

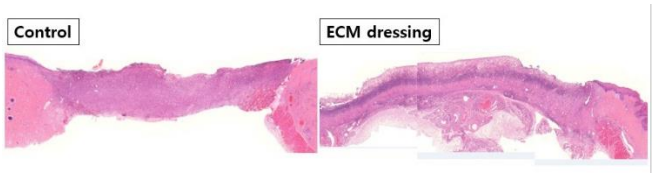
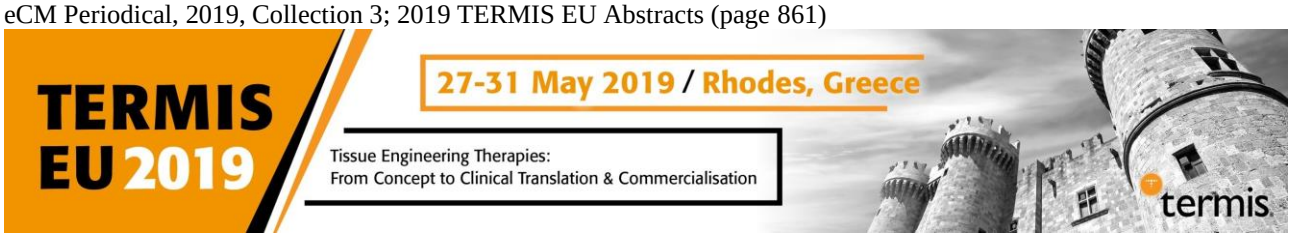


Figure 1: Histological evaluation of wound sections in the ECM sheet, control groups

ACKNOWLEDGEMENTS: This work was financially supported by the Ministry of Trade Industry and Energy of Korea (10062127 and 10063334) and by the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017M3A9E2060428).

REFERENCES

[1] Hassan WU et al. Wound Repair Regen.2014;22:313–25
[2] Han S et al. Crit Rev Eukaryot Gene Expr.2015;25:145–52
[3] De Francesco F et al. Tissue Eng Part B Rev.2015;21:572–84



The effect of Lactoferrin delivered in absorbable collagen sponge on bone regeneration in a rat calvarial defect model

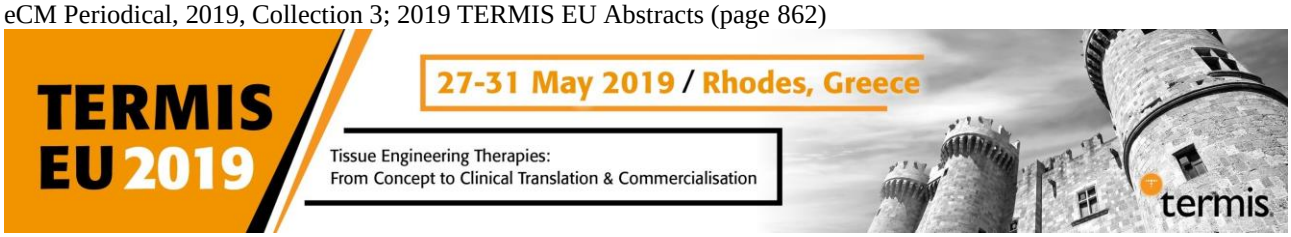
Y.-E. Park[1], M. Watson[1], K. Callon[1], M. Zhu[1], D. Tuari[1], B. Matthews[2], D. Naot[1], J. Cornish[1], D.S. Musson[1]
Presenting author: Young-Eun Park, y.park@auckland.ac.nz
[1]Bone and Joint Research Group, Department of Medicine, University of Auckland, Auckland, New Zealand [2]Department of Molecular Medicine and Pathology, University of Auckland, New Zealand

INTRODUCTION: Lactoferrin (LF) is a large glycoprotein that has been shown to have positive effects on bone. Previous in vivo work has shown that bovine LF, delivered in a collagen gel, increases bone formation in a rat calvarial defect model. Recombinant human bone morphogenetic protein-2 (rhBMP2) is a clinically approved growth factor for bone regeneration, and is delivered in an absorbable collagen sponge (INFUSE® Bone Graft kit, Medtronic). In this study, we used INFUSE® collagen sponges to deliver LF to rat calvarial bone defects, and compared the efficacy of LF in promoting bone regeneration to that of rhBMP2.

METHODS: Calvarial defects, five mm in diameter, were created over the left parietal bone in 30 Sprague–Dawley rats, which were randomised into three groups: i) empty defects (control), ii) defects grafted with LF/collagen sponge (100 ug/sponge), and iii) defects grafted with rhBMP2/collagen sponge (10 ug/sponge). Twelve weeks post-operation, calvariae were dissected and scanned by microCT (Skyscan 1172, Bruker). Bone volume (BV), tissue mineral density (TMD), porosity, volume, surface area and number of pores were compared between the groups. In addition, the calvariae in the rhBMP2 group were compared with intact calvariae from age-matched rats of the same strain. For histology, samples were fixed in 10% neutral buffered formalin, decalcified in 10% formic acid, paraffin embedded, and 10 µm thick slices were stained with haematoxylin and eosin.

RESULTS & DISCUSSION: There was no difference in the mean BV between the control and LF groups, whereas the BMP2 group had significantly higher mean BV than the control ($p<0.0001$) and LF ($p<0.001$) groups. The mean TMD was similar between all three groups. The BMP2 group had higher mean percentage of closed pores within the regenerated bone than the control ($p<0.01$) group, and higher mean volume of closed pores than the control ($p<0.0001$) and LF ($p<0.001$) groups. Histological analysis showed the presence of a fibrous-like structure within the regenerated calvariae of all rats treated with LF or rhBMP2, likely to be the collagen sponge still present at 12 weeks after operation. Also, all the calvariae in the rhBMP2 group had large cavities within the regenerated bone which were filled with blood cells and a large number of adipocytes. These large cavities and adipocytes were not present in the control and LF groups. Interestingly, reconstructed images from microCT suggested that BMP2-induced bone growth in excess of the normal thickness of the calvariae, when the rhBMP2 group was compared with age-matched calvariae.

CONCLUSIONS: LF delivered in INFUSE® collagen sponge had no effect on bone regeneration in this experiment, so further study is required to find a suitable delivery system for LF. BMP2 grafted in the collagen sponge improved bone regeneration, however, rhBMP2 induced excessive bone growth, which is generally not desirable in the clinical setting. Also, the regenerated bones had unusual features, including the presence of large cavities filled with numerous adipocytes, which might have a negative effect on bone health and strength. Therefore, although rhBMP2 promotes bone regeneration, there is still a need for further exploration of factors that might induce the formation of bone of better quality.



Differentiation of human amniotic fluid stem cells cultured on biomaterials having nanosegments

Y.R. Huang[1], N.Y. Ku[1], A. Higuchi[1,2]
Presenting Author: Yu Ru Huang, destination8411@gmail.com
[1]Department of Chemical and Materials Engineering, National Central University (NCU), Zhongli, Taiwan [2]Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama, Japan

INTRODUCTION: Human amniotic fluid stem cells (hAFSCs) are pluripotent fetal cells, which are capable to differentiate into multiple lineages. However, when we are culturing hAFSCs, it takes a long time for hAFSCs to become confluence. Therefore, we designed a culture method of hAFSCs where the different substrates were coated on tissue culture polystyrene (TCPS) dishes, such as TCPS coated with Synthemax II (oligo-vitronectin based substrate), human recombinant-vitronectin (rVN), poly(N-isopropylacrylamide) (PolyNIPAAm) and poly(N-isopropylacrylamide-co-butylacrylate) (PolyNIPAAm-BA). Furthermore, we have investigated the differentiation ability of hAFSCs into multiple lineages such as osteoblasts, chondrocytes and retinal pigment epithelium cells. Thus, hAFSCs may become a more suitable source of stem cells in tissue engineering and regenerative medicine in future.

METHODS: We used different substrates coated on TCPS dishes. Especially polyNIPAAm or polyNIPAAM-BA were coated on cell culture dish conjugated with different ECMs (rVN) and Synthemax II. The optimal condition for hAFSCs attached on dishes by evaluating the doubling time was investigated. After the cells became confluence, the differentiation medium was introduced to induce the cells to differentiate into each cell lineage that we intended.

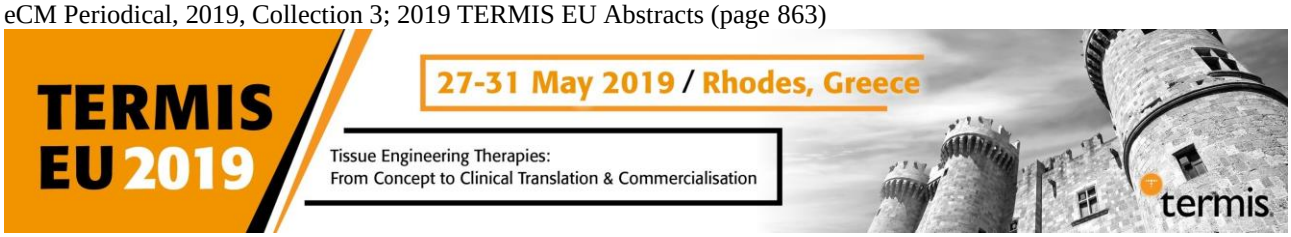
RESULTS & DISCUSSION: We found that hAFSCs had stronger adhesion on dishes which were coated with extracellular matrices such as rVN and Synthemax II than TCPS. Particularly, polyNIPAAm and polyNIPAAM-BA dishes coated with ECMs showed better cell proliferate ratio than conventional TCPS dishes. After the cells became confluence, hAFSCs differentiated into osteoblasts. At day 14, we detected the ALP (Alkaline Phosphatase) activity for the early stage of osteogenic differentiation. After day 28, we could evaluate the late stage of osteogenic differentiation by alizarin red S and von Kossa staining.

CONCLUSIONS: Our culture method shows that hAFSCs can grow faster than culture on conventional TCPS dishes and also has higher differentiation ratio on especially polyNIPAAm and polyNIPAAM-BA dishes coated with several ECMs. hAFSCs may become suitable source in tissue engineering and regenerative medicine in future.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Science and Technology (MOST 107-2119-M-008-002).

REFERENCES

[1] A. Higuchi, et al., Differentiation Ability of Amniotic Fluid-Derived Stem Cells Cultured on Extracellular Matrix-immobilized Surface, Current Nanoscience, 9, 893-901 (2011).



Implantable cervix with protein sustained release functionality by 3D printing

Y. Pang[1], C.J. Zhao[1], W. Sun[1]
Presenting Author: Yuan Pang pangyuan31@mail.tsinghua.edu.cn
[1] Biomanufacturing Center, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China

INTRODUCTION: The cervix is one of the most important organs in the female reproductive system. But cervical cancer, induced by human papillomavirus (HPV) infection, has become the second death threat for women. Traditional treatment of cervical conization leads to partial tissue defect with significant pain points, and increases the risk of reproductive tract infection. More dangerous is leading to miscarriage and premature rupture of membranes [1]. In view of the worldwide cervical disease and the inevitable tissue damage caused by surgery, we aim at the engineering of implantable cervix constructs with drug-laden based on 3D printing technology.

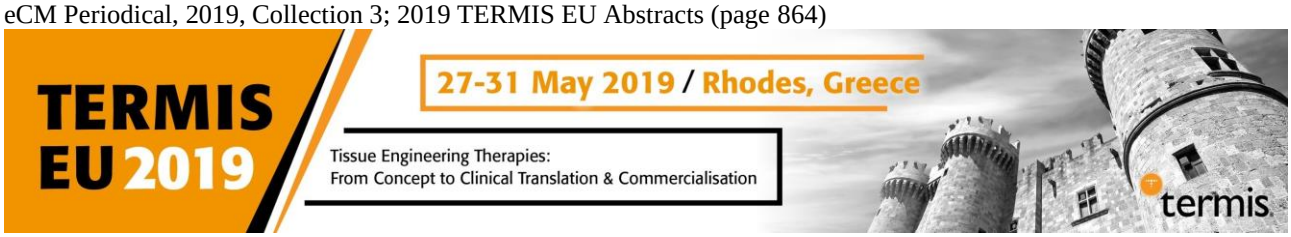
METHODS: The proposed cervix construct was designed as a cone-shaped scaffold according to the excised tissue part in conization: 3000 μm in bottom diameter, 1500 μm in height with a hollow channel of 500 μm in diameter. Polyurethane (PU), a FDA-approved implantable material, was selected as the raw material for fabrication using low-temperature deposition manufacturing (LDM), with the concentration at 12.5% (w/v, PU/1,4-dioxine). Scaffolds with three different wire spacing (1000 μm, 1200 μm, 1500 μm) were printed to evaluate the effect of porosity on scaffold performance. Anti-HPV protein was loaded onto the porous scaffold through negative pressure. The biocompatibility, structural stability and protein release of the 3D printed PU-based structure was analyzed.

RESULTS & DISCUSSION: Tailored cervix construct was engineered by LDM followed with freeze drying process. The construct is a gradient porous structure with pore size varying from 1 to 100 μm. The elasticity modulus of PU is 11.75MPa and the elastic limit is 13.5%. The elongation at break is greater than 250%, which indicates that PU has good elasticity. The cytotoxicity test shows that HUVECs grow well in PU extract with a relative increase rate of 88%. Anti-HPV protein was successfully loaded into the pore structure, which endowed the construct protein sustained release functionality.

CONCLUSIONS: Implantable cervix constructs was designed according to the conization and fabricated using PU via the 3D printing technology of LDM. The characteristics of the gradient pore distribution facilitated the loading of anti-HPV protein into the constructs. The results of the biocompatibility, structural stability and protein release suggest the feasibility of current cervix constructs function as accurate, structured and tailored tissue elements as well as controlled-release drug delivery systems towards individual treatment.

REFERENCES

[1] Freitas A C D, et al. Gynecologic Oncology, 2012, 126(2):0-0.



A hybrid-membrane migration method to isolate high-purity of adipose-derived stem cells from fat tissues through membranes coated with extracellular matrices

Y.C Lee[1], N.J Ku[1], A. Higuchi[1,2]
Presenting Author: Yu-Chun Lee, jk70655374@gmail.com
[1]Department of Chemical and Materials Engineering, National Central University (NCU), Zhongli, Taiwan [2]Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama, Japan

INTRODUCTION: Human adipose-derived stem cells (hADSCs) exhibit heterogeneous characteristics, indicating various genotypes and differentiation abilities. The isolated hADSCs can possess different purity levels and divergent properties depending on the purification methods used. Hence, we developed a membrane migration method to isolate the hADSCs which can extemsively reduce the purification time. Furthermore, the isolated hADSCs are expected to have high pluripotency and high differentiation ability into chondrocytes, osteoblasts, and adipocytes.

METHODS: We are developing a new membrane migration method using Nylon mesh membranes having optimal pore sizes, 11 and 20 μm, and PLGA/silk membranes where optimal extracellular matrix (ECM) is coated on the membranes, which can purify hADSCs. We isolated hADSCs from adipose tissue by the membrane migration method where different membranes are used, e.g., (a) Nylon mesh (pore size=11 or 20 μm) and PLGA/silk membrane, (b) Nylon mesh (pore size=11 or 20 μm) and PLGA/silk membrane coated with collagen type I, (c) Nylon mesh (pore size=11 or 20 μm) and PLGA/silk membrane coated with human recombinant-vitronectin, (d) Nylon mesh (pore size=11 or 20 μm) and PLGA/silk membrane coated with human fibronectin. Collagen type I is xeno-containing materials, other extracellular matrices (ECMs) are xeno-free materials.

RESULTS & DISCUSSION: hADSCs are successfully isolated by membrane migration method with different kind of membranes coated with extracellular matrices. After separating the hADSCs, we also counted the cell ratio of the isolated hADSCs (Fig. 1). Finally, the isolated hADSCs differentiated into osteoblasts. At 14 days, we detected the (alkaline phosphatase) ALP activity assay for the early stage of osteogenic differentiation. We also had the late stage of osteogenic differentiation detection with alizarin red and von Kossa staining after 14 days.

CONCLUSIONS: Membrane migration method can quickly purify and isolate the different types of hADSCs. In the late stage, migrated cells have the higher osteogenic differentiation ratio than those on TCPS (Fig. 2).

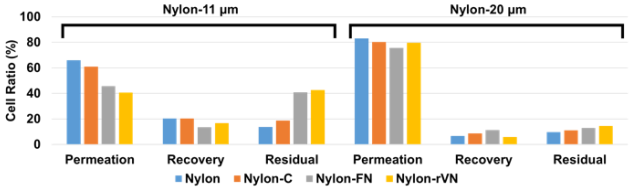


Figure 1: (Left) Cell ratio of the isolated hADSCs.

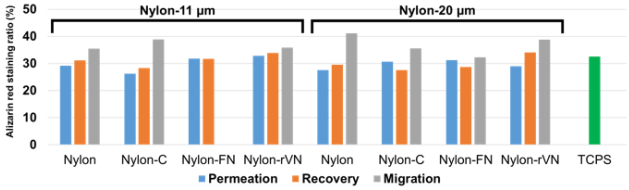
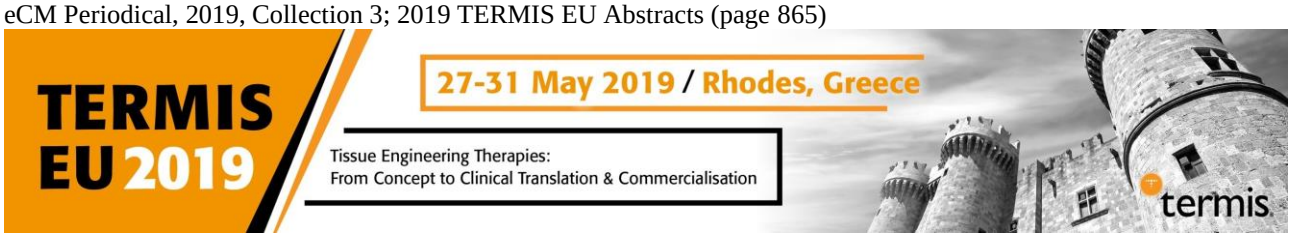


Figure 2: (Right) Alizarin red staining ratio of the isolated hADSCs.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Science and Technology (MOST 107-2119-M-008-002).



Cell-selective peptide functionalized polysaccharide-collagen hybrid material for medical application

Y. Wang[1], K. Kanie[1], K. Kaneko[1], A. Ogata[2], Y. Narita[2], T. Takezawa[3], R. Kato[1,4]*
Presenting Author: Yue Wang, 1129wangyue@gmail.com
[1]Graduate School of Pharmaceutical Sciences, Nagoya University, Japan [2]Graduate School of Medicine, Nagoya Univesity, Japan [3]National Agriculture and Food Research Organization, Japan [4]Division of Micro-Nano Mechatronics, Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University, Japan

INTRODUCTION: For more safe and therapeutic effect of medical implants, their surface functionalization to enhance their regenerative performance is an effective approach. Especially with the medical implants for circulatory system, surface modification and its effect have critical effect. For the enhancement of regenerative effect on medical implants, our group have been exploring short peptides with cell-selective adhesion/growth [1,2]. These peptides are screened from combinatorial peptide library by evaluating the dual effect on different types of cells, which enhances the adhesion/growth of cells that enhance the regeneration and inhibits the cells with negative affect on implanted area. For the application of these peptides, we have been investigating bio-compatible scaffold material for medical applications. In this work, we have developed a hybrid material of polysaccharide and collagen processed by vitrification method [3]. We here report the mechanical property and its peptide-modified effect for evaluating its medical usage performance.

METHODS: Ten different types of polysaccharides were evaluated to make vitrified collagen hybrid film. Their mechanical properties and cell-selective adhesion performance was evaluated with endotherial cells, fibroblasts, and smooth muscle cells, which mainly consists of blood vessel and to be controlled in the regeneration of vascular tissue.

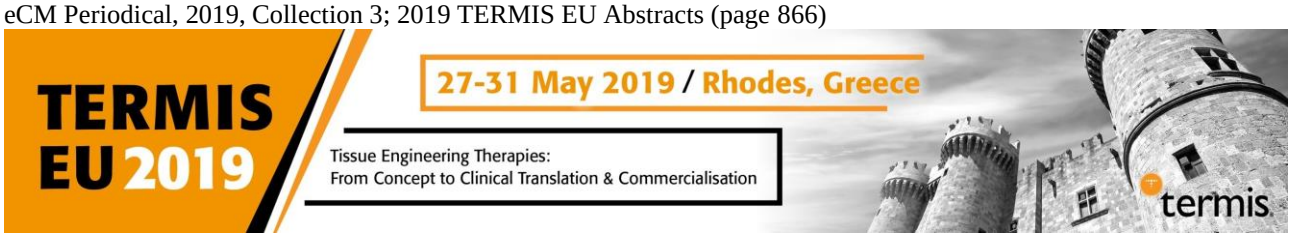
RESULTS & DISCUSSION: We found optimized condition of polysaccharide property together with the vitrification protocol and condition to maximize the cell-selective adhesion peptides. As a result, we found that our cell-selective peptides can provide cell-selective enhancement of endothelialization on newly designed polysaccharide and collagen hybrid material.

CONCLUSIONS: Our hybrid material combined with cell-selective material can serve as new regeneration enhancing material in medical usages.

ACKNOWLEDGEMENTS: This research was funded JSPS Grant-in-Aid for Scientific Research (18K14061) and Research Fund from Toyoaki Foundation.

REFERENCES

[1] Kanie et al, Materials. 2016; 9(9) 730.
[2] Kanie et al, Biotechnol Bioeng. 2012; 109:1808-16.
[3] Takezawa T, Cell Transplant. 2004;13:463-73.



Synergistic effect of surface elasticity and topology of nerve implants on peripheral nerve regeneration

F. Liu[1], G. Li[1], Y. Yang[1]
Presenting Author: Yumin Yang, yangym@ntu.edu.cn
[1]Jiangsu Key Laboratory of Neuroregeneration, Nantong University, 226001, Nantong, P.R. China

INTRODUCTION: A variety of synthetic and natural biological materials have been used in the repair of peripheral nerve injury, and the surface properties are found to play an important role in tissue regeneration, however, it is still unclear about the effect of synergistic surface physical properties, especially for the elasticity and topography, on peripheral nerve regeneration.

METHODS: In this study, the chitosan/acrylamide hydrogel with different surface elasticity and topology was prepared by in-situ radical polymerization and micromolding methods. The physical and chemical properties of the hydrogel were characterized respectively. The effects of these materials on nerve regeneration are evaluated using dorsal root ganglion cell model, and the priority parameters for regulating cell growth were confirmed, including the morphology, number of protrusions and growth rate. Meanwhile, the transcriptome sequencing technology was employed to analyze the impact of the materials with different surface properties on the gene expression profiles. Moreover, the involved molecular mechanisms were also elucidated by the combination use of bioinformatics methods and experimental validation.

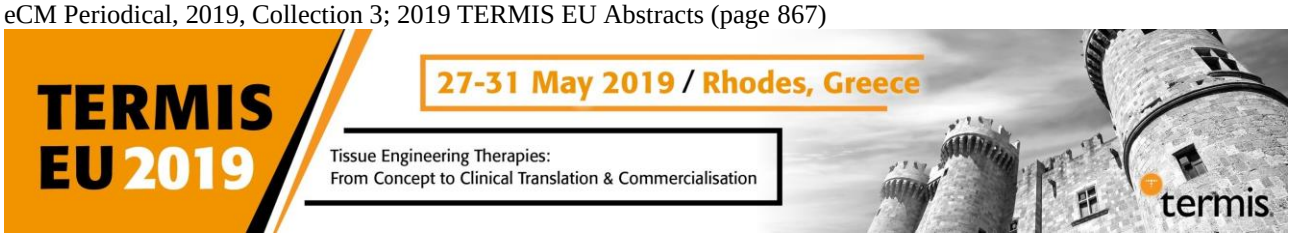
RESULTS & DISCUSSION: The results showed the chitosan/acrylamide hydrogel with different surface elasticity and topology could be well fabricated. The study reveals the structure-activity relationship between the growth of neurons and anterior horn motor neurons, respectively, yielded a range of favorable surface properties (elasticity and topology) for nerve regeneration.

CONCLUSIONS: The study demonstrates that the design of surface elasticity and topology of nerve implants will provide a potential and effective selection for biomaterials surface modification and shows great potential for the application in peripheral nerve regeneration.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge the financial support of the National Key Research and Development Program of China (2017YFA0701304, 2016YFC1101600, 2018YFC11056), National Natural Science Foundation of China (31830028, 31771054,81671823).

REFERENCES

[1] Wu R et al. 2016, Neurol Res,16: 1-6
[2]. Ashur H et al. 1987; Exp Neurol 97:365-374
[3]. Kang KB, et al. 2017; Invest Ophth Vis Sci 58: 6388-6398



Nanostructured scaffolds based on bioresorbable polymers and graphene oxide to promote neurodifferentiation of dental pulp-derived stem cells

Y. Polo[1,2], J. Luzuriaga[3], I. Irastorza[3], J.M Encinas[4,5], J.R. Pineda[4], G. Ibarretxe[3], F. Unda[3], A. Larrañaga[1], J.R. Sarasua[1]
Presenting Author: Yurena Polo, ypolo@polimerbio.com
[1]Department of Mining, Metallurgy and Materials Science, University of the Basque Country (UPV/EHU), Bilbao, Spain [2]Polimerbio SL, Donostia – San Sebastián, Spain [3]Cell Biology and Histology Department, UPV/EHU, Leioa, Spain [4]Achucarro Basque Center for Neuroscience, UPV/EHU Scientific Park, Leioa, Spain [5]IKERBASQUE, The Basque Foundation for Science. Bilbao, Spain

INTRODUCTION: Regeneration of the nervous system is challenging due to its limited tissue plasticity and poor ability to regenerate after injury [1,2]. Stem cell-based therapies, even if promising, have faced important limitations in terms of cell survival and integration into the neural circuitry. To overcome this, we have developed two-dimensional nanostructured scaffolds based on bioresorbable polyesters and graphene oxide (GO) to promote the neurodifferentiation of dental pulp-derived stem cells (DPSCs).

METHODS: Poly (D, L-lactide) (PDLLA) scaffolds were first nanopatterned with grating of 350 nm linewidth and subsequently functionalized with polydopamine, which acted as an adlayer for the final immobilization of GO. Films were extensively characterized by means of scanning electron microscopy (SEM), atomic force microscopy, X-ray photoelectron spectroscopy and Raman spectroscopy. Human DPSCs were seeded on these films and after 1, 3 and 7 days, cells were fixed and immunostained for proliferation (Ki67), morphology (Rhodamine-Phalloidin), neuronal markers (NeuN, DCX) and glial markers (GFAP, S100b). Metabolic activity of cells was assessed using alamarBlue.

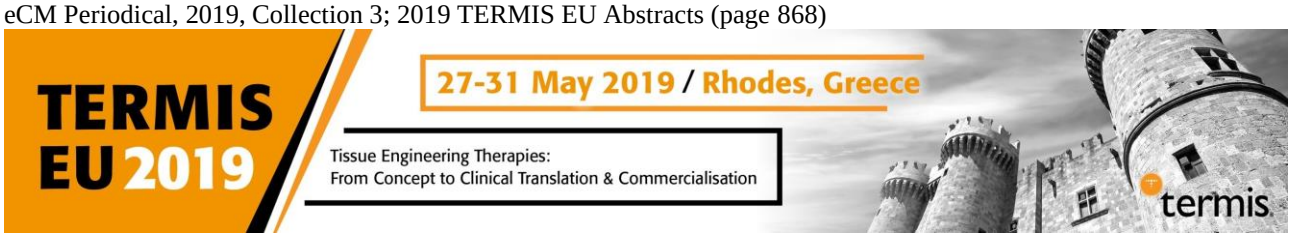
RESULTS & DISCUSSION: DPSCs adhere and proliferate in vitro on the developed scaffolds, as demonstrated by rhodamine-phalloidin and Ki67 immunostaining. Moreover, DPSCs were elongated and aligned in the nanograting axis. Immunohistochemistry analyses to cells incubated in Neurocult media show a higher expression of neuronal markers (i.e., NeuN and DCX) and a lower expression of the glial markers (i.e., GFAP and S100b) when seeded on nanostructured films functionalized with GO with respect to the control (i.e., flat surface without GO).

CONCLUSIONS: The combination of GO with nanoscale topographical cues provided by the polymeric structure would serve as functional mimics to drive neuronal commitment and directional growth of neurites, thereby enabling a simple and scalable method for a spatial alignment of DPSCs committed to neuronal fate for future clinical use.

ACKNOWLEDGEMENTS: Government of the Basque Country for the financial support. (GIC 15/52, IT-927-16) and to Bikaintek PhD grants.

REFERENCES

[1] Ahuja CS et al. Neurosurgery. 2017 Mar 1;80(3S):S9-S22.
[2] Hilton BJ et al. Development. 2017 Oct 1;144 (19):3417-3429.



The influence of hyperbaric oxygen therapy on the biology of adipose tissue mesenchymal stem cells

Y. Yoshinoya[1], A. H. Böcker [1,2], T. Ruhl[1], B.-S. Kim[1], J. P. Beier [1]
Presenting Author: Yuriko Yoshinoya, yuriko.yoshinoya@rwth-aachen.de
[1]Department of Plastic Surgery, Hand Surgery – Burn Center, University Hospital RWTH Aachen, Aachen, Germany [2]current affiliation: Department of Hand-, Plastic- and Reconstructive Surgery, Burn Center - BG Trauma Center Ludwigshafen, Hand and Plastic Surgery, University of Heidelberg, Germany

INTRODUCTION: The treatment of chronic wounds represents a great challenge in Plastic and Reconstructive Surgery with limited options for efficient treatment. Hyperbaric oxygen therapy (HBOT) has gained interest as an experimental strategy to promote wound healing. HBOT means prolonged exposure to 100% O_2 under increased atmospheric pressure (ATM). Mesenchymal stem cells (MSCs) play a major role in wound repair due to their multilineage differentiation capacity and paracrine function. In the present study, we studied the effect of HBOT on the biology of adipose tissue (AT-)MSCs in an in vitro setting.

METHODS: AT-MSCs from seven healthy donors were treated in a custom-made HBO chamber for five consecutive days with either 2 or 3 ATM. Metabolic activity was measured immediately before and after HBOT via PrestoBlue® staining. Proliferation was determined using Crystal violet staining. The effect of HBOT on adipogenic and chondrogenic differentiation was measured by Oil Red O and Alcian-PAS staining, respectively. Lastly, the secretion of cytokines in cell media was quantified by ELISA.

RESULTS & DISCUSSION: Treatment with 3 ATM enhanced metabolic activity 24 hours after treatment in a saw-tooth progress, and it increased proliferation over to the untreated control group and the 2 ATM treated cells. HBOT promoted adipogenic differentiation, while it did not affect chondrogenic differentiation. EGF, HGF and TNF-α were not detected in the cell supernatants, while secretion of TGF-β decreased 24 hours after the time points of the highest metabolic activity.

CONCLUSIONS: HBOT influences MSC metabolic activity and drives adipogenesis, supporting its effectivity in the process of wound healing, especially in the field of soft tissue regeneration. However, 3 ATM seemed to be more effective than 2 ATM.

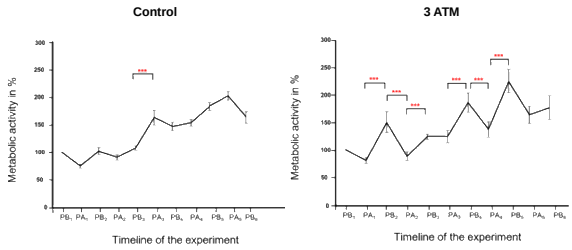
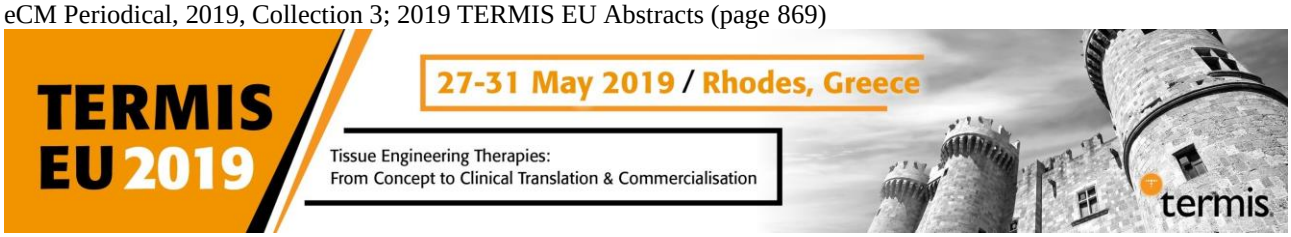


Figure 1: Metabolic activity measured with PrestoBlue® before (PB) and after (PA) each HBOT (1-5) for 3 ATM (right) and untreated control (left). Metabolic activity is increased 24 hours after treatment; note the zig-zag course for 3 ATM between PB and PA.

ACKNOWLEDGEMENTS: Financial support was received from “START”, a program for young scientist of the Medical Faculty at the RWTH University Aachen.



In-process evaluation of cell manufacturing process using morphology-based image analysis

Y. Imai[1], K. Yoshida[1], K. Kanie[1], R. Kato[1, 2]
Presenting Author: Yuta Imai, imai.yuuta@a.mbox.nagoya-u.ac.jp
[1]Department of Basic Medicinal Sciences, Graduate School of Pharmaceutical Sciences, Nagoya University, Japan [2]Division of Micro-Nano Mechatronics, Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University, Japan

INTRODUCTION: Industrial-scale manufacture technologies for producing Mesenchymal Stem cells (MSCs) are expected to widely distribute cells for therapies [1, 2]. However, one of the bottlenecks is the quality monitoring difficulties for the stable and efficient cell manufacturing. By the lack of effective in-process measurement technology, cellular status monitoring during the total process of complex and long cell culture has been relied on manual microscopic observations. However, standardization and control of manual observation is difficult, and quantitative data understanding has been more difficult. To breakthrough such manual-based cell monitoring, we here propose the computational automatic concept to detect and visualize the irregular operation troubles that occur in the cell culture process only by the image-based cellular morphological information.

METHODS: Twenty-one lots of human mesenchymal stem cells (MSCs), were cultured under 5 conditions (one standard and 4 types of intentional errors, such as clear failure of handlings and machinery malfunctions). Using time-course images, cell morphological profiles were quantitatively measured and utilized for visualization and prediction modeling. For visualization, modified principal component analysis (PCA) was used. For prediction modeling, linear regression analysis and the MT method were applied.

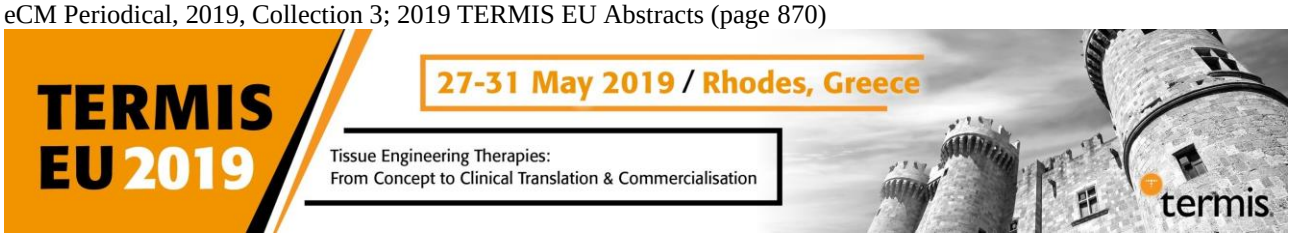
RESULTS & DISCUSSION: By modified PCA visualization, the differences in cellular lots and culture conditions were illustrated as traits on a morphological transition line plot and found to be effective descriptors for discriminating the deviated samples in a real-time manner. In the prediction modeling, both the cell growth rate and error condition discrimination showed high accuracy (>80%), which required only 2 days of culture.

CONCLUSIONS: Morphological information that can be quantitatively acquired during cell culture has great potential as an in-process measurement tool for quality control in cell manufacturing processes.

ACKNOWLEDGEMENTS: This research was funded by the JST Program for Creating STart-ups from Advanced Research and Technology (START) program, and NEDO AI project.

REFERENCES

[1] Wang Y, et al. Stem Cell Int 2012; 2012:1e4.
[2] Jossen V, Appl Microbiol Biotechnol 2018; 102:3981-94.



The utilisation of bioprinted tissue constructs oriented with phosphate glass fibres for repair of neural injuries

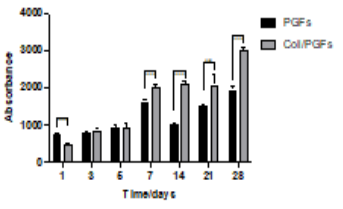
Z. Keskin-Erdogan[1], J. C. Knowles[1,2]
Presenting Author: Zalike Keskin Erdogan, zalike@gmail.com
[1]Biomaterials and Tissue Engineering Division, Eastman Dental Institute, University College London, London, UK [2]The Discoveries Centre for Regenerative and Precision Medicine, UCL Campus, London, UK

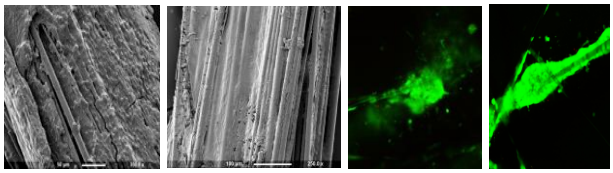
INTRODUCTION: After severe trauma there can be significant nerve injury and loss of function in the nervous systems. Due to the various challenges in regeneration like slow axonal regeneration and inhibitory effects of injury environment, nerve recovery is one of the most complex biological phenomena [1]. This study presents preliminary results that focusing to nerve recovery by developing TiO_2 phosphate glass fibers (PGFs) based bioprinted hydrogels.

METHODS: Phosphate glass fibers were produced (P_2O_5–$CaCO_3$–NaH_2PO_4–TiO_2) with the molar percentage of elements ($0.5P_2O_5$-$0.35CaO$-$0.1Na_2O$-$0.05TiO_2$). Cell line C6 (ATCC-CCL-107) rat glial cells were used at $5x10^{5-6}$ cells/ml concentration. The hydrogel for 3D printing was GelMA and also preliminary results were obtained by using Type-1 Collagen gel. Cellink Inkredible^{TM} 3D printer was used for bioprinting.

RESULTS & DISCUSSION: SEM, confocal microscopy and viability assessments (AlamarBlue) showed biocompatibility of PGFs and its directional guidance potential for growth of neural cells. Bioprintability of prepared GelMA hydrogels showed promising results regarding to cell viability and aligned growth after printing.

CONCLUSIONS: Thus far, data collected, together with expected results would be able to illustrate that PGFs with 3D printed GelMA may have a great potential to provide axonal regeneration for neural tissue engineering.





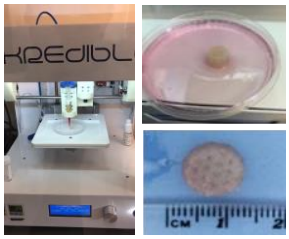
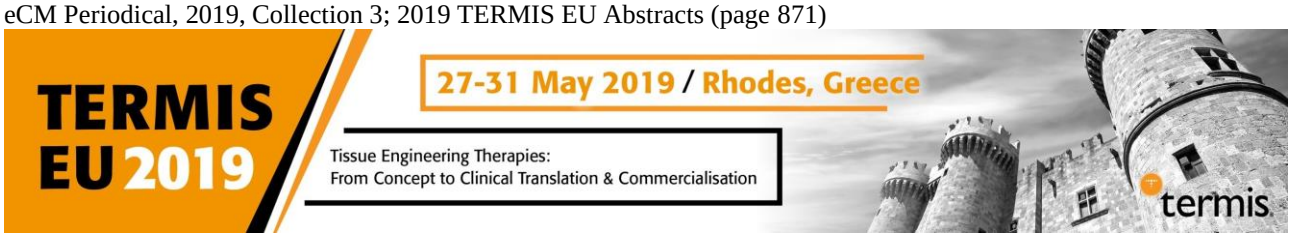


Figure 1: (left) Metabolıc actıvıty results of C6 cells on PGFs and Coll/PGFs. **Figure 2:** (middle) SEM &live and dead images of C6 cells on PGFs/Collagen **Figure 3:** (right) Printing process and various 3D printed scaffolds

ACKNOWLEDGEMENTS: Financial support for ZKE has been received from Turkish Ministry of National Education.

REFERENCES

[1] Ghorbani, F., & Zamanian, A. (2016). J. of Tissue Science & Engineering, 07(03).



A novel composite of nanohydroxyapatite (nHAp), synthetic polymer (PLA-PEG) and bone morphogenetic protein 2 (rhBMP-2) for bone regeneration

Z. Bal[1], K. Takashi[1], H. Ishiguro[1], R. Okada[1], J. Kushioka[1], J. Kodama[1], P. Korkusuz[2], E. Ciftci Dede[3], Hideki Yoshikawa[1], F. Korkusuz[4]
Presenting Author: Zeynep Bal, zeynepbal@gmail.com
[1]Department of Orthopaedic Surgery, Osaka University Graduate School of Medicine, Osaka, Japan [2]Department of Histology & Embryology, Medical Faculty, Hacettepe University, Ankara, Turkey [3]Department of Bioengineering, Hacettepe University, Ankara, Turkey [4]Department of Sports Medicine, Medical Faculty, Hacettepe University, Ankara, Turkey

INTRODUCTION: Proper sustainable release of BMP needed for bone regeneration was reported to be decreased [1,2]. Compared to conventional hydroxyapatite, the nano-sized hydroxyapatite (nHAp) has superior osteoblast adhension properties [3] and osteoinductive ability. Herein, we combined PLA-PEG with nHAp as carrier/scaffold system for BMP in order to investigate the bone regenerative capacity of newly established biomaterial in a rat spinal fusion model.

METHODS: BMP-2/PLA-PEG (5mg) /nHAp (12.5mg) composites containing different dosage of rhBMP-2 (0µg/3µg/10µg) were created. The release kinetics of rhBMP-2 from the composite was investigated by ELISA. For in vivo bone formation, same composites were placed on both sides L4-5 transverse processes and bone formation was assessed by µCT and histology at post-op. 8 weeks

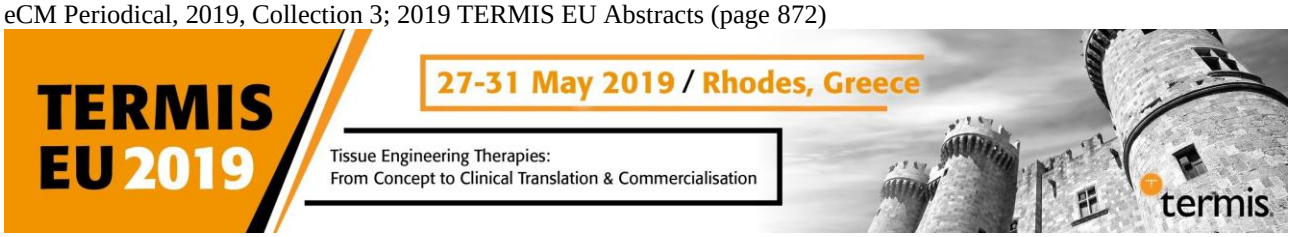
RESULTS & DISCUSSION: The composite showed a burst-release (69% of the total release) in the initial 24 hours and the release was continued for 25 days. µCT and histology demonstrated that spinal fusion was achieved either one or both sides of the L4-5 transverse processes in all BMP3µg and BMP10µg groups (bilateral non-union / unilateral fusion / bilateral fusion, BMP0µg group; 10/0/0, BMP3µg group; 1/0/9, BMP10µg group; 0/1/7).

CONCLUSIONS: We developed a new biomaterial for bone regeneration with a novel composite of BMP-2/PLA-PEG/nHAp. The reduction in the required dosage of BMP-2 for 100% bony fusion in rat spinal fusion was achieved.

ACKNOWLEDGEMENTS: Financial support This study is supported by TUBITAK and JSPS (Project no: 215S834).

REFERENCES

[1] Kaito T et al. Biomater. 2005; 26(1):73-79
[2] Yoshikawa H et al. J R Soc Interface. 2009;6(Suppl 3):S341-S348
[3] Webster T J et al. Biomater. 2000;21(17):18



Cell seeded collagen-polyurethane scaffold supplemented with TGFβ for annulus fibrosus repair

J. Du[1], R.G. Long[1,2], D. Sakai[3], L.M. Benneker[4], J.C. Iatridis[5], M. Alini[1], S. Grad[1], Z. Li[1]
Presenting Author: Zhen Li, zhen.li@aofoundation.org
[1]AO Research Institute, Switzerland [2]Department of Genetics, Harvard Medical School, USA [3]Tokai University, Japan [4]Inselspital, University of Bern, Switzerland [5]Icahn School of Medicine, USA

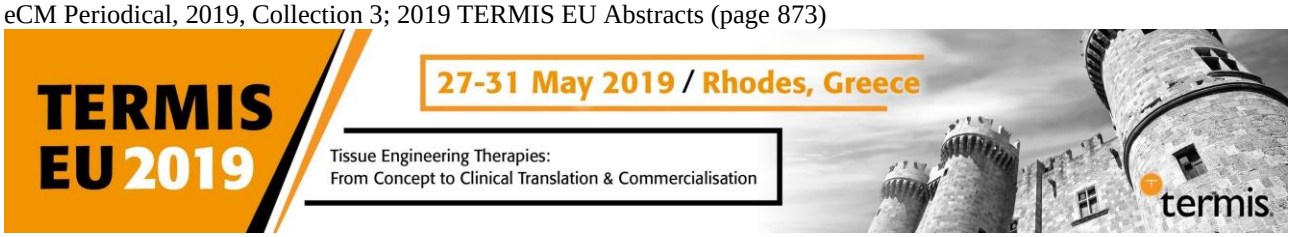
INTRODUCTION: Intervertebral disc (IVD) herniation causes low back pain, which leads to a high socioeconomic burden. Large annular rupture after discectomy is associated with an increased rate of reherniation. In this study a tissue engineered construct, composed of polyurethane (PU) scaffold with TGFβ supplemented type I collagen hydrogel and TGFβ pre-treated human AF cells, was evaluated in vitro and ex vivo for the repair of annulus fibrosus (AF) rupture.

METHODS: AF cells were isolated from human AF tissue of trauma patients, then expanded in αMEM with 10% FBS at 2% O_2. In vitro, AF cells were cultured with or without 5 ng/ml TGFβ for 4 days, then evaluated by flow cytometry, gene expression analysis and cell contraction assay. TGFβ treated AF cells were seeded into PU scaffold only (PU-AFCs), or combined with collagen I hydrogel and treated with (PU-Col-AFCs-TGFβ) or without 5 ng TGFβ (PU-Col-AFCs). The cells were seeded into a PU scaffold (diameter 3.5 mm, length 4 mm) at a cell density of 0.2×10^6 per scaffold. After 7 days culture, scaffolds were collected for gene expression and DNA and GAG content. **Ex vivo**, a bovine caudal IVD AF defect organ culture model created by biopsy punch (diameter 3 mm, length 4 mm) was used in this study. An acellular PU scaffold with collagen hydrogel (PU-Col), or PU-Col with TGFβ pre-treated AF cells with (PU-Col-AFCs-TGFβ) or without (PU-Col-AFCs) 5 ng TGFβ were implanted into annulotomized IVD for AF defect repair. After 14 days culture with physiological loading, discs were evaluated by Safranin O/Fast Green staining; implanted scaffolds were evaluated by gene expression.

RESULTS & DISCUSSION: In vitro, TGFβ treated AF cells had greater contractility, higher gene and protein expression of AF marker CD146, and higher SM22α and SCX gene expression compared with non-treated cells. When AF cells were seeded into PU scaffolds, gene expression of collagen type II, SM22α, SCX and MKX were increased while the metalloproteinase ADAMTS5 was decreased after 7 days culture in PU-Col-AFCs-TGFβ and PU-Col-AFCs groups compared with PU-AFCs group. Besides, higher DNA and GAG/DNA ratio were detected in PU-Col-AFCs-TGFβ group. Ex vivo, Implanted AF cells were present and started matrix production in both PU-Col-AFCs-TGFβ and PU-Col-AFCs groups after 14 days organ culture with physiological loading. However, PU-Col-AFCs-TGFβ group had greater cell number, collagen matrix production (Fig.1), and higher collagen I and CD146 gene expression.

CONCLUSIONS: TGFβ upregulated AF marker gene expression, and increased contractility in AF cells, indicating that TGFβ pre-treated cells may be a superior cell source for AF tissue engineering. Collagen type I hydrogel as a cell carrier in the PU scaffold maintained the functional phenotype. TGFβ treatment within the collagen hydrogel further promoted matrix production of AF cells both in vitro and ex vivo. PU-Col-AFCs-TGFß constructs have a good potential for generating tissue engineered AF, and may therefore be used to repair AF defects after discectomy.

ACKNOWLEDGEMENTS: AFR CRP AO Foundation, AOSpine International, NSFC 81772333 & 51873069.



Poster

Poly(diol citrates) as versatile biomaterials: synthesis, surface properties and cytocompatibility studies

A. Flis[1], F. Koper[2], W. Kasprzyk[2] E. Pamuła[1]
Presenting Author: Agata Flis, flis@agh.edu.pl
[1]Department of Biomaterials and Composites, AGH University of Science and Technology, Kraków, Poland [2]Faculty of Chemical Engineering & Technology, University of Technology, Kraków, Poland

INTRODUCTION: Poly(diol citrates) (PDC) are considered one of the most promising biomaterials for the fabrication of vascular tissue scaffolds. PDCs are thermoset and amorphous at 37^{o}C and their mechanical properties and degradation kinetics can be adjusted. Moreover they are easy to synthesize, inexpensive and non-toxic [1]. By using various diols, the mechanical properties and degradation characteristics of the obtained PDCs can be tuned. The aim of this study was to synthesize poly(1,8-octanediol citrate)s of various molar ratios and to characterize their surface properties and cytocompatibility. These polymers, in our further studies, are intended to be modified with panthenol and/or glutathione to provide them with luminescent and antioxidative properties, what is relevant for vascular tissue engineering.

METHODS: Citric acid was reacted with 1,8-octanediol in molar ratio 1:1 and 2:3, creating poly(1,8-octanediol citrate). Reagents were added to 30-ml flask and melted at 140 ^{o}C for 40 min under stirring to synthesize prepolymers. Then, prepolymers were dissolved in ethanol, precipitated in water, lyophilized and post-polymerized for 2, 3, 4 or 5 days at 80^{o}C. The surface roughness (Hommer-Werker profilometer) and the water and diodomethane contact angles of PDCs were measured by sessile drop method (DSA10, Kruss). The surface free energy was calculated by the Owens-Wendt equation. FTIR-ATR (Tensor 27, Bruker) analysis permitted to determine the surface chemical composition of the materials. Cytocompatibility studies with endothelial cells were tested in extracts from synthesized PDCs.

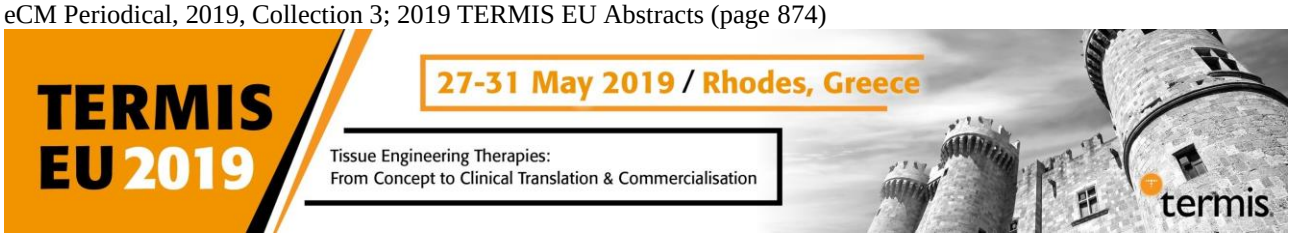
RESULTS & DISCUSSION: FTIR-ATR results show that PDCs with all studied molar ratios have similar spectra, differing only in peak intensities depending on composition. The samples were hydrophobic (water contact angle from 85^{o} to 105^{o}) and smooth (R_a in the range 0.16 - 0.40 µm). The surface free energy varied depending on polymer composition and post-polymerization time. Metabolic activity of endothelial cells cultured in the extracts from PDCs was not significantly different as compared to cells cultured in control conditions.

CONCLUSIONS: Two PDCs with different molar ratio were synthesized and their surface properties were characterized. They were found cytocompatible with endothelial cells, what paves the way of their further application in vascular tissue engineering.

ACKNOWLEDGEMENTS: This study was financed by National Science Center, Poland (2018/28/C/ST5/00461) and Polish National Agency for Academic Exchange (PPI/PRO/2018/ 1/00026/U/001).

REFERENCES

[1] Yang J et al. Tissue Eng. 2005; 11(11/12): 1876-86



A modified hyaluronic acid hydrogel for enhancing trabeculectomy recovery in glaucoma patients

M. Lemoine[1, 2, 3], D. Brennan[4], C. O'Brien[5, 6], F. O'Brien[1, 2, 3], A. Hibbitts[1, 2, 3]
Presenting Author: Alan Hibbitts, alanhibbitts@rcsi.ie
[1]Tissue Engineering Research Group, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland [2]Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Dublin [3]Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI and TCD, Dublin [4]Anatomy, School of Medicine, Health Sciences Centre, University College Dublin, Belfield, Dublin 4 [5]Institute of Ophthalmology, Mater Misericordiae University Hospital, Dublin [6]Clinical Research Centre, UCD School of Medicine, Mater Misericordiae University Hospital, Dublin

INTRODUCTION: Glaucoma is the second-leading cause of blindness with patient numbers to reach 80 million by 2020. There is no cure for glaucoma and progression often leads to intervention via trabeculectomy. Unfortunately, 30%–50% of these procedures fail due to formation of post-operative fibrotic fistulas [1, 2]. The formation of these fistulas is known to follow a two-stage process with inflammation and angiogenesis over the first 1-7 days followed collagen deposition over a 4-week period [3].

METHODS: To target these events, a modified hyaluronic acid (HyA) hydrogel was developed capable of controlled anti-inflammatory/fibrotic drug release profiles. Modified hydrogels were assessed for changes in mechanical strength, degradation and ease of insertion into cadaveric sheep eyes. Furthermore, anti-inflammatory/anti-fibrotic drug release was assessed using cell culture and a chick embryo model of angiogenesis.

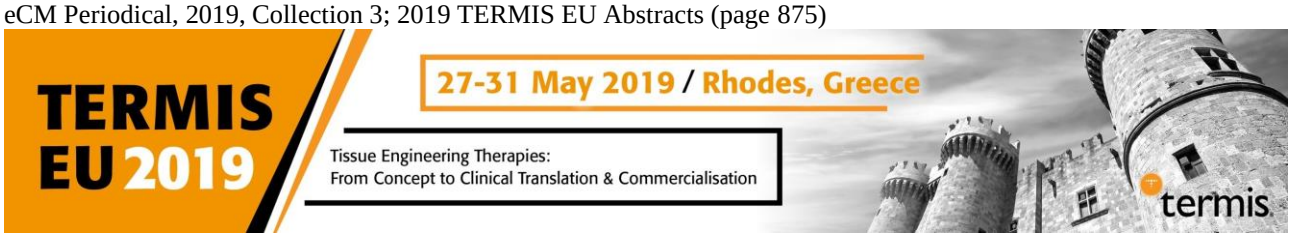
RESULTS & DISCUSSION: On analysis, it was found that HyA hydrogels were capable of rapid swelling (<30 min) and were stable in physiological conditions up to a minimum of 8 weeks but demonstrated dose dependent enzymatic degradation. Secondary modification allowed for an immediate anti-fibrotic effect through a 50% drop in collagen deposition in vitro at 24hrs. Furthermore, drug-loaded gels demonstrated visible decreases in angiogenesis up to day 7.

CONCLUSIONS: This work demonstrates a highly controllable hydrogel matrix capable of contact inhibition and anti-fibrotic drug release. It is now expected to be assessed in in vivo glaucoma models.

ACKNOWLEDGEMENTS: This work was funded by Science Foundation Ireland through the TIDA award 17/TIDA/5098.

REFERENCES

[1] Li, E.Y., et al. Invest Ophthalmol Vis Sci, 2013. **54**(5): p. 3394-9.
[2] Grover, D.S. and O. Smith. Curr Opin Ophthalmol, 2012. **23**(2): p. 127-34.
[3] Van Bergen, T., et al. Clin Ophthalmol, 2014. **8**: p. 857 67.



Impact of hydrogel stiffness on differentiation of human adipose-derived stem cell microspheroids

S. Zigon-Branc[1,2], M. Markovic[1,2], J. Van Hoorick[3,4] S. Van Vlierberghe[3,4], P. Dubruel[3], E. Zerobin[5,2], S. Baudis[5,2], A. Ovsianikov[1,2]
Presenting Author: A. Ovsianikov, Aleksandr.Ovsianikov@tuwien.ac.at
[1]Institute of Material Science and Technology, Technische Universität Wien (TU Wien), Vienna, Austria [2]Austrian Cluster for Tissue Regeneration (www.tissue-regeneration.at) [3]Polymer Chemistry and Biomaterials Group, Ghent University, Belgium [4]Brussels Photonics, Department of Applied Physics and Photonics, Vrije Universiteit Brussel, Belgium [5]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria

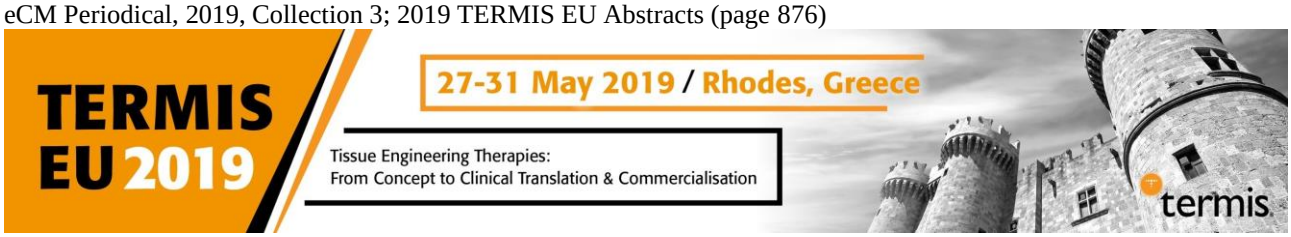
INTRODUCTION: Hydrogels represent versatile 3D biomimetic cell culture matrices, with their mechanical properties influencing proliferation, migration and differentiation of stem cells. In this study, we investigated how the stiffness of methacrylamide-modified gelatin (Gel-MOD) hydrogels affects proliferation and differentiation of immortalized human adipose-derived stem cell (hASC/hTERT) microspheroids in control (Ctr), chondrogenic (Ch) and osteogenic (Ost) medium.

METHODS: Microspheroids containing 1000 (hASC/hTERT) were produced employing MicroTissues® 3D Petri Dishes® (Sigma) and re-suspended in control medium containing 5, 7.5 or 10% (wt%) Gel-MOD and 0.6 mM photoinitiator. Volumes of 30µL (containing 81 microspheroids) were dispensed on pre-methacrylated glass-bottom dishes and crosslinked by exposure to UV-A light (10 min at 365 nm and 25 mW/cm^2). After 3 – 5 weeks of culture in Ctr, Ch or Ost media, cell viability, gene expression, matrix production or mineralization were monitored using confocal microscopy (Live/Dead staining), quantitative polymerase chain reaction and histological analyses, respectively. The stiffness of 1mm thick Gel-MOD sheets was determined by rheology.

RESULTS & DISCUSSION: Regardless of the medium used, cells in microspheroids remained viable for 3-5 weeks in all hydrogels. Ctr medium supported fast sprouting and inter-connection of cells in softer hydrogels, which was slower and very limited in Ost and Ch media, respectively. High expressions of SOX9, COL2A1 and ACAN and a positive Alcian blue glycosaminoglycan staining were detected in all tested hydrogels, cultured in Ch medium. Although expressions of RUNX2, ALPL, BGLAP and COL1A1 were not conclusive in the samples subjected to Ost medium, Von Kossa staining showed a strong calcium presence in the two softer hydrogels. The storage modulus G` of investigated formulations 5, 7.5 and 10% Gel-MOD concentrations corresponded to 538, 3584 and 7263 Pa, respectively.

CONCLUSIONS: In the presence of a corresponding differentiation medium, Gel-MOD hydrogels support moderate osteogenic and excellent chondrogenic differentiation of encapsulated hASC/hTERT microspheroids, the extent of which depends on hydrogel stiffness. The hydrogel-microspheroid strategy proved exceptionally successful in promoting chondrogenesis, which was confirmed at the gene and protein levels.

ACKNOWLEDGEMENTS: Financial support was received from European Research Council (Starting Grant-307701, A.O.), Research Foundation Flanders Belgium (FWO-SB PhD Grant J.V.H) and Research Foundation Flanders-Austrian Science Fund (FWOAL843, #I2444N28).



The application of gaseous nitric oxide and nitric oxide donors for wound healing

A.V. Butenko [1], A.V. Pekshev [2], A.F. Vanin [1], A.L. Fayzullin.[1], T.G. Rudenko[1], A.B. Shekhter[1]
Presenting author: Aleksandra Butenko, butenko.aleksa@mail.ru
[1]Sechenov University, Moscow, Russia [2]Bauman Moscow State Technical University, Moscow, Russia

INTRODUCTION: Nitric oxide facilitates wound healing and protects the wounds from bacterial infection. The treatment of wounds with gaseous NO and/or with NO donors was shown to be effective [1, 2]. The aim of our research was to study the effects of gaseous nitric oxide (Plason device) and low-molecular dinitrosyl iron complexes (DNIC) with thiol ligands (cysteine or glutathione).

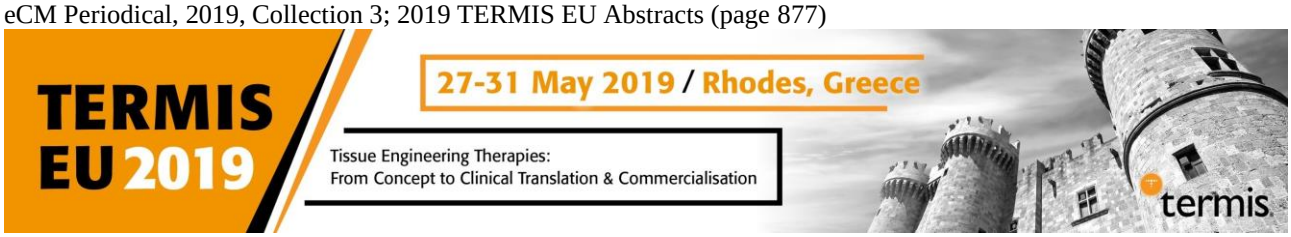
METHODS: Experiments were performed in 40 male albino rats (August, 120–140 g) kept under standard conditions. A circle of 8–10 mm diameter was excised down to the fascia from depilated skin between the shoulder blades of the animals. There were 2 groups of 20 animals. In the study with the nitric oxide donors (group 1), there were 4 subgroups of 5 animals. The injections were on the first and the second day after surgery. In the 1st subgroup (control) 0.6 ml of saline was syringed once a day; in the 2nd subgroup: DNIC-Cys 5 μmol; in the 3d: DNIC-GS (2.5 μmol), total 5 μmol; in the 4th: S-nitrosothiol (GSNO) (5 μmol), total 10 μmol. In the study with gaseous nitric oxide (group 2), in the 1st subgroup (control), the wounds were left without treatment. In the 2 subgroup the temperature of NO-containing gas flow (CGF) (t_{NO-CGF}) was 60°, NO containing (NOc) – 560 ppm; in the 3rd one t_{NO-CGF} was 22°, NOc – 560 ppm; in the 4th one - t_{NO-CGF} was 22°, NOc – 1120 ppm during 90 seconds on Day 1, Day 2 and Day 3 after surgery. On Day 4 the animals were removed from the experiment and wound tissues were analyzed with morphological, immunohistochemical, morphometric and statistical methods.

RESULTS & DISCUSSION: Histological data showed that dimeric DNIC with cysteine or glutathione were beneficial for wound healing at optimal amounts (cumulative 5 μmol per wound). Application of DNIC reduces inflammation and facilitates the growth and maturation of the granulation tissue. However, the same amount of NO with GSNO (10 μmol) worsened the condition of the wound. In all the experimental subgroups the study of the gaseous nitric oxide demonstrated that the intensity of inflammation was statistically lower and the ratio of regeneration (vascularization, fibroblast proliferation, synthesis and fibrillogenesis of collagen) was statistically higher than in control one. The effect was more expressed in the 4th subgroup with a two-fold increase in the content of nitric oxide at the distance of l=65mm. According to the morphometric study, the application of the NO-CGF at l=130 mm led to a thinner layer of fibrin and at l=65 mm – to the increase in content of collagen types I and III. The temperature factor of the NO-CGF did not affect the course wound healing.

CONCLUSIONS: Application of NO-CGF and NO-donors demonstrated beneficial effect on microcirculation, induction of phagocytosis, angiogenesis, proliferation of fibroblasts and activation of antioxidant protection through oxidative stress. The application of NO reduced inflammation and stimulated regeneration in wounds.

REFERENCES

[1] Shekhter, A.B. et al, 2015. Eur. J. Pharm. Sci. 44 78, 8-18.
[2] Shekhter, A.B. et al, 2005. Nitric Oxide 47 12, 210-219



Microvesicles released by human endothelial progenitor cells and adipose tissue mesenchymal stem cells support cells involved in wound healing process

A. Krawczenko, A. Bielawska-Pohl, M. Paprocka, H. Kraśkiewicz, A. Klimczak
Presenting Author: Aleksandra Klimczak, aleksandra.klimczak@hirszfeld.pl
Laboratory of Biology of Stem and Neoplastic Cells, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

INTRODUCTION: Endothelial progenitor cells (EPC) as well as mesenchymal stem cells (MSC) produce a number of biologically active substances secreted in a form of microvesicles (MVs). Microvesicles transfer and release their cargo; thus changing microenvironment in a way that could promote regenerative processes.

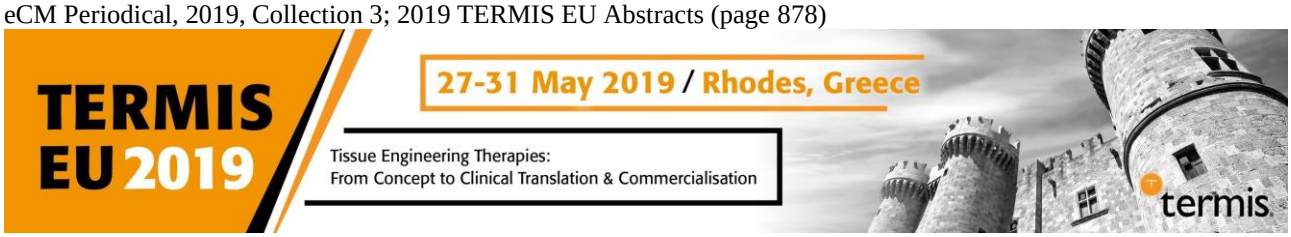
METHODS: MVs were isolated from human EPC cell line HEPC-CB.1 and human MSC cell line HATMSC1 using a serial centrifugation method. The protein content of MVs was examined using Human Angiogenesis Array C1000 (RayBiotech). The presence of hypoxia- and angiomiRs in MVs was investigated in real-time RT-PCR with TaqMan technique. Cell proliferation (human microvascular dermal endothelial cell line HSkMEC.2, human fibroblast cell line MSU-1.1 and human keratinocyte cell line HaCaT) was investigated using the standard MTT test. Angiogenic properties of MVs were examined in Matrigel angiogenic assay with the use of HSkMEC.2 cell line.

RESULTS & DISCUSSION: MVs of EPC expressed 28 proteins, and MVs of AT-MSC expressed 12 proteins among 43 examined. Both EPC and MSC MVs transport growth factors (e.g. bFGF, EGF) and proangiogenic factors (e.g. IL-8, VEGF, TIMP-1, TIMP2). MVs isolated both from EPC and MSC are enriched in all examined miRNA such as: miR 210, miR 296, miR 126 and miR 378 as compared to the parental cells. The effect of isolated MVs on the proliferation and angiogenic properties of the cells involved in wound healing was examined. Both EPC and MSC derived MVs increased proliferation of human endothelial cells and this effect was dose-dependent. MVs also improved angiogenic properties of endothelial cells in a dose-dependent manner. Pseudotubule formation was visible only for a ratio of 100:1 (100 MVs per 1 cell), not for the ratio of 10:1. The effect was observed for MVs of both EPC and MSC origin (Fig.1).

CONCLUSIONS: This result suggests that MVs released by mesenchymal stem and endothelial progenitor cells influence biological activity of endothelial cells in the wound microenvironment.

Figure 1: Pseudovessels creation. HSkMEC.2 cells were incubated 24 h in the presence of MVs from HEPC-CB.1 or HATMSC1 cell lines, at a ratios of 10:1 and 100:1. Picture acquisition in an Olympus CKX41 microscope, magnification 40x.

ACKNOWLEDGEMENTS: Studies supported by The Leading National Research Center (KNOW) for the years 2014-2018 (project ID 56/2017/KNOW/IITD).



Osteogenic potential and deposition of ovine bone marrow-derived mesenchymal stem cells onto 3D-scaffold for bone repair

S. Gromolak[1], A. Krawczenko[1], A. Antonczyk[2], J. Biezynski[2], B. Liszka[2], Z. Kielbowicz[2], A. Klimczak[1]

Presenting Author: Aleksandra Klimczak, aleksandra.klimczak@hirszfeld.pl

[1]Laboratory of Stem and Neoplastic Cells, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland [2]The Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences

INTRODUCTION: Mesenchymal stem cells (MSCs) are one of the most promising tools for regenerative medicine. The "gold standard" in the cell-based treatment of damaged tissues is the direct transplantation of cells, but tracking them and keeping in one particular place of injured organ is difficult. This problem could be solved by application of MSC attached to 3D scaffolds. We investigated the biological properties and osteogenic potential of sheep bone marrow-derived MSCs (BM-MSCs) and efficacy to seed them onto biocompatible scaffold.

METHODS: Bone marrow (8-10ml) was collected from sheep iliac crest (n=6). Flow cytometry for stem cell markers expression was performed on a BD FACS Calibur. MSCs differentiation into the osteogenic lineage in dedicated medium (PromoCell) was followed by Alizarin Red S, anti-osteocalcin, anti-collagen type I immunofluorescence staining, and osteopontin (OPN) gene expression by PCR. Cells were seeded at the density $1x10^6$/ml medium onto a polymeric scaffold. The deposition and morphology of sheep MSCs growth on the 3D scaffold was examined on day 3, 7 and 12 by PKH26 Red and DAPI staining.

RESULTS & DISCUSSION: Sheep bone BM-MSCs were positive for CD73, CD90 and CD105 and negative for hematopoietic markers CD34, CD45 and MHC class II. Cells incubated in osteogenic medium for 21 days exert bone mineralization capacity confirmed by Alizarin Red S staining. Moreover, the osteogenic markers: osteocalcin and type I collagen were detected by immunofluorescence and OPN by PCR.

CONCLUSIONS: Sheep BM-MSCs with characteristic naïve stem cell markers expression, were able to differentiate into osteogenic lineage and to proliferate on 3D scaffold. Thus, sheep bone marrow may be use as a potential source of MSCs for the studies of bone regeneration and the sheep can serve as a large animal preclinical model for bone engineering.

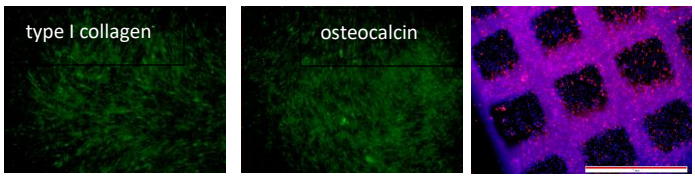
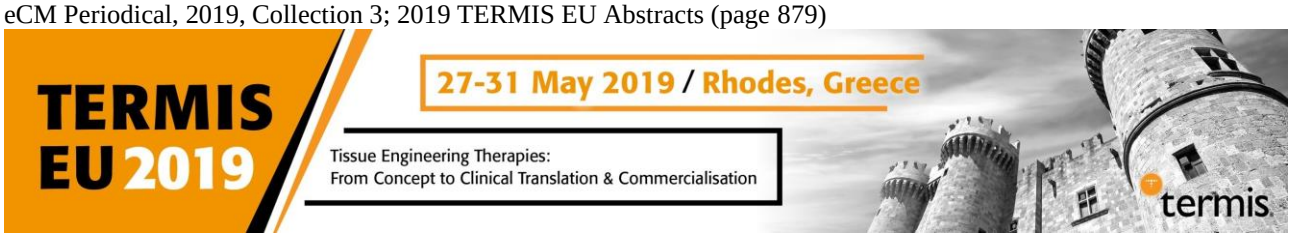


Figure 1: Immunofluorescence staining of collagen I and osteocalcin after 21 days of differentiation. Sheep MSC were seeded onto biocompatible 3D-scaffold to construct bioengineered bone. The deposition, morphogenetic and viability of seeded cells were determined by PKH26 Red and DAPI staining. After 12 days of culture, BM-MSC were attached and spread well on the scaffold. PKH26 (red) and DAPI staining sheep MSCs attached on the 3D scaffold after 12 days of incubation.

ACKNOWLEDGEMENTS: Supported by grant STRATEGMED 3/306888/3/NCBR/2017



A patient-based 3D breast cancer cell model to tackle ER signaling

A.L. Cartaxo[1,2*], M.F. Estrada[1,2*], G. Domenici[1,2], C. Pinto[1,2], R. Roque[3], S. André[3], C. Brito[1,2]
Presenting Author: Ana Cartaxo, acartaxo@ibet.pt
[1]iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal [3]Instituto Português de Oncologia Francisco Gentil Lisboa, Portugal * these authors contributed equally

INTRODUCTION: Breast cancer (BC) is one of the leading causes of death worldwide. Available models to study estrogen receptor α (ER)-positive BC and to evaluate drug response fail in recapitulating specific disease features, such as the ER signaling [1]. Herein, we propose a 3D ex vivo patient-derived model of ER+ BC based on alginate encapsulation and orbital-shaking, previously validated in our group for cell lines [2,3]. This strategy allows cellular crosstalk, retention of ECM components and promotes uniform distribution of both nutrients and cell-secreted factors.

METHODS: >80 tumor samples were collected during surgery and transported to the laboratory within 4 hours, where they were mechanically dissociated, digested and encapsulated with 2% alginate. Encapsulated explant cultures were kept under orbital shaking. Cell viability was assessed along culture, using fluorescein diacetate (FDA) and propidium iodide (PI) to stain live and dead cells, respectively. Encapsulated explants were collected after 1 month in culture and paraffin blocks were prepared and sectioned for immunohistochemistry and hematoxylin and eosin (H&E) staining. To assess ER presence and functionality, explant cultures were challenged at day 30 with estradiol, a natural ER agonist, and the expression of downstream effector genes evaluated by RT-qPCR.

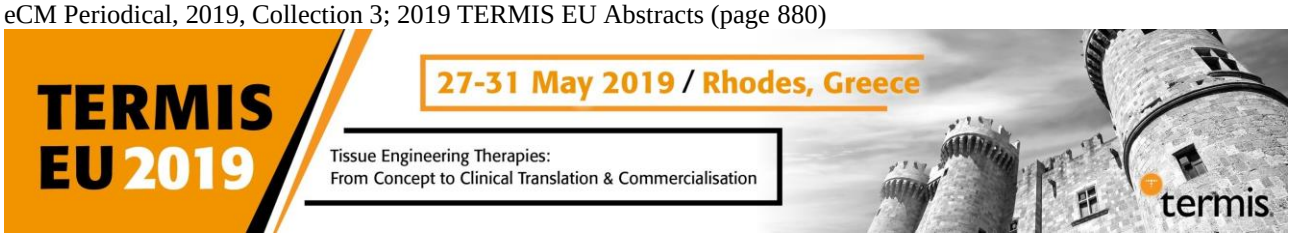
RESULTS & DISCUSSION: Alginate encapsulation and dynamic culture conditions showed to be an efficient strategy for long-term maintenance of breast cancer explants with high cell viability and retention of tissue architecture, including epithelial and stromal phenotypes and ratios. Importantly, after 1 month in culture, ER protein was still detected in the explants. ER functionality was demonstrated by an increase in the expression of downstream effectors of ER signaling (progesterone receptor: PR; amphiregulin: AREG; trefoil factor 1: PS2) upon stimulation.

CONCLUSIONS: Here we propose a new tissue-based 3D ex vivo cell model suitable for studying and interrogating ER signaling and evaluation of ER targeted compounds.

ACKNOWLEDGEMENTS: Financial support was received from FCT (PD/BD/114047/2015) and iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-007344), co-funded by FCT / MCES, through national funds, and by FEDER under the PT2020 Partnership Agreement.

REFERENCES

[1] Klitgaard MC, et al. Nat Commun. 2015;6(8786).
[2] Rebelo SP, et al. Biomaterials. 2018;163:185-197. doi:10.1016/j.biomaterials.2018.02.030.
[3] Estrada MF, et al. Biomaterials. 2016;78:50-61. doi:10.1016/j.biomaterials.2015.11.030.



Evaluation of encapsulated mesenchymal stem cell joint migration in horses

V. H. Santos[a]; J. P. Pfeifer[a]; J. B. Souza[a]; M. A. Golim [b], E. Deffune[c], C.A. Hussni[a], A. L.G. Alves[a*].
Presenting Author: Ana Liz Garcia Alves, ana.liz@unesp.br
[a] Department of Veterinary Surgery and Anesthesiology, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), District of Rubião Júnior, s / n, 18618-681, Botucatu, SP, Brazil

INTRODUCTION: Osteoarthritis is characterized clinically by pain and a progressive decrease in joint mobility. The implantation of synovial membrane mesenchymal stem cells (MSC_{SM}) has shown promising results for its treatment. The challenge of the technique is to keep the cells at the site of action to increase their therapeutic potential [1]. The aim of this study was to evaluate the effectiveness of the Qtracker® 655 nanocrystal marking on allogeneic MSC_{SM}, encapsulated in alginate hydrogel, evaluating the migration of these cells to the synovial joint.

METHODS: Five horses were submitted to arthroscopic surgery (D0), chondral lesion was induced in the radial carpal bone of both thoracic limbs. The 10 radiocarpal joints were divided into two groups (GA, GB). The chondral defect was treated according to the group: GA received free-labelled MSC_{SM} and GB received labelled MSC_{SM} microcapsules[2]. Seven days after lesion induction and implantation of labelled cells, biopsies of the lesion site were performed in two animals, and fragments of SM and joint capsule were collected, which were frozen and later processed in histological sections for fluorescence microscopy visualization. The synovial fluid of the other three animals was analyzed by flow cytometry three times – 3, 7 and 21 days after application.

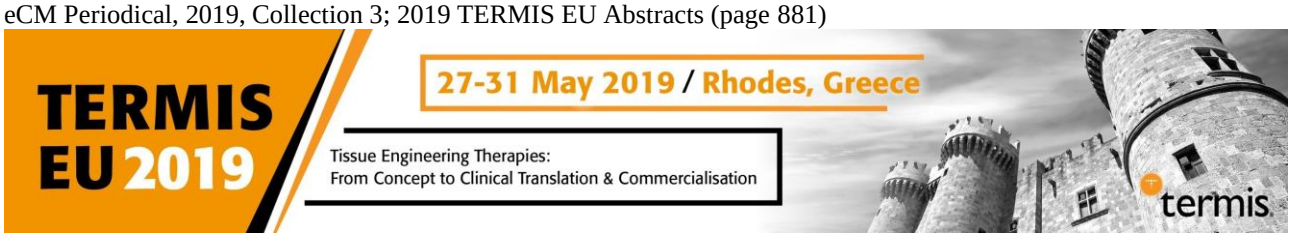
RESULTS & DISCUSSION: The viability obtained prior to cell labelling was 90 ± 2% and after incubation (24 hours) with the Qtracker 655 nanocrystal [2] continued with 90 ± 2.5%. The cellular marking with the nanocrystals allowed the visualization of the cells in cartilage, synovial membrane, synovial fluid and articular capsule, but with a predilection for the synovial membrane. Flow cytometry of synovial fluid was also used to evaluate the migration of MSC_{SM}, where we observed that the cells survived and remained marked for up to 21 days. The labelled MSC_{SM} inserted in microcapsules were scarce in the synovial fluid and could be related to the small quantity of MSCs leaving the pores of the microcapsules, since the MSCs of horses have a mean diameter of 19.8 and 20.4 μm, derived from marrow bone and umbilical cord, respectively.

CONCLUSIONS: The MSC_{SM} labelled with the Qtracker 655® nanocrystals did not show a decrease in their viability in the in vitro analyses, suggesting that the nanocrystal is safe and has no toxic effect on the cells. There was an evident presence of marked cells in the synovial membrane and the observation of these cells at the lesion site was scarce. Cells encapsulated in less quantity are also favorable, because the cells release paracrine effects acting for a long period until differentiation.

ACKNOWLEDGEMENTS: This research was supported by São Paulo Research Foundation (FAPESP), São Paulo, SP, Brazil

REFERENCES

[1] Li M, Luo X, et al. In vivo human adipose-derived mesenchymal stem cell tracking after intra-articular delivery in a rat osteoarthritis model., 2016. Stem Cell Res Ther. 7:160.
[2] Carvalho AM et al. Evaluation of mesenchymal stem cell migration after equine tendonitis therapy, 2014. Equine Vet J. 46(5):635-8



Changes in mechanical properties of chondrogenically differentiating MSC spheroids

A.V. Tsvetkova[1], I.V. Vakhrushev[1,2], A.A. Gryadunova[2,4], Y.B. Basok[3], A.M. Grigoriev[3], S.A. Khorkova[5], E.K. Nezhurina[2], E.V. Koudan[2], E.A. Bulanova[2], Y.G. Hesuani[2], V.A. Mironov[2,4], K.N. Yarygin[1]

Presenting Author: Anastasia Tsvetkova, anastasiatsvetkova@hotmail.com

[1]V.N. Orekhovich Institute of Biomedical Chemistry, Moscow, Russia [2]Private Institution Laboratory for Biotechnological Research «3D Bioprinting Solutions», Moscow, Russia [3]V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Ministry of Health of the Russian Federation, Moscow, Russia [4]Institute for Regenerative Medicine, I.M. Sechenov First Moscow State Medical University [5]Skolkovo Institute of Science and Technology, Moscow, Russia

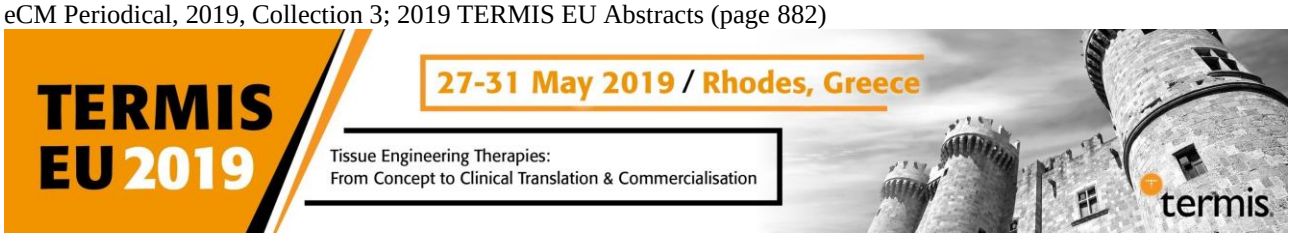
INTRODUCTION: Damaged cartilage does not heal easily because of low intrinsic regenerative capacity and vascularization of cartilaginous tissue. Stem cell tissue engineering is an advantageous treatment strategy, but optimal cell sources and cell expansion as well as production of a functionally similar construct, that is ideally able to seamlessly integrate into the existing tissue, all pose challenges. Most current strategies use scaffolds to regulate construct properties with cells seeded on them. However, within cartilage, extracellular matrix (ECM) is responsible for its unique mechanical properties, and in that aspect scaffold-free approach, where cell aggregates are used as bio-ink, also relies on the produced ECM. Both approaches aim at eventual integration of construct into damaged tissue. Cartilaginous tissue functions under constant dynamic load, and uniformity of mechanical properties across the repaired cartilage surface is crucial for equal load distribution and therefore must have uniform resistance and elasticity. This study evaluated mechanical properties and ECM production in spheroids of differentiating chondro-progenitors.

METHODS: Human MSCs were derived from Wharton's jelly, dental pulp, and adipose tissue. Primary chondrocyte culture served as positive control. Cells were first expanded as a 2D culture in DMEM/F12+10%FBS, then spheroids of each cell type were formed by aggregation of 8,000 cells per well in a 96-well ULA plate in 100 ul of the growth medium replaced by chondrogenic medium after 24 h. Spheroid fusion was initiated on days 1, 7 and 14 by placing two spheroids into one well. Spheroid formation and fusion were monitored using IncuCyte Live Cell Analysis System. The MicroSquisher was used to measure Young modulus of spheroids every three days for two weeks. ECM production in spheroids was evaluated by using alcian blue and Masson's trichrome staining after 14 days of incubation in chondrogenic medium.

RESULTS & DISCUSSION: Elasticity modulus of spheroids of chondrogenically differentiating MSCs was increasing compared to spheroids of control MSCs and was similar to that of chondrocyte spheroids. It also positively correlated with glycosaminoglycan and collagen production (higher levels observed in spheroids of adipose tissue-derived MSCs). Spheroid fusion initiated on days 7 and 14 after differentiation induction was impaired, but control spheroids of all the cell types tested fused equally well at all time points.

CONCLUSIONS: Young modulus together with molecular marker levels is a useful indicator for assessing ECM formation and construct applicability. Spheroid fusion is adversely affected by cell differentiation. Nevertheless, the results indicate that spheroids of chondro-differentiated MSCs are potentially suitable for building tissue constructs for treatment of damaged cartilage.

ACKNOWLEDGEMENTS: Russian Foundation for Basic Research (grant № 16-29-07322).



Z-disc streaming and incorrect sarcomere assembly in Drosophila col4a1 mutants

A. A. Kiss[1], N. Popovics[1], Z. Boldogkői[1], K. Csiszár[2], M. Mink[1]
Presenting Author: András A. Kiss, kiss.andras.attila@gmail.com
[1]Institute of Medical Biology, University of Szeged, Somogyi B. u. 4, H-6720 Szeged, Hungary [2]John A. Burns School of Medicine, University of Hawaii, 1960 East West Road, Honolulu, HI 96822, USA

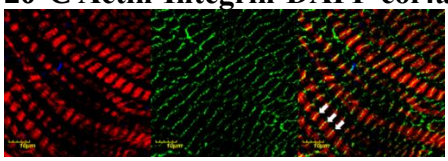
INTRODUCTION: The Z-disc streaming is a feature of defect in cytoskeletal muscles, where the Z-disc in the sarcomeres is fragmented or localized to become confused. In our col4a1 mutants, the Z-disc streaming rate was observed as a result of increased temperature.

METHODS: Common oviducts were removed under carbon dioxid anesthesia from adults that were grown at both permissive and restrictive temperature for 14 days. Dissected common oviducts were fixed in 4% paraformaldehyde dissolved in phosphate buffered saline (PBS) for 10 min, washed tree times in PBS, permeabilized for 5 min in 0,1% Triton X dissolved in PBS and washed tree times in PBS. Blocking was achieved for in 5% BSA dissolved in PBS for 1 hour, and washed tree times in PBS.

RESULTS & DISCUSSION: In order to determine the exact position of the Z-discs in the muscle fibers of the oviduct, we used antibodies against the scaffold protein kettin as a morphological marker. Immunohistochemistry using anti-integrin antibodies provided the same staining pattern in close localization as observed for kettin, confirming integrin localization to the Z-discs in muscle fibers of the oviduct. In col4a1 mutant, we observed aberrant integrin expression in the epithelial cells of the Malpighian tubules, and also surmised irregular integrin deposition in muscle fibers, consistent with the muscle pathology of Z-disc streaming.

CONCLUSIONS: The disrupted sarcomeric cytoarchitecture, Z-disc streaming collectively suggest a role of COL4A1 in integrin mediated adhesion. In conclusion, our Drosophila mutant series may serve as an effective model to uncover the mechanisms by which COL4A1 mutations result in disrupted myofiber-basement membrane interactions and inproper muscle function.

20°C Actin-Integrin-DAPI col4a1^{G552D1}mutant



29°C Kettin-Integrin col4a1^{G552D2}mutant

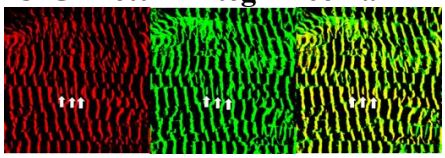
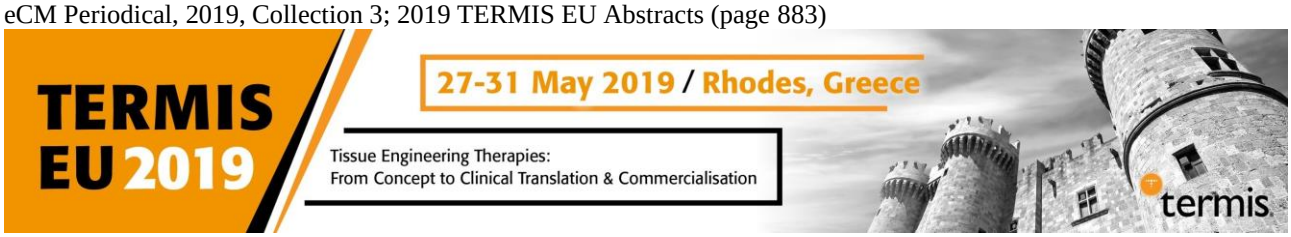


Figure 1: Photomicrographs were made in col4a1 mutants after two weeks of incubation. The arrows indicate the wrong position of the Z-disc.

ACKNOWLEDGEMENTS: This research was supported by the Hungarian Scientific Research Fund OTKA, Contract no. NN 108283 to Mátyás Mink, and by the New National Excellence Program, Contract no. UNKP-18-3-IV-SZTE-23 to András A. Kiss, and the New National Excellence Program, Contract no. UNKP-18-3-IV-SZTE-43 to Somlyai-Popovics Nikoletta



Anisotropic properties of a hyaluronic acid collagen biomaterial ink to control cellular behavior

A. Schwab[1], L. Ambrosio[1], M. Alini[1], D. Eglin[1], M. D`Este[1]
Presenting Author: Andrea Schwab, andrea.schwab@aofoundation.org
[1]AO Research Institute, AO Foundation, Davos Platz, Switzerland

INTRODUCTION: 3D (bio-) printing is an additive manufacturing method to fabricate structured, multi-layered hybrid scaffolds that mimic zonal orientation of extracellular matrix.
Here, tyramie modified hyaluronic acid (THA) was combined with collagen I to investigate cell instructive properties for application in 3D printing of anisotropic cartilage scaffolds.

METHODS: THA (2.5% w/v) hydrogels were prepared via enzymatic cross-linking using horseradish peroxidase (0.3 – 1.0 U/ml) and hydrogen peroxidase [1]. Composite hydrogels were prepared by functionalizing THA with acidic dissolved collagen I (0.5% w/v), isolated from rat tail, in equal volumes of both precursor gels (THA-col). Structural organization of collagen I in composite hydrogels were evaluated by turbidity measurement (313 nm) over 60 min at 37°C and via polarized light microscopy of cryo-sections. Cell attachment on THA and THA-col was evaluated after seeding with bone marrow derived hMSCs by life-dead staining. To evaluate cell behavior, hMSCs were encapsulated in THA-col and cultured up to 21 days. Immune histochemistry, GAG and DNA assay as well as viability assay were conducted over time.

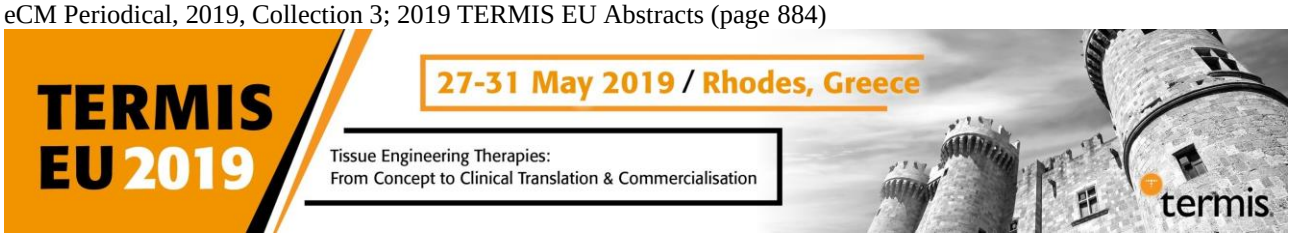
RESULTS & DISCUSSION: Turbidity measurement resulted in the presence of collagen fibers correlating to increase in absorbance. The presence of thin collagen fibers within the double network hydrogels were visualized by polarized light microscopy and confirmed by immune histological staining for collagen I. DN hydrogels induced hMSC attachment and spreading. For cell encapsulation similar trend was observed: Cells were spindle shaped and attached to THA-col within 1 day whereas MSCs stayed round and did not attach in THA gel.

CONCLUSIONS: THA with its property to adhere to cartilage ECM can overcome the limited integration of scaffolds in cartilage repair. Combining collagen and THA results in cell instructive properties by means of cell adhesion and spreading since collagen naturally contains cell adhesion ligand RGD [2]. Whether anisotropic properties of this biomaterial ink induce cell alignment, migration and cell differentiation is going to be evaluated.

ACKNOWLEDGEMENTS: This work is part of the osteochondral defect collaborative research program supported by the AO foundation. The Graubünden Innovationsstiftung is acknowledged for its financial support

REFERENCES

[1] Loebel C et al. Carbohydr Polym. 2015; 115: 325-33
[2] Baniasadi, M and Minary-Jolandan M Materials 2015; 8(2): 799-814



Building a standardized human hematopoietic stem cell niche in vitro

T. Klein[1*], A. Garcia-Garcia[1*], PE. Bourgine[2] and I. Martin[1]
Presenting Author: Andres Garcia-Garcia, andres.garcia-garcia@unibas.ch
[1]Tissue Engineering, Department of Biomedicine, University Hospital Basel, University of Basel, 4031 Basel, Switzerland [2]Cell, Tissue and Organ engineering, Department of Clinical Sciences, Lund University, SE-221 00 Lund, Sweden

INTRODUCTION: In vitro 3D-engineered bone marrow microenvironment based on human mesenchymal stromal cells (hMSCs) has been shown to successfully maintain functional human hematopoietic stem and progenitor cells (HSPCs) (1). However, the availability and variability of hMSC material remain as major limitations to go further in the in vitro HSPCs expansion challenge. We now propose to substitute primary hMSCs by an immortalized cell line exhibiting MSC properties (MSOD) (2) to generate a standardized hematopoietic stem cell niche.

METHODS: 10^6 hMSCs were seeded and cultured within collagen scaffolds (Ultrafoam, 8 x 4 mm) under direct perfusion flow. After 3 weeks of culture in osteogenic medium, the resulting microenvironment was seeded with either cord blood-derived or buffy coat-derived $CD34^+$ cells and cultured for 1 week. The capacity of the MSOD engineered niche to maintain HSPCs was assessed by flow-cytometry (CD34 CD38 CD45RA CD90 markers), while their functionality was evaluated with Colony Formation Unit Assays (CFU-Cs).

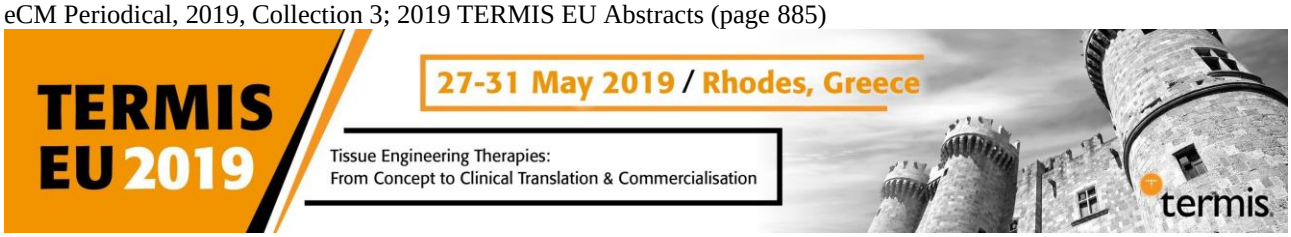
RESULTS & DISCUSSION: Contrasting the 60% of hematopoietic cells that could be retrieved from hMSCs-derived engineered niches, only 7% of cord blood-derived cells and 3% of buffy coat-derived cells could be recovered from niches engineered with MSOD cells. Furthermore, less than 5% of these blood cells remained in the engineered niche (the vast majority of them were retrieved from the supernatant fraction). Intriguingly, MSOD cells exhibited a 8.7-fold expansion during the culture, while our previous results showed that primary hMSCs presented only a 1.7-fold expansion. MSOD niches did not expand primitive HSCs ($CD34^+CD38^-$) in comparison with unseeded collagen scaffolds, but promoted a 2-fold expansion on more committed hematopoietic progenitors ($CD34^-CD38^+$ and $CD34^+CD38^+$). Furthermore, in vitro CFU-C assays revealed that cord blood-derived and buffy coat-derived HSPCs cultured in MSOD niches maintain their differentiation properties since they could give rise to more committed progenitors (CFU-GEMM, CFU-GM and BFU-E).

CONCLUSIONS: The use of MSOD cell line allows the standardization of engineered hematopoietic stem cell niches, which promote HSPC expansion and differentiation. However, its efficiency to host hematopoietic cells is dramatically reduced in comparison with hMSC-derived niches. The massive expansion of MSOD cell counts during the culture might suggest an uncontrolled MSOD proliferation, which eventually could saturate the niches and impair HSPC maintenance. Therefore, in order to improve their functionality, engineering MSOD niches require further research to readjust culture settings according to MSOD proliferation

ACKNOWLEDGEMENTS: Financial support was received from the Swiss National Science Foundation (Grant 310030-133110).

REFERENCES

[1] Bourgine, P. E., Klein et al. PNAS. 2018. 201805440.
[2] Bourgine, P. E., et al. Adv. Funct. Mater. 2017.



Piezoelectric polymeric nanofibers as smart scaffolds for tissue engineering

A. Zaszczynska[1], P.Ł. Sajkiewicz[1], A. Gradys[1]
Presenting Author: Angelika Zaszczyńska, azasz@ippt.pan.pl
[1]Institute of Fundamental Technological Research Polish Academy of Sciences, Laboratory of Polymers and Biomaterials, Warsaw, Poland

INTRODUCTION: As regards the field of biomedical engineering, in recent decades there is observed an increasing scientific and technical interest in research related to development of Smart Materials (SM). Such materials are generally designed to react to external stimuli (physical, chemical, mechanical) and behave similarly to natural body tissues. One type of such SM are piezoelectric scaffolds, which can generate electrical signals in response to the applied stress [1,2].

METHODS: Polyvinylidene fluoride (PVDF) nanofibers formed by electrospinning were subjected to in-vitro cellular studies. In the stimulation experiments, fibroblasts L929 cells cultured on the piezoelectric PVDF scaffolds collected at various rotational speeds of the collector, were exposed to ultrasounds for 30 minutes, one time per day, for 7 days. Ultrasound stimulus with power 20mW, 80mW and frequency 1,7 MHz were applied. In order to confirm the piezoelectric effect of the PVDF scaffolds on fibroblasts activities, piezoelectric PVDF scaffolds without ultrasonic stimulation were used as a control.

RESULTS & DISCUSSION: Enhanced viability and activity of cells dynamic culture have been observed and are invoked as a proof of suitability of the piezoelectric effect (Fig. 1). Cell culture studies demonstrated that the viability and growth of fibroblasts on PVDF fiber scaffolds were comparable over a 7-day period. The observations using SEM verified the attachment and proliferation of the cells on the fiber scaffolds. Moreover, the cell morphology on the fiber scaffolds was different when the 1-day culture and 7-day culture images were compared: on day 1, the cells had more rounded morphology while for 7 days their morphology was more elongated and spread-out (Fig. 1).

CONCLUSIONS: The use of ultrasounds stimulation, in combination with piezoelectric polymers, is advantageous for cellular studies. Exhibiting good cellular response show potential to be used in tissue engineering as a scaffold material.

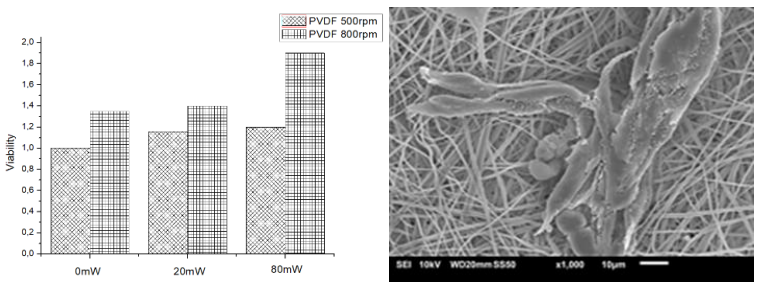
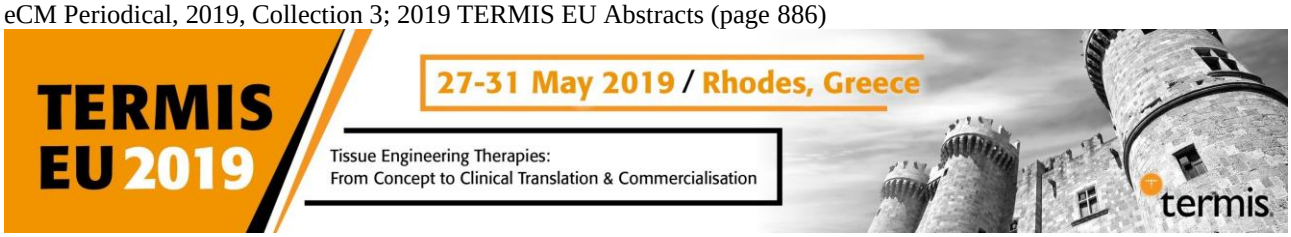


Figure 1: Viability of fibroblasts L929 using ultrasound stimulation with different power. SEM images of PVDF nanofibers with fibroblasts L929 cell culture on day 7.

REFERENCES

[1] Sajkiewicz P et al. Eur. Polym. J. 1999;35 (3):423-29.
[2] Kim YT et al. 2009, Biomaterials 2009;30 2582-25090.



Bone augmentation using non-setting injectable materials

A. Diez-Escudero[1], J. Bohlin[2], P. Procter[1], J. Hilborn[2], C. Persson[1]
Presenting Author: Anna Diez-Escudero, anna.diez@angstrom.uu.se
[1]BioMaterial Systems, Department of Engineering Sciences, Uppsala University, SE-751 21, Uppsala, Sweden [2]Polymer chemistry, Department of Chemistry - Ångström, Uppsala University, SE-751 21, Uppsala, Sweden

INTRODUCTION: Bone screws are a standard technique to stabilize bone fragments in orthopaedic fracture repair. The risk of screw loosening may be reduced by augmenting the fixation with self-setting calcium phosphate cements, however in the fractured femoral neck these impair healing [1]. A non-setting mix of a gel and hydroxyapatite (HA) has, on the other hand, been reported to result in rapid mineralization in vivo [2]. More recently it has also been shown that a non-setting gel-HA combination increased screw pull-out strength in cancellous bone granting instant grip [3]. The present study aims to further explore this unexpected reinforcing effect arising from particulate fillers in hydrogel matrices, and investigate a possible optimization of their particle size as enhancement of this bone augmentation.

METHODS: Gels consisting of 2% w/v agar in calcium-free PBS were used as base material and compared to 40%w/v hydroxyapatite (HA) reinforced gels. Two different HA sizes were used, varying by one order of magnitude (3µm to 300nm, denoted mHA and nHA, respectively). Strain and frequency sweeps were used to determine the viscoelastic properties of the gels (37°C, parallel 19mm Ti plate, 650µm gap). Screw pull-out tests were performed using cancellous titanium screws inserted 10mm (HB 4x16mm) in femoral condyle rabbit bones as previously described [3].

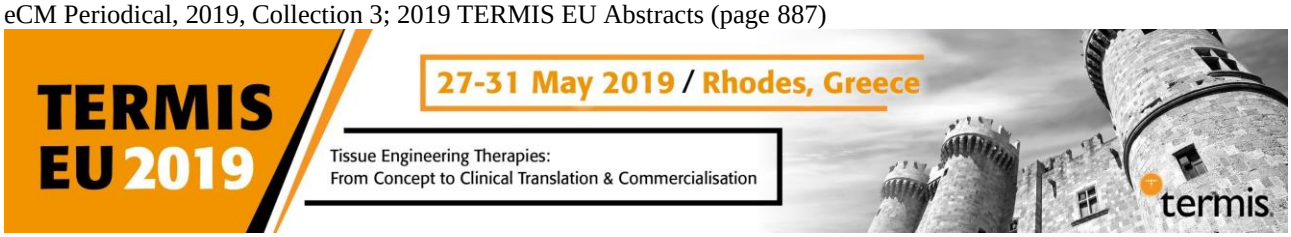
RESULTS & DISCUSSION: All materials showed a steady solid-like behavior (G'>G'') and presented a linear behavior up to 0.5% strain from the strain sweep tests. Frequency sweeps depicted a clear nHA reinforcement with higher values for both G' and G'' moduli ($4.5 \cdot 10^5$ and $1 \cdot 10^5$ Pa, respectively) compared to agar ($8 \cdot 10^3$ and $1 \cdot 10^3$ Pa, respectively) and agar with mHA ($2 \cdot 10^5$ and $6 \cdot 10^4$ Pa, respectively) relating to an increase in particle-gel interactions. The pull-out forces correlated with this reinforcing effect derived from the rheological evaluation, which might be explained by stronger surface interactions due to the smaller particulate size with higher specific surface area.

CONCLUSIONS: The decrease in particle size and increase in specific surface area showed an enhancement in bone augmentation by fostering particle-gel-bone interactions. These results make this material combination a promising one as it also carries the potential to minimize interference with the bone healing cascade compared to self-setting materials.

ACKNOWLEDGEMENTS: The authors are grateful for financial support from the Göran Gustafsson Foundation.

REFERENCES

[1] Mattsson & Larsson Acta Orthop. 2006 ;77(2):251-6
[2] Kettenberger et al. J Tissue Eng Regen Med. 2017 Jul;11(7):1974-1985
[3] Solana Munoz et al. Clin. Biomech. 2018; 59: 174-78.



Surface functionalization of poly(L-lactide-co-glycolide) membranes with amphiphilic poly(2-oxazolines)

A.M. Tryba[1], M. Krok-Borkowicz[1], C. Paluszkiewicz[2], E. Pamuła[1]
Presenting Author: Anna-Maria Tryba, amtryba@agh.edu.pl
[1]AGH University of Science and Technology, Faculty of Science and Ceramics, Department of Biomaterials and Composites, Kraków, Poland [2]Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland

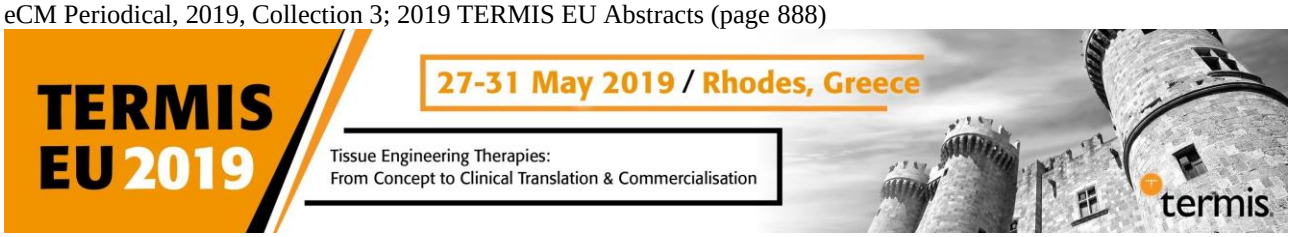
INTRODUCTION: Poly(2-oxazolines) (Pox) are amphiphilic, non-ionic polymers used in medical and drug delivery applications. They can be conjugated with drugs, peptides and proteins. In this study we intend to use model Pox to modify surface of poly(L-lactide-co-glycolide) (PLGA) membranes. The membranes were produced by one-step phase separation process between poly(ethylene glycol) (PEG), PLGA and Pox, dissolved in dichloromethane (DCM) followed by PEG leaching.

METHODS: PLGA (85:15, Mn=100kDa, d=1.9), PEG (400 Da) and POx (methyl-P[MeOx37-b-BuOx-23-b-MeOx37-pipeidine(P2-P2) (Mn=8 kDa, d=1.14) were dissolved in DCM, casted on Petri dishes, dried and immersed in UHQ-water for PEG leaching to obtain membranes (M_Pox). As a reference PLGA membranes (M) and PLGA foils without Pox (Foil) were made. Micro-structure of the samples was observed under scanning electron microscopy (SEM). Water contact angle and FTIR spectroscopy was used to characterise the materials. Cytocompatibility tests were performed with osteoblast-like MG-63 cells for 24 h, 3 and 7 days. Viability was measured by resazsurin reduction; phalloidin/DAPI and live/dead staining tests were done.

RESULTS & DISCUSSION: Phase separation between PLGA and PEG followed by PEG leaching was found useful in producing porous membranes. Addition of Pox influenced topography of the M-Pox, by decreasing average pore size on the upper side of the membrane and decreasing water contact angle (data not shown). Cells cultured on all membranes were better spread and had better developed actin fibres as compared to cells cultured on foils for 24 h. Cell viability on days 3 and 7 was also significantly higher on the membranes than on the foils.

CONCLUSIONS: One-step phase separation process between PLGA, PEG and Pox, dissolved in DCM followed by PEG leaching resulted in membranes, which were found cytocompatible with osteoblast-like cells.

ACKNOWLEDGEMENTS: This study was supported from Interfaculty Environmental Doctoral Studies (FCB) at the Faculty of Materials Science and Ceramics, AGH University of Science and Technology. and Polish National Agency for Academic Exchange (PPI/PRO/2018/1/00026/U/001).



Proteomic approach for bone tissue engineering

A. Consalus[1, 2], J. Arivalagan[1, 2], F. Poirier [1, 2], A. Boccon[3], H. Guibourt[2, 3] D. Lutomski[1, 2]
Presenting Author: Anne Consalus, consalus.anne@gmail.com
[1]University Paris 13-UMR CNRS 7244-Tissue Engineering and Proteomic Team-UFR SMBH, Bobigny, France [2]Proteobios ANR joint laboratory (Labcom program), France [3]Ost Developpement, Clermont Ferrand, France

INTRODUCTION: When bone fracture occurs, body can repair itself. But, if the size of the defect is superior to a critical size, bone substitutes can be used [1]. The aim of bone substitute is to repair bone defect. Chemical treatments are applied on bone substitutes to ensure a high level of safety. But these treatments can affect the quality and quantity of proteins contained in bone substitute. The aim of Proteobios Lab is to analyse as much as possible proteins present in bone substitutes of natural origin and evaluate their effect on stem cells.

METHODS: Bone substitute is finely ground to produce a homogeneous powder using grinder. Bone substitute powder is decalcified with acidic solution overnight, incubated with extraction buffer and proteins are digested with trypsin. Then peptides are identified using mass spectrometers. Identified proteins are classified according biological processes and molecular functions, and proteins interactions analyzed using STRING online proteins database.

RESULTS & DISCUSSION: More than 80 proteins were identified byNanoLC/MS-MS. With STRING online proteins database, interactions between proteins can be studied.

CONCLUSIONS: Proteomic is a smart and efficient tool to analyze the composition of bone substitute. Protein composition, biological process, molecular function and interactions between proteins were analyzed.

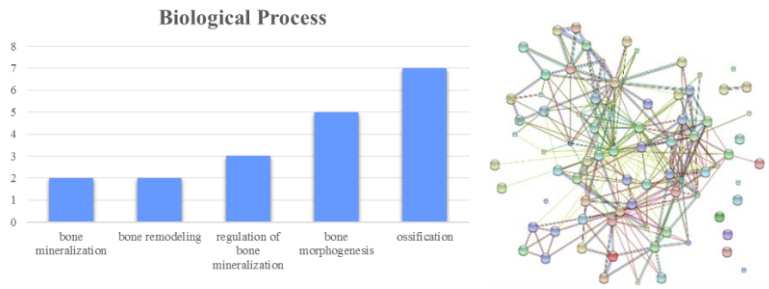
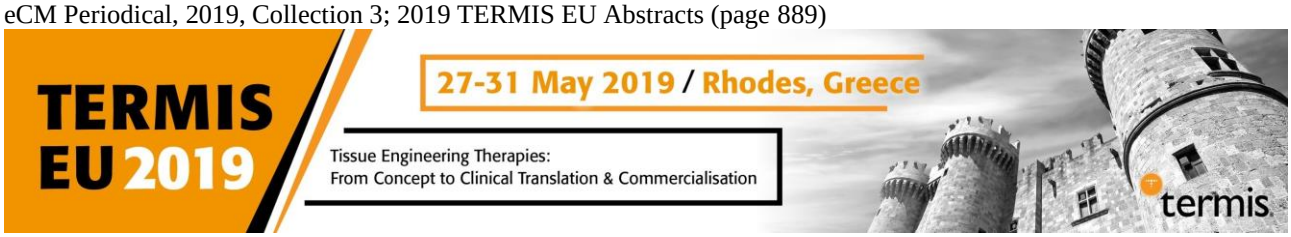


Figure 1: Identified proteins of natural biomaterial are involved in biological processes such as ossification, bone morphogenesis, regulation of bone mineralization and are also implicated in molecular functions such as calcium binding and glycosaminoglycan binding. The network view summarizes the network of predicted associations of groups of proteins. Nodes represent proteins and edges represent the predicted functional associations.

ACKNOWLEDGEMENTS: Financial support from French ANR (Labcom program) and Association nationale de la recherche et de la technologie (ANRT)

REFERENCES

[1] Gavazzoni A et al. J Mater Sci Mater Med2018 Nov 3;29(11):167



Recent advances on application of small molecules as osteogenic inducers

A. Ahmadi[1,2†], S.S. Ebadi[1,2†], R. Mazloomnejad-Meybodi[1,2], N. Halvaei[1,2], H. Niknejad[1]
Presenting Author: Armin Ahmadi, arminahmadi@sbmu.ac.ir
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran [†]Co-first author

INTRODUCTION: high morbidity rates alongside with significant costs for the healthcare system are some of the cons of current treatment methods of bone defects [1]. Stem cell-based methods are considered to be a possible substitute. Since the discovery of stem cells, guiding them towards osteogenic lineages has been an area of great attention. Agents administered to differentiate stem cells have generally been natural growth factors including BMPs and IGFs combined with genetic engineering methods and small molecules. Due to expensiveness and difficulty of access, growth factors do not provide a widely practical clinical application. Small molecules are subjects of a growing body of experiments because of not having the mentioned problems. Therefore, we suggested here that knowledge of small molecule mediated osteogenic differentiation is necessary in order to investigate clinical applications of tissue engineering methods regarding treatment of bone defects.

METHODS: Keywords searched included "stem cell differentiation", "small molecules", "osteogenic differrntiation" and "in vitro", "in vivo" and "clinical application". The main databases used were "google scholar" and "Pubmed". More than 200 papers were found, from which 120 were selected based on their relevance, year of publishment and number of citations.

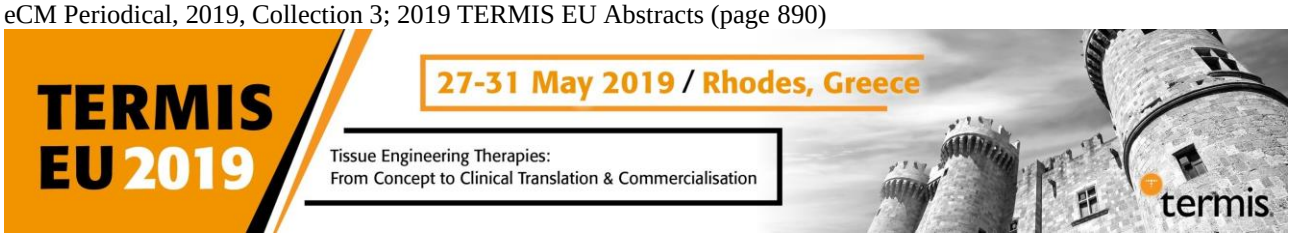
RESULTS & DISCUSSION: Many molecules, including Statins, metformin and Phenamil widely known to operate through specific cellular pathways have also been found to affect other pathways within the cells resulting in complex intracellular signalings leading to osteogenic differentiation.[2] Bisphosphonates and Dexamethasone are also known to induce osteogenesis. The importance of these molecules as well as non-pharmacologic molecules synthesized solely for the purpose of experimentation, like T63, SAG and THQ-1a are of no question.[3] Simvastatin, Phenamil, Adenosine, T63, Alendronate and Stronium ranelate are among the agents that have promoted osteogenic differentiation in vivo. Recently, Simvastatin, in a clinical study has been shown to induce osteogenesis in surgically removed third molars in a faster motion. Further investigations are needed to elucidate whether or not these molecules can replace natural growth factors totally in this matter.

CONCLUSIONS: All in all, this study states that small molecule-based osteogenic differentiation is proposed as a promising treatment method of bone defects including nonunion fractures and osteoporosis in the future.

ACKNOWLEDGEMENTS: Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran. We also like to thank Dr. Tahereh Tayebi for her many assists.

REFERENCES

[1] Perez et al. Front, Bioeng, Biotechnol, 2018;6:105
[2] WL Yu et al. Sci. Rep. 2017;7:44129
[3] Xiao-li Zhao et al Sci. Rep. 2017;7:10397



Electrospun nanofiber yarns as sutures to repair wounds

A. Richard[1], R. S. Verma[1]
Presenting Author: Arthi Richard, arthihard@gmail.com
[1]Department of Biotechnology, Indian Institute of Technology, Madras, India

INTRODUCTION: Sutures are the most common medical devices used for surgical procedures [1]. The twisted strand of Electrospun polymer nanofiber yarn has gained attention due to the advantages of high surface area, increased mechanical strength and drug release properties for improved suture material [2,3].

METHODS: Electrospun Polylactic acid nanofibers were modified to form yarns using a funnel collector rotating at high speed placed perpendicular to the needle connected to high voltage supply. The polymer nanofiber yarns were initiated by drawing using a grounded metal rod and wound to bobbin. The formation of continuous yarns was optimised at rotating speed of 400,500 and 600 rpm. Mechanical testing was performed according to ASTM D226

RESULTS & DISCUSSION: Physical characterisation of Polymer nanofiber yarns spun at different rotating speed were analysed by Scanning Electron Microscopy. the diameter of the yarn was in the range of 170-190µm. The increase in speed leads to decrease in diameter of the yarn and increase in twist. Mechanical characterisation of the yarn at different rotating speed were tested by ASTM D226 with Tensile stress in the range of 0.05MPa.

CONCLUSIONS: SEM data and tensile testing data clearly illustrate the high surface area of nanofibers twisted together as nanoyarns and appropriate mechanical strength provides cues for use of nanofiber yarns as a building block to fabricate suture in twisted or braided form to repair wounds.

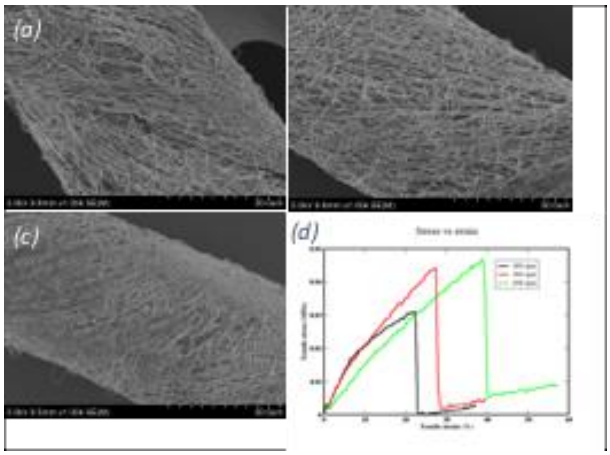
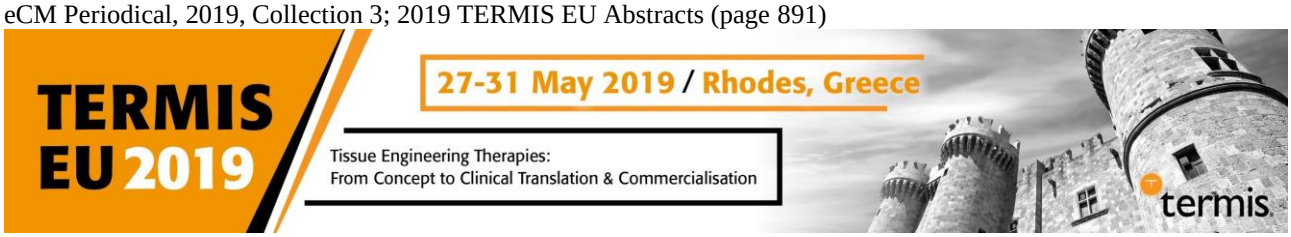


Figure 1: SEM micrographs and mechanical characterization.

ACKNOWLEDGEMENTS: Financial support was received from MHRD and IIT Madras

REFERENCES

[1] Sooneon Bae et al., ACS Applied Materials & Interfaces 2018 10 (10), 8426-8435.
[2] Smrithi Padmakumar et al., ACS Appl. Mater. Interfaces 2016, 8, 6925−6934.
[3] Ya-Jing Ye et al., Macromol. Biosci. 2018, 18, 1800041



Translational study on dentin regeneration by means of bio-inspired scaffolds and stem cells

G. Vagropoulou [1, *], M. Trentsiou [1, *], A. Bakopoulou[1], A. Georgopoulou [2, 3], M. Chatzinikolaidou [2, 3], P. Koidis [1]
Presenting Author: Athina Bakopoulou, abakopoulou@dent.auth.gr
[1]Department of Prosthodontics, School of Dentistry, Aristotle University of Thessaloniki, Greece [2]Department of Materials Science and Technology, University of Crete, Heraklion, Greece [3]IESL-FORTH, Heraklion, Greece, *equally contributing first authors

INTRODUCTION: Chitosan (CS)/Gelatin (G)/nanohydroxyapatite (nHAp) blends have been applied as promising scaffold materials for mineralized tissue - primarily bone - regeneration [1, 2]. However, little is known whether they could be used as suitable scaffolds to stimulate the differentiation of dental pulp stem cells (DPSCs) into odontoblastic lineages to induce dentin regeneration. Aim of this study was to develop biomimetic CS/G/nHAp scaffolds to be used as carriers of DPSCs for targeted dentin regeneration.

METHODS: Scaffolds were prepared by keeping the ratio 3:1:1 of the three solid components, i.e. nHAp, CS, and G and dissolving into a 3 w/v % thoroughly stirred nHAp powder in deionized water dispersion, a 2 w/v % CS in 1 v/v % acetic acid solution and a 2 w/v% G in ultrapure water solution and stirring for 2 h at 50 °C. The mixture was casted in 24 well plates, lyophilized for 24 h, crosslinked with 0.1 % glutaraldehyde and lyophilized again. The scaffolds were characterized by means of SEM, EDS, TGA, FTIR and XRD. DPSC cultures were established from healthy donors and characterized for stem cell markers with flow cytometry. Scaffolds were seeded with DPSCs in complete culture medium (CCM: α-MEM with 15% FBS, 100 mM L-ascorbic and antibiotics/antimycotics). SEM was used to evaluate cell attachment/morphology within the scaffolds. Live/dead staining visualized by confocal microscopy was used to evaluate cell viability, and real time PCR for the expression of odontogenic genes.

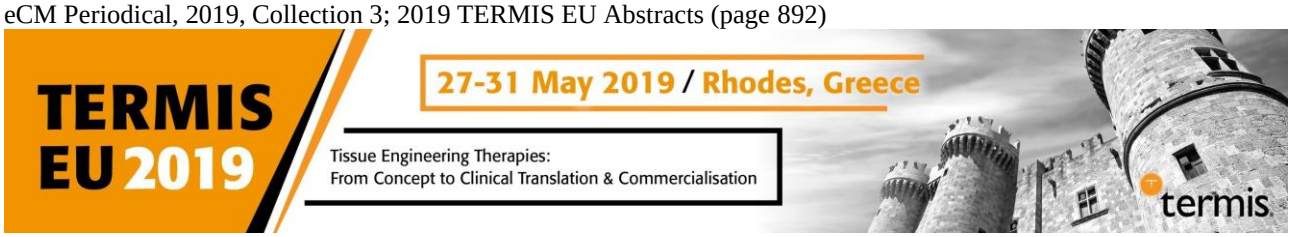
RESULTS & DISCUSSION: Scaffolds with interconnective pores with an average size 100 μm were prepared and physicochemically characterized. SEM analysis up to day 14 revealed that CS/G/nHAp scaffolds favour the attachment of DPSCs. Live/dead staining confirmed that most cells (>90%) remained viable throughout the entire culture period in both types of scaffolds. Significant and time-dependent ($p<0.05$) upregulation of odontogenic differentiation genes, including alkaline phosphatase, bone morphogenetic protein 2, dentin sialophosphoprotein, and osteocalcin was observed in DPSC-seeded scaffolds over a period of 14 days.

CONCLUSIONS: CS/G/nHAp hybrid scaffolds supported attachment, viability and odontogenic differentiation of DPSCs in vitro revealing a promising strategy for their application in targeted dentin regeneration.

ACKNOWLEDGEMENTS: This study was conducted under the action Excellence II (Project: Osteobiomimesis, 3438) and funded by the European Union (EU) and National Resources.

REFERENCES

[1] Chen J et al. Biomaterials. 2011; 32(21):4793-805
[2] Bakopoulou et al. Dent Mater. 2018; pii: S0109-5641(18)30550-5.



Perfusion bioreactor enhances fibrochondrogenic differentiation of Dental Stem Cells/Chitosan-Gelatin constructs under hypoxic conditions

M. Bousnaki[1], A. Bakopoulou[1], E. Vereroudakis[2,3], A. Georgopoulou[2,3], A. Kritis[4], M. Chatzinikolaidou[2,3], P. Koidis[1]
Presenting Author: Athina Bakopoulou, abakopoulou@dent.auth.gr
[1]Department of Prosthodontics, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece [2]Department of Materials Science and Technology, University of Crete, Heraklion, Greece [3]Institute of Electronic Structure and Laser, Foundation for Research and Technology Hellas (FORTH), Heraklion, Greece [4]Department of Physiology and Pharmacology, Faculty of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

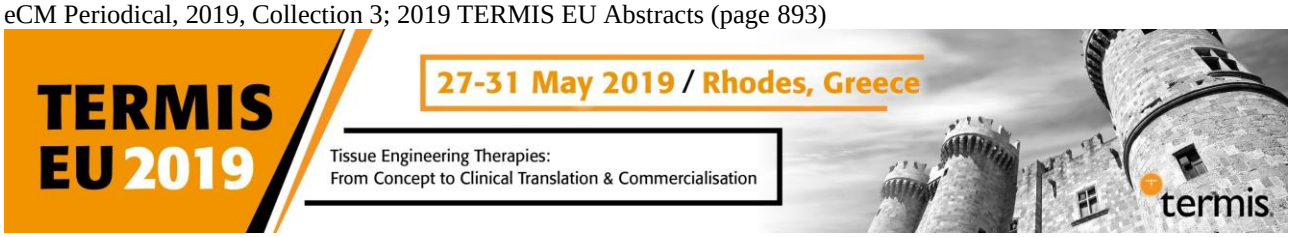
INTRODUCTION: Tissue engineering (TE) may provide effective alternative treatment for challenging temporomandibular joint (TMJ) pathologies associated with disc malpositioning or degeneration leading to severe masticatory dysfunction. Aim of this study was to evaluate the potential of chitosan/gelatin (Ch/Gel) scaffolds seeded with dental pulp stem cells (DPSCs) and cultured in a perfusion bioreactor to promote fibro/chondrogenic differentiation and production of fibrocartilage tissue, serving as a replacement of the natural TMJ disc.

METHODS: Porous Ch/Gel scaffolds, with a composition of 40%-60% chitosan-gelatin, were fabricated by chemical crosslinking with 0.1% glutaraldehyde and lyophilization. DPSCs were isolated from third molars and seeded onto the Ch/Gel scaffolds ($2x10^6$ cells/scaffold). DPSC/scaffold constructs were cultured under normoxic (20% O_2) or hypoxic (5% O_2) conditions. For the viability assay constructs were cultured with standard culture medium, while for the assessment of differentiation constructs were cultured with chondrogenic medium. Live/dead staining was used to evaluate cell attachment and viability after 3, 7 and 14 days in culture. Real time PCR was used to evaluate the expression of specific fibro/chondrogenic markers (Collagen I-COLI, Collagen X-COLX, Sox9-SOX9) after 7 and 14 days in culture. After 4 weeks in static culture, one group of DPSC/scaffold constructs was placed in the perfusion bioreactor (10 ml/min) and further cultured for 4 weeks. DPSC/scaffold constructs were assessed for extracellular matrix production by means of histology and dynamic mechanical analysis after 4 and 8 weeks.

RESULTS & DISCUSSION: Live/dead staining showed that more than 90% of the cells remained viable inside the scaffolds in both conditions. DPSCs cultured into Ch/Gel scaffolds under hypoxic conditions demonstrated a significant increase of gene expression of fibrocartilaginous markers (COLI, COLX, SOX9) after 2 weeks in culture compared to normoxic conditions. Histological data after 8 weeks indicated that only the constructs cultured in the perfusion bioreactor support abundant fibrocartilaginous tissue formation. Dynamic mechanical analysis revealed increased yield strain of the constructs cultured under dynamic conditions compared to static ones.

CONCLUSIONS: Collectively, these data provide evidence of a promising strategy for TMJ disc TE-based replacement, by application of natural biomaterials combined with dental-tissue derived mesenchymal stem cells.

ACKNOWLEDGEMENTS: Te European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY).



Stromal cells behavior during artificial wound healing via a novel 3D microgap model

D. S. D. C. Avelino[1,2], I.-S. Choi[3], K. Park[1,2]
Presenting Author: Dos Santos Da Costa Avelino, 613515@kist.re.kr
[1]Center for Biomaterials, Korea Institute of Science and Technology, Seoul, Korea [2]Division of Bio-Medical Science and Technology, KIST School, University of Science and Technology (UST), Seoul, Korea [3]Department of Materials Science and Engineering, Seoul National University, Seoul, Korea

INTRODUCTION: During wound healing process, proliferation of stromal cells is one of the important stages for granulation, contraction and re-epithelialization. Proliferation occurs in three-dimensional (3D) environment in vivo and granulation involves cell crawling of fibroblasts to fill the wound bed. The purpose of this study is to understand the working mechanism of fibroblasts migration via our 3D wound model and to investigate the way how cells migrate and close the gap on varying geometries of 3D microgap.

METHODS: 3D microgap with varying gaps from 100-300 μm was fabricated by pouring polydimethylsiloxane (PDMS) on patterned mold. After solidification process, PDMS was peeled off and then coated with polydopamine (PDA). NIH 3T3 cells were used in this work. Both 25 μM of blebbistatin and Y-27632 were added to the cell culture media for inhibition of contractility of myosin II and Rho-associated protein kinase (ROCK), respectively. Live cell microscopy was used to monitor the migration of cells closing the gaps while confocal microscopy was employed for immunofluorescence images.

RESULTS & DISCUSSION: Phase contrast live cell and confocal microscopies reveal that cells close the gap on 3D microgap through contractility of myosin II including ROCK and adherens junction (Figure 1). These lead to supracellular actomyosin contractility, a mechanism that was mostly found on 2D planar substrate using epithelial cells [1]. Decay of area over time shows that even at larger gaps the closure of gaps is linear (Figure 1).

CONCLUSIONS: Our study show a complete dependence of 3D microgap closure on both cellular contractility of myosin II and ROCK.

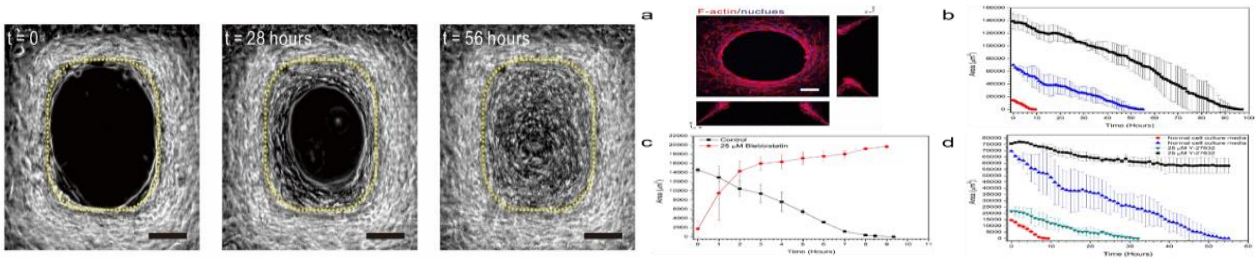
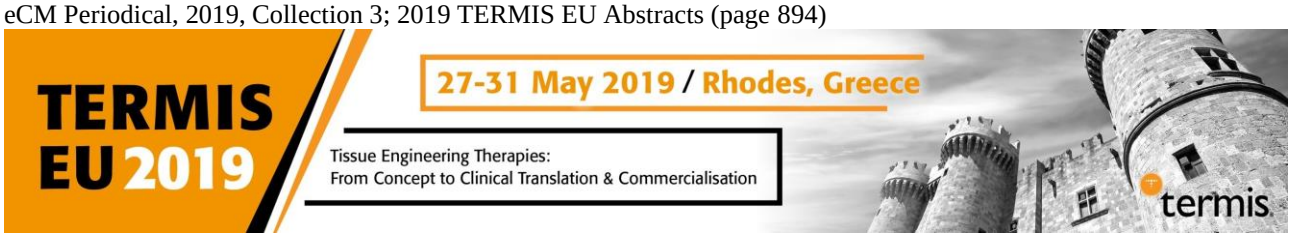


Figure 1: Phase contrast images show that NIH 3T3 cells close a microgap with time. Yellow line is the boundary of the 3D microgap. Scale bars are 100 μm. a. Immunofluorescence of F-actin and nucleus on 3D microgap during gap closure. Scale bar is 100 μm. b. Decay of area over time with different gap diameters (100-300 μm). c and d. Gap closure is mediated by contractility of myosin II and ROCK.

REFERENCES

[1] Brugués, A. et al. Nature physics 2014;10: 683-690



Practical guide to extrusion-based 3D bioprinting

L. Ouyang[1, 2], A. Moore [2], J.A. Burdick[3], W. Sun[1]
Presenting Author: Axel Moore, axel.moore@imperial.ac.uk
[1]Department of Mechanical Engineering, Tsinghua University, 100084, Beijing, China [2]Department of Materials, Imperial College London, South Kensington, SW7 2AZ, London, UK [3]Department of Bioengineering, University of Pennsylvania, 19104, Philadelphia, PA, USA

INTRODUCTION: Despite numerous extrusion-based approaches developed in the field of 3D bioprinting, there is still a lack of understanding of the general process of this technology. To better understand the nature of extrusion-based bioprinting, we aimed to investigate the general criteria through progressively developing feasible bioprinting processes for different bioinks.

MATERIALS & METHODS: All the case studies followed the similar route as showed in Fig 1 (left), where bioink was extruded to form gel filaments for 3D construct fabrication. Bioinks with various gelation properties were studied, including guest-host self-assembly, thermal gelation and photo-crosslinking. The bioprinting process was optimized corresponding to specific bioink based on either home-made or commercial (e.g., SunP Biotech) bioprinters. For examples, temperature and light were well controlled for printing thermo-responsive and photo-crosslinkable hydrogels, respectively. General characterizations of the bioprinting outcome include 3D printability, construct stability, cell viability, and functionality.

RESULTS & DISCUSSION: Three cases were studied independently, starting with gel filament generation and ending with cell viability maintenance. The shear-thinning and self-healing formulation (guest-host hyaluronic acid) was demonstrated to support consistent extrusion and maintenance of hydrogel shape, and additional secondary crosslinking helped to further stabilize the structure (Fig 1, right A). With careful control of temperature in the nozzle and printing platform, gelatin-based bioink could be successfully printed, maintaining good 3D printability and cell viability (> 90%) (Fig 1 right B). By simultaneously introducing light to a light-permeable nozzle during printing, various non-viscous photo-crosslinkable bioinks could be printed into 3D constructs (Fig 1 right C).

CONCLUSIONS: General requirements for bioink and bioprinting process include biocompatibility, consistent injectability, filament maintenance, co-adhesion between layers, further stability and low shear force on cells.

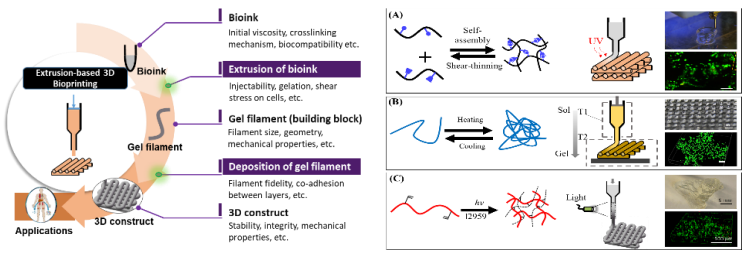
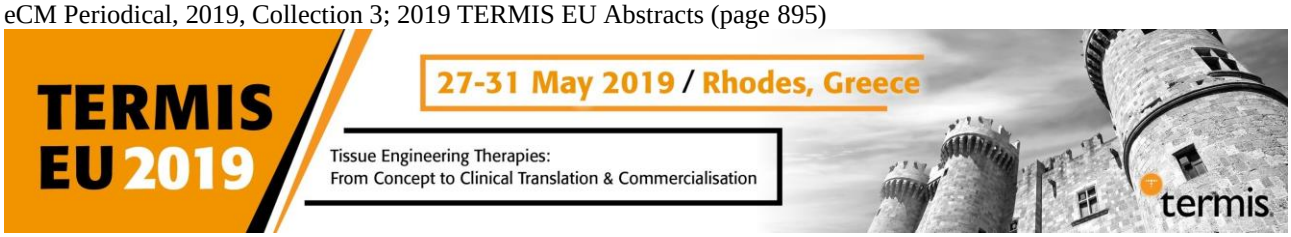


Figure 1: (left) Schematic of extrusion-based bioprinting process, showing general criteria in each step. (right) Bio-3DP examples based on (A) shear-thinning and self-assembly, (B) thermo-responsive, and (C) photo-crosslinkable hydrogel formulations.

ACKNOWLEDGEMENTS: The authors thank the support from NSFC, CSC and SunP Biotech.



Sonocoating of polymeric orthopaedic textiles with bioactive nanoparticles

B. Woźniak[1], J. Rogowska-Tylman[1,2], A. Chodara[1,2], U.Szałaj[1,2], W. Łojkowski[1], R. Mrugas[3]
Presenting Author: Bartosz Woźniak, b.wozniak@labnano.pl
[1]Institute of High Pressure Physics (Laboratory of Nanostructures), Polish Academy of Sciences, Poland [2]Faculty of Materials Science and Engineering, Warsaw University of Technology, Poland [3]Ortholigaments Ltd., Poland

INTRODUCTION: There is a number of patients suffering from problems caused by torn ligaments or tendons. In 20% of cases, treatment with traditional methods, does not produce lasting results. It has very negative consequences for patients' life. We have used sonocoating method to deposit hydroxyapatite nanoparticles on ligament's fibers. It will cause bone ingrowth between the fibers of ligament, firmly attaching to the bone tissue and strengthening the connection between ligament and bone tissue. We are developing sonocoating technology to be brought into the clinical phase level of the study.

METHODS: We were investigating the influence of coating conditions (coating duration, suspension concentration) in order to obtain homogeneous layer on the fiber's surface. Four types of nanohydroxyapatite particles (nHAP) were selected. Textile fabric was fixed on holder and immersed in ultrasonic chamber with nHAP suspension. Subsequently, ultrasonic waves were induced nearby material in order to deposit nHAP on its surface. Research are based on SEM investigations and weight change analysis.

RESULTS & DISCUSSION: Homogeneous layers of nHAP were produced using sonocoating method. It was proved that nHAP layer structure depends on nanoparticles size, coating duration and suspension concentration. Lengthening coating time and increasing suspensions concentration results in higher amount of hydroxyapatite deposited on the surface. Sonocoating method have beneficial conditions as it occurs in water, room temperature and short coating duration.

CONCLUSIONS: The novel method of nHA deposition on the polymeric fibers can serve as an alternative for currently known orthopaedic implants modification solutions. Material testing revealed good stability and bioactivity of the deposited nHA coating in SBF. The immersion test of the in vivo bone bioactivity prediction in SBF can reduce the number of animals used in and the duration of future animal experiments.

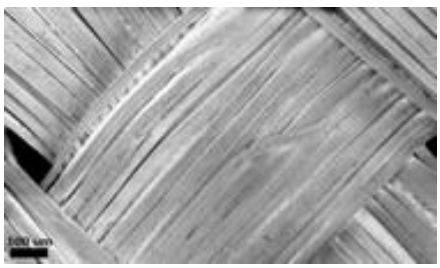
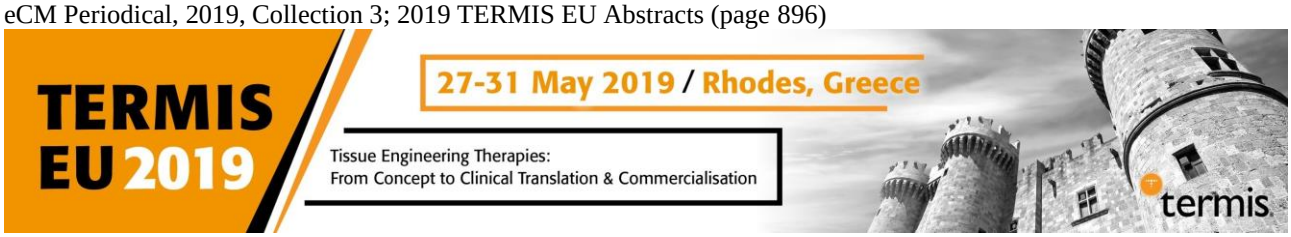


Figure 1: SEM picture of sonocoated textile fiber's with nano-hydroxyapatite particles.

ACKNOWLEDGEMENTS: This work is supported by the polish National Centre for Research and Development (NCBR) project: NanoLigaBond (POIR.04.01.02-00-0016/16) Artificial tendons and ligaments fixation to bone tissue using nanotechnological approach.



Methylcellulose as a smart thermosensitive scaffold material for tissue engineering

B. Niemczyk, P. Sajkiewicz, A.Gradys
Presenting Author: Beata Niemczyk, bniemczyk21@gmail.com
Laboratory of Polymers and Biomaterials, Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Injectable thermosensitive hydrogels can be rapidly introduced into the body by injection, thus help to avoid complex and long surgeries. Methylcellulose (MC) aqueous solution, while heated up to near 37°C, as an effect of dehydration, undergoes physical two-step crosslinking [1]. Injectability of such hydrogel system at room temperature and crosslinking during subsequent heating to physiological temperature make such approach attractive for tissue engineering. The objective of this study was to investigate the crosslinking kinetics, biological properties and injection ability of MC aqueous solution, what was studied at various concentrations.

METHODS: Methylcellulose METHOCEL A15LV (Sigma Aldrich) was prepared at various (w/w %) concentrations in demineralized water.
The cross-linking kinetics was studied by differential scanning calorimetry (DSC) at the heating rate of 2 K/min, in the temperature range 20-80°C. In order to prevent water evaporation, hermetic pans were used. Biological tests of hydrogels were performed using L929 fibroblasts. MTT cytotoxicity test was carried out on extracts. Injectability tests allowed determination of the maximum force (should be lower than 30 N [2]), which is needed to carry out injection of the hydrogel into medium that simulates native tissue at 37°C.

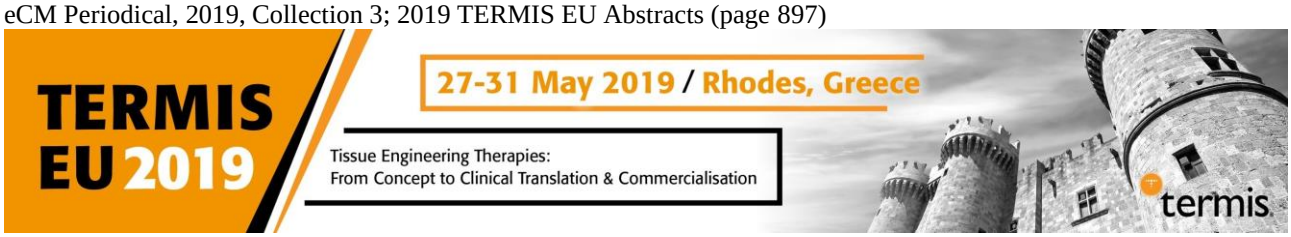
RESULTS & DISCUSSION: DSC results show complex thermal behavior with upmost two small endothermic effects, shifting to lower temperatures with increase in MC concentration. For MC concentration higher than 6%, low temperature peak disappears, leaving only the high temperature peak. Analysis of the kinetics of the effects indicates that the rates of the two cross-linking processes increase with temperature and decrease with MC concentration. MTT cytotoxicity tests showed that this type of materials are not cytotoxic. For all MC concentrations the injectability tests showed the maximum force below 30 N.

CONCLUSIONS: Depending on MC concentration, the MC cross-linking may be two- or one-step process. Two-step cross-linking process, which takes place at lower MC concentrations, seems to proceed at higher rates, which is probably due to higher chain mobility of diluted solutions and due to higher temperature. Injectability tests prove that all of the investigated hydrogel concentrations can be easily injected.

ACKNOWLEDGEMENTS: Financial support was received from Polish National Science Center (NCN) (2018/29/N/ST8/00780).

REFERENCES

[1] Joshi S et al. J. Appl. Polym. Sci. 2006;101;1620-1629.
[2] Kim, M et al. Int. J. Biol. Macromol. 2018;109;57-64.



An insight into the bioadhesives of the ixodid ticks Amblyomma hebraeum and Dermacentor marginatus

B. Engel[1], J. Suppan[2], S. Nürnberger[2], M. Marchetti-Deschmann[1]
Presenting Author: Benedikt Engel, benedikt.engel@tuwien.ac.at
[1]Institute of Chemical Technologies and Analytics, TU Wien, Vienna, Austria [2]Department of Orthopedics and Trauma Surgery, Medical University of Vienna, Austria

INTRODUCTION: Ticks' salivary glands produce a sticky secretion called attachment cement. This biological adhesive is a mainly proteinaceous substance and hardens rapidly when secreted. However, the functions of the cement are not fully understood yet, but it is suggested that besides other functions it mainly strengthens the attachment of the ticks' mouthparts to the vertebrate host during their blood meal [1]. In this work we present bioanalytical results for tick cement to get a deeper insight into its biochemical composition.

METHODS: Tick cement was collected from in vitro fed Amblyomma hebraeum and Dermacentor marginatus, both ixodid ticks. After homo-genization by grinding, portions of the cement were used for amino acid analysis by GC-EI-MS/MS (gas chromatography electron ionization tandem mass spectrometry). SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was carried out to separate proteins and subsequently identify them from respective gel bands. Proteolytic peptides resulting from in-gel digestion were analysed by LC-ESI-MS/MS (liquid chromatography electrospray ionization tandem mass spectrometry). Data evaluation of the obtained MS and MS/MS spectra was carried out with PLGS (ProteinLynx Global Server, Waters) in order to identify tick cement proteins.

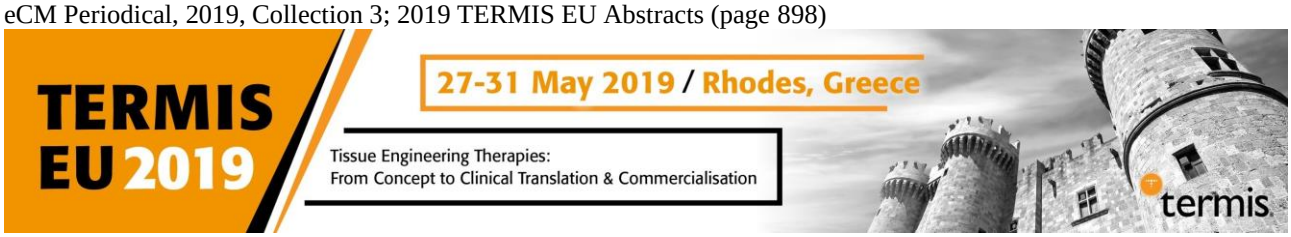
RESULTS & DISCUSSION: Amino acid analysis after acid hydrolysis revealed that the cement mainly consists of non-polar amino acids and glycine as the major component. Our findings are in good agreement with amino acid compositions theoretically calculated from genomic data [2]. Different buffer systems allowed solubilisation of proteins, however some insoluble debris was always observed. Proteins between 20 and 80 kDa were separated by SDS-PAGE. The identification of bovine proteins confirms that the feeding material, containing bovine blood, contaminates the cement. Therefore tick specific protein identification was challenging. In addition to that the lack of reviewed database entries impedes protein identification. Nevertheless we were able to identify cement specific proteins (e.g. cement protein RIM36). The identification of proteins named glycine rich proteins confirms the high amount of glycine found by amino acid analysis.

CONCLUSIONS: The ability of ticks to adhere to their host skin makes the secreted cement a very interesting field of research as such an adhesive could be used in medicine for various applications. This work allowed to get an insight into the amino acid and protein composition of the cement of two tick species which in future might help to understand the attachment mechanism.

ACKNOWLEDGEMENTS: This work was financially supported by the Austrian Science Foundation (Project Number P 28962), COST-ENBA (CA15216) and by Shimadzu Austria by the loan of the TQ8040 to the Metabolomic and Bioprocess Analytics Laboratory (TU Wien).

REFERENCES

[1] Suppan J et al. Biol Rev Camp Philos Soc. 2018; 93(2):1056-1076
[2] Bullard R et al. Ticks Tick borne Dis. 2016; 7(5):880-892



FACS reveals more Pluripotent Intervertebral Disc Progenitor Cells compared to MACS and pluriSelect

D.A. Frauchiger[1], R.D. May[1], E. Džafo[1], X. Zhang[1], J. Stoyanov[2], A. Bertolo[2], L.M. Benneker[3], D. Sakai[4], S. Grad[5], M.A. Tryfonidou[6], B. Gantenbein[1,3]
Presenting Author: Benjamin Gantenbein, benjamin.gantenbein@dbmr.unibe.ch
[1]Department for BioMedical Research, University of Bern, Bern, CH [2]Spinal Injury Research, Swiss Paraplegic Research, Nottwil, CH [3]Department of Orthopaedic Surgery, Inselspital, Bern University Hospital, University of Bern, Bern, CH [4]Department for Orthopaedic Surgery, Tokai University School of Medicine, Isehara, Kanagawa, JP [5]AO Research Institute Davos, Davos, CH [6]Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, NL

INTRODUCTION: Nucleus Pulposus Progenitor Cells (NPPCs), positive for the angiopoietin-1 receptor (Tie2), were demonstrated in human, mouse, canine and bovine NP tissue [1,2,3]. Tie2+ NPPCs possess a multi-lineage differentiation potential, and regeneration potential is attributed to them. However, the isolation of Tie2+ NPPCs can be cumbersome. Hence, three isolation methods were compared.

METHODS: Bovine NP cells were isolated from 10-14-month-old animals. Cell sorting was performed with an antibody against Tie2 (bs-1300R, Bioss) using FACS, magnetic-activated cell sorting (MACS) and pluriSelect, a size-based sorting method. Outcomes were evaluated by cell yield of Tie2+ cells, the ability of sorted cells to form colonies and tri-lineage differentiation assays.

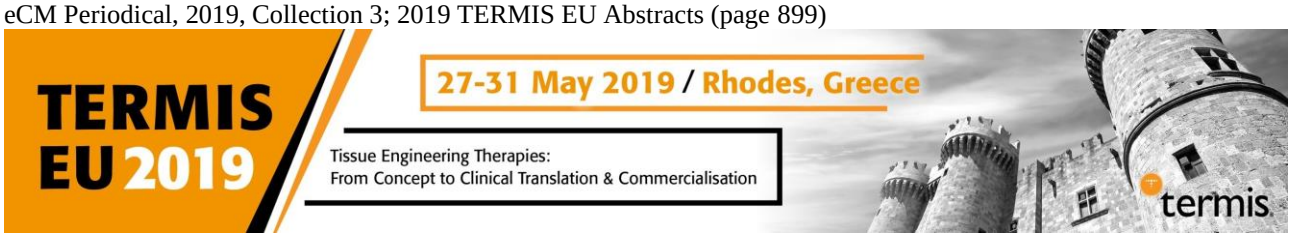
RESULTS & DISCUSSION: FACS resulted in the highest Tie2+ cell yield (5.0 ± 4.0%) followed by MACS (1.6 ± 2.9%) and pluriSelect (1.1 ± 1.4%). Colony forming ability did not differ between Tie2+ and Tie2- cells for any isolation method. However, Tie2+ cells obtained by MACS tended to have more colonies than FACS and pluriSelect. Osteogenic and adipogenic differentiation of Tie2+ and Tie2- cells did not result in a clear distinction for MACS and pluriSelect; Tie2+ FACS-sorted cells demonstrated superior osteogenic and adipogenic differentiation over Tie2- cells. Also for chondrogenesis, the Tie2+ FACS-sorted Tie2+ NPPCs tended to produce more proteoglycan versus Tie2- NPPCs, whereas for MACS and pluriSelect no difference was found.

CONCLUSIONS: Based on the parameters tested, isolation of NPPC is possible with all three methods. However, cell yields differed widely. FACS although most invasive, appears to be the most specific sorting method for these $Tie2^+$ cells among the tested methods as $Tie2^+$ cells do not demonstrate osteogenic and adipogenic differentiation. As for cell yield MACS seems to reveal the most, possibly this is due to inclusion of cells expressing Tie2 less strongly.

ACKNOWLEDGEMENTS: We thank Eva Roth and Selina Steiner for their assistance. Financial support was received from the Horizon2020 Project “iPSpine” # 825925.

REFERENCES

[1] A. Tekari et al. Stem Cell Res Ther 2016; 7(1):75.
[2] D. Sakai et al 2012; Nat Commun 31264.
[3] D. Sakai et al 2018; JOR Spine 2018e.



Investigation of the effect of nicotinamide riboside on primary human bone-marrow derived mesenchymal stromal cells in vitro

E. Džafo[1], R. D. May[1], E. J. Müller[1,2,3], Stefan Baertschi[3], O. Naveiras[4], L. M. Benneker[5], B. Gantenbein[1,5]

Presenting Author: Benjamin Gantenbein, benjamin.gantenbein@dbmr.unibe.ch

[1]Department for BioMedical Research, Medical Faculty, University of Bern, Bern, Switzerland [2]Department of Dermatology, Insel University Hospital, University of Bern, Bern, Switzerland [3]CELLnTEC Advanced Cell Systems AG, Bern, Switzerland [4]Interfaculty Institute of Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland [5]Department of Orthopaedic Surgery and Traumatology, Insel University Hospital, University of Bern, Switzerland

INTRODUCTION: Mesenchymal stromal cells (MSC) have been identified as important cell-based therapy candidates for cartilage, bone and intervertebral disc diseases [1]. During prolonged in vitro expansion prior to administration, MSC become senescent which impairs their therapeutic potential [2]. Here, we aimed to investigate whether extracellular nicotinamide riboside (NR) is beneficial for MSC expansion with respect to delaying the senescence, improving cellular activity and growth kinetics.

METHODS: MSC were isolated from human bone marrow aspirates by gradient centrifugation of the bone marrow and subsequent expansion in α-MEM + 10% fetal bovine serum + 2.5 ng/ml bFGF-2. The cytotoxicity of NR was measured at day 4 for 29 concentrations in a range from 5 nM to 4 mM. The long-term effects of NR were tested at concentrations of 10, 100 and 1,000 µM by measuring the population doubling level (PDL), relative confluency (real-time live-cell imaging with IncuCyte S3®), mitochondrial activity by resazurin reduction, senescence-associated β-galactosidase assay (SA-β-gal) and NAD/NADH ratio.

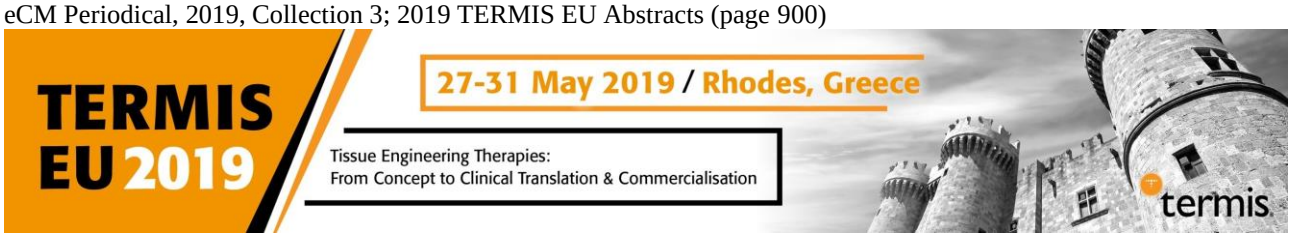
RESULTS & DISCUSSION: MSC treated with 3 and 4 mM NR had a significantly higher mitochondrial activity at day 4 than the negative control ($p=0.0027$ and $p<0.0001$ respectively, N=3). However, in the weeks 3 to 8, cells treated with ≥100 µM NR died reaching a maximum PDL of 13.43 (N=4). In two donors, the experimental group with 10 µM NR reached a 2-fold higher PDL than the negative control. The relative confluency at passage (P) 2 after 6 days in culture was higher with 10 µM NR compared to the negative control (35.00 ± 9.29% and 26.19 ± 5.41% respectively, mean ± SD, N=2). The mitochondrial activity was significantly higher with 10 µM NR at P4, P8 and P10 ($p<0.01$, N=4). At all passages, the percentage of SA-β-gal positive cells was under 5%, except in the negative control medium at P11 (18.17% ± 18.18%, mean ± SD, N=1). All experimental groups treated with NR had a higher NAD/NADH ratio which exhibited a dose-dependent trend (N=1).

CONCLUSIONS: Extracellular NR elevated the intracellular NAD/NADH ratio. NR is not cytotoxic within 4 days of culture at concentrations up to 4 mM. Long-term culture with 10 µM NR improved the growth kinetics markedly in two donors.

ACKNOWLEDGEMENTS: Financial support was received by the Competence Center for Applied Biotechnology and Molecular Medicine (CABMM) start-up grant to BG.

REFERENCES

[1] Vedicherla S et al. J Orthop Res. 2017; 35(1):8-22
[2] Turinetto V et al. Int J Mol Sci. 2016;17(7):1164.



Development of Emulsion Templated Scaffolds Manufactured from Photocurable Polycaprolactone

B. A. Dikici[1], G.C. Reilly[2], F. Claeyssens[1]
Presenting Author: Betül Aldemir Dikici, baldemirdikici1@sheffield.ac.uk
[1]University of Sheffield, The Kroto Research Institute, Department of Materials Science and Engineering, Sheffield, UK [2]University of Sheffield, INSIGNEO Institute for in silico medicine, Department of Materials Science and Engineering, Sheffield, UK

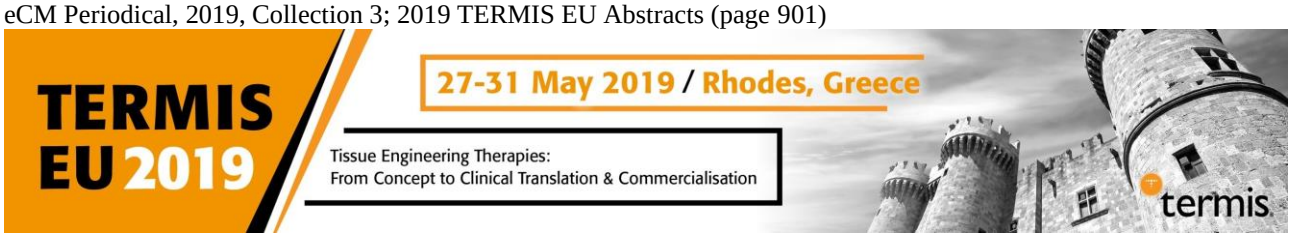
INTRODUCTION: Emulsion templating is an emergent manufacturing route for producing highly porous scaffolds (PolyHIPEs) with interconnected porosity. In this paper, we present the development of PolyHIPEs made of photocurable 4-arm polycaprolactone methacrylate (4PCLMA) which is one of the most extensively used synthetic, biocompatible and bioresorbable polymers, used as a scaffold material for both hard and soft tissues. The viscosity of the PCL pre-polymer is high which can lead to destabilisation of the emulsion. Here solvent dilution was used to reduce the viscosity of PCL to enable creation of an emulsion. The effect of solvent composition and volume on emulsion stability and morphology was investigated and the interplay between solvent and oil phase densities was analysed.

METHODS: By using various solvents/solvent blends PolyHIPE compositions were created and their morphologies, stabilities and mechanical properties were tested. Human dermal fibroblasts were cultured on both the produced PolyHIPEs and a control scaffold (commercial polyHIPE scaffold, Alvetex®). Cytotoxicity was evaluated comparing cell viability. Cell attachment and infiltration were investigated via SEM and histological staining, respectively.

RESULTS & DISCUSSION: Stable PolyHIPE foams consist of fully PCL was developed successfully. Chloroform was found to be a better porogenic solvent to dilute the PCL and creating porous scaffolds. Alternatively, when toluene was used to dilute the oil phase, HIPEs were found to be more stable. Both open porous and comparably stable HIPEs were successfully created when solvent blends of both chloroform and toluene were used to dilute the oil phase. Pore and window size of the 4PCLMA PolyHIPEs were shown to be tuneable by adjusting the chloroform and toluene ratios in the solvent blend. These changes in morphology have an effect on mechanical properties. 4PCLMA PolyHIPE scaffolds were shown to be capable of supporting cell attachment, growth, and migration within the scaffold.

CONCLUSIONS: Understanding effect of solvent volume and composition on morphology and the mechanical properties of the scaffold, enable us to control scaffold properties. By controlling the conditions, a scaffolds architecture and mechanical characteristic can be tailored for various tissue engineering applications.

ACKNOWLEDGEMENTS: The author BA was funded by Turkish Ministry of Education.



Optical coherence guided laser based preparation of tissue, scaffolds and biomaterials for analysis in regenerative medicine

F. Will, H. Richter, I. Bleeker, B. Stolze
Presenting Author: Birgitta Stolze, b.stolze@lls-rowiak.de
LLS ROWIAK LaserlabSolutions GmbH, Hannover, Germany

INTRODUCTION: In the fast growing field of regenerative medicine, it is more important than ever to analyze tissue structures or implant-interface interaction. Beyond, easy and gentle preparation of scaffolds or biomaterials for use in tissue engineering is desirable. Current technologies for cutting hard tissue, scaffolds or biomaterials lack of preciseness, 3D function or the ability to process the material at a sufficient quality. Femtosecond laser based cutting combined with Optical Coherence Tomography (OCT) imaging offers new roads for material processing as demonstrated at different applications out of a broad range of applications in regenerative medicine.

METHODS: a) For histology sections from orthopedic or cardiovascular resin embedded samples were cut using a laser microtome, hard tissue without prior decalcification. Stainings were applied as indicated in the results. b) For site-specific 3D-sectioning of native tissue, regions of interest at bone-titanium interfaces of a fresh rat tibia were determined by OCT. Then small volumes were cut (~300x300x300 µm). Harvested tissue was further processed for TNF-alpha gene expression analysis. c) Laser preparation of native or fixed pericardium, hard valves tissue layers or cornea was performed as indicated in the results.

RESULTS & DISCUSSION: a) A broad range of histological stainings and even immunohistochemistry can be successfully applied to thin sections performed by laser microtomy. The sections show tissue architecture and cellular details clearly. b) Image-guided collection of native tissue samples along an implant could be demonstrated successfully (Fig.1). A fresh rat tibia with a dental implant was imaged by OCT to identify new formed bone along the implant. This bone and control samples from surrounding areas were cut out and subsequently analyzed for TNF-alpha expression. Enhanced site specific expression at the interface was observed. c) Separation of heart valve elastin from collagen layer was successfully performed, as also separation of cornea epithelium. Laser sectioning of pericardium for further tissue engineering purposes resulted in precise cutting without any visible detriments.

CONCLUSIONS: Collectively, these data clearly illustrate that image guided femtosecond laser based tissue and material sectioning opens new dimensions for tissue processing, histological and biochemical tissue analysis in regenerative medicine.

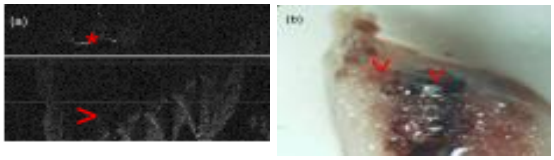
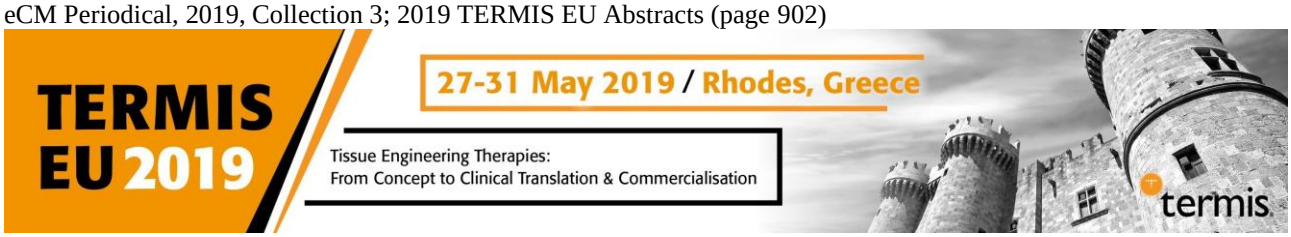


Figure 1: Gentle laser based isolation of new formed bone along a dental implant in a rat tibia. a) OCT of rat tibia with dental implant (*). New formed bone along implant (>). b) Areas of interest was cut out successfully (arrow).

ACKNOWLEDGEMENTS: We thank Dr. O. Omar (University Gothenburg) for provision of sample and performing molecular biological analysis.



Scarring in an arecanut-induced fibrotic skin tissue environment

B. Ghosh[1], J. Chatterjee[1]
Presenting Author: Biswajoy Ghosh, biswajoy101@gmail.com
[1]School of Medical Science and Technology, IIT Kharagpur, Kharagpur, India

INTRODUCTION: Phytochemicals present in the areca nut extract (ANE) such as arecoline along with high Cu^{2+} has a strong fibrotic effect on tissues. Chewing of areca nuts is a habit among people in the Indian subcontinent triggering precancer condition such as the oral submucous fibrosis. Post-operative/post-biopsy healing on a stiff fibrotic tissue ambiance is thus important. Here we document the behavior of post-injury fibrotic scarring on a stiffened skin with arecanut induced fibrosis.

METHODS: Arecoline was extracted by methanol based solvent extraction from arecanut. The powder was obtained by sequential removal of solvents by rotary-evaporation and freeze drying. The extract was applied on mouse skin dorsum (35-40 gm, all males, 6 months age, Mus musculus) to cause fibrosis and a wound was made at the fibrotic site. Optical Coherence Tomography (OCT, Thorlabs, USA) was used to image the wound healing cross-sections in vivo. Animal studies were performed as per the Institute Animal Ethical Committee guidelines.

RESULTS & DISCUSSION: The extract showed a high content of arecoline (Fig 1a). On treatment with ANE, within 30 days, the dermis showed contraction and hypodermis started to disappear (Fig 1 b, c). Upon withdrawal of the ANE, the hypodermis layer started to appear (Fig 1 d). The scar in the normal skin contracted more than the surrounding giving a hammock-like appearance (Fig 1 e). Scars in fibrotic skin, the optical intensities closely matched the fibrotic ambiance (Fig 1 f, g) without a hammock-like appearance of the scar. We found that the optical scattering intensity ratio between the scar and the ambiance was highest in normal skin, followed by the fibrotic bed with continued and withdrawn treatments of ANE (Fig 1 h).

CONCLUSIONS: We found that upon healing, the scar tissue blends better with the fibrotic tissue ambiance after the ANE treatment was withdrawn.

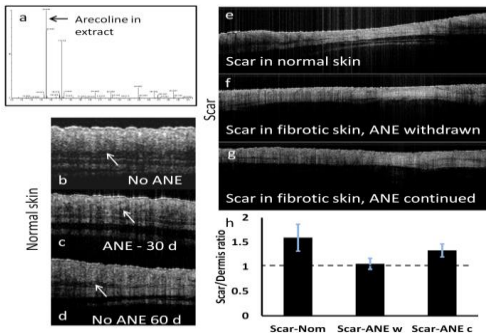
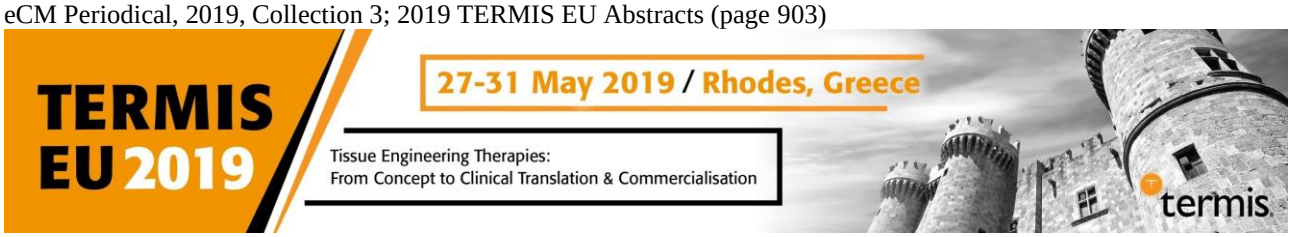


Figure 1: Effect of scarring in a fibrotic skin tissue bed. (a) Characterization of arecoline in the arecanut extracts using mass spectrometry. (b) OCT image of mice skin (arrow shows fat rich hypodermis) (c) fibrosis of 30 days ANE treatment and (d) 60 days withdrawal. (e-g) scars in normal and fibrotic beds, (h) optical intensity ratio of the scar and ambient tissue (w-treatment withdrawn, c-continued).

ACKNOWLEDGEMENTS: Funded by Dept. of Biotechnology, Govt. of India.



Direct reprogramming of human cardiac fibroblasts to cardiomyocytes using microRNA mimics

C. Paoletti[1], C. Divieto[2], F. Di Meglio[3], D. Nurzynska[3], V. Chiono[1]
Presenting Author: Camilla Paoletti, camilla.paoletti@polito.it
[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy [2]INRIM, Metrology for Life Quality, Turin, Italy [3]Department of Public Health, University of Naples "Federico II", Naples, Italy

INTRODUCTION: The combination of four different microRNAs (miR-1, 133, 208 and 499), named "miRcombo", has been used for the direct reprogramming of murine fibroblasts into cardiomyocytes (CMs) for myocardial infarction (MI) treatments.[1,2] Here, we evaluated miRcombo mediated reprogramming of human adult cardiac fibroblasts (AHCFs) into CMs in 2D and 3D culture.

METHODS: For digital droplet PCR (ddPCR) analysis, $3x10^5$ AHCFs were plated in 6-well plates, for Immunocytochemistry (ICC) $5x10^4$ cells were plated in 24-well plates. AHCFs were transfected with miRcombo (mirVana) using DharmaFECT1 (Dharmacon). Untrasfected and NegmiR (mirVana) transfected AHCFs were used as controls. After 24 hours, medium was changed to medium with 1 μM Jak1 Inhibitor for 4 days for 2D experiments; for 3D experiments, cells were cultured in fibrin-based hydrogels.

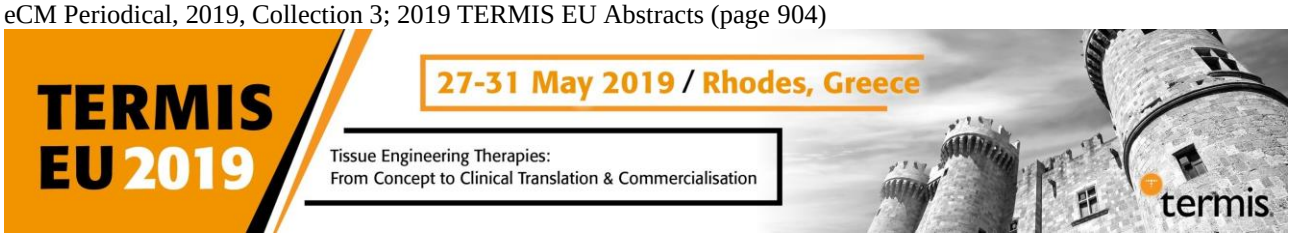
RESULTS & DISCUSSION: ddPCR analysis showed significant increase expression of early cardiac transcription factors (TFs) Hand2 and Mef2c ($p < 0.005$) slight increase expression of Tbx5 and Nkx2.5, although non-significant ($p > 0.05$), and reduced Vimentin expression ($p < 0.05$) in miRcombo-transfected AHCFs compared to controls after 4 days in 2D culture. ICC analysis showed increased expression of late cardiac markers α-sarcomeric actinin and cTnT in miRcombo-transfected AHCFs after 10 and 20 days of culture in 2D. However, ddPCR showed no significant differences of late cardiac markers Myh6 and cTnI expression between the groups after 15 days in 2D culture. On the other hand, cells cultured in 3D fibrin-based hydrogels showed enhanced cardiac TFs expression compared to 2D. However, miRcombo transfection did not significantly enhance cardiac gene expression in AHCFs cultured in 3D hydrogels respect to controls after 4 days. After 15 days, AHCFs cultured in 3D hydrogels showed a strongly enhanced expression of cardiac genes such as cTnI and Myh6 compared to 2D.

CONCLUSIONS: Together, these results showed that a 3D environment was found to play a key role in enhancing direct reprogramming of AHCFs into CMs.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 772168; BIORECAR project).

REFERENCES

[1] Jayawardena TM et al. Circ Res. **2013**, 110, 1465–1473
[2] Li Y et al. Sci. Rep. **2016**, 6, 1–11



Gene repression of the CREB transcription factor promotes the healing of wounded human tissue-engineered corneas

C. Couture[1,2], E. Gillard[1,2], P. Desjardins[1,2], L. Germain[1,2], S.L. Guérin[1].
Presenting author: Camille Couture, camille.couture.1@ulaval.ca
[1]Département d'ophtalmologie, Université Laval, CUO-recherche/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada [2]Département de chirurgie, Université Laval, Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada

INTRODUCTION: The cornea composes the outer surface of the eye and its transparency is required to allow light transmission to the retina. However, because of its position, the cornea is subjected to chemical and mechanical injuries that may lead to blindness. In our laboratory, we demonstrated that activation of the transcription factor cAMP-response element binding protein (CREB) is reduced during corneal wound healing in human tissue-engineered corneas (hTECs) and that addition of the CREB inhibitor C646 considerably accelerates wound closure [1]. To alter CREB gene expression in a more specific manner, the dCas9-KRAB system was used to suppress or reduce its expression in human corneal epithelial cells (hCECs).

METHODS: Four different single guide (sg) RNAs complementary to four distinct regions from the CREB gene bearing DNA target sites for transcription factors reported to contribute to CREB gene expression were designed and cloned into the pLV hU6-sgRNA hUbC-dCas9-KRAB-T2a-GFP plasmid [2]. These plasmids, each containing one of the four sgRNAs, were transfected by electroporation into HCECs. The capacity of each sgRNA to reduce or suppress the expression of the CREB protein when transfected into hCECs was then evaluated by Western Blot and Electrophoretic Mobility Shift Assay (EMSA). HCECs in which expression of CREB has been reduced to various levels were then used for the production of hTECs that were used in wound healing assays.

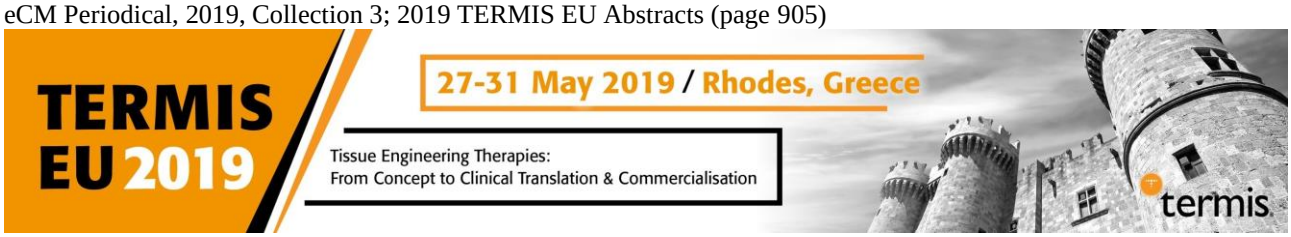
RESULTS & DISCUSSION: Transfection of the sg1 RNA allowed for the complete suppression of total CREB expression whereas the sg2, sg3 and sg4 RNAs reduced its expression to varying levels (42,20%, 93,83% and 94,81%, respectively). Phosphorylated CREB expression was also considerably reduced (between 90% and 100% for each sgRNA). These results were further supported by the demonstration that each sgRNA considerably impaired the binding of CREB to its DNA target site in EMSA. The production of hTECs with CREB-deficient hCECs and their use in wound healing studies is actually underway.

CONCLUSIONS: The dCas9-KRAB system is particularly effective at reducing the expression of CREB in hCECs. We believe that the functional characterisation of CREB in corneal wound healing will help develop therapeutic strategies aimed at improving corneal wound healing in patients.

ACKNOWLEDGEMENTS: C. Couture received financial support from the Fonds de recherche du Québec – Santé (FRQS) (Doctoral Training). L. Germain is the recipient of a Tier 1 Canadian Research Chair on Stem Cells and Tissue Engineering and a Research Chair on Tissue-Engineered Organs and Translational Medicine of the Fondation de l'Université Laval.

REFERENCES

[1] Couture C et al. Acta Biomater. 2018;73:312-325
[2] Thakore PI et al. Nat Methods. 2015;12(12):1143-9



Development of a microgel-based extracellular vesicle delivery system for heart repair

C. Marijon[1,2], J.P.K. Armstrong[1], S.C. Skaalure[1], I. Mylonaki[1], R. El Laham[1], D.J. Stuckey[3], S.E. Harding[2], M.M. Stevens[1]
Presenting author: Camille Marijon, c.marijon15@imperial.ac.uk
[1]Department of Materials, Department of Bioengineering, and Institute for Biomedical Engineering, Imperial College London, London, U.K [2]National Heart and Lung Institute and BHF Centre for Research Excellence, Imperial College London, London, UK [3]Centre for Advanced Biomedical Imaging, University College London, London, UK

INTRODUCTION: Cardiovascular diseases are the primary cause of death globally, responsible for approximately 17.5 million deaths per year. In particular, myocardial infarction caused by the obstruction of a coronary artery can lead to heart failure. A growing number of studies have demonstrated that extracellular vesicles (EVs) exhibit great potential as cell-free cardioprotective agents, which could overcome many of the technical, ethical and regulatory hurdles associated with cell-based therapies [1-3]. However, the beneficial effects of administered EVs are short-lived; a major limitation that could be circumvented using a material-based approach for increased retention and sustained release. Therefore, the main objective of this study was to develop a feasible approach for repairing the infarcted heart, in the form of injectable, 'off-the-shelf' EV-loaded synthetic microgels. This platform was designed to improve EV stability and retention time at the site of administration, with sustained and environmentally-responsive release of EVs to the surrounding damaged tissue.

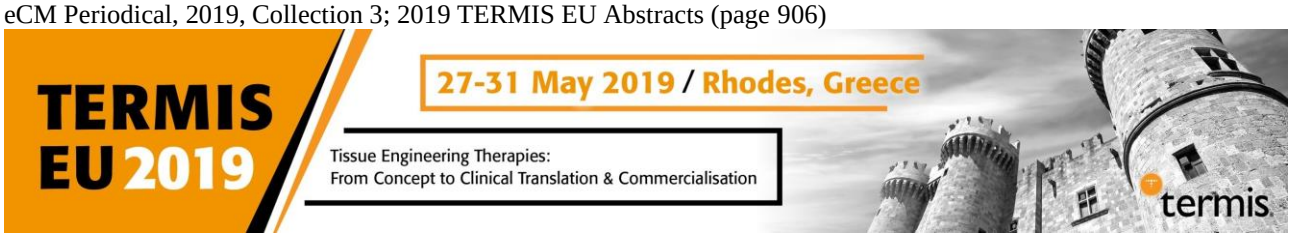
METHODS: We developed a droplet-based microfluidic platform enabling high-throughput fabrication of monodisperse, injectable EV-loaded microgels composed of multi-arm poly(ethylene glycol) (PEG) crosslinked with enzyme degradeable peptides.

RESULTS & DISCUSSION: This hydrogel composition enabled controlled microgel degradation and EV release over a period of at least four days in the presence of specific matrix metalloproteinases that are upregulated in heart tissue after myocardial infarction. This delivery system was validated by monitoring the release of EVs containing a Bioluminescence Resonance Energy Transfer construct. This method enabled EV quantification and validation of sustained bioactivity both in vitro and in vivo.

CONCLUSIONS: Taken together, these results demonstrate that EVs can be successfully encapsulated and controllably released via an injectable and biodegradable microgel system. Future work will focus on verifying EV stability in the microgels over time, and ultimately delivering therapeutic EVs in vivo for repair of the infarcted heart.

REFERENCES

[1] Ibrahim A et al. Stem Cell Rep. 2014; 2:606–619.
[2] Bian S et al. J Mol Med Berl Ger. 2014; 92:387–397.
[3] Kervadec A et al. J Heart Lung Transplant. 2016;35(6):795-87.



The potential of biomaterial-based approaches as therapies for ischaemic stroke: A systematic review of preclinical studies

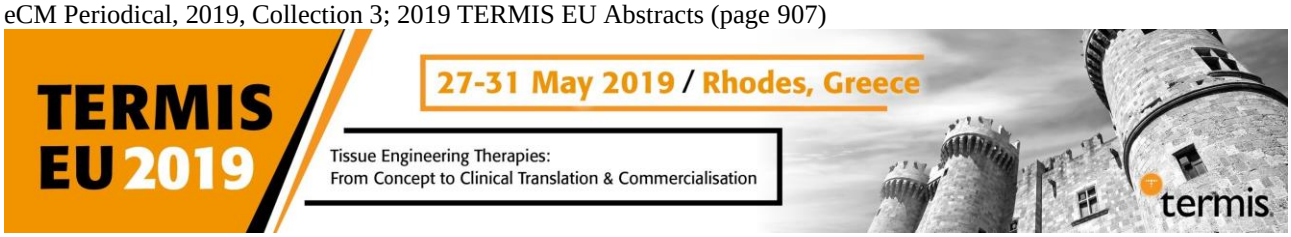
F. Bolan[1], I. Louca[1], C. Heal[2], C.J. Cunningham[1]
Presenting Author: Catriona Cunningham, catriona.cunningham@manchester.ac.uk
[1]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom
[2]Centre for Biostatistics, Faculty of Biology, Medicine and Health, Academic Health Sciences Centre, University of Manchester, Manchester, United Kingdom

INTRODUCTION: Stroke is a major health problem with limited treatment options. According to the World Health Organisation, stroke is the second leading cause of death worldwide accounting for 6.7 million deaths annually and for surviving patients, nearly two thirds will be left with some disability. In recent years, interest in the development of biomaterial-based therapies to promote brain repair and functional recovery has increased exponentially. A broad range of strategies have been investigated including nanoparticles or liposomes as delivery vehicles for drugs and scaffolds as structural support for tissue regeneration. Despite interest in this area, no systematic review and meta-analysis has so far been conducted assessing the efficacy of these biomaterial-based approaches.

METHODS: Studies were identified by searching electronic databases and reference lists of relevant review articles. Studies reporting lesion volume (brain damage) and/or neurological score as outcome measures were included. Standardised mean difference (SMD) and 95% confidence intervals were calculated using DerSimonian and Laird random effects. The CAMARADES (Collaborative Approach to Meta-analysis and Review of Animal Data in Experimental Studies) checklist was used to assess study quality. Publication bias was then visualised by funnel plots followed by trim and fill analysis of missing publications.

RESULTS & DISCUSSION: Sixty six publications were included in the systematic review, of which 48 (88 comparisons) were assessed in the meta-analysis. Overall, biomaterial-based interventions reduced stroke damage (SMD: -1.56, 95% CI: -2.0, -1.13) and improved neurological scores (SMD: -2.3, 95% CI: -2.85, -1.76). The median score on the CAMARADES checklist was 5.5/10 (IQR 4.25-6). In particular, reporting of blinding (50%) and randomisation (35%) was low. There was pronounced asymmetry in the funnel plots of both lesion volume and neurological score data indicating publication bias. Furthermore, trim and fill analysis estimated there were 25 unpublished studies reporting negative or neutral lesion volume data which, when adjusted for, reduced the effect size to -0.40 (95% CI: -1.57, 0.77).

CONCLUSIONS: Biomaterial-based interventions decrease brain damage and promote recovery in pre-clinical stroke models. However, it should be noted there is a high risk of publication bias and there were limitations in study design including lack of blinding. We believe this study provides valuable insight to preclinical researchers interested in developing biomaterial-based interventions and informs on future directions for the translation of such therapies to the clinic.



Influence of hypoxic environment on mesenchymal stem cell pro-angiogenic and immunomodulatory profile

L. Aubert[1], M. Dubus[1], H. Rammal[1], C. Mongaret[1], J. Lesieur[2], S. C. Gangloff[1], C. Chaussain[2], C. Mauprivez[1], H. Kerdjoudj[1]
Presenting Author: Cedric Mauprivez, cedric.mauprivez@univ-reims.fr
[1]EA 4691 « Biomatériaux et inflammation en site osseux » (BIOS), Université de Reims Champagne Ardenne, REIMS, France [2]EA 2496 « Pathologies, Imagerie et Biothérapies orofaciales et Plateforme d'Imagerie du Vivant », Université Paris Descartes, France

INTRODUCTION: Along with their differentiation potential, mesenchymal stem cells (MSCs) are thought to exert regenerative effects through immunomodulation including the ability to control inflammatory responses during tissue damage. Pre-seeding MSCS into 3 dimensional (3D) scaffolds may promote immune cell chemoattraction and would effectively provide localized control of inflammation and improve wound healing [1]. Low oxygen (O_2) concentration plays a crucial role in maintaining undifferentiated state and proliferation [2]. Taken together, we hypothesized that hypoxia might promote the immunomodulatory potential of MSCs cultured in 3D through paracrine regulation. We have previously reported that the efficiency of collagen based-medical device (Hemocollagene®) as biomimetic scaffold mimicking MSCs niche [3]. Here, the aim of this study has been to evaluate the hypoxic (3% O2) effects on the proliferation of MSCs derived from various tissue sources and the production of cytokines and growth factors required for tissue healing.

METHODS: Human MSCs from bone marrow (BM-MSCs), dental pulp (DP-MSCs) and Wharton's jelly (WJ-MSC) were cultured within the foam for 4, 7 and 10 days under 3% and 21% of O_2. The secretory profile of these cells was analyzed by ELISA quantification.

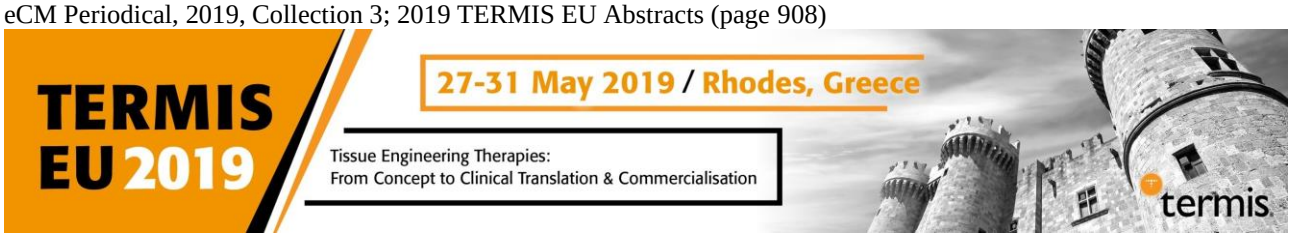
RESULTS & DISCUSSION: MSCs cultured in 3D secreted angiogenic factors without prior activation, such as IL-6, IL-8 and VEGF, whatever their origin. TGF-β, HGF and PGE-2 were also secreted by MSCs creating an immunosuppressive environment. In addition, neither major pro- and anti-inflammatory cytokines (TNFα, IL1-β and IL-10 respectively) nor b-FGF were detected in our experiments. 3% O_2 do not change the amount of cells containing in foams over the time, except for DP-MSCs for which a decrease has been reported. Hypoxia enhances both angiogenic and immunomodulatory capacities of MSCs. We noticed that profiles of mediators release varied according to MSCs source, the proportion of oxygen and the duration of cell culture.

CONCLUSIONS: The outcomes show that 3% O2 for 10 days under 3D culture may enhance MSCs therapeutic effects before transplantation for cell therapy or regenerative medicine. It would be interesting to study the behavior of endothelial cells and immune cells in regard of immunomodulatory and pro-angiogenic capacities of MSC after preconditioning.

ACKNOWLEDGEMENTS: This work was partially supported by SEPTODONT.

REFERENCES

[1] Aktas E, et al., J Orthopaedic Res 2016.
[2] Ahmed et al., Sci Rep, 2016.
[3] Aubert L et al., Int J Mol Sci, 2017.



Combined effects of co-culture and substrate mechanics on 3D tumor spheroid formation within microgels prepared via flow-focusing microfluidic fabrication

D. Lee[1], C. Cha[1,2]
Presenting Author: Chaenyung Cha, ccha@unist.ac.kr
[1]School of Materials Science and Engineering [2]School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan 44919, Republic of Korea

INTRODUCTION: In this study, tumor spheroids were developed within size-controlled microgels as a 3D cell culture platform via a flow-focusing microfluidic fabrication. Mechanical properties of microgels and co-culture with supporting cells were controlled to influence 3D cancer spheroid formation.

METHODS: A double flow-focusing microfluidic device coupled with photocrosslinking was used to fabricate spherical microgels encapsulated with breast cancer cells (e.g. MCF-7). For co-culture experiments, MCF-7 cells. The mechanical stiffness of microgel was controlled, with elastic modulus ranging from 0.7 to 30 kPa, by MGel concentration. The viability, proliferation and spheroid formation of encapsulated cells were monitored over time by LIVE/DEAD assay (Thermo Fisher Scientific).

RESULTS & DISCUSSION: The MCF-7 cells encapsulated inside the microgels showed high viability throughout the cell culture regardless of the mechanical properties of microgel. However, the rate of proliferation was highly dependent on their mechanical properties; the cells proliferated faster within microgels with higher mechanical stiffness. MCF-7 cells alone did not lead to a mature spheroid within a microgel, in which all the cells form a large, compact, and well-defined spherical cell cluster, but rather a collection of smaller cell aggregates was formed regardless of the microgel stiffness. However, when MCF-7 cells were co-cultured with supporting cells, macrophages or fibroblasts, well known to be involved with tumor progression, the cells turned into a mature spheroid regardless of the microgel stiffness, though their rate and extent of spheroid formation was dependent on the microgel stiffness.

CONCLUSIONS: The 3D tumor spheroids developed in this study could be applied as platform for drug screening applications as well as fundamental biological investigation.

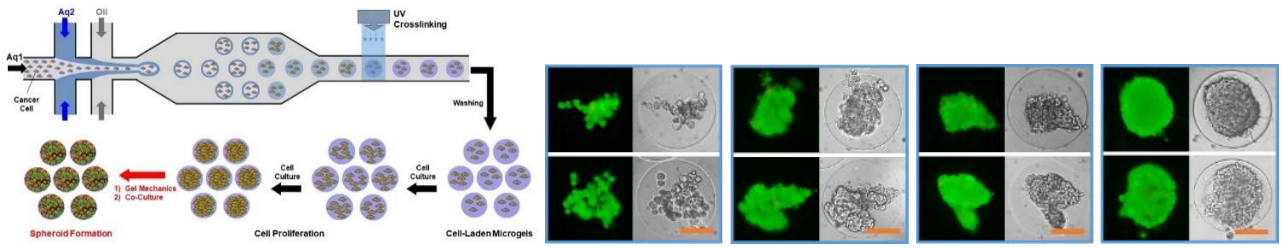
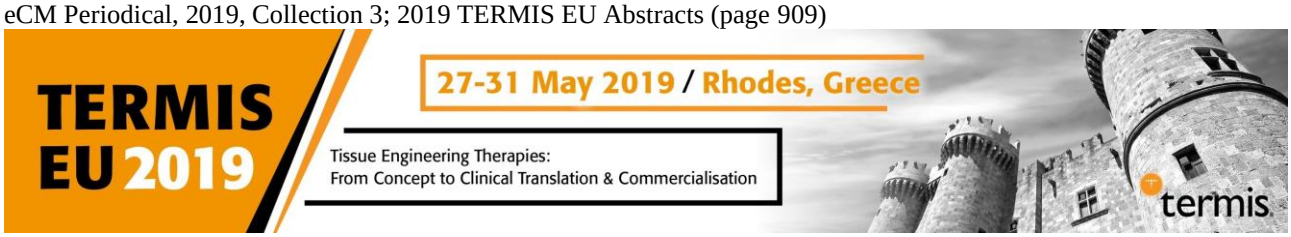


Figure 1: (Top) Schematics of a flow-focusing microfluidic fabrication of cell-laden microgels. (Bottom) Tumor spheroid formation within the microgels over time (up to 7 days).

ACKNOWLEDGEMENTS: This study was supported by the 2019 Research Fund (1.190048.01) of UNIST (Ulsan National Institute of Science and Technology), and Bio & Medical Technology Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (2017M3A9C6033875).



PRMT6 inhibits the migration ability of stem cells from the apical papilla

M. Li[1,2], C. Zhang[2], Z. Fan[1]
Presenting Author: Chen Zhang, zhangchen710cool@qq.com
[1]Laboratory of Molecular Signaling and Stem Cells Therapy, Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Capital Medical University School of Stomatology, Beijing, China [2]Department of Endodontics, Capital Medical University School of Stomatology, Beijing, China

INTRODUCTION: Enhanced cell homing is a basic and essential ability for dental mesenchymal stem cells to promote dental pulp regeneration [1]. Multiple studies have indicated the crucial role of PRMT6 in cells migration capacity [2-3]. Hence, we used stem cells from the apical papilla (SCAPs) to investigate the effect of PRMT6 on the migration ability.

METHODS: SCAPs at passages 3-5 were used in experiments. Real-time RT-PCR and Western blot were conducted. Scratch migration assays: SCAPs were cultured in six-well plates at a density of 2×10^5 cells/ well. Wound tracks were introduced by scraping the cell monolayer on the well with a 1000μl pipette tip (Axygen® Corning, NY, USA). Images after incubation for 24 hours and 48 hours were also taken. The upper chamber with an 8.0 μm pore size membrane (Corning, Costar, MA, USA) was seeded with 2×10^4 SCAPs. Transwell chemotaxis assays: The plate was cultured in an incubator at 37°C for 24 hours and 48 hours. The images of each well were randomly selected by using microscopy (Olympus Corporation, Tokyo, Japan) and the transferred cell numbers were counted.

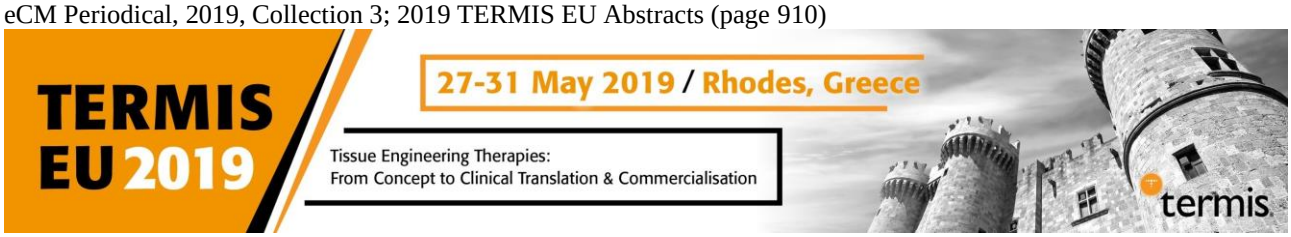
RESULTS & DISCUSSION: PRMT6 was knocked down in SCAPs, as confirmed by real-time RT-PCR and Western blot. The scratch-simulated wound migration assay results showed that PRMT6 knockdown enhanced the migration ability of SCAPs at 24 hours and 48 hours. Transwell chemotaxic assay results showed that PRMT6 knockdown enhanced the chemotaxis ability of SCAPs compared with the control group at 24 hours and 48 hours.

CONCLUSIONS: Collectively, these data indicated that PRMT6 inhibits the migration and chemotaxic ability of SCAPs.

ACKNOWLEDGEMENTS: Financial support was received from National Natural Science Foundation of China (81200783).

REFERENCES

[1] Belema-Bedada F et al. Cell Stem Cell. 2008;2:566-575.
[2] Almeida-Rios D et al. Oncotarget, 2016, 7(33):53018-53028.
[3] Manling L et al. International Journal of Molecular Sciences, 2015, 16(12):29467-29481.



The ability of conditioned media from stem cells to repair vocal fold injuries

C.-S. Kim[1]; H. Choi[2]; S. W. Kim[1]; D.-I. Sun[1]
Presenting Author: Choung-Soo Kim, drchoung@catholic.ac.kr
[1]Department of Otolaryngology-Head and Neck Surgery, College of Medicine, The Catholic University of Korea [2]Clinical Research Institute, Daejeon St. Mary's Hospital, Daejeon, Republic of Korea

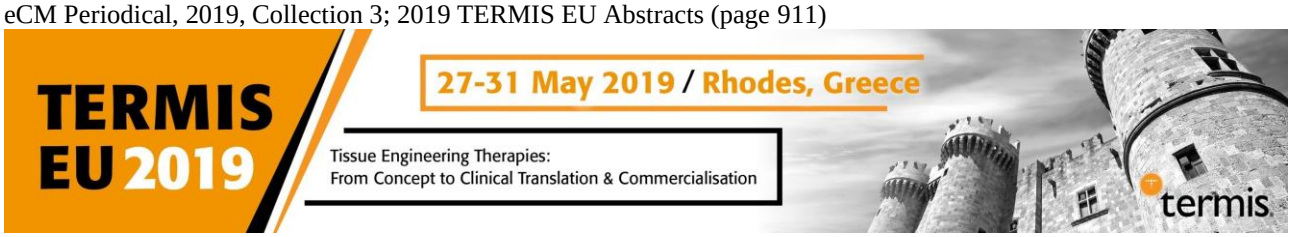
INTRODUCTION: This study investigated the ability of hypoxia-induced 25-fold concentrated conditioned media (hCM) from human nasal inferior turbinate-derived mesenchymal stem cells (hTMSC) to repair injured vocal folds in early phase of wound healing process.

METHODS: The right-side lamina propria of the vocal fold was injured in 60 Sprague-Dawley rats. Next, hCM from hTMSC (the hCM group) or hTMSC (the hTMSC group) were injected to the injured vocal folds. As a control, phosphate-buffered saline (the PBS group) or 25-fold concentrated media (the media group) was injected the in the same manner. Injections were performed twice weekly in both hCM and media group, and the vocal folds were harvested for quantitative real-time polymerase chain reaction (PCR) at 1 week and 2 weeks after injury. Histologic evaluation was done at three weeks post-injury.

RESULTS & DISCUSSION: In the hCM group at 1 week after injury. PCR showed that the gene encoding hyaluronan synthase (HAS) 1, HAS 2 were significantly upregulated compared the media and normal group. The gene encoding procollagen III was significantly downregulated compared with the media group. Nearly identical results were obtained for the hTMSC group at 1 week after injury However, the mRNA expression levels of several genes did not differ among the groups at 2 weeks after injury. Histological examination showed that the hCM group was similar or better than the hTMSC group in collagen deposition and hyaluronic acid production.

CONCLUSIONS: The injection of hCM into injured vocal folds twice per week produced an antifibrotic effect in the early phase of wound healing. The results were equivalent to those with hTMSC for up to 2 weeks. These results provide a foundation for future clinical use of hCM for vocal fold regeneration.

ACKNOWLEDGEMENTS: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT and Future Planning (No. 2014R1A2A2A01004325) Korea Health Industry Development Institute funded by the Ministry of Health and Welfare (HI14C3228, HI16C0133)



Investigation of endothelial cell viability and growth on 3D printed GelMa vascular networks

C. Chappell[1], C. Deans[1, 2], O. Tsigkou[1, 2] , B. Derby
Presenting Author: Chrisoula Chappell, Chrisoula.chappell@postgrad.manchester.ac.uk
[1]School of Materials, University of Manchester, Oxford Road, Manchester, M13 9PL, UK

INTRODUCTION: A major limitation for the development of successful three-dimensional (3D) engineered tissues is the absence of viable and perfusable blood vessels [1-3]. 3D filament networks of pluronic were printed using the I&J 7300-LF printer, the filaments were then coated with a hydrogel (gelatin methacrylate, GelMa) forming cylindrical channels. Human umbilical vein endothelial cells (HUVECs) were cultured and lined with the hydrogel channels to consequently encourage vascularization of adipose tissue constructs.

METHODS: GelMa was synthesized using the one pot synthesis method. The hydrogel was then characterized using NMR, surface tension, contact angle and DMA. Pluronic filaments were printed using a robotic printer onto glass slides. The diffusion of substances onto the gel was also investigated by using food dyes. HUVECs were cultured on top of the GelMa substrate. Live/Dead and Alamar Blue assays were used to assess the cells' viability and proliferation respectively. Phalloidin staining was used to assess actin cytoskeleton organization.

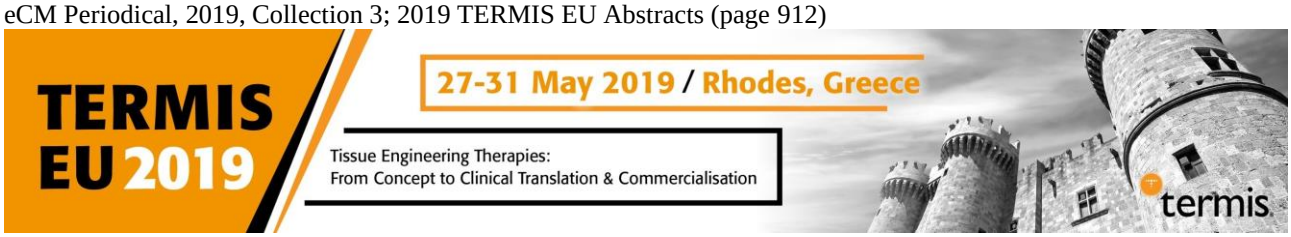
RESULTS & DISCUSSION: The GelMa NMR showed the changes that occurred before and after crosslinking. Furthermore due to its high hydrophilic and adhesive properties, viability assays confirmed that the cells were viable after 48 hours culture. The alamar blue data indicated an increase in cell metabolism over a 7 day period which was further complimented with a pico green assay. The pico green assay showed that there was a rapid cell number increase after 96 hrs using a standard curve equation y=mx+c. Phalloidin staining demonstrated good organisation of the actin cytoskeleton of HUVECs on GelMa.

CONCLUSIONS: Collectively, these data clearly illustrate that HUVEC cells could potentially grow and differentiate within those channels. However investigations are still ongoing.

ACKNOWLEDGEMENTS: Financial support was received from the EPSRC DTA doctoral award.

REFERENCES

[1] Loessner D et al. A Nat Protoc. 2016; 4:727-46
[2] Shirahama H et al. Tissue Scientific Reports. 2016; 6:31036
[3] Kolensky D et al. Adv Mater. 2014; 26:3124-3130



Chondrogenic hydrogel for cartilage bioprinting

L. Caballero-Aguilar[1, 3], C. Onofrillo[2, 3], C. O'Connell[3], S. Duchi[2, 3], S. Moulton[1, 3], C. Di Bella[2, 3]
Presenting Author: Claudia Di Bella, claudia.dibella@unimelb.edu.au
[1]Swinburne University of Technology, Melbourne, Australia [2]Department of Surgery, The University of Melbourne, Melbourne, Australia [3]Biofab 3D, Melbourne, Australia

INTRODUCTION: During in vitro stimulation of stem cells differentiation, the amount of chondrogenic factors is precisely administered from the external cell media over time. In vivo this condition cannot be replicated. The ideal chondro-regenerating bioscaffold needs to be carefully fabricated to allow the self-differentiation of stem cells laden into the biomaterial implanted in vivo [1]. We have identified a system that can be applied for the delivery of chondrogenic factors directly within the scaffold itself. This strategy consists on the fabrication of microspheres distributed homogeneously within the 3D printed bioscaffold, encapsulating the differentiating factors with a slow and controlled release across the sample.

METHODS: Alginate spheres loaded with chondrogenic growth factors TGFβ-3 and BMP-6 separately were fabricated using a microfluidic device. The release of growth factors from Chondrogenic Alginate Spheres (CAS) was tested in cell culture media for 20 days. The amount of growth factors released was quantified using ELISA tests. In vitro, CAS were tested by preparing three different concentrations of growth factors per sample (31.25, 62.5 or 125 ng/sample) incorporated within human adipose derived mesenchymal stem cells laden hydrogel material (GelMA/HAMA). DNA analysis, GAG content, gene expression (PCR) and Immunostaining analysis was performed at 0, 14 and 21 days.

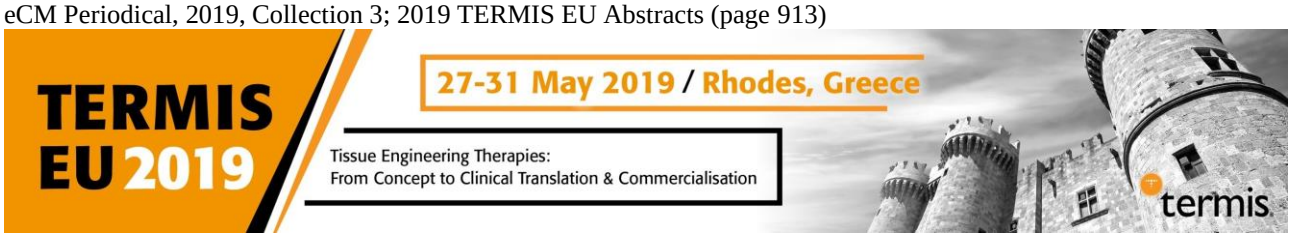
RESULTS & DISCUSSION: The release profile from CAS demonstrated a controlled sustained release over 21 days. The GAG/DNA content showed a dependency on the loading concentration of the growth factors within CAS. In vitro, the CAS induced the expression of Collagen II gene after 21 days of culture. Immunostaining analysis showed collagen I and collagen II formation across all the samples treated with CAS.

CONCLUSIONS: The incorporation of CAS within a bioprinted hydrogel containing stem cells can induce chondrogenesis. This approach has the potential to drive cellular differentiation in surgically printed hydrogels in vivo.

ACKNOWLEDGEMENTS: Financial support was received from Arthritis Australia, Australian Orthopaedic Association (AOA) and The Sylvia and Charles Viertel Charitable Foundation. Dr Di Bella has received a Senior Lecturer Fellowship from the Royal Australasian College of Surgeons to perform this work.

REFERENCES

[1] Song L. et al., Cytotherapy,2004; 6(6), 596-601



In situ bioprinting for cartilage regeneration is superior to standard bench-based biofabrication techniques

C. Di Bella[1, 2], C. Onofrillo[1, 2], S. Doyle[2], C. O'Connell[2], S. Duchi[1,2]
Presenting Author: Claudia Di Bella, claudia.dibella@unimelb.edu.au
[1]Department of Surgery, The University of Melbourne, Melbourne, Australia [2]Biofab 3D, Melbourne, Australia

INTRODUCTION: Various surgical techniques have been proposed for joint cartilage injuries, but regardless of the regenerative technique, securing good integration at the host–bioscaffold interface is a long-standing technical hurdle that leads to an unstable junction, unable to withstand the physiologic stresses placed across it. The eventual result is deterioration and disintegration of the attempted repair. In this work we demonstrate that in situ bioprinting and crosslinking of a chondrogenic hydrogel in an osteochondral ex-vivo model greatly improves the immediate adhesion at the host cartilage-scaffold interface.

METHODS: GelMa/HAMA hydrogel with human adipose-derived stem cells were used as chondrogenic scaffold for cartilage regeneration[1] and bioprinted in human ex-vivo osteochondral defect models [Duchi et al, Tissue Eng part C, submitted for publication]. Four groups were compared: 1) Control: defect left empty; 2) Ex situ: bioscaffold pre-fabricated in a mold of the same size of the defect and implanted; 3) Ex situ + Glue: bioscaffold pre-fabricated in a mold of the same size of the defect, implanted and fixed with fibronectin glue (commercially available surgical glue); 4) In situ: bioscaffold printed and crosslinked directly in the defect, using a hand-held extrusion printer [2,3]. Samples were cultured for 8 weeks in a perfusion system with chondrogenic media. We performed immunohistology with hyaline-like cartilage markers, confocal microscopy, second harmonic generation, light sheet microscopy imaging, and mechanical tests (including confined and unconfined compression test + pull out test).

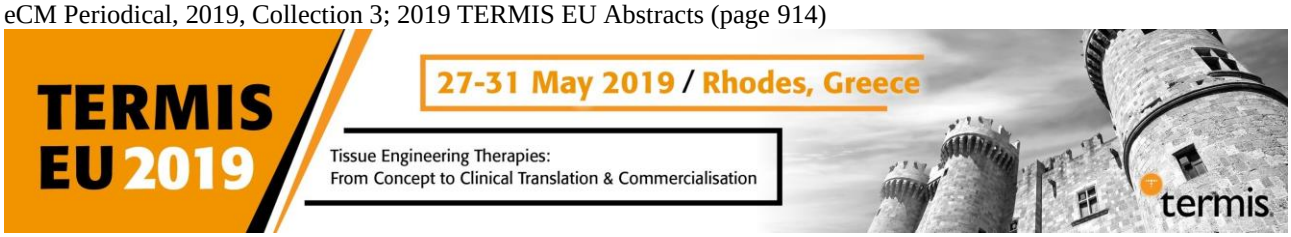
RESULTS & DISCUSSION: Collagen II is accumulated in the in situ bioscaffolds after 8 weeks of chondrogenic stimulation by TGFb-3 and BMP6. The in situ bioscaffolds presents regions of integration with the Osteochondral Unit. In the Control condition no spontaneous regeneration was present. The ex situ approach did not allow a proper implantation. In presence of Fibrin glue no evidences of lateral integration were observed. The in situ bioscaffold is better integrated with the host tissue. In situ bioprinting and crosslinking demonstrated superior adhesion to the surrounding cartilage, defined by an increased pull out resistance.

CONCLUSIONS: Surgical bioprinting can improve adhesion and integration of cartilage scaffolds, therefore solving one of the main challenges in articular cartilage regeneration.

ACKNOWLEDGEMENTS: Financial support was received from The CASS Foundation, Arthritis Australia and the University of Melbourne Seeding Grant. Dr Di Bella has received a Senior Lecturer Fellowship from the Royal Australasian College of Surgeons.

REFERENCES

[1] Onofrillo C et al, Biofabrication 2018
[2] O'Connell C et al, Biofabrication 2016
[3] Di Bella C et al, J Tissue Eng Reg Med 2018



α-TCP bone cements for bone tissue regeneration

D. Loca, M. Skrinda, Z. Irbe, J. Locs
Presenting Author: Dagnija Loca, dagnija.loca@rtu.lv
Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Pulka 3, Riga, LV-1007, Latvia

INTRODUCTION: The most prospective materials for the bone tissue replacement and regeneration are calcium phosphates (CaP) due to their biocompatibility, osteoconductivity and similarity to the natural bone mineral phase. Among CaP biomaterials, CaP bone cements (CPCs) have attracted extended attention because of their ability of self-setting in vivo, moldability and injectability, opening the new opportunities for minimally invasive surgical procedures [1]. Cements based on α-TCP are expected to have intermediate resorbtion properties in-between stoichiometric hydroxyapatite ($Ca_5(PO_4)_3OH$) cements and brushite ($CaHPO_4 \cdot 2H_2O$) cements. The reaction between water and α-TCP is slow and must be accelerated by adding the phosphate ions to the liquid phase, for cement to set in a reasonable time. The impact of α-TCP preparation method on the resulting CPC was evaluated in terms of setting time, porosity and mechanical properties.

METHODS: Two approaches were applied for an α-TCP preparation: high temperature synthesis at 1300 °C and low temperature synthesis <1000°C. The phase composition of prepared powders was analyzed using XRD, the specific surface area of α-TCP was determined using the BET method (ISO 9277:2010). The value of specific surface area found was used to calculate the average α-TCP particle size. CPC samples were prepared by mixing the α-TCP with sodium salts and setting time as well as mechanical properties and morphology of resulting CPCs were determined.

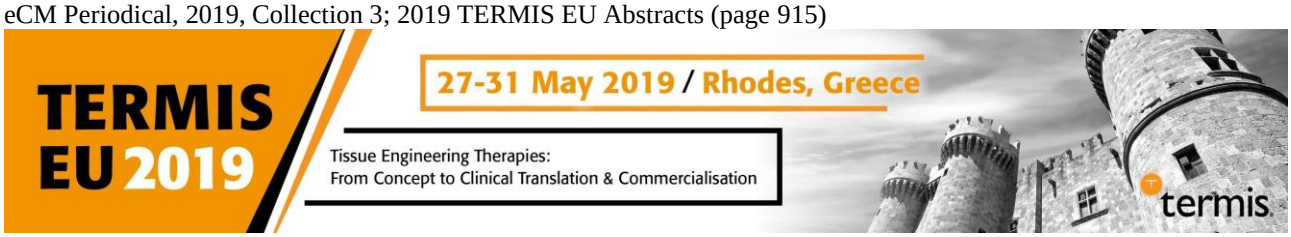
RESULTS & DISCUSSION: It was determined that high temperature α-TCP particles have irregular morphology, specific surface area of 1.5 g/m2 and average particle size (calculated from BET results) of 1.38 μm. Hence the setting reaction of α-TCP is too slow for the clinical applications; phosphate salt solution was used to obtain the cement setting time of 17.5 ± 1.2 min. Impact of solid to liquid phase ratio on CPS mechanical properties was investigated and it was found that by increasing the volume of liquid phase open porosity of the samples increased, decreasing the mechanical properties of calcium phosphate bone cements from 9.61 ± 2.31 MPa down to 4.51 ± 0.38 MPa. As the particle size and surface area of the solid phase are mainly responsible for the cement final properties, low temperature synthesis of α-TCP was performed, obtained particles characterized and CPC prepared.

CONCLUSIONS: During the research it was established that by low temperature synthesis approach it is possible to obtain α-TCP particles with decreased particle size, increased specific surface area and overall reactivity.

ACKNOWLEDGEMENTS: The project "Drug Delivering 3D Printed Scaffold Strategy Brings Human Body Implants to Next Level of Personalization" (DD-SCAFF) has partly received funding from the European Regional Development Fund under Agreement No.1.1.1.5/ERANET/18/01.

REFERENCES

[1] Loca D et al. Mater. Sci. Eng. C. 2015; 49(1):106-113



A double-crosslinking approach for high-fidelity 3D bioprinting of cell-laden scaffolds

D. Akilbekova[1], H. David[1], M. Rubert[1], X.H. Qin[1], R. Müller[1]
Presenting Author: Dana Akilbekova, dana.akilbekova@hest.ethz.ch
[1]Institute for Biomechanics, ETH Zurich, Zurich, Switzerland.

INTRODUCTION: Over the last decade, work on development of tissue organoids using 3D bioprinting (3DP) has been at the forefront of medical research [1]. High fidelity 3DP of cell-laden scaffolds depends on the bioink that possesses favorable physical characteristics such as shear thinning to be printable and at the same time the ability to retain its shape after printing. Here, we describe a double crosslinking approach (ionic and covalent) for 3DP of alginate (Alg) and gelatin (Gel) based bioink with phototunable mechanical properties for creating 3D engineered bone tissues.

METHODS: Human mesenchymal stem cells (hMSCs) (Lonza, Walkersville, MD, USA) were isolated from bone marrow aspirate. The cells were expanded and then mixed with the bioinks containing 0.8% Alg, 0.2% photoinitiator (Irgacure D-2959) and various Gel and methacrylated Gel (GelMA) concentrations. During printing, a lattice-rod model was extruded at the pressure of 30-50 kPa. The scaffolds were photo-crosslinked with UV for 5 min and 2% (w/v) $CaCl_2$ solution for 10 min. Printability, mechanical properties, in situ photocrosslinking and in vitro cell viability of Alg/Gel/GelMa bioinks were measured.

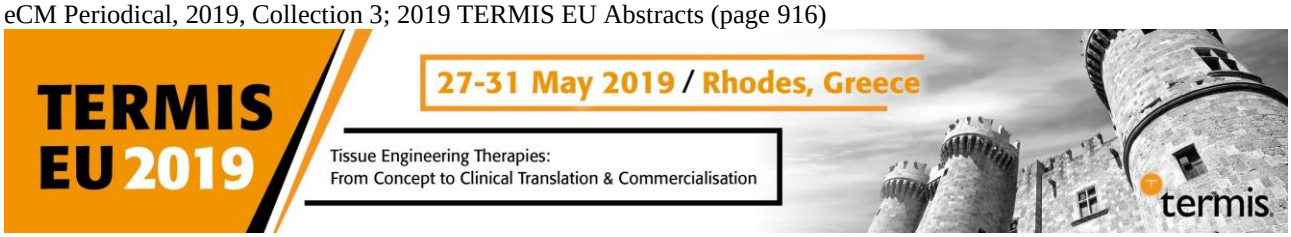
RESULTS & DISCUSSION: Rheology studies showed the shear-thinning behavior for Gel/GelMA bioinks, where inks with higher Gel concentration had more pronounced shear-thinning behavior. Addition of GelMA significantly increased the relative weight after a 14-day incubation in culture medium from 0.447±0.05 to 1.192±0.053. Compressive moduli of the double-crosslinked gels resulted in significant increase of stiffness ~20 times for 10% GelMA compared to 2.5%. Material stiffness was found to be important for cell viability. Gels with lower compressive moduli had lower number of viable cells (47%), while the stiffest gel also resulted in lower viability (63%). Formulations with 6/5 and 4/5 Gel/GelMA showed viability of around 80%.

CONCLUSIONS: In summary, these data demonstrate that combining ionic crosslinking (Alg/Ca^{2+}) and covalent UV crosslinking (GelMA) can help to create more stable bioprinted constructs. It was shown that only a GelMA concentration of 5% was suitable for bioprinting purposes. Gel presence helped to modulate the viscosity and provided initial stability during the printing due to a higher gelation rate.

ACKNOWLEDGEMENTS: Swiss Government Excellence Scholarship – Postdoctoral Fellowship.

REFERENCES

[1] Kang H.W. et al. Nat. Biotechnol. 2016;34(3):312-319



Cell surface glycan engineering of CD44 potentiates the immunomodulatory properties of adipose mesenchymal stem cells

M. García-Arranz[1], AI. García-Guillén[2], CM. Martínez[3], D. García-Olmo[1], AG. Zapata[4], R. Sackstein[5], JM. Moraleda[2], D. García-Bernal[2]
Presenting Author: David García-Bernal, david.garcia23@um.es
[1]Foundation Health Research Institute-Fundación Jiménez Díaz University Hospital, Madrid, Spain [2]Hematopoietic Transplant and Cellular Therapy Unit, IMIB-Arrixaca, University of Murcia, Spain [3]Experimental Pathology Unit, IMIB-Arrixaca, University of Murcia, Spain [4]Department of Cell Biology, Faculty of Biology, Complutense University of Madrid, Spain [5]Departments of Dermatology and Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

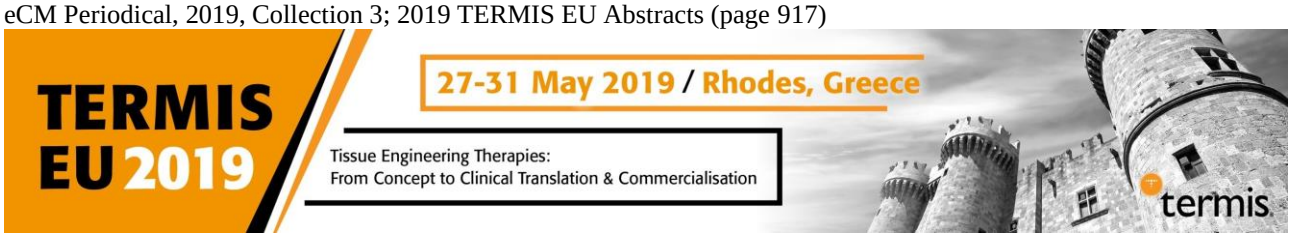
INTRODUCTION: Immunoregulatory features of mesenchymal stem cells (MSCs) have raised great hope for its clinical use in the treatment of various immune-mediated disorders. However, all MSCs lack molecular effectors of cell migration, thereby limiting their ability to enter inflamed tissue(s) following systemic administration. Ex vivo fucosylation of the molecule CD44 on MSCs generates the potent E-selectin ligand HCELL, enabling in vivo MSC migration to endothelial beds that express E-selectin. However, the inherent biochemical and functional properties of fucosylated MSCs has not been tested.

METHODS: In the present work we performed a direct comparison between unmodified murine adipose tissue MSCs (UmASCs) and fucosylated mASCs (FucmASCs) by analyzing: 1) cell morphology in culture; 2) proliferation kinetics; 3) MSC immunophenotype; 4) multipotent differentiation capacity; 5) secretome profile; and 6) expression of anti-inflammatory molecules.

RESULTS & DISCUSSION: UmASCs and FucmASCs displayed similar plastic adherence and a fibroblastic-like appearance typical of MSCs in culture. Both UmASCs and FucmASCs showed similar exponential cell growth curves, no significant different expression of MSC surface markers compared to UmASCs along time of culture, neither significant differences in the ability to differentiate to adipocytes, osteoblast and chondroblasts. However, we found that FucmASCs-conditioned medium contained significant greater amounts of several chemokines including KC, eotaxin, MIP-1, MCP-1 and RANTES. Analysis of expression of anti-inflammatory molecules by qPCR revealed that FucASCs expressed significant higher levels of heme-oxygenase-1 (Hmox-1), IL10, galectin-1, COX-1 and TGF compared to UmASCs. Importantly, IFN-stimulated FucmASCs showed a significant upregulated expression of HGF, Hmox-1, IDO, COX-2 and TGF compared to UmASCs.

CONCLUSIONS: Apart from their demonstrated improved migration to inflamed tissues, fucosylated ASCs displayed a significantly different secretome profile and an enhanced anti-inflammatory potency compared to unmodified ASCs, indicating that fucosylated ASCs may be a new safe and more effective cell therapy product for the treatment of immune-mediated disorders.

ACKNOWLEDGEMENTS: Financial support was received from Instituto de Salud Carlos III (ISCIII), projects PI16/01341 and PI16/01455 (co-funded by European Regional Development Fund-ERDF) and the Spanish Net of Cell Therapy (TerCel), projects RD16/0011/0001, RD16/0011/0002 and RD16/0011/0013 funded by ISCIII and co-founded by FEDER “una manera de hacer Europa”.



Antibacterial and bioactive nanofibrous membranes for tissue engineering applications

D. Porrelli[1], M. Mardirossian[1], F. Vecchies[1], M. Pacor[1], F. Berton[1], C. Stacchi[1], R. Di Lenarda[1], G. Turco[1]
Presenting Author: Davide Porrelli, dporrelli@units.it
[1]Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy

INTRODUCTION: Electrospinning (ELS) is a technique used for the production of polymer based nanostructured membranes suitable for biomedical applications, in particular in the field of tissue engineering. The present work reports the production and the characterization of polycaprolactone (PCL) based membranes, implemented with a bioactive chitosan derivative (CTL) and silver nanoparticles (nAg).

METHODS: A solution of PCL 12% w/V in DCM:DMF 7:3 was electrospun for 1 h by means of a custom made device with the following parameters: 17 kV of potential, flow rate of 0.6 mL/h, 27G needle, 15 cm of distance. Scanning Electron Microscopy (SEM) was used to analyze the nanofibers and micro-Computed Tomography for the analysis of membrane thickness. An air-plasma treatment was used to expose hydrophilic groups on membrane surface, in order to adsorb CTL and CTL-nAg. Ag was quantified with Inductively Coupled Plasma Mass Spectrometry ICP-MS. Wettability and biocompatibility of membranes were tested.

RESULTS & DISCUSSION: The ELS process used in this work allowed to obtain PCL membranes with an average thickness of 215 μm and an average nanofiber diameter of 600 nm. The efficacy of CTL adsorption process was confirmed with confocal microscopy using FITC labelled CTL (Fig. 1A). Contact angle measurements showed the poor wettability of PCL membrane (as prepared), and the increased hydrophilicity of air-plasma and CTL coated membranes. Biocompatibility was tested using MG63 cells cultured on membrane surface; Fig. 1B shows that CTL-coated membranes were able to support cell adhesion and proliferation. In contrast, both as-prepared membranes and air-plasma treated membranes exhibited poor cell adhesion and proliferation.

CONCLUSIONS: The data here presented proved the hydrophilicity and biocompatibility of ELS membranes made of CTL-nAg. These data are promising for potential application such as wound healing or Guided Bone Regeneration.

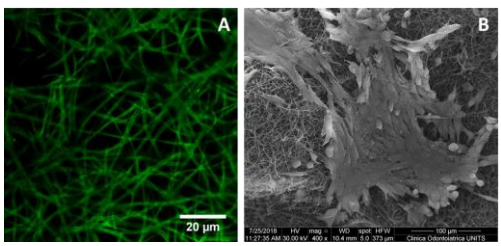
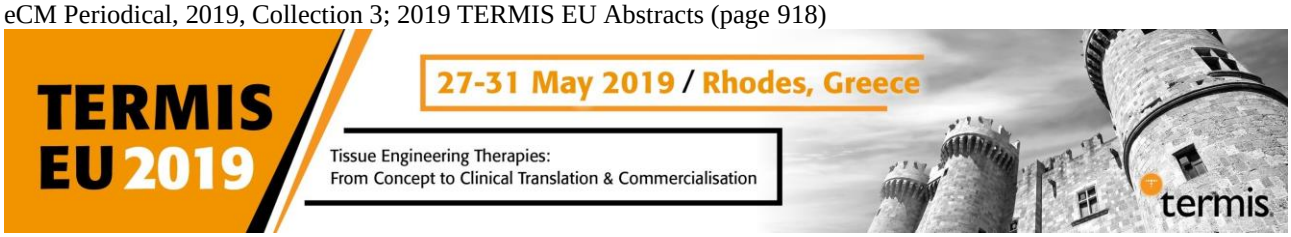


Figure 1: A) Confocal microscopy image, which shows the fluorescence of CTL-FITC adsorbed on PCL nanofibers. B) SEM micrograph of MG63 cells, which proliferated for 7 days on the surface of CTL coated PCL membrane.

ACKNOWLEDGEMENTS: Financial support was received from University of Trieste fund program: FRA 2016. The authors thank Dr. Denis Scaini (SISSA, Trieste, Italy) for his help with air-plasma and Dr. Matteo Crosera (University of Trieste) for his kind support with ICP-MS.



Development of 3D Tumour Models for Ameloblastoma

D. Bakkalci[1], S. Fedele[1,2], U. Cheema[1,2]
Presenting Author: Deniz Bakkalci, deniz.bakkalci.16@ucl.ac.uk
[1]Division of Surgery and Interventional Sciences, University College London (UCL)

INTRODUCTION: Ameloblastoma (AM) is a rare odontogenic neoplasm, which is associated with bone resorption (1, 2). The underlying mechanisms of how bone destruction occur are not well known. Aim of this project is to develop a 3D tumour model for ameloblastoma to look at possible bone resorption mechanisms.

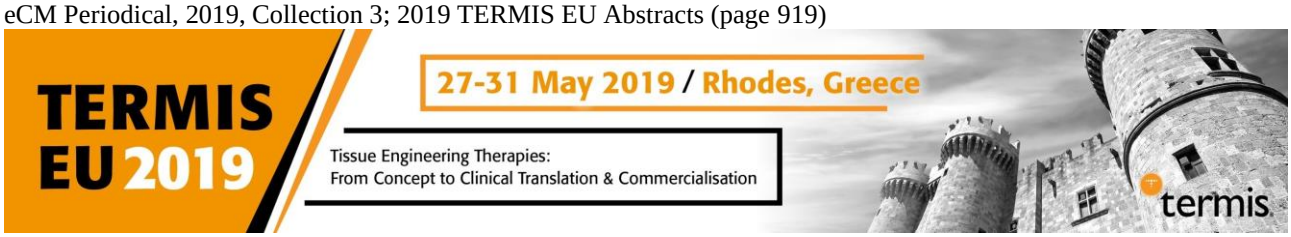
METHODS: Ameloblastoma cells (AM-1 and AM-3 cells) and osteosarcoma cells (MG-63) were cultured using RAFT^{TM} 3D Cell Culture system. 50 000 cells for each cell line were seeded per ACM. The gels were compressed via RAFT absorbers to remove excess fluid. To measure invasion distance and determine invasion patters, images were taken for Day 1, 7, 14, and 21 at four different positions (Figure 1). The tumouroids were stained for Immunofluorescence imaging for different time points. Invasion represents invading the surrounding stroma from ACM, thereby, invasion is the distance from stroma.

RESULTS & DISCUSSION: Previously, it was reported that highly metastatic cancers invade aggressively to the surrounding stroma within tumoroids. (3) showed that, a highly metastatic colorectal cancer cell line HCT116 was found to invade as cell sheets. MG-63 and AM-1 cells also showed the similar sheet pattern. Exclusive invasion of MG-63 can be due to the fact that its association with poor prognosis upon metastasis and recurrence of the disease (4). (3) also highlighted that a less metastatic colorectal cell line HT29 invades limited distances due to its invasion pattern. Similar to HT29, AM-3 cells form bud-shape cell aggregates around ACM and invades to stroma by keeping this shape. When invasion distances of AM cells and MG-63 cells were compared over 21 days, AM-1 and MG-63 cells can invade greater distances than AM-3. This might be due to cell sheet invasion pattern as both AM-1 and MG-63 cells have the same pattern. Whereas, AM-3 cells tend to form cell aggregates budding out from ACM and these aggregates are conserved for 21 days.

CONCLUSIONS: 3D tumour models enables to investigate cell characteristics, future works will focus on the association between ameloblastoma cells and proteins involved in bone resorption.

REFERENCES

[1] Becelli R, Carboni A, Cerulli G, Perugini M, Iannetti G. Mandibular ameloblastoma: analysis of surgical treatment carried out in 60 patients between 1977 and 1998. J Craniofac Surg. 2002;13(3):395-400; discussion
[2] Siar CH, Tsujigiwa H, Ishak I, Hussin NM, Nagatsuka H, Ng KH. RANK, RANKL, and OPG in recurrent solid/multicystic ameloblastoma: their distribution patterns and biologic significance. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119(1):83-91.
[3] Magdeldin T, Lopez-Davila V, Pape J, Cameron GW, Emberton M, Loizidou M, et al. Engineering a vascularised 3D in vitro model of cancer progression. Scientific reports. 2017;7:44045.
[4] Lindsey BA, Markel JE, Kleinerman ES. Osteosarcoma Overview. Rheumatology and therapy. 2016;4(1):25-43.



Differentiation of stem cells from minor salivary glands of oral mucosa into insulin-producing b-cells of pancreas. Science fiction or reality?

A.M. Pavlou[1], D. Andreadis[1] A. Bakopoulou[2], I. Bonovolias[2], A. Kritis[3], A. Poulopoulos[1]
Presenting Author: Dimitrios Andreadis, dandrea@dent.auth.gr
[1]Department of Oral Medicine/Pathology, School of Dentistry [2]Department of Prosthodontics, School of Dentistry and [3]Department of Physiology, School of Medicine, Aristotle University of Thessaloniki, Greece

INTRODUCTION: The isolation and trans-differentiation of salivary gland stem cells (SGSCs) could be of critical for salivary gland- or other glands' regeneration in case of pathology. Recently, stem cells from major salivary glands were reported to trans-differentiate to other cell lines including hepatocytes [1-3]. This study aims to investigate the capacity of SCs derived from human minor salivary gland (mSGSCs) to trans-differentiate to b-cells of pancreas.

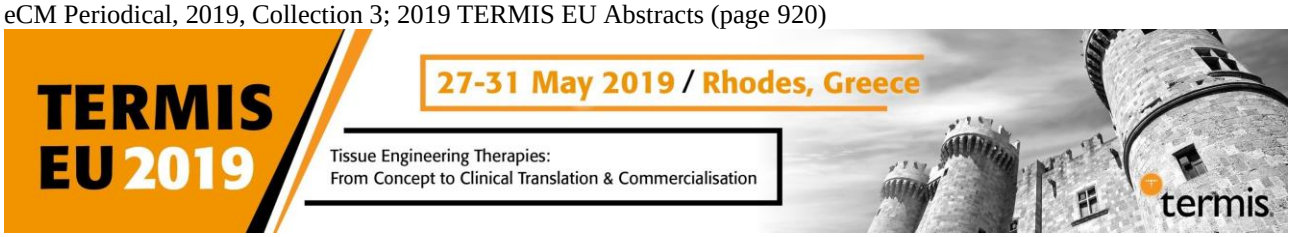
METHODS: mSGSCs from the lip were isolated and characterized using mesenchymal, epithelial and stem cell biomarkers by flow cytometry. Cells were cultured in DMEM/F-12 medium, supplemented with 10% FBS, antibiotics / antimycotics, in addition to activin-A, retinoic acid and GLP-1 (glucagon-like peptide1). The gene expression of pancreatic transcriptional factors Pdx1 (pancreatic duodenal homeobox factor-1), Ngn3 (neurogenin 3), Pax4 (paired box 4) and Nkx6.1, pancreatic SCs markers including c-Met (HGF receptor) and carbonic anhydrase II, as well as insulin, Glut2 (glucose transporter 2) and AFP (alpha-fetoprotein) was evaluated by Real time RT-PCR.

RESULTS & DISCUSSION: MSGSCs expressed embryonic stem cell marker SSEA-4 (32.6 %), mesenchymal markers CD90/Thy1 (92.9 %), CD105 (86.4 %), nestin (84.9 %) but not hematopoietic markers CD34 (1.9 %) or CD45 (1.4 %), and epithelial markers cytokeratins (CK) 14 (66.0 %), CK 7/8 (53.9 %), and CK 18 (26.3 %). Microscopically, a mixed population with fibroblast-like cells (majority) and only few epithelial-like cells was observed. This mixed population strongly expressed pancreatic transcriptional factors Pdx1 and Nkx6.1, as well as pancreatic stem cell markers (c-Met and carbonic anhydrase II).

CONCLUSIONS: For the first time stem cells derived from human minor salivary glands from oral mucosa, an easily accessible source of stem cells, were able to differentiate towards cells expressing pancreatic transcriptional factors and pancreatic stem cells markers. This provides a promising strategy for their autologous application in pancreatic regeneration of Type II diabetic patients.

REFERENCES

[1] Okumura K, et al. Hepatology. 2003;38(1):104-13.
[2] Sato A et al. Cloning Stem Cells. 2007;9(2):191-205.
[3] Baek H et al. J Tissue Eng Regen Med. 2014;8(9):717-27.



Avascular necrosis of the femoral head: retrospective analysis of tissue-engineered bone equivalent applications

R. Vasyliev[1,2], V. Oksymets[2], A. Rodnichenko[1,2], A. Zlatska[1,2], O. Gubar [2,3], I. Gordiienko[2,4], V. Khadzhinova[2], N. Oliinyk[2], D. Zubov [1,2]
Presenting Author: Dmytro Zubov, d.zubov@ilaya.ua
[1]State Institute of Genetic & Regenerative Medicine NAMSU, Kiev, Ukraine [2]Medical Company ilaya®, Kiev, Ukraine [3]Institute of Molecular Biology & Genetics NASU, Kiev, Ukraine [4]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NASU, Kiev, Ukraine

INTRODUCTION: Avascular necrosis of the femoral head (ANFH) is accompanied by severe pain and results in gradual failure of the femoral head and substantial joint function disorder. We defined the following purposes of an experimental study: (1) to develop a regenerative medicine technology for the surgical treatment of ANFH with use of 3D tissue-engineered osteoprogenitor equivalent/graft (3D-OPG) based on autologous bone marrow-derived multipotent mesenchymal stromal cells (BM-MSCs) and fibula-derived periosteal progenitor cells (PPCs) seeded within bone chips, as applied to disease stages III-IVC; (2) to assess the safety of surgical treatment and its effect on pain in the hip, the functional state of the hip joint, and the anatomical condition of the femoral head in the near and distant post-treatment timeframes. The purpose of the analysis was to assess in retrospective the clinical outcomes of 26 patients aged 21 to 52 years who were treated with use of 3D-OPG during 2004-2013 in two hospitals in Ukraine.

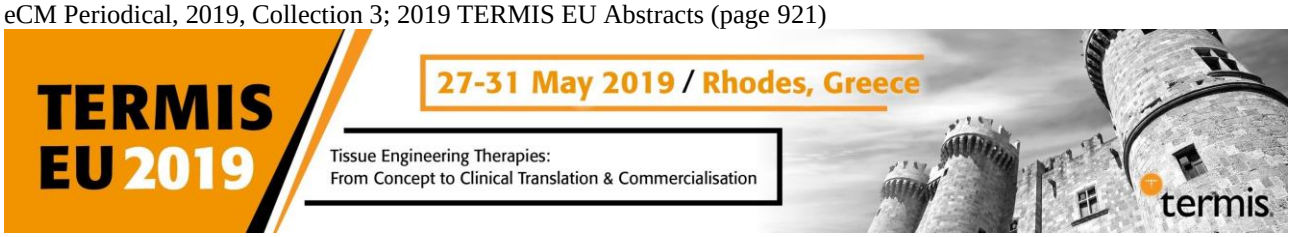
METHODS: For cell and bone equivalent manufacturing, tissue engineering and cell culture methods were applied. For clinical results' assessment the X-ray and MRI examinations were used.

RESULTS & DISCUSSION: After completion of treatment with the use of 3D-OPG, all patients either had no pain or reduced pain ($p = 0.001$). Six months after the completion of treatment, 36% of patients had no pain, 52% had pain after a long-term load on the joint and 12% after a minor load. After 5 years, 24% of patients had no pain, 52% had pain after a long-term load, 20% after minor load, and in one patient (4%), pain occurred within 10 min after imposition of the load. The functional state of the joint in all patients was better than before treatment ($p = 0.001$) for the entire observation period. After 6 months, 64% of patients were able to function independently. By the end of the fifth year, 72% of patients were able to function independently, while one patient (4%) required constant external help. Six months after the authorization of a full load on the joint, 36% had no increase in the deformation of the head; 52% had a slight (less than 5%) deformation. In the remaining 12%, this deformation did not exceed 10%. After 5 years, the deformation of the head had not increased in 24%; in 52% head deformation exceeded 5% and in one patient (4%) the deformity of the head had increased by more than 10%. By the end of the fifth year follow-up, 56% of patients had good results, 40% had satisfactory results, and one (4%) had poor results.

CONCLUSIONS: The surgical treatment of ANFH III-IVC, according to our original 3D-OPG technology, was revealed to be safe and effective. This provided therapy reduces the severity of pain ($p = 0.001$), improves the functional condition of the hip ($p = 0.001$), allows the arrest of the process of bone tissue destruction in the femoral head [1].

REFERENCES

[1] Vasyliev R et al. Exp Oncol. 2017; 39(3):191-196



Analysis of human Cultured endometrial mesenchymal stromal cell properties under xeno-free conditions

A. Zlatska[1,2], A. Rodnichenko[1,2], I. Gordiienko[2,3], O. Gubar[2,4], L. Litvinova[5], V. Shupletsova[5], K. Yurova[5], N. Todosenko[5], D. Zubov [1,2], R. Vasyliev [1,2]
Presenting Author: Dmytro Zubov, d.zubov@ilaya.ua
[1]State Institute of Genetic & Regenerative Medicine NAMSU, Kiev, Ukraine [2]Medical Company ilaya®, Kiev, Ukraine [3]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NASU, Kiev, Ukraine, [4]Institute of Molecular Biology & Genetics NASU, Kiev, Ukraine [5]Immanuel Kant Baltic Federal University, Kaliningrad, Russia

INTRODUCTION: Human endometrium is a new potential source for MSCs isolation due to significant regenerative potential and ability to full self-renewal throughout the woman's reproductive life [1, 2]. However, the potential therapeutic use of endometrial MSCs (eMSCs) evokes a number of challenges, e.g. ex vivo cell large-scale expansion under xeno-free conditions with use of platelet lysate (PL).

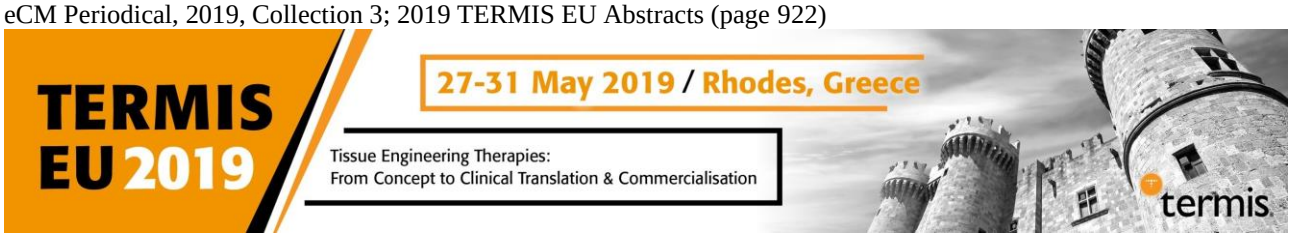
METHODS: Endometrium samples were obtained from 5 donors with endometrial hypoplasia (34 ± 3.3 years) by pipelle sampling in a proliferative phase. Patient's voluntary informed consent was signed. The endometrium biopsies were dissociated by enzymatic method. To study the immunophenotype and cytokine production cell cultures at P3 were used. The PDT value was assessed at P3, using standard formula. There was control versus two culturing condition groups of comparison (2% PL vs 5% PL).

RESULTS & DISCUSSION: It was observed a significant difference between the control group and 2% PL group. However, the PDT values in the groups of control and with 5% PL were comparable. The analysis of the typical MSC immunophenotype did not reveal a significant difference by markers (CD90+ CD105+ CD73+ CD34- CD45- HLA-DR-), both in the control and in PL groups. An increase in the expression of CD140a in the comparison groups against the background of reduced CD166 expression was observed to the control group. The expression of CD140b, CD146 were maintained at a consistently high level, while CD106 CD184 CD271 CD325 were expressed at a low level. Expression levels of SUSD2, ESR-1 and PGR were comparable in PL groups. Although the expression level of ESR-2 increased in 2% PL group. High levels of growth factors' expression were observed in the control and 5% PL groups, when in 2% PL group it was consistently low. The functional analysis of eMSCs showed the basal level production of IL-1ra, IL-8, IL-9, IL-10, IL-15, IL-17, FGF, G-CSF, GM-CSF, IFN-β, MCP-1, TNF- α in all groups. The production of IP-10 was significantly reduced in experimental groups in comparison with a control. It should be noted that in the group with 2% PL it was observed the highest level of IL-6 and eotaxin production. Production of VEGF, PDGF-bb, RANTES, IL-12 was increased in dose-dependent manner in PL groups.

CONCLUSIONS: Cultured under xeno-free conditions eMSCs meet minimal ISCT criteria [3]. Their proliferative potential and secretome profile make them a perspective object for use in regenerative medicine.

REFERENCES

[1] Gargett C et al. Hum Reprod Update 2016; 22(2):137-163
[2] Zlatska A. et al. Exp Oncol. 2017; 39(3):197-202
[3] Dominici M. et al. Cytotherapy 2006; 8:315-317



Hybrid scaffold to anterior cruciate ligament regeneration

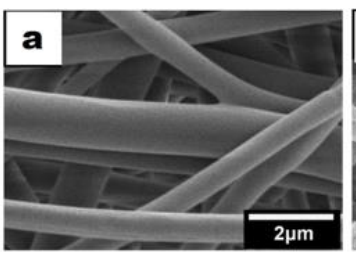
D. Kolbuk-Konieczny[1], O. Urbanek-Swiderska, O. Jeznach[1]
Presenting Author: Dorota Kolbuk-Konieczny, dkolbuk@ippt.pan.pl; dorotakolbuk@gmail.com
[1]Institute of Fundamental Technological Research Polish Academy of Sciences, Laboratory of Polymers and Biomaterials, Warsaw, Poland

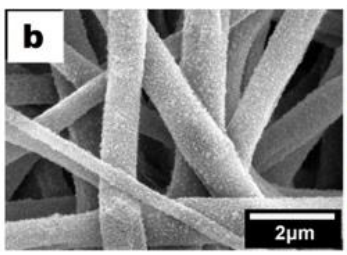
INTRODUCTION: Anterior cruciate ligament rupture is ~~on~~ the main injury of sportsmen and young people. Despite the relatively long time of recovery, professional footballers cases indicate that after 3 years from the end of treatment, only 65% of the players returned to professional activity [1]. The aim of this poster is to show hybrid scaffold which is able to regenerate ligament and ~~regenerate~~ bone in which it is fixed.

METHODS: Different types of polyesters nonwovens were formed by electrospinning. Their surface was modified with growth factors and hydroxyapatite. Scaffold morphology was illustrated by SEM (scanning electron microscopy); chemical structure was evaluated by FTIR (Fourier-transform infrared spectroscopy) and EDS (energy dispersive spectroscopy). In-vitro cellular studies were carried out on fibroblasts and osteoblasts. Cellular morphology was analyzed by SEM and FM (fluorescence microscopy); cytotoxicity and proliferation were determined by MTT and Presto Blue test respectively.

RESULTS & DISCUSSION: FTIR and EDS data confirm effectiveness of chemical modification. SEM illustrates hydroxyapatite nanoparticles on electrospun fibres surface (Fig. 1). Cell culture studies demonstrated that the fibroblasts are spread on the nonwovens, they indicate proper morphology in comparison to cells seeded on TCP (tissue culture plastic- control) (Fig. 2). Cytotoxicity test indicates nontoxic character of nonwovens before and after modification; cellular proliferation is higher on nonwovens modified by TGF and BMP growth factors.

CONCLUSIONS: Nonwovens modified by growth factors and hydroxyapatite enhance cellular spreading, migration and proliferation due to similarities to natural extracellular matrix in chemical and morphological properties.





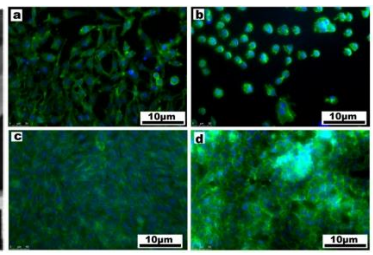
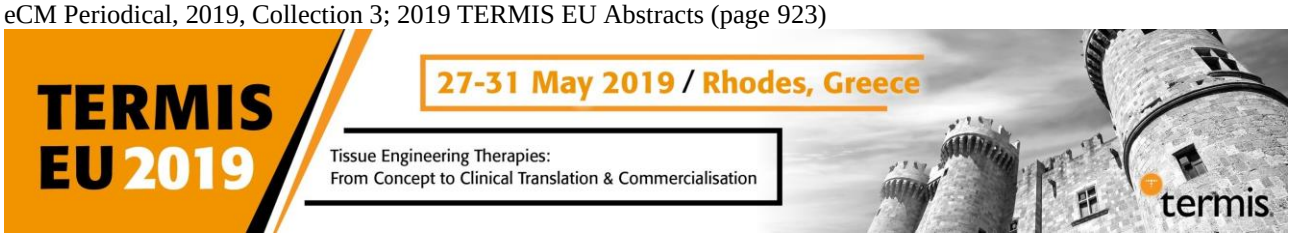


Figure 1: (left) Nonwovens morphology before (a) and after HA (b) coating. **Figure 2:** (right) Morphology of cells seeded on a) TCP, b) PLGA with TGF, c) PLCL with RGD d) PLCL with TGFβ. Images were collected with FM.

ACKNOWLEDGMENTS: This work was supported by the National Centre of Research and Development under grant No. 388/L-6/2014.

REFERENCES

[1] Waldén M et al. Br J Sports Med 2016;50:744–50.



Mathematical modeling of magnetically targeted stem cell delivery

E. Yeo[1], H. Markedies[2], J. Oliver [1], A. El Haj [2], S. Waters[1],
Presenting Author: Edwina Yeo, yeo@maths.ox.ac.uk
[1]Mathematical Institute, University of Oxford [2] University of Birmingham, Birmingham

INTRODUCTION: In stem cell therapies used in regenerative medicine a key challenge is to deliver the cells to the site of injury. One approach is to label cells with magnetic particles and use externally applied magnetic fields to guide the cells to the target site. To optimise delivery protocols it is essential to understand the interplay between the fluid mechanics, magnetic fields and stem cell properties. We develop mathematical models alongside in vitro and in vivo experimental models to provide mechanistic understanding.

METHODS: Magnetic nanoparticles (MNPs) are utilised to tag mesenchymal stem cells (MSCs) which can then be targeted using an external magnetic field. This was carried out in vivo and in vitro. In vitro we used a custom model and MSCs in concentrations of red blood cells (RBCs) from 0% to 40% for various flow rate and degrees of magnetic tagging. This has shown that magnetic tagging increases trapping, with a trapping of up to 31% (versus 17.5% for untagged cells) in a blood concentration of 40% [1]. In our mathematical model we have assumed steady fully developed Poiseuille flow in the pipe. The magnetically tagged MSC experiences Stokes drag from the fluid and a magnetic force [2]. Haematocrit is included Figure 1: Comparison of experimental data to model simulation for varying RBC percentage. through modifying the viscosity of the fluid. We have computed trajectories of cells in the pipe as a function of the initial position, flow rate, magnetisation of the particles and properties of the fluid.

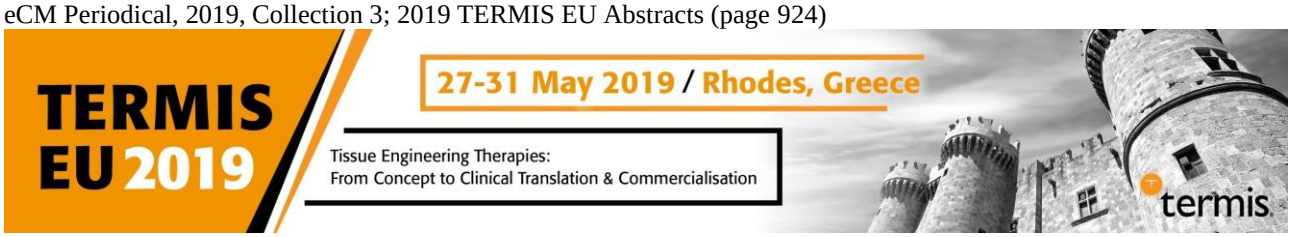
RESULTS & DISCUSSION: Mapping the flow theoretically we have been able to predict deposition of stem cells on the vessel wall. The model reproduces the damping effect of RBCs on capture of stem cells. Realistic magnetic fields allow us to quantify effect of magnet strength and geometry on capture of stem cells.

CONCLUSIONS: Mathematical models can allow us to gain cheap and quick insights into potential treatments. This can allow the rapid exploration of parameter space, calculating optimal regimes for trapping as well as the boundaries the space where capture is feasible. Through this model we can predict the optimal flow rate and minimum magnetic field strength required for a desired percentage of trapping. This model can be readily adapted to include variability in tagging with MNPs, whereas current studies have assumed heterogeneity of tagging. It also offers the flexibility to include time dependent effects such as pulsatile fluid flow or the extravasation of cells.

ACKNOWLEDGEMENTS: AJE and SLW gratefully acknowledge funding from EPSRC in the form of a Healthcare Technologies Discipline Hopping Award, and SLW the award of a Royal Society Leverhulme Trust Senior Research Fellowship.

REFERENCES

[1] El Haj A J et al. 2015. J. Tiss Eng & Regen Med. 9(6):724-33
[2] Grief A et al. 2005. J. Mag. & Mag. Mat. 293(1):455-63



Generation of 3D vascular units for tissue engineering applications

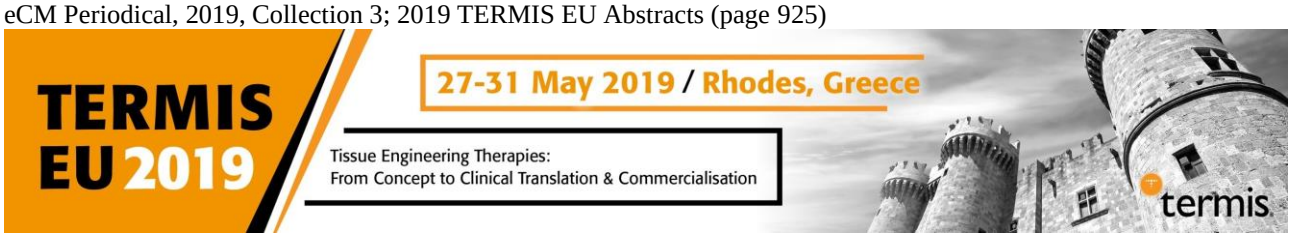
M. Markou[1,2], D. Kouroupis[1], E. Barka[1], F. Badounas[3], D. Stellas[4], T. Fotsis[1,2], E. Bagli[1*], C. Murphy[1*]
Presenting Author: Eleni Bagli, elenibgl@hotmail.com
[1]Division of Biomedical Research, Institute of Molecular Biology and Biotechnology-FORTH, Ioannina, Greece [2]Laboratory of Biological Chemistry, Medical School, University of Ioannina, Ioannina, Greece [3]Department of Immunology, Inflammation Group, Transgenic Technology Laboratory, Hellenic Pasteur Institute [4]Department of Cancer Biology of the BRFAA, Athens, Greece

INTRODUCTION: Large regenerating tissues (100–200μm) show limited nutrient supply and waste removal capacity by diffusion, and thus they require an intimate supply of vascular networks. Vascularization from the surrounding tissue however, is a slow process and therefore, it remains a critical obstacle in engineering multicellular, metabolically demanding organs. Since Endothelial (ECs) and Mural (MCs) cells are the cellular components of blood vessels and their interactions are crucial for neovascularization, both cell types and their arrangement into correct spatial organization are needed in order to rescue tissue engineered constructs from critical ischemia and to form a functional vascular network in vivo. Based on this context and in order to overcome the limitations concerning the isolation and expansion of human MCs, we initially induced the differentiation of human pluripotent stem cells (hPSCs) to MCs. hPSCs-MCs were then co-grafted with hECs as 3D vascular units (VUs) into a matrix to rapidly give rise to a complex three-dimensional functional network of human neovessels in vivo.

METHODS: We developed a simple, efficient and rapid strategy to induce the differentiation of human PSCs to defined MC populations (contractile and synthetic hPSCs-MCs) by using feeder-free and low serum conditions in vitro. Cells were extensively characterized concerning their phenotype (expression of CD44, CD73, CD105, NG2, PDGFRβ, aSMA, calponin, SM22α, SMHC) and function. We generated then VUs (spheroids), consisting of 1000 cells (hECs and hPSCs-MCs) using a methylcellulose-based (MC) hydrogel system. VUs were extensively characterized regarding cell phenotype and cell-cell interactions and their ability to form a three-dimensional capillary network in vitro as well as in vivo (matrigel plug assay). Furthermore, we explored the vascularization potential of these units, when they were embedded in hydrogels composed of defined extracellular components (collagen/fibrinogen/fibronectin), which could be used as scaffolds for tissue engineering applications.

RESULTS & DISCUSSION: hPSC-MCs were phenotypically and functionally stable for at least 8 passages whereas they could stabilize vessel formation and inhibit vessel network regression, when co-cultured with hECs in vitro. Furthermore, hECs/hPSCs-MCs spheroids served as focal starting points for the sprouting of capillary-like structures in vitro, using defined matrices with variable concentration and different fragments of extracellular matrix components. Finally, VU hECs/hPSCs-MCs delivery in vivo led to rapid generation of a complex functional vascular network.

CONCLUSIONS: We developed a robust method for the generation of defined hMCS phenotypes from hPSCs. Fabrication of hECs/hPSCs-MCs VUs embedded in chemical defined matrices is a significant step forward in tissue engineering and regenerative medicine.



Controlled release gentamicin delivery systems for bone regeneration applications

D. Loca, E. Tracuma, J. Locs
Presenting Author: Eliza Tracuma, eliza.tracuma@rtu.lv
Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Pulka 3, Riga, LV-1007, Latvia

INTRODUCTION: For the treatment of musculosceletal disorders, traumas and diseases a large number of highly efficient tools including load bearing metallic implants, numerous calcium phosphate bone regenerative materials and various antimicrobial agents are available on the biomaterial market and are widely used in the clinical practice. Although calcium phosphate bone cements are osteoconductive [1] and could be used through minimally invasive surgical procedures, it carries potential inflammation and bone infection risks, usually precluded through systemic antibiotic therapy. The conventional treatment requires much higher drug doses, that could be overcome by prolonged local antibiotic drug delivery [2]. Gentamicin sulphate (GENTA), is commonly used antibiotic as a vehicle for drug delivery, it has a wide antibacterial spectrum against gram-negative and gram-positive bacteria with a minimum inhibitory concentration (MIC) of ~ 0.5 μg/ml [3]. The aim of the current study is to develop the method for the preparation of GENTA containing microparticles able to release the active ingredient for 14 days, possessing MIC of ~ 0.5 μg/ml. For this purpose, GENTA was microencapsulated in poly-L-lactic acid (PLA) matrix through 3 different emulsification routes.

METHODS: Microcapsules were prepared by water-in oil-in water ($W_1/O/W_2$) double emulsion technique using three different emulsification approaches: mechanical mixing, high speed homogenization and ultrasound homogenization. Prepared GENTA/PLA microcapsules were evaluated towards total drug content, repeatability of the microencapsulation process, drug encapsulation efficiency, microcapsule average size distribution as well as drug release profiles in vitro.

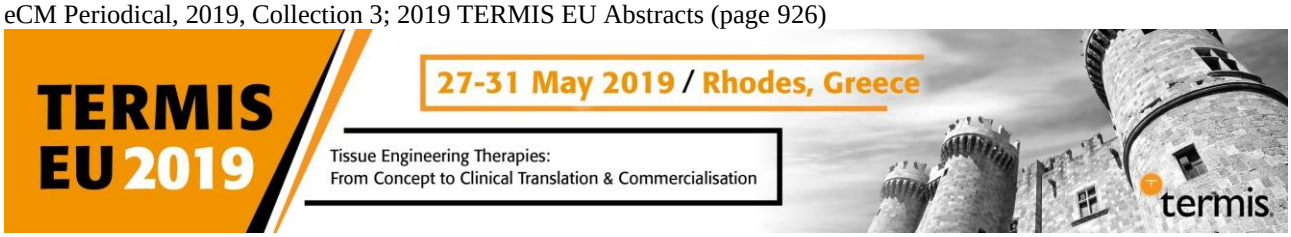
RESULTS & DISCUSSION: Analysis of the prepared microcapsules showed that upon encapsulation of GENTA in the PLA matrix it is possible to obtain microcapsules in which the total GENTA content equals up to 5.5 ± 0.4 wt.%, the encapsulation efficiency of the active ingredient reaches 50%, and the average microcapsule size (d_{50}) are in the range from 4.27 ± 0.02 μm to 220 ± 36 μm.

CONCLUSIONS: During the research it was established that emulsification approach significantly affected not only the active substances mean particles size and total drug load, but also facilitated the preparation of drug delivery system with variable and controllable GENTA delivery profile.

ACKNOWLEDGEMENTS: The project "Drug Delivering 3D Printed Scaffold Strategy Brings Human Body Implants to Next Level of Personalization" (DD-SCAFF) has partly received funding from the European Regional Development Fund under Agreement No.1.1.1.5/ERANET/18/01.

REFERENCES

[1] Chen D et al. Mater. Sci. Eng. C. 2011; 31(2):334-341
[2] Loca D et al. Mater. Sci. Eng. C. 2015; 49(1):106-113
[3] Colpo J et al. J. Mater. Sci. 2018; 53(10):7112-7124



Comparison of pre-osteoconductive characteristics of commercially available xenografts and a novel xenograft

K. Shankar[1], E. Dory[2], A. Hegglin[1], J, Gao[3,4], C. Sfeir[3,4,5,6]
Presenting Author: Emilie Dory, emilie.dory@wishbone-biotech.com
[1]Nobel Biocare Services AG, Kloten, Switzerland [2]Wishbone SA, Belgium, Dental Biomaterials Research Unit (d-BRU) [3]Department of Periodontics and Preventative Dentistry, University of Pittsburgh, Pittsburgh, USA [4]The Center for Craniofacial Regeneration, University of Pittsburgh, Pittsburgh, USA [5]Department of Bioengineering, University of Pittsburgh, Pittsburgh, USA [6]The McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, USA

INTRODUCTION: Commercial bovine xenografts are used for hard tissue augmentation in dentistry for various indications. However, very little is known about how these materials result in bone formation. In this study we have compared two commercially available and a novel bovine xenograft in a series of in vitro experiments to assess their biological characteristics claimed to impact osteoconductivity.

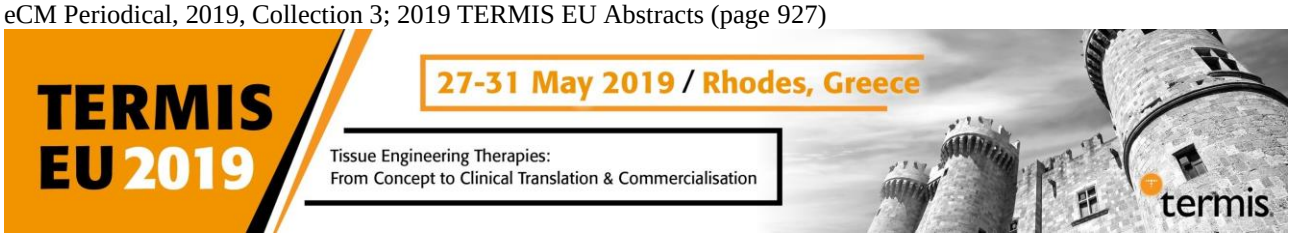
METHODS: Two commercially available xenografts, Bio Oss (BO, Geistlich Pharma AG, Wolhusen, Switzerland), creos xenogain (CXG, Nobel Biocare, Göteborg, Sweden) were compared with a novel xenograft (HA, Wishbone SA, Belgium). Physical properties including surface area and porosity of the materials were measured with BET. Dissolution of the materials were investigated with DMEM cell culture medium supplemented with 10% fetal bovine serum (FBS) in cell culture plates. The total & mono protein adsorption were compared with FBS and albumin. Chemotaxis of cells was investigated with macrophage cell lines, RAW 264.7 cells in a Transwell chamber (Corning, USA) while cell proliferation, differentiation was investigated with 1 x 10^5/ cm^2 murine osteoprogenitor cells, MC3T3-E1 cells for total period of 7 or 14 days.

RESULTS & DISCUSSION: HA material showed significant differences in comparison with BO & CXG (Table 1). The surface area of BO was significantly higher than CXG and HA ($p < 0.05$). Total protein adsorption and albumin adsorption illustrated significant increase ($p < 0.01$) in HA in comparison with BO at time periods of 1and 4 days. BO & CXG chemotaxis assay revealed significant number of macrophages in the BO & CXG group compared to the HA group.

CONCLUSIONS: The protein adsorption, progenitor cell proliferation & differentiation data clearly indicates the importance of unique surface properties in dictating the cascade of events leading to osteoconduction in xenografts. Further in vivo studies are needed to identify whether these differences impact the material osteoconductivity in the clinical setting.

Properties	Time period	P value	
		WH vs BO	WH vs CXG
Total protein adsorption	1 day	0.0004	0.1207
	2 days	0.1479	0.4775
	4 days	0.001	0.222
Chemotaxis	24 hrs	0.0001	0.0001
Proliferation	1 day	0.002	0.048
	3 day	0.0001	0.0001
	7 day	0.0001	0.0001
Differentiation	10 days	0.93	0.7
2D dissolution (Mg^{2+} release)	5 day	0.0001	0.0001
	20 days	0.0001	0.0001

Table 1: Statistical comparison of results from investigations & resulting p value between xenografts at different time periods.



COMP's derived potential matricryptins show no role on specific aspects of joint regeneration

E. Andrés Sastre[1], G.J.V.M van Osch[2], J. W. Bouma[1], E. Farrell[1]
Presenting Author: E. Andrés Sastre, e.andressastre@erasmusmc.nl
[1]Department of Oral and Maxillofacial Surgery, Erasmus MC, University Medical Center Rotterdam, the Netherlands [2]Departments of Orthopaedics and Otorhinolaryngology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

INTRODUCTION: Matricryptins are biologically active extracellular matrix fragments produced by limited proteolysis [1]. Recently, three peptides derived from the Cartilage Oligomeric Matrix Protein (COMP) were found to be secreted by wounded zones of cartilage into synovial fluid [2]. Here we asked if any of the three peptides could be involved in the regulation of specific aspects of joint regeneration. For this purpose, we tested the peptides in vitro for known functions of the COMP C-terminal domain from which they derive: TGFβ signaling, vascular homeostasis and inflammation [3].

METHODS: Peptides were produced by chemical synthesis [Peptide2.0, Brookfield], and used separately at a concentration of 100 nM. In order to test if any of the three peptides could modulate the TGFβ signaling pathway, primary MSCs in monolayer were stimulated with either 0.1 ng/ml TGFβ3 or 100 ng/ml BMP2, with or without the peptides for 24h. Then, the expression of downstream target genes of TGFβ (COMP) and BMP2 (MSX2) was measured by qPCR. Angiogenesis was assessed by tube formation assay. In short, endothelial cells (HUVECs) were seeded on 96 well plates coated with reduced growth factor basement membrane [Geltrex] in combination with the peptides. After 24h, tube-like structures were imaged and quantified. Finally, synovial explants obtained from knee replacement surgeries were cultured in presence of the peptides for 48h and the expression of the enzymes MMP1, MMP3, MMP13, ADAMTS4 and ADAMTS5 was assessed by qPCR.

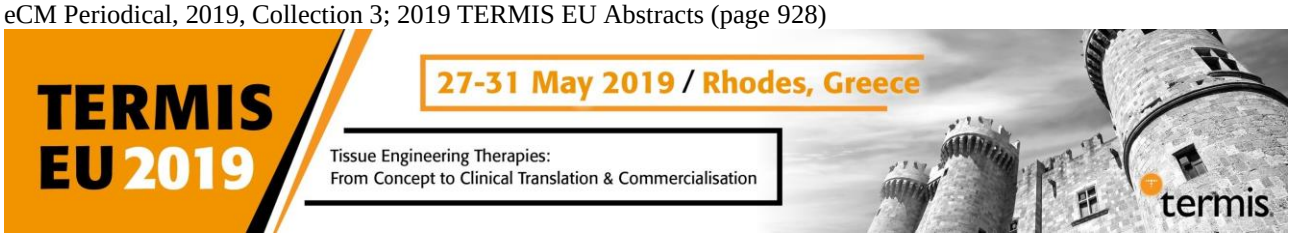
RESULTS & DISCUSSION: None of the peptides affected the downstream expression of target genes of BMP2 and TGFβ3 ($p>0.05$). We observed no effects of the peptides in the tube formation assay ($p>0.05$). No differences on the expression of synovial catabolic enzymes were found when peptides were added to the culture medium ($p>0.05$).

CONCLUSIONS: Joint homeostasis does not appear to be regulated by the peptides tested, at least on the concentrations and assays tested.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 721432.

REFERENCES

[1] Ricard-Blum S. et al. Exp Dermatol. 2014; 23(7):457-63
[2] Calamia V. et al, Osteoarth cartilage. 2016; 24(1)S160
[3] Fu Y et al. Curr Vasc Pharmacol. 2017;15(3):186-196



3D bioprinting of soft tissue models using nanocellulose-based cell instructive bioinks

E. Karabulut[1,2], L. Stridh Orrhult[1], P. Gatenholm[1,2]
Presenting Author: Erdem Karabulut, erdemk@chalmers.se
[1]Chalmers University of Technology, 3D Bioprinting Center, Göteborg, 413 46, Sweden [2]Wallenberg Wood Science Center, Chalmers University of Technology, Göteborg 412 96, Sweden

INTRODUCTION: 3D Bioprinting is a revolutionary technology for the bottom up fabrication of human tissue and organs [1]. The use of biocompatible inks in 3D Bioprinters enables the rapid manufacturing of 3D tissues and organs in vitro and the precise control over microarchitecture and functionality of cell-laden constructs. The printing versatility of bioinks together with living cells diminishes the risk for poor cell viability and cell accessibility throughout the 3D network. A typical bioink consist of cells mixed with hydrogels to generate a good distribution of cells inside the 3D scaffold. Here we summarize examples of cell instructive bioinks prepared with chemically modified cellulose nanofibrils (CNFs) [2]. The CNFs were bioconjugated covalently with a cell adhesive peptides (RGD) and laminin (LN521) in order to promote human dermal fibroblast adhesion and viability in the scaffolds.

METHODS: CNF-based inks modified with the tripeptide Arg-Gly-Asp (RGD) (CNF-RGD) have been investigated and compared with CNF-Alginate ink in terms of 3D printability. The CNF-Alginate and NFC-RGD inks were mixed with alginate and Human dermal fibroblasts and 3D bioprinted in grid architecture with a 3D Bioprinter. After bioprinting the hydrogels were crosslinked in a 100 mM CaCl2 solution. Cell viability and morphology was investigated.

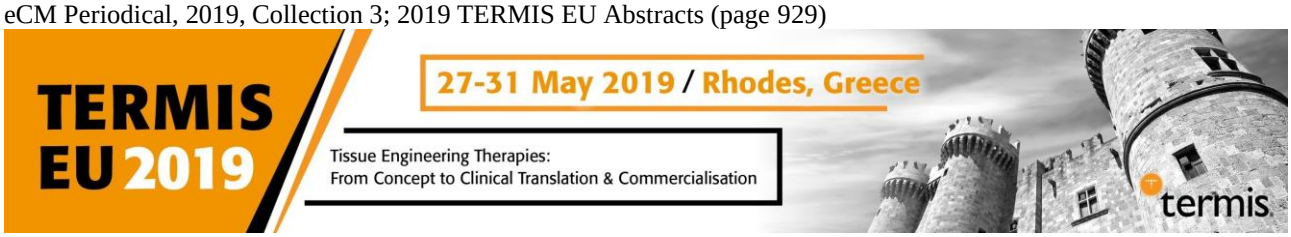
RESULTS & DISCUSSION: The RGD peptides were coupled to the CNFs covalently. The formulations of the bioinks were evaluated in terms of reology, printability and cross-linkability. CNF-Alginate and CNF-RGD demonstrate analogous rheological properties when measuring shear rate-viscosity. The results showed that the adhesion, viability and proliferation of fibroblasts were promoted significantly in the 3D constructs bioprinted with RGD modified bio-inks. Improved cartilage-like formations were achieved in the laminin LN521-CNF/Alginate bioinks after two weeks of differentiation of human-derived induced pluripotent stem cells (iPSCs).

CONCLUSIONS: The RGD peptides and LN521 were succesfully conjugated to the nanofibrils with various charge densities. The rheological performance and printability of the bioinks were ideal to be printed in 3D printer.

ACKNOWLEDGEMENTS: The financial support was received from the Wallenberg Wood Science Center (WWSC).

REFERENCES

[1] Murphy SV, Atala A, Nature Biotechnology, 32 (8), 773-785, 2014.
[2] K. Markstedt, A. Mantas, I. Tournier, H. Martínez Ávila, D. Hägg, P. Gatenholm, Biomacromolecules, 2015, 16 (5), 1489–1496.



3D printed conductive biopatches for cardiac conduction

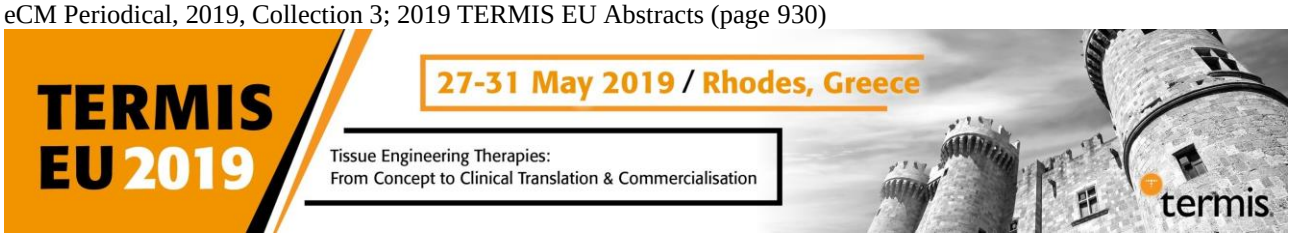
E. Karabulut[1], D. M. Pedrotty[2,3], V. Kuzmenko[4], A. M. Sugrue[2], C. Livia[2], V. Vaidya[2], C. J. McLeod[2], S. J. Asirvatham[2], Paul Gatenholm[1,5], S. Kapa[1]
Presenting Author: Erdem Karabulut, erdemk@chalmers.se
[1]Department of Chemistry and Chemical Engineering, 3D Bioprinting and Wallenberg Wood Science Center, Chalmers University, Gothenburg, Sweden [2]Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN [3]Department of Medicine, Division of Cardiovascular Medicine, University of Pennsylvania, Philadelphia, PA [4]Department of Microtechnology and Nanoscience and Wallenberg Wood Science Center, Chalmers University, Gothenburg, Sweden [5]CELLHEAL

INTRODUCTION: The present work relies on the development of a conductive nanocellulose-based ink for the 3D printing of neural guidelines. Reentrant ventricular arrhythmias (VA) is a major cause of sudden death in patients with structural heart disease. Current treatments focus on electrically homogenizing regions of scar contributing to VA with ablation or altering conductive properties using antiarrhythmic drugs. The high conductivity of carbon nanotubes may allow restoration of conduction in regions where impaired electrical conduction results in functional abnormalities. We propose a new concept for arrhythmia treatment using a stretchable, flexible biopatch with 3D printed pattern with electrically conductive inks to restore conduction across regions in which activation is disrupted.

METHODS: Carbon nanotube patches composed of nanofibrillated cellulose CNF/single-walled carbon nanotube (SWCNT) ink 3D-printed in conductive patterns onto bacterial nanocellulose (BNC) sheets were developed and evaluated for conductivity, flexibility, and mechanical properties. The patches were applied on 6 canines to epicardium before and after surgical disruption. Electro-anatomical mapping (EAM) was performed on normal epicardium, then repeated over surgically disrupted epicardium and finally with the patch applied passively.

RESULTS & DISCUSSION: We developed a 3D printable carbon nanotube ink complexed on bacterial nanocellulose that was expressable through 3D printer nozzles, electrically conductive, flexible, and stretchable. 6 canines underwent thoracotomy and, during epicardial ventricular pacing, mapping was performed. We demonstrated disruption of conduction after surgical incision in all 6 canines based on activation mapping. The patch resulted in restored conduction based on mapping and assessment of conduction direction and velocities in all canines.

CONCLUSIONS: We have demonstrated that 3-D custom printed electrically conductive patches with carbon nanotube inks can be surgically manipulated to improve cardiac conduction when passively applied to surgically disrupted epicardial myocardium in canines.



Design and development of a melt electrowritten in vitro radial migration assay

E. Bakirci[1], O. Dahri[1], E. Cebellos[1,2], A. Hrynevich[1], J. Groll[1], N. Schäfer[2], C. Villmann[2], P. D. Dalton[1]

Presenting Author: E. Bakirci, ezgi.bakirci@fmz.uni-wuerzburg.de

[1]Department for Functional Materials in Medicine and Dentistry, University Clinic Würzburg, Pleicherwall 2, D-97070 Würzburg, Germany [2]Department for clinical Neurobiology University Clinic Würzburg, Versbacherstr 5 97078 Würzburg, Germany

INTRODUCTION: In vitro models are essential for biomedical research since they provide an insight into cell behavior in a controlled environment. Several studies have been dedicated to establish an in vitro 3D neuronal model but the use of such experimental models remains limited [1]. Mimicking the physiological environment in in vitro studies has been always a challenging issue for the scientific community. Recent advances in additive manufacturing technologies, however, could improve and result in more accurate in vitro models. This study establishes a melt electrowritten (MEW) 3D culture system to monitor neural cell migration which allows simultaneously to test the effect of different hydrogels on cell and neurite migration.

METHODS: In vitro culture systems were melt electrowritten directly onto freshly prepared 6-arm, NCO-terminated star-shaped poly(ethylene oxide-stat-propylene oxide) (sP(EO-stat-PO))-coated glass slides as previously described [2]. This adhered the poly(ε-caprolactone) (PCL) fibers (Purasorb PC-12) to the slide and permitted numerous washing steps associated with in vitro culture. The MEW structure, printed in a radial shape, includes 8 different chambers in which specific hydrogel formulation can be dispensed and a central cell depot. Different Matrigel concentrations were dispensed into each chamber, and mouse dorsal root ganglia (DRG) loaded into the cell depot. Radial migration was monitored at 3 different time points for 7 days. The mechanical behavior of different matrix concentrations was tested with a rheometer.

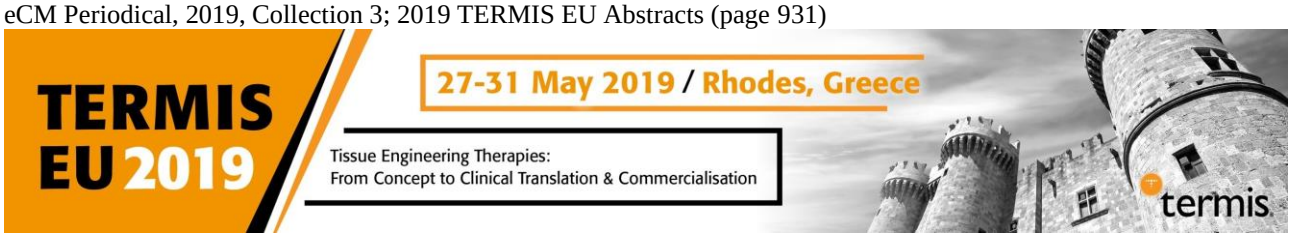
RESULTS & DISCUSSION: The PCL fibers strongly adhered to the NCO-terminated sP(EO-stat-PO)-coated slides and allowed extensive printing parameter and design reiteration. The sealing of the various chambers was determined through the use of added dyes (Figure 1). The system was investigated from the visualization perspective for DRG cell migration into the different matrices. The impact of the height, dimensions and printing design on the feasibility of the radial migration assay was performed.

CONCLUSIONS: The design and development of a 3D radial migration assay is presented that combines MEW with reactive macromers and a selection of matrices so that multi-combinatorial approaches can be assessed for cell or neurite migration.

ACKNOWLEDGEMENTS: This work was funded by the German Research Foundation # 326998133, TRR 225 (subproject B01).

REFERENCES

[1] Frega, M, et al. Scientific reports 4 (2014): 5489.
[2] Groll, J, et al. Biomed Mater Res 74A



Bioactive composite polyurethane/hydroxyapatite (PU/HA) for orbital floor repair

F. Alhamoudi [1], A.A. Chaudhry [3], G.C. Reilly [2], I. U. Rehman [1]
Presenting Author: Fahad Alhamoudi, f.alhamoudi@lancaster.ac.uk
[1]Engineering Department, Lancaster University, UK [2]INSIGNEO institute for in silico Medicine, University of Sheffield, UK [3] Interdiscplinary Research Center in Biomedical Materials (IRCBM), Pakistan

INTRODUCTION: The demand for more bioactive and biocompatible materials for orbital floor repair is increasing tremendously. Therefore, alloplastic materials are attracting attention worldwide. PU/HA composite has been introduced in the material science as a promising material for biomedical applications. Few investigations have been carried out to identify the effectiveness of such materials for bone regeneration, but little attention has been paid to the biodegradable polyurethane for orbital floor regeneration.

METHODS: The materials used were Poly-ester-urethanes (Avalon 85AB), dimethylformamide (DMF) and Commercial HA (P218R). The HA was heat treated at 750, 950 and 1100 C° respectively. The PU solutions were obtained by 10 wt% PU in DMF. 25wt% HA powders were added to PU solution to obtain PU/HA solution. Particles leaching method was used to synthesize porous scaffolds by mixing salt particles with both PU and PU/HA solution in ratio of 0.6 ml/1 g respectively, and solvent casted (evaporated) at 37 C°. Moreover, the scaffolds were characterised using FTIR (ATR and PAS), Mechanical testing (tensile), SEM and cell viability.

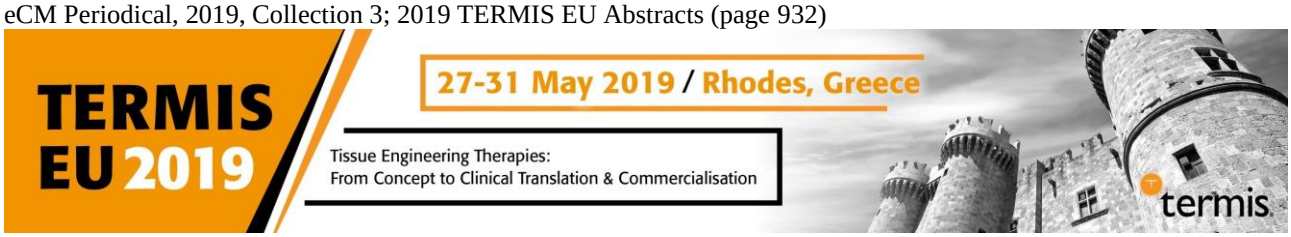
RESULTS & DISCUSSION: Chemical characterization confirmed the presence of HA in composite scaffolds while ATR confirmed the significant amount of HA at the scaffolds top surface. The spongy reaction of scaffolds under pressure and SEM micrographs confirmed the pore interconnectivity. The 25% scaffold survived more in tensile test compared to PU one. However, cell viability (using MG63) did not show significant difference between PU and PU/HA scaffold. Additionally, the heat treated HA (750 C°) showed significant difference in the cell viability results.

CONCLUSIONS: The porous PU and PU/HA scaffold with active and non-active surface was obtained. PU/HA scaffolds could be better for orbital floor repair due to the tensile test results. Moreover, CAM assay showed promising results with PU and PU/ HA which demonstrate potential to induce angiogenesis. Heat treated HA (750 C°) could be the most suitable choice regarding the results of cell viability.

ACKNOWLEDGEMENTS: Financial support was provided by King Khalid University; Ministry of Education; Kingdom of Saudi Arabia. Cell Viability test was conducted in Kroto Research Institute; Sheffield University.

REFERENCES

[1] Khan, A. S., Ahmed, Z., Edirisinghe, M. J., Wong, F. S. L., & Rehman, I. U. (2008). Preparation and characterization of a novel bioactive restorative composite based on covalently coupled polyurethane–nanohydroxyapatite fibres. Acta Biomaterialia, 4(5), 1275-1287.
[2] Tonsuaadu, K., Gross, K. A., Plūduma, L., & Veiderma, M. (2011). A review on the thermal stability of calcium apatites. Journal of Thermal Analysis and Calorimetry, 110(2), 647-659.



Signaling pathways involved in regulation of keratinocyte differentiation of stem cells

F. Biniazan [1], F. Rajaei [1], A. Manzari-Tavakoli[2], F. Safaeinejad, [3] H. Niknejad [3]
Presenting Author: Felor Biniazan, felorbiniazan@yahoo.com
[1]Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran [2]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Iran [3]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran

INTRODUCTION: Skin plays an important role in protecting and supporting the body in life time. The epidermis is composed of various functional layers, which are mainly characterized by the keratinocyte properties. During epidermal development, proliferative keratinocytes in a single layer give rise to multiple differentiating layers to provide skin surface. Controlling keratinocyte adhesion, migration, proliferation and differentiation is crucial for skin homeostasis. Signaling pathways play significant roles in the various stages of epidermal differentiation. In the present study, we discussed the signaling pathways and molecules involved in epidermal differentiation.

METHODS: Original research articles about cell differentiation signaling pathways of the keratinocytes were searched in the PubMed, Cochrane and google scholar databases from 2010 to the end of 2018.

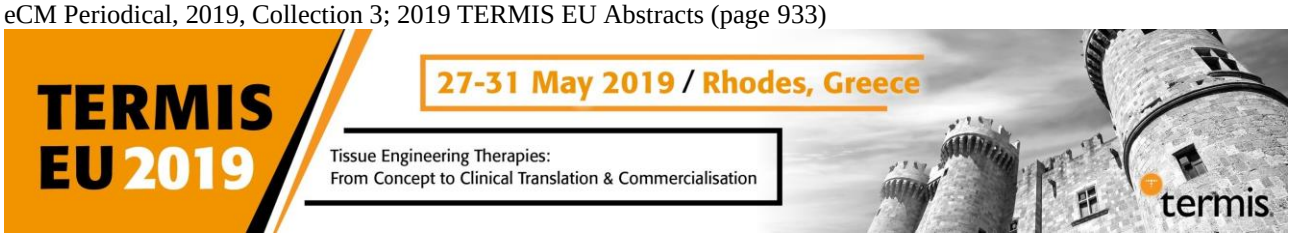
RESULTS & DISCUSSION: The signaling pathways Notch, WNT and TGFβ play an important in the process of keratinocytes differentiation through their various kinases, phospholipases and critical molecules [1-3]. The TGF-β superfamily dose dependently induces stem cells to have keratinocyte fate. Notch signaling pathway takes part in a number of major developmental decisions, promotes keratinocyte differentiation and down regulates their proliferation. On the other hand, Wnt signaling pathway suppresses the differentiation of epidermal keratinocytes via activation of β-catenin.

CONCLUSIONS: There are several signaling pathways involved in keratinocyte differentiation of stem cells. However, the selection of these signaling pathways depends on the type of stem cells, cross-talk between the signaling and the special temporal effects of the key molecules of signaling cascades.

ACKNOWLEDGEMENTS: The authors would like to thank to Dr. A. Jafari, Dr. T. Tayebi, Dr. B. Noushin and Dr. B. Farhadihosseinabadi in the Department of Pharmacology. Research reported in this publication was supported by Elite Research Grant Committee from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Meyers JM et al. PLoS Pathog. 2017; 13(1):e1006171
[2] Wang W et al. Int J Med Sci. 2017; 14(10):1002-1007
[3] Asrani K et al. J Clin Invest. 2017; 127(11):4001-4017



BMP Signaling cross-talk with other pathways in neural differentiation

A. Manzari-Tavakoli [1], F. Biniazan[2], A. Moghimi[1], H. Niknejad[3], F. Safaeinejad[3]
Presenting Author: Felor Biniazan, felorbiniazan@yahoo.com
[1]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Iran [2]Cellular and Molecular Research Center, Qazvin University of Medical Science, Qazvin, Iran [3]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

INTRODUCTION: Bone morphogenetic proteins (BMP) functions exist in both vertebrates and invertebrates and play important roles in developmental processes such as cell proliferation, differentiation and apoptosis. BMP signaling is involved in different stages of nervous system development. In the early stages of development, BMP inhibition is required to create neuroectoderm. However, at later development stages, BMP signaling are required for specifically induction of neurogenesis, migration of neural cells and the spinal cord patterning [1-3]. The objective of this study was to evaluate the cross-talk of BMP signaling with the pathways involved in neural differentiation.

METHODS: A review of literature was conducted to identify studies regarding cross-talk of BMP signaling with other signaling pathways in regulation of neural differentiation. Databases used for finding the articles included PubMed, Google Scholar, MEDLINE and EMBASE.

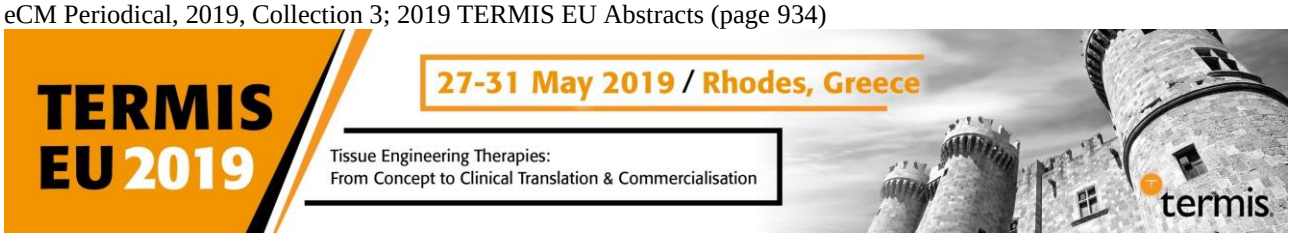
RESULTS & DISCUSSION: Neural induction might have more complexity than simple inhibiting BMP signaling. Additional signals FGF, Notch, SHH and WNT signaling pathways are involved in the specification of neural fate. A key regulatory mechanism involved in neural induction is the inhibition of BMP signaling. The other mentioned signaling pathways display additional regulatory influences through BMP-related mechanisms for commitment to neural lineage.

CONCLUSIONS: BMPs signaling can induce either neurogenesis or glial differentiation at different stages of embryonic development that depend on source and the age of the target cells. The proper functioning of the BMP pathway depends on its constitutive and extensive communication with other signaling pathways, leading to synergistic or antagonistic effects and eventually desirable biological outcomes.

ACKNOWLEDGEMENT: The authors are grateful to Dr. M. Manzari-Tavakoli in the Medicinal Plants and Drugs Research Institute and Dr. A. Jafari, Dr. T. Tayebi, Dr. B.Noushin and Dr. B. Farhadihosseinabadi in the Department of Pharmacology. Also would like to thank to Mr. H. Najarzadeh for their invaluable assistance. Research reported in this publication was supported by Elite Research Grant Committee from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Niknejad et al. Neuroscience Letters. 2012; 506 (1); 22–27.
[2] Gámez B et al. Frontiers in cellular euroscience. 2013; 7:87.



In vivo biocompatibility analysis of crosslinked fibrin-agarose hydrogels for tissue engineering applications

F. Campos[1,2], M. Carmona[3], M.A. Martín-Piedra[1,2], A. Irastorza[1], D. Sánchez-Porras[1], S. Oyonarte[1,2,4], I. Garzón[1,2], A. Campos[1,2] M. Alaminos[1,2], V. Carriel[1,2]
Presenting Author: Fernando Campos, fcampos@ugr.es
[1]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [2]Instituto de Investigación Biosanitaria ibs.GRANADA, Spain [3]Division of Dermatology, Hospital General Universitario de Ciudad Real, Spain, [4]Blood and Tissue Bank of Granada-Almería, Spain

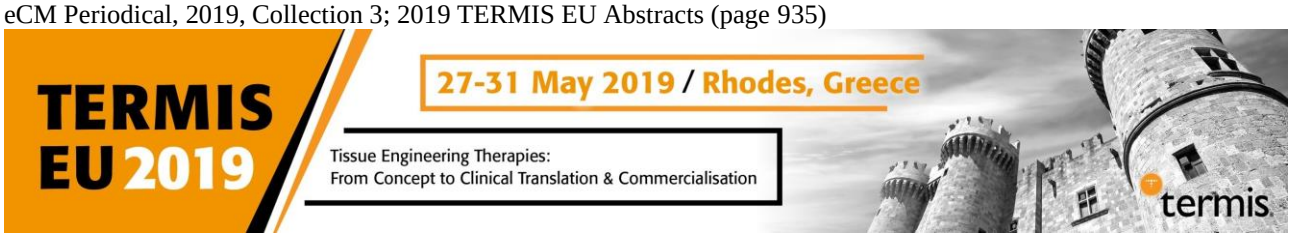
INTRODUCTION: The objective of tissue engineering is the synthesis of bioengineered tissues for clinical use. A requirement for these tissues is biocompatibility, and these structures should induce no inflammatory response once implanted in a living tissue. In addition, bioengineered tissues should be biomimetic and fulfill a series of biomechanical properties. New biomaterials have recently been developed by combining techniques such as nanostructuration and cross-linking applied to fibrin-agarose hydrogels. The main objective of this work is to analyze the in vivo biocompatibility of these biomaterials subjected to cross-linking techniques in order to increase their biomechanical properties.

METHODS: Fibrin-agarose hydrogels (FAH) were treated with the cross-linking agent Genipin at 0.1% and 0.2%. Native FAH and crosslinked FAH (FAH-GP) were subcutaneously implanted in 5 adult mice and results were analyzed after 12 and 26 days of in vivo follow-up. For histological analysis, animals were perfused with 10% formalin solution and post-fixed in the same solution for 24 h, dehydrated and embedded in paraffin. 5 µm thickness sections were stained with hematoxylin-eosin (H&E) for histological evaluation. PAS histochemical analyses were carried out to evaluate the glycogen content of each tissue, whereas picrosirius red histochemistry was used to identify collagen fibers in the analyzed samples.

RESULTS & DISCUSSION: We observed an initial inflammatory response in all the experimental groups (FAH, FAH-GP 0.1% and FAH-GP 0.2%). This reaction was moderate and showed a predominance in lymphoplasmacytic cells after 12 days. After 26 days, an increase in the involvement of macrophages was found, coinciding with an evident biodegradation of the FAH and FAH-GP 0.1% biomaterials. However, FAH-GP 0.2% implants did not tend to biodegradation at both evaluation times. No complications such as infection signs, necrosis or granulomas were detected in any of the groups.

CONCLUSIONS: In vivo evaluation of the biocompatibility of these biomaterials is essential in order to foresee their future performance in the human body. Our results suggest that these tissues are safe once grafted in vivo, which a minimum initial inflammatory reaction, and support the future clinical application in regenerative medicine protocols.

ACKNOWLEDGEMENTS: Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) from Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grants FIS PI17-391 and FIS PI17-393 (co-financed by ERDF-FEDER, European Union), and grant AC17/00013 (NanoGSkin) by ISCIII thorough AES 2017 and within the EuroNanoMed framework.



Biomechanical characterization of human skin substitutes developed by tissue engineering

J. Chato-Astrain[1,2], F. Campos[1,2], O.D. García-García[1,2], D. Durand-Herrera[1,2], Martín-Piedra[1,2], D. Sánchez-Porras[1], A. Irastorza[1], M. Alaminos[1,2], V. Carriel[1,2], I. Garzón[1,2]
Presenting Author: Fernando Campos, fcampos@ugr.es
[1]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [2]Instituto de Investigación Biosanitaria ibs.GRANADA, Spain [3]Division of Dermatology, Hospital General Universitario de Ciudad Real, Spain [4]Blood and Tissue Bank of Granada-Almería, Spain

INTRODUCTION: The goal of skin tissue engineering is to produce functional artificial skin tissues able to reproduce the biological functions of this organ. The mechanical properties of these skin substitutes should mimic the properties of the native human skin in order to provide its required anatomical and physiological functions. In this context, analysis of the mechanical properties of artificial skin substitutes is of great importance in the context of the quality control analyses of these bioengineered organs. Ensuring that the mechanical properties of these products are similar to the native skin is essential before clinical use [1, 2]. The purpose of this study was to perform a biomechanical analysis of human artificial skin substitutes obtained by tissue engineering techniques.

METHODS: Normal human skin biopsies were used to obtain primary cell cultures of human keratinocytes and fibroblasts. Once primary cell cultures were stablished, bioengineered skin tissues were generated using fibrin and agarose biomaterials and skin cells [3]. In this work, 4 experimental groups were analyzed: 1) acellular scaffolds; 2) dermal skin substitutes; 3) complete skin substitutes; 4) human native skin. All skin substitutes were kept in culture for 7, 14, 21 and 28 days. Biomechanical properties (Young's modulus, break load, traction deformation, stress at fracture-break and strain at fracture break) were analyzed using an Instron mechanical testing analyzed. Results were analyzed using Kendall tau and Mann-Whitney statistical tests.

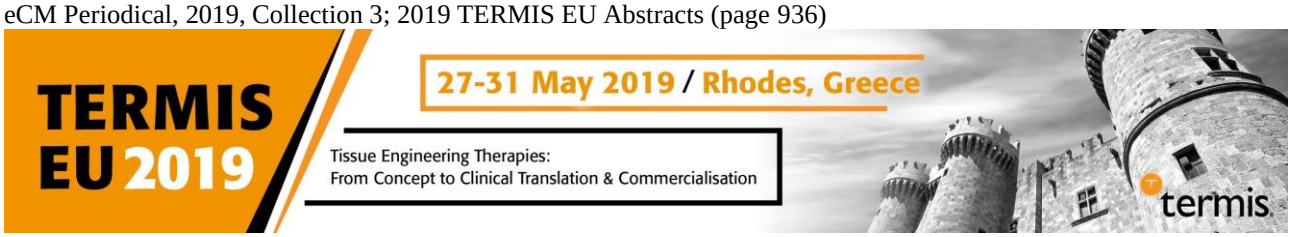
RESULTS & DISCUSSION: First, we found a significant correlation of the biomechanical properties of the different samples and the type of cells used, with the acellular samples showing the lowest values for all parameters. Complete skin substitutes with fibroblasts and keratinocytes did not differ from human control skin for traction deformation, stress at fracture-break and strain at fracture break. No correlation was found between time in culture and biomechanical properties ($p>0.05$) for most study groups.

CONCLUSIONS: Our results suggest that the biomechanical properties of bioengineered skin substitutes directly depend on the type of cells used. In general, complete substitutes with fibroblasts and keratinocytes had the most appropriate biomechanical parameters as compared to native skin independently of the time of development in culture.

ACKNOWLEDGEMENTS: Supported by grant AC17/00013 (NanoGSkin) by ISCIII thorough AES 2017 and within the EuroNanoMed framework and grant PE-0393-2018 from FIBAO foundation, Granada, Spain.

REFERENCES

[1] Pedram R et al. Mater Sci Eng C Mater Biol Appl. 2018;1(93):356-366
[2] Griffin M et al. J Vis Exp. 2016;13(118): 54872
[3] Carriel V et al. Cells Tissues Organs. 2012;196(1):1-12



Printing spatio-temporally defined patterns of growth factors for controlled tissue regeneration

F.E. Freeman[1,2], P. Pitacco[1,2], J. Nulty[1,2], D.C. Browe[1,2], J. Shin[3], E. Alsberg[3] D.J. Kelly[1,2]
Presenting Author: Fiona Freeman, freemanf@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland [2]Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Ireland [3]Departments of Biomedical Engineering and Orthopaedic Surgery, Case Western Reserve University, Cleveland, OH, USA

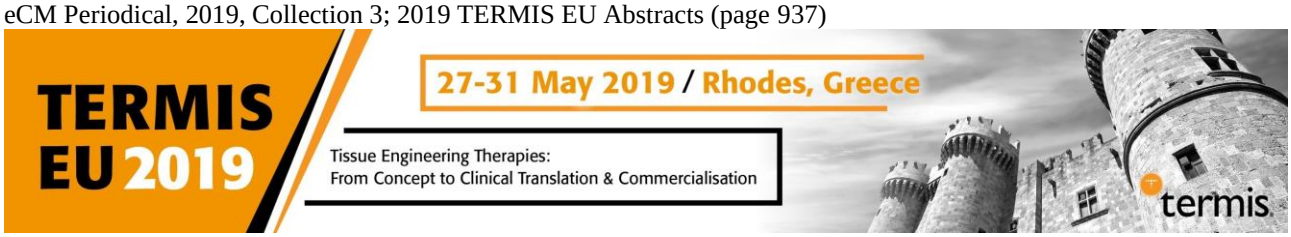
INTRODUCTION: Successful tissue or organ regeneration requires the induction of angiogenesis followed by the appropriate differentiation of progenitor cells to produce a functional repair tissue. The aim of this study was to 3D bioprint implants containing spatio-temporally defined patterns of growth factors to direct the regeneration of large femoral bone defects. These bioprinted constructs were designed to introduce a short-term (~10 days) gradient in vascular endothelial growth factor (VEGF) to enhance angiogenesis, followed by a delayed but sustained localization of bone morphogenic protein-2 (BMP-2) to regions of the femur where new bone formation is required.

METHODS: Bioinks were prepared by dissolving RGD functionalized, γ-irradiated alginate (3.5%w/v) and methylcellulose (1:2%w/w) in αMEM, to generate the base bioink. To develop a vascular bioink that allows for controlled short-term release of VEGF, nano hydroxyapaptite (nHA) (1:1%w/w) was added. To develop an osteogenic bioink which slowly releases BMP-2 over time, laponite (6:1%w/v) was added. Bioinks were printed within networks of PCL filaments to generate 3 experimental groups [1] **VEGF Gradient** – the vascular bioink loaded with 500ng/ml of VEGF in the centre of the implant and base bioink in the periphery; [2] **BMP-2 gradient** - the osteo bioink loaded with 8µg/mL BMP-2 in the implant periphery, with the base bioink in the centre; [3] **Composite** (VEGF+BMP-2) - the osteo bioink in the periphery with the vascular bioink in the centre. Constructs were either implanted subcutaneously into nude mice or into rat a 5mm segmental bone defect and compared to an empty defect. In vivo microct (µCT) was performed at 4, 8, 10 & 12 weeks. Repair was assessed histologically at weeks 2 & 12.

RESULTS & DISCUSSION: Printed gradients of VEGF were found to enhance angiogenesis of implants following subcutaneous implantation compared to implants homogenously patterned the same VEGF. 2 weeks after implantation into large bone defects, regeneration in all treatment groups was proceeding via an endochondral pathway. A significant increase in bone healing was only observed in the VEGF gradient group after 12 weeks, but occurred after 10 weeks in the BMP-2 gradient group, and as early as 8 weeks in the composite group. µCT reconstructions revealed that the composite group had the most consistent bone regeneration, with little ectopic bone formation. Bone preferentially formed in the annulus of the defect.

CONCLUSIONS: Using 3D bioprinting we were able to design constructs with spatio-temporal gradients in growth factors. The appropriate patterning of VEGF enhanced angiogenesis, and when coupled with defined BMP-2 localization, also enhanced bone healing with little ectopic bone formation. In conclusion, printing of distinct growth factor gradients allows for controlled tissue regeneration.

ACKNOWLEDGEMENTS: Funding from ERC grant 647004 & the IRC grant GOIPD/2016/324.



Identification of candidate adhesive proteins in Drosophila melanogaster saliva

F. Borne[1], V. Courtier-Orgogozo[1]
Presenting Author: Flora Borne, flora.borne@ijm.fr
[1]Institut Jacques Monod, CNRS UMR7592, Université Paris-Diderot, 75013 Paris, France

INTRODUCTION: Drosophila salivary glands produce a proteinaceous glue that allows the animal to adhere to a substrate for several days during metamorphosis. Our aim is to use the powerful genetic tools of the model organism Drosophila melanogaster to explore the molecular basis for fly glue adhesion. We identified a set of proteins present in the glue and now plan to examine their role in adhesion.

METHODS: Available transcriptomic data [1] were analysed using bioinformatic tools in order to find proteins that are likely responsible for the glue adhesive properties [2] such as predicted peptide signal, amino acid enrichment bias, repeated sequences and basic isoelectric point. Preliminary tensile tests were performed with a universal testing machine (Ametek model LS1S/H/230V) on a pupa naturally attached to a glass slide and show that pupa adhesion is in the order of 10^3 kPa. As the glue is water soluble, we also collected it by incubating pupae in water. We then placed the collected liquid on a glass slide covered by a cover slip and let it dry. We estimated adhesion by adding loads to the cover slip until detachment.

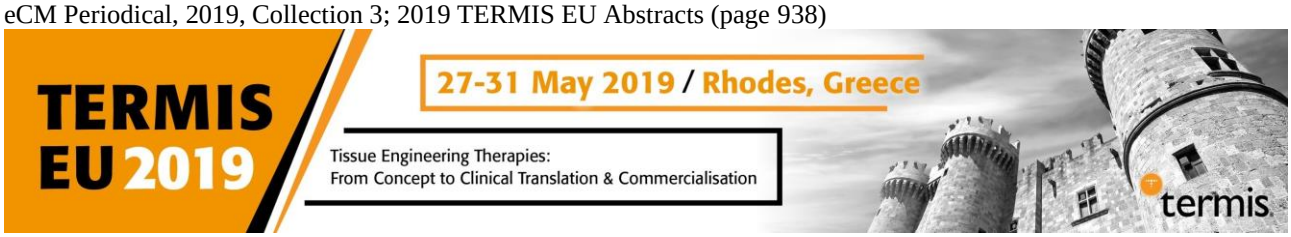
RESULTS & DISCUSSION: Among the 27 genes that have been identified as differentially expressed in the salivary glands and up-regulated before pupation [1], 25 code for proteins with predicted peptide signals. 12 of them present a high content of serine and threonine (more than 25% of the amino acids is serine or threonine), an isoelectric point superior than 8 except for one and repeated sequences, suggesting a potential role of these proteins in adhesion [2].NRNAi lines are available for all of these candidate genes except one in D. melanogaster. Candidate genes will be tested by crossing the corresponding available RNAi mutant lines with a Gal4 line that drives expression in the salivary gland. Adhesion tests are being developed to phenotype the mutants. Preliminary results show that adhesion for wild-type glue is in the order of 10^2 kPa for a protein concentration of 10^2 µg/ml.

CONCLUSIONS: Using transcriptomic data, we identified candidate genes that could be involved in pupa adhesion. These candidate genes will be analysed further for their impact on adhesion using an adhesion test specifically designed for our system.

ACKNOWLEDGEMENTS: This project is funded by the European Research Council under the European Community's Seventh Framework Program (FP7/2007-2013 Grant Agreement no. 337579) to V.C.-O. Financial support was received from ENBA COST Action CA15216.

REFERENCES

[1] Li T.R & White K.P Dev Cell. 2003;5:59-72
[2] Hennebert E et al. Interface Focus. 2015;6;5:20140064



Digestive fistula healing via a thermoresponsive hydrogel (and adipose stromal cell-derived extracellular vesicles): from pre-clinical to clinical investigation

A. Silva,[1] G. Perrod[2], L. Pidial[2], C. Wilhelm,[1] O. Clément,[2] F. Gazeau,[1] G. Rahmi [2]
Presenting Author: Florence Gazeau, florence.gazeau@univ-paris-diderot.fr
[1]Laboratoire Matière et Systèmes Complexes, CNRS UMR 7047 Université Paris Diderot, 10 rue Alice Domon et Léonie Duquet, Paris, France [2]Hopital Européen Georges Pompidou, APHP and PARCC, INSERM U970, Université Sorbonne Paris Cité (USPC), Université Paris Descartes, 56 rue Leblanc, 75015, 75015, Paris, France

INTRODUCTION: Digestive fistulas are disabling and challenging conditions urgently requiring innovative therapeutic approaches. We investigated herein the potential therapeutic benefit of a thermoresponsive Poloxamer 407 gel as a fistula occlusive and the combined action of extracellular vesicles (EVs) derived from porcine adipose tissue stromal cells (ADSCs), as cell-free regenerative effectors. We expect the thermoresponsive gel to be advantageous due the possibility of injection in its liquid form <20°C filling all the fistula tract, while gelling in situ at body temperature to facilitate retention, occlusive effect and sustained EV release. We explored, in a porcine fistula model, the healing potential of the combined product while the gel alone was also tested for one patient. Indeed, poloxamer 407 gel is authorized for clinical use as a vessel occlusive agent. Herein, we investigated, for the first time, its repurposing as a fistula occlusive agent.

METHODS: Esophageal fistulas were surgically created in pigs by placing two plastic stents during 30 days into their neck. Then, animals were randomized in 3 groups: control untreated one, a group treated with the gel at 20% alone (gel group), and a group treated with the gel at 20% containing 1.3×10^{11} EVs/ml (gel plus EVs group). Clinical, endoscopic and radiological evaluation of pig fistula healing was performed at day 30 and day 45, before histological assessment. A patient with colocutaneous fistula received an off-label treatment with a thermoresponsive Poloxamer 407 gel (20%). A total of 14 administrations during 7 weeks were performed. Fistula orifice size and secretion output were analyzed.

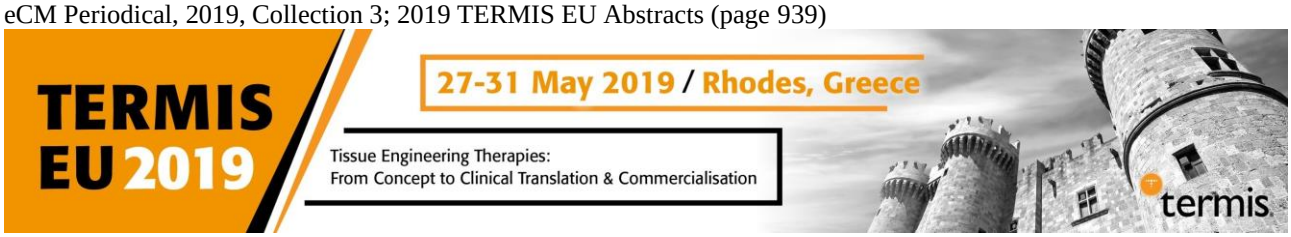
RESULTS & DISCUSSION: In the porcine study, complete fistula healing was reported to be 100% for the gel + EVs group, 67% for the gel group and 0% for the control. Only the combination of gel and EVs resulted in a statistically significant (i) reduction of fibrosis, (ii) decline of inflammatory response, (iii) decrease in the density of myofibroblasts and (iv) increase of angiogenesis. The gel treatment in one patient resulted in reduced fistula orifice diameter from 4.8 ± 0.5 to 1 mm and fistula output decreased from 425 ± 65 to 10 ± 4 mL, when comparing the months before and after therapy.

CONCLUSIONS: ADSC-EVs delivered into a thermosensitive gel induced total fistula healing in a pre-clinical swine fistula model. The gel itself was able to induce a therapeutic fistula occlusive effect in pigs and also in one patient, reducing fistula diameter and output. This study opens up new prospects for (i) local minimally-invasive EV delivery based on a thermo-actuated administration strategy in fistula therapy and (ii) the efficient repurposing of poloxamer 407 gel as a fistula occlusive [1].

ACKNOWLEDGEMENTS: CNRS, France.

REFERENCES

[1] Silva et al. ACS Nano 2018; 12(10):9800-14.



Improvement of a 3-layered in vitro skin model for topical application of irritating substances

F. F. Schmid [1], A.-C. Volz [1], F. Atzinger[1], S. Nowakowski, P.J. Kluger[1]
Presenting Authors: Franziska Atzinger, Franziska.Atzinger@Reutlingen-University.DE
[1]Smart Biomaterials, Reutlingen University, Reutlingen, Germany [2] University of Hohenheim, Stuttgart, Germany

INTRODUCTION: The skin is the outermost layer of the human and serves as the initial impermeable barrier that protects the human body from external harmful agents as well as restricting the loss of body fluids. Thus, the skin attracts much focus in the assessment of chemicals and pharmaceuticals. As an ethical alternative for skin toxicity testing, in-vitro-generated organ-like reconstructed human skin models have been developed by tissue engineering techniques. Although these models are applicable for many endpoints such as skin corrosion or skin irritation (OECD TG431/TG439), the only approved in vitro test methods are using epidermal models due to insufficient development of a permeability barrier of full-thickness skin equivalents [1]. The lacking barrier is caused by shrinkage of the skin equivalent [2]. The aim of this work was to improve the barrier function of a 3-layered in vitro skin model by adapting the culture conditions.

METHODS: The 3-layered skin model is comprised of primary human epidermal keratinocytes, primary human dermal fibroblasts and mature adipocytes. Cells were isolated from human skin and human subcutaneous adipose tissue. For 3D constructs fibroblasts and mature adipocytes were encapsulated in hydrogels based on collagen I. Both layers were casted underneath a transwell. Thereafter keratinocytes were seeded inside of a transwell for epidermal development.
The improved model was compared to the standard setup. The epidermal shrinkage was measured over the culture time of 15 days. The barrier was quantified by an indirect barrier function test with triton X-100 and a permeability assay with sodium fluorescein and FITC-dextran. Furthermore, characterization of both models were conducted by immunofluorescence staining.

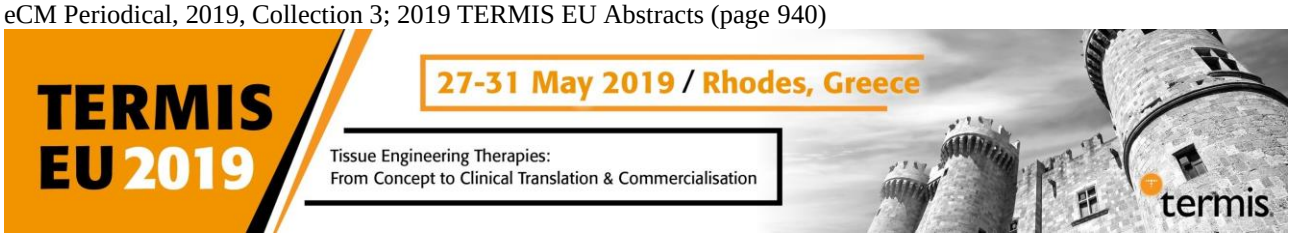
RESULTS & DISCUSSION: The improved setup led to a significant increase of the barrier function due to the prevented epidermal shrinkage. Primary human epidermal keratinocytes differentiated to a multi-layered epidermis with a well-formed basal layer and a dense stratum corneum. The differentiation was shown by immunofluorescence staining against cytokeratin 10 and 14, and fillagrin. Adipocyte specific proteins, perilipin A, laminin γ-1 and collagen IV (colIV) were verified by immunofluorescence staining.

CONCLUSIONS: The improvement auf the barrier of full-thickness skin equivalents is of great importance. Taken together, the results show that the modification of culture conditions lead to an improvement of the barrier function and to enhanced permeability properties without the loss of morphology features. The results show that the improved setup has great potential for meeting the challenges of alternative methods for animal testing.

ACKNOWLEDGEMENTS: Financial support was received from the Ministry of Rural Affairs, Food and Consumer Protection Baden-Wuerttemberg (0 391 E).

REFERENCES

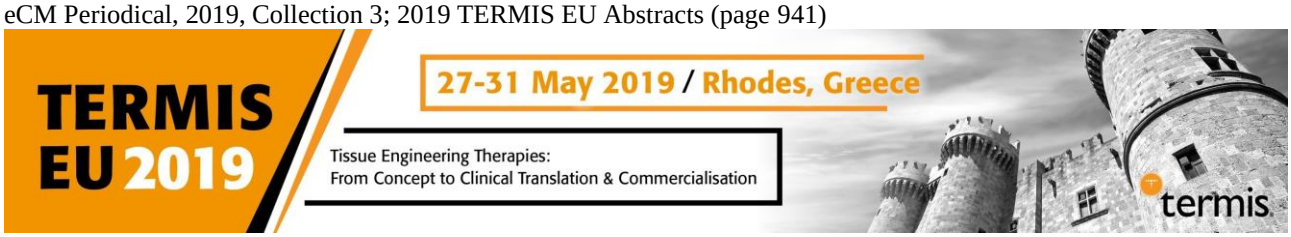
[1] Groeber F et al. Toxicol In Vitro. 2016; 36:254-261.
[2] Lotz C et al. ACS Appl. Mater. Interfaces. 2017; 9(24):20417-20425



Fabrication of silica nanocage incorporated composite scaffolds coated with drug loaded chitosan/MMT nanospheres for tissue regeneration at bone-cartilage interface

S. Tamburaci[1], C. Kimna[1], S. Deger[1], F. Tihminlioglu[1]
Presenting Author: Funda Tihminlioglu, fundatihminlioglu@iyte.edu.tr
[1]İzmir Institute of Technology, Department of Chemical Engineering, Gülbahçe Campus, İzmir, Turkey

Inflammation is still a problem in bone tissue transplantations due to the limitations of conventional antibiotic administration. Thus, traditional treatments have been replaced with local antibiotic therapies to overcome their limitations. Recently, there has been a significant increase on design of controlled drug releasing systems for the treatment of bone infections. Polymer based composite scaffolds have been used for controlled drug release at the defect area to treat the infection as well as regenerate the tissue. In this study, it is aimed to develop nanocomposite scaffold with functional POSS nanocage reinforcement (CS/POSS) and to immobilize the drug loaded chitosan/MMT composite nanospheres by coating the scaffold surface to provide controlled antibiotic delivery at the defect site. Gentamicin and vancomycin were selected as model antibiotic drugs and drug loaded chitosan/MMT nanospheres were fabricated with electrospray technique and characterized within the scope of morphology, hydrodynamic size, surface charge, FT-IR, in vitro drug release, antimicrobial activity and cytotoxicity. CS/POSS nanocomposites were fabricated via freeze-drying technique and characterized with mechanic, swelling test, SEM and micro CT analyses. Positively charged nanospheres with uniform morphology were obtained. High drug encapsulation efficiency (80-95%) and sustained release profile up to 25 days were achieved with a cumulative release of 80-90%. In addition, the release media of the nanospheres (6 h, 24 h and 25 days) showed a strong antimicrobial activity against S.aureus and E.coli, and did not show any cytotoxic effect to 3T3 and SaOS-2 cell lines. CS/POSS nanocomposites were obtained with high porosity (89%) and 223.3±55.2µm average pore size. POSS reinforcement increased the compression modulus from 755.7 to 846.1Pa for 10 % POSS addition. In vitro studies of nanosphere coated bilayer scaffolds were found cytocompatible with osteosarcoma and chondrosarcoma cell lines. Both of the cell lines successfully proliferated on chitosan/POSS scaffolds. ALP activity and osteocalcin secretion results showed that POSS nanoparticle incorporation significantly increased the osteogenic activity of Saos-2 cells. In addition, GAG and total collagen secretion were investigated with SW1353 cells cultured on scaffolds. In conclusion, these bilayer nanocomposite scaffolds can be considered as a potential candidate for infection preventive bone-cartilage interface with enhanced physico-chemical properties as well as biological activities.



Influence of the human cornea post-mortem time on human corneal epithelial cells used for tissue-engineered cornea

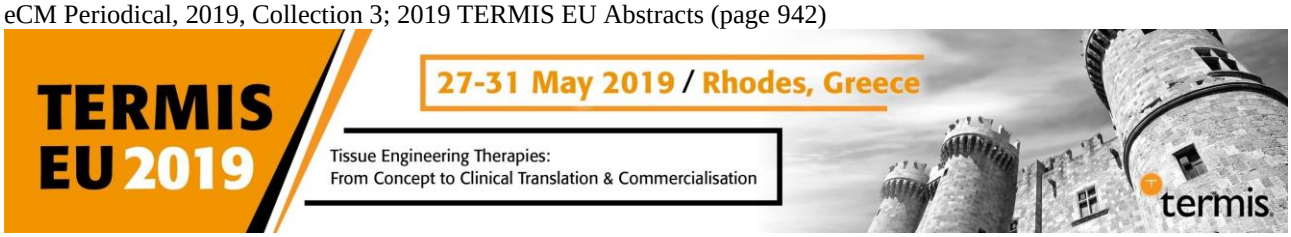
G. Le-Bel[1,2], P. Desjardins[1], C. Couture[1,2], S. Cortez Ghio[2], E. Gillard[1], L. Germain[1,2], SL. Guérin[1].
Presenting Author: Gaëtan Le-Bel, gaetan.le-bel.1@ulaval.ca
[1]Département d'ophtalmologie, Université Laval, CUO-recherche/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada [2]Département de chirurgie, Université Laval, Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada

INTRODUCTION: Over the last decade, we developed a human tissue-engineered cornea (hTECs) that may serve as a model to study several biological processes, such as corneal wound healing. To produce a human cornea by tissue-engineering, self-renewal of the corneal epithelium through the maintenance of a sub-population of corneal stem cells is required in order to preserve its functionality. Human corneal epithelial cells (hCECs) that are isolated from human donor's corneas should therefore be of a high quality. The goal of the present study was to determine whether long post-mortem times before cell isolation may impact on the hCECs' proliferative capacity and wound healing properties using both monolayers of hCECs and hTECs as in vitro models.

METHODS: Ten populations of hCECs were isolated from human corneas at different times after donor's death and separated in two groups: short (0, 1, 2, 3, 4 days after death) and long post-mortem times (15, 16, 17, 18, 19 days after death). Each population was grown as monolayers on several passages and the impact of the post-mortem time on hCECs' proliferative potential analysed by colony-forming efficiency assays (P1 and P2) and growth rate measurements (P1 to P4). hTECs were produced by the self-assembly approach using hCECs from both post-mortem groups and wounded with a 8-mm biopsy punch. Wound healing was monitored until complete closure. Masson's trichrome staining (histological section) and indirect immunofluorescence against collagen IV, laminin V, fibronectin, ZO-1 and tenascin-C were performed to evaluate the impact of both short and long post-mortem times on the hTECs quality.

RESULTS & DISCUSSION: hCECs cultured from donor's eyes with short post-mortem times have a higher growth rate and clonogenicity when compared to the long post-mortem time group as a result of a decreased doubling time at passages P1 to P4 and increased colony-forming efficiency with more holoclones at P1 and P2. However, no difference in the wound closure dynamic could be observed between hTECs prepared using hCECs with low and high post-mortem times. Furthermore, all hTECs had normal histological characteristics and stained positive for the extracellular matrix (ECM) components collagen IV and laminin V, as well as for the tight junction protein ZO-1. However, hTECs produced using low post-mortem time hCECs had a lower expression of the ECM components fibronectin and tenascin-C.

CONCLUSION: Our results suggest that there is a reverse relationship between post-mortem time and the proliferative capacity of hCECs. However, when used for the production of tissue-engineered corneas, these post-mortem, time-dependent proliferative variations of hCECs do not translate into any significant difference in the wound healing capacity of hTECs. Our study therefore provided evidence that donor's eyes with long post-mortem times may be used as source of biological material for culturing hCECs that are to be used for the production of hTECs in vitro.



Controlled Delivery of hBMP-2 within a 3D Matrix Bioengineered with human-Mesenchymal Stem Cells: a FEM approach for growth factor mass transfer simulation

G. Della Porta, N. Maffulli
Presenting author: Giovanna Della Porta, gdellaporta@unisa.it
[1]Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi (SA), Italy

INTRODUCTION: Ordinate deposition of cells and biopolymer microcarriers for the controlled delivery of bioactive molecules within a 3D hydrogel can simulate an ordinate synthetic extracellular matrix [1-2]. In this work, human-Bone Morphogenetic Protein-2 (hBMP-2) release profiles from poly-lactic-co-glycolic acid (PLGA) microcarriers have been experimentally measured and a Finite Element Modelling approach was used for the prediction of hBMP-2 mass transport within a 3D scaffold bioengineered with Human-Mesenchymal Stem Cells (hMSCs).

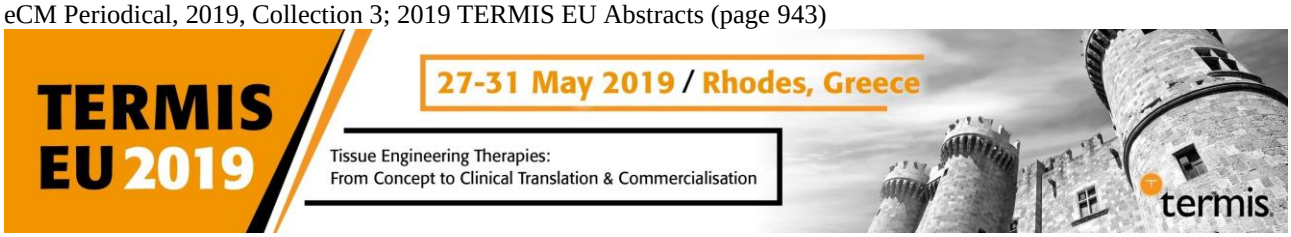
METHODS: PLGA microspheres (800±100 nm) for the controlled release of hBMP-2 were fabricated by a proprietary technology (US Patent US/8628802 B2) and their release profile experimentally measured in DMEM at 37°C. hBMP-2 mass transfer was simulated into a 3D system using COMSOL Multiphisics®, taking into account its release profile and cell binding data [3]. hBMP-2 degradation rate was also taken into account in the simulations.

RESULTS & DISCUSSION: A simulation over a section of scaffold with volume of 3.4 mm^3 was performed as snapshots of the whole geometry at 7, 14 and 21 days. The sensitivity analysis proposed allowed the identification of the optimum hBMP-2/PLGA microcarriers/hMSCs ratio within the 3D scaffold at to assure hBMP-2 concentration values within the range of 10-20 ng/cm^3, as the minimum effective for hMSCs osteogenic stimulation. Moreover, hBMP-2 concentration profiles taken at different distances from the 3D scaffold center are superimposed and there is basically no radial profile within the considered system due to the very low alginate resistance to mass transfer Indeed, the calculated hBMP-2 diffusion coefficient (D) in Alginate was of 4^{-13} cm^2/s; whereas, in PLGA was of 6^{-20} cm^2/s.

CONCLUSIONS: The developed tool can be useful to predict the effective microenvironment formulation to assure effective bio-signal concentrations within the designed hydrogel microenvironment.

REFERENCES

[1] Della Porta G et al. Biomed Mater Res A. 2015; 103(6): 2161-2171.
[2] Govoni M et al. Tissue Eng Part A. 2017; 23(15-16): 811-822.
[3] Ribeiro FO et al. PLoS One. 2015; 10(6):e0127722.



Quality of life in urogenital congenital malformations with existing lack of tissue

G. Reinfeldt Engberg[1,2], M. Fossum[1,2], A. Nordenskjöld[1,2]
Presenting Author: Gisela Reinfeldt Engberg, gisela.reinfeldt.engberg@ki.se
[1]Department of Women's and Children's Health and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden [2]Department of Highly Specialized Pediatric Surgery and Pediatric Medicine, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden

INTRODUCTION: Urogenital congenital malformations affect every ethnicity worldwide. These malformations may manifest as lack of urinary bladder, vaginal, uterine, or penile urethral tissue, and both environmental and genetic mechanisms are involved. Without reconstructive surgery these malformations may have a high impact on quality of life, such as multiple infections, increased demands of medical care and infertility. These factors may cause negative psycho-social effects with impact on education, occupation and family building. Our research group has focused on analyzing risk-factors and psycho-social effects of bladder exstrophy, affecting 3:100 000 live born, in register-based studies, as well as performing tissue engineering research within the field of reconstructive urology [1-2].

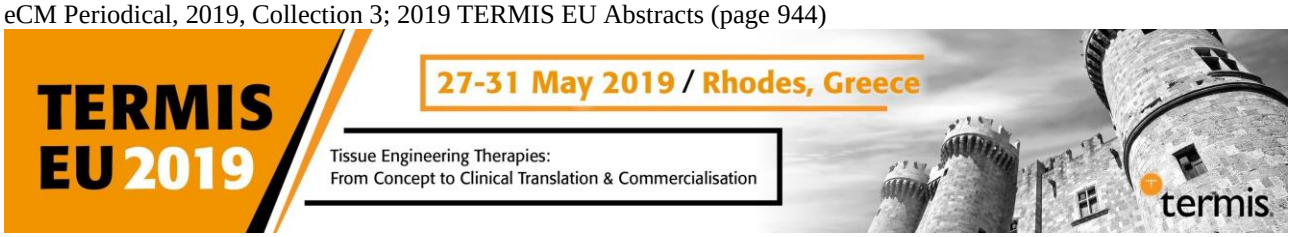
METHODS: Matched cohort studies nested within the entire pool of live births in Sweden between 1952 and 2011 were conducted regarding bladder exstrophy patients. Complete nationwide health, birth and social registers were used, and cases were matched with 5 controls each. The objective was to study proxies for quality of life such as maternal risk factors, birth descriptive data, comorbidity, fertility, level of education, partnership and number of biological children.

RESULTS & DISCUSSION: Studies demonstrated no significant maternal risk factors and low birth descriptive impact. Most children born with bladder exstrophy had an isolated malformation. Comorbidity, such as inguinal hernia and non-descended testis requiring surgery were more common. Overall, educational and occupational levels were high and partnership formation was comparable to controls. Fertility and the number of biological children was, however, significantly decreased [3].

CONCLUSIONS: The clinical aspect of infertility and fewer biological children demonstrate that the surgical techniques in lack of tissue must be addressed in further research in basic science as well as clinical and patient related studies. Introduction of better surgical techniques and progress in application of regenerative medicine and tissue engineering contribute to higher quality of life for patients with urogenital congenital malformations. There is, however, still a need for more advanced applications of tissue engineering and tissue regeneration methods which would reduce the need for use of autologous transplantation tissue from other organs. Targeted tissue engineering and regenerative reconstruction techniques could minimize existing post-surgical comorbidity with positive influence on quality of life. Further studies will address psychiatric impact on these patients and the risk of developing urinary bladder cancer.

REFERENCES

[1] Reinfeldt Engberg G et al. Biomed Res Int. 2013; 2013:212734
[2] Reinfeldt Engberg G et al. Biomed Res Int. 2016; 2016:5415012
[3] Reinfeldt Engberg G et al. J Pediatr Urol. 2016 Oct;12(5):304



Dental pulp stem cells and their extracellular vesicles as proangiogenic tool for tooth regeneration

G. Merckx[1], B. Hosseinkhani[2], S. Kuypers[2], J. Irobi[2], L. Michiels[2], I. Lambrichts[1], A. Bronckaers[1]
Presenting Author: Greet Merckx, greet.merckx@uhasselt.be
[1]Morphology Research Group, Biomedical Research Institute, UHasselt, Diepenbeek, Belgium
[2]Bionanotechnology Research Group, Biomedical Research Institute, UHasselt, Diepenbeek, Belgium

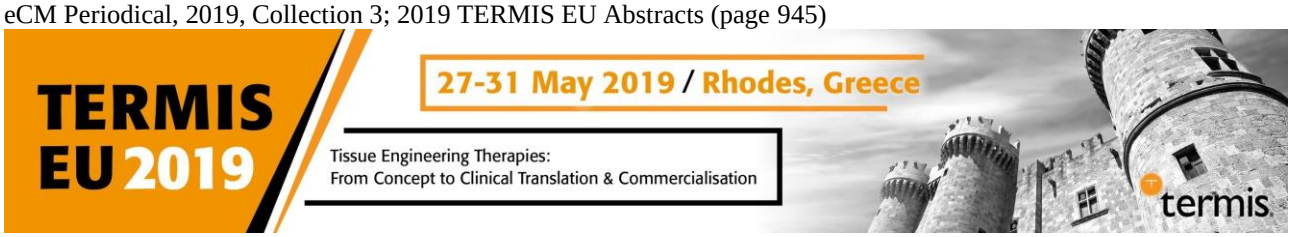
INTRODUCTION: Tooth loss remains a major health problem since current therapies are not able to regenerate damaged dental tissues including pulp and enamel. Successful pulp regeneration requires angiogenesis to supply nutrients and oxygen to newly formed tissue. Proangiogenic properties have already been assigned to dental pulp stem cells (DPSCs), mesenchymal stem cells (MSCs) residing in the dental pulp. For a long time, paracrine factors (e.g. VEGF) have been considered responsible angiogenic mediators. However, recent studies demonstrate that extracellular vesicles (EVs) derived from bone marrow-derived MSCs (BM-MSCs) also have the potential to enhance neovascularization. Therefore, our goal is to investigate the proangiogenic capacities of EVs produced by DPSCs in comparison with those of BM-MSC EVs.

METHODS: EVs were isolated from serum-free conditioned medium (CM) of DPSCs and BM-MSCs by differential ultracentrifugation. EV size and concentration were measured by Nanoparticle Tracking Analysis (NTA) and purity was confirmed by Western blot with enrichment of classical EV markers (CD9, CD63, CD81 and Annexin II) and absence of non-EV marker (mitochondrial Bax). The functional effect of EVs on the migration of human umbilical vein endothelial cells (HUVECs), as a key step in angiogenesis, was studied in a transwell system. The angiogenic profile of EVs was mapped by an antibody array. EV uptake by HUVECs was measured in time by fluorescence microscopy.

RESULTS & DISCUSSION: DPSC-derived EVs significantly induce HUVEC migration (n=8). However, this effect was less compared to BM-MSC EVs (n=6), which might be caused by the lower EV yield from DPSCs as measured by NTA. An antibody array revealed lower expression of proangiogenic factors (e.g. VEGF, MCP-1 and angiopoietin-1) and enrichment of antiangiogenic factors (e.g. TIMP-1) in EVs compared to EV-depleted CM of both cell types. A time-dependent EV uptake by HUVECs was observed.

CONCLUSIONS: Our preliminary data show promising in vitro proangiogenic effects of DPSC EVs. In the future, the potential of DPSC and BM-MSC EVs to induce blood vessel growth in ovo will be tested and ultrastructural analysis of both EV types will be performed. Acquired insights have positive implications for pulp tissue regeneration and diseases associated with insufficient angiogenesis, including stroke and myocardial infarction.

ACKNOWLEDGEMENTS: This research was supported by 'Bijzonder Onderzoeksfonds' of Hasselt University (BOF, BOF16DOC06).



Elastomer reinforced decellularized myocardium constructs

G. Günal[1], A. Tevlek[1], H.M. Aydin[1,2]
Presenting Author: Gülçin Günal, ggunal07@hacettepe.edu.tr
[1]Bioengineering Division, Institute of Science, Hacettepe University, Ankara, Turkey [2]Centre for Bioengineering, Hacettepe University, Turkey

INTRODUCTION: Unlike heart valves, heart muscle has no replacement alternatives. The most challenging goal in the field of cardiac tissue engineering is to reestablish the structure and function of injured myocardium [1]. This study offers a newly tissue engineered hybrid cardiac patch that has promising mechanical properties for ventricular wall reconstruction.

METHODS: The left ventricular wall of fresh sheep hearts were dissected parallel to the epicardial plain into 3 mm-thick tissue slices. Myocardial slices were exposed to 1% sodium dodecyl sulfate (SDS) for 72 hours. Poly(glycerol-sebacate) (PGS) pre-polymer was synthesized via microwave irradiation and then, acellular myocardial tissue fragments embedded into the pre-polymer [2]. The cross-linking of PGS were performed in vacuum at 150 ^{o}C. To evaluate the cellular remnants and ECM components, native and decellularized myocardial tissues were stained with H&E, Masson's trichrome and DAPI. PicoGreen dsDNA quantification assay, hydroxyproline assay and DMMB test were applied to evaluate residual DNA, collagen and sGAG content. Structural characterization of cardiac patches was analyzed by FT-IR, DSC and NMR. Mechanical characterization was evaluated by tensile tests and SEM. Hybrid constructs were recellularized with Human Cardiomyocytes (Promocell, Germany). Cell viability and cytotoxicity analyses were also performed.

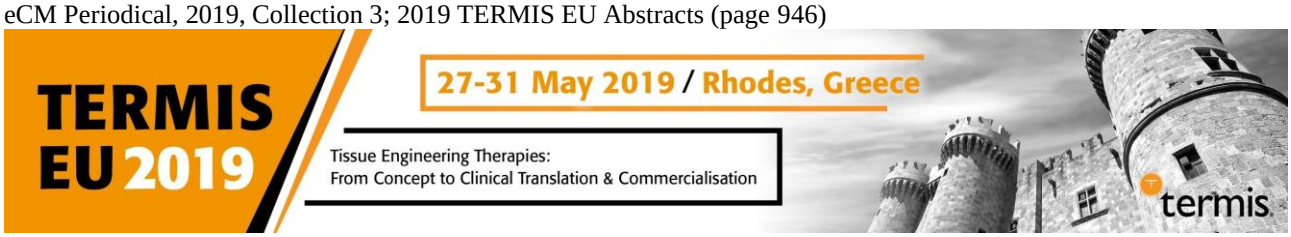
RESULTS & DISCUSSION: Following the 72-hour of decellularization process with 1% SDS, no residual cells were observed in H&E and DAPI staining. The DNA content was significantly reduced in the decellularized matrix compared to the native myocardium (96 ± 3.5 %). Analysis of the hybrid construct with SEM, FT-IR and DSC revealed significant alterations in compositional properties of the ECM. Mechanical properties of decellularized tissues were improved via in situ crosslinking of PGS elastomer.

CONCLUSIONS: We emphasis that PGS elastomers can be used to modify soft tissues to provide sufficient strength to decellularized matrices.

ACKNOWLEDGEMENTS: The authors would like to thank BMT Calsis Co for equipment support and TUBITAK for 2211-C Domestic Priority Areas PhD scholarship program and 1001 research grant (No:118M356).

REFERENCES

[1] Satyam A et al. Adv Mater. 2014; 26(19):3024-34.
[2] Aydin HM et al. Biomat Sci. 2013; 1:503-509.



Designing of cell laden nanofibers reinforced peptide conjugated hydrogel for vascularized bone tissue

G. Onak[1], H.K. Bilgili[1], O. Karaman[1*]
Presenting Author: Günnur Onak, gunnur.onak@ikc.edu.tr
[1]Department of Biomedical Engineering, Izmir Katip Celebi University, Izmir, Turkey

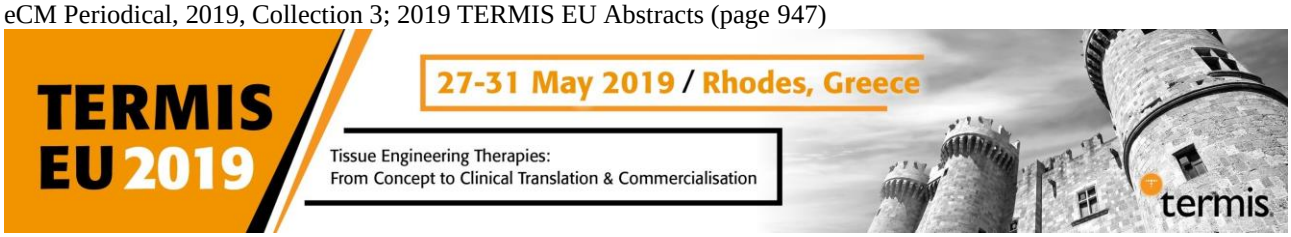
INTRODUCTION: One of the major challenges in reconstruction of bone defects is the inadequate ability of designing biomimetic scaffolds that induces differentiation of osteoprogenitor cells by cell-matrix interaction at the nanoscale, as well as ensuring microvascular structure. Due to the inadequate mechanical properties, there are only few studies on building bone matrix mimetic scaffold by hydrogels. Therefore, there is still tremendous demand for new approaches and strategies for developing vascularized bone tissue. The objective of this study is to fabricate in vitro vascularized bone tissue model by using mesenchymal stem cell (MSC) laden nanofibers (NFs) reinforced hydrogels, which can support osteogenic and vasculogenic differentiation. In this study, NF reinforced vascularized bone tissue model was fabricated in vitro by using IKVAV and YIGSR peptide conjugated PEG-DA (Poly (ethylene glycol) diacrylate) hydrogel and EEEEE and EEGGC peptide conjugated NFs. Peptide conjugated NF and hydrogels that can induce osteogenesis and vasculogenesis will be developed and characterized.

METHODS: All peptide sequences were manually synthesized on 4-Methylbenzhydrylamine (MBHA) resin. After producing NFs with electrospinning, EEEEE and EEGGC peptides were conjugated to NFs with EDC/NHS chemistry. Next, 10% PEGDA was dissolved in 100 mM PBS and C-IKVAV and C-YIGSR peptides were added to this solution with a 1.1 molar ratio to conjugate with PEGDA. Human bone marrow MSCs ($5x10^4$ cells/cm^2) were seeded on fibers and incubated for 1 hours at 37°C. After the hydrogel solution was filtered, HUVEC (10^6 cells/mL) was encapsulated to solution. PEGDA solution was added to mold in 10mm diameter and crosslinked by UV. Layer by layer structure was produced with NFs and hydrogels. PEGDA solution and NF without peptide conjugation was used as negative control. MTT analysis of negative control group were evaluated at 1, 4, 7 days (d). Next, cell seeded fiber reinforced hydrogels incubated in osteogenic medium. Calcium Assay, ALP Assay and DNA quantification assay were evaluated on groups by 7d, 14d, 21d and 28d to compare the effect on calcium phosphate nucleation and osteogenic differentiation of MSCs.

RESULTS & DISCUSSION: Cell number was significantly increased after 7 days. Cell attachment and proliferation on fiber reinforced hydrogel model was successfully performed. Osteogenic differentiation studies are still in process and results will be presented in the conference.

CONCLUSIONS: Bone tissue model by using stem cell laden NF reinforced hydrogels was successfully fabricated in vitro. After osteogenic differentiation results are obtained, vasculogenic differentiation studies will be performed to develop vascularized bone tissue. This biomimetic approach to develop NF reinforced vascularized bone tissue model in vitro by using peptide conjugated hydrogel could be used for healing of metabolically demanding bone defects.

ACKNOWLEDGEMENTS: This study was financially supported by BAP (Research Projects Fund of Izmir Katip Celebi University Scientific) through the research project of 2016-ONP-MUMF-0022.



Generation of human induced pluripotent stem cell lines with cytochrome P450 enzyme polymorphisms (CYP2C19*2/CYP3A5*3C) and defective CYP activities

G. Kim[1], J. Lee[1, 2], D. H. Woo[2], H. J. Park[3], K. Park[1], D. S. Ko[1], J. H. Kim[1,*]
Presenting Author: Gyeongmin Kim, kkkjjj0815@naver.com
[1]Laboratory of Stem Cells and Tissue Regeneration, Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea [2]Laboratory Stem Cells, NEXEL. Co., Ltd, Seoul, Republic of Korea [3]Predictive Model Research Center, Korea Institute of Toxicology, Daejeon, Republic of Korea

INTRODUCTION: Genetic polymorphisms, including single nucleotide polymorphisms (SNPs) in CYP genes lead to interindividual differences in hepatic metabolism, affecting on individual drug effects and toxicities. Here, we generated human induced pluripotent stem cell (hiPSC) lines having particular CYP genotypes (CYP2C19*2 / CYP3A5*3C) for the development of personalized drug therapy.

METHODS: 1,000,000 cells of LCLs were electroporated with 3 ug of each plasmid (pCXLE-hOCT3/4-shp53, pCXLE-hSK and pCXLE-hUL), which were used at 1600 V for 3 pulses of 10 ms each using Electroporator MP-100 (NanoEntek). Cell were seeded on matrigel-coated 6-well plates in mTeSR1 medium (STEMCELL Technologies) with 10uM Y-27632 (Merck Millipore) for 24 h and were changed every day with mTeSR. After 21-28 days of electroporation, iPSC-like colonies were appeared, then, each colony was transferred onto matrigel-coated plates and cultured.

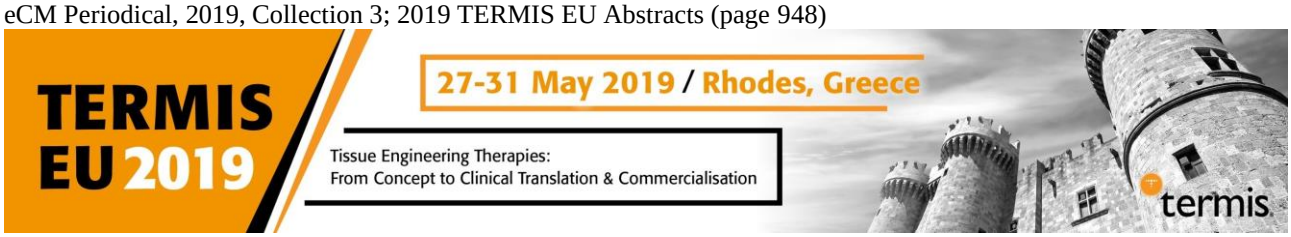
RESULTS & DISCUSSION: Considering that LCL-derived iPSCs represented donor-specific traits [1], we generated iPSC from LCLs having a specific CYP polymorphisms in CYP2C19 or CYP3A5 or both, and wild type LCLs. We established LSCTR-LiPSC-6 (CYP2C19*2/CYP3A5*3C), LSCTR-LiPSC-2 (CYP3A5*3C), and LSCTR-LiPSC-3 (CYP2C19*2). They showed typical morphology of pluripotent stem cells, as seen in LSCTR-LiPSC-10 (Normal control). The expression of pluripotent stem cell markers (OCT4 and SSEA-4) and alkaline phosphatase (AP), together with three germ layer markers in the differentiated EBs were confirmed in these iPSC lines. All iPSC cell lines displayed normal karyotypes in the karyotyping analysis, and their SNPs in CYP genes were confirmed by comparing the SNPs with CYP-patient control, CYP-normal control, and negative control.

CONCLUSIONS: The established iPSC lines could be a valuable source for predicting individual drug responses and optimizing therapeutic drug doses for patients with defective CYP activities.

ACKNOWLEDGEMENTS: This study was provided with bioresources from National Biobank of Korea, the Centers for Disease Control and Prevention, Republic of Korea. This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science & ICT (No. 2017M3A9B4042581 and No. 2018M3A9H1019504).

REFERENCES

[1] Koki Fujimori et al. Molecular Brain. 2016; 9(1):88



Micro/nanoscale biochemical characterization of human stem cells mechanobiologically induced bone-like nodules

H. Rammal[1], M. Dubus[1], N. Bercu[2], S. C. Gongloff[1], F. Quilès[3], H. Kerdjoudj[1]
Presenting Author: Halima Kerdjoudj, Halima.Kerdjoudj@univ-reims.fr
[1]EA 4691 BIOS, Université de Reims Champagne Ardenne, Reims, France [2]EA 4682 LRN, Université de Reims Champagne Ardenne, Reims, France [3]CNRS, UMR 7564, LCPME, Université de Lorraine, Nancy, France

INTRODUCTION: Advances in material sciences have garnered interest in providing intrinsic material features; allowing stem cells commitment into desired phenotype [1]. Bone is a highly textured composite of submicrometric inorganic particles embedded in organic matrices. In vitro bone engineered tissue from stem cells differentiation was successfully achieved and reported. However, stem cells are a heterogeneous population with small proportion of osteoblast-committed cells, compromising thereby the use of standard molecular biology techniques to justify an effective differentiation [2]. Herein, imaging techniques are gathered in order to carry out bone engineered tissue analysis on micro/nanoscales

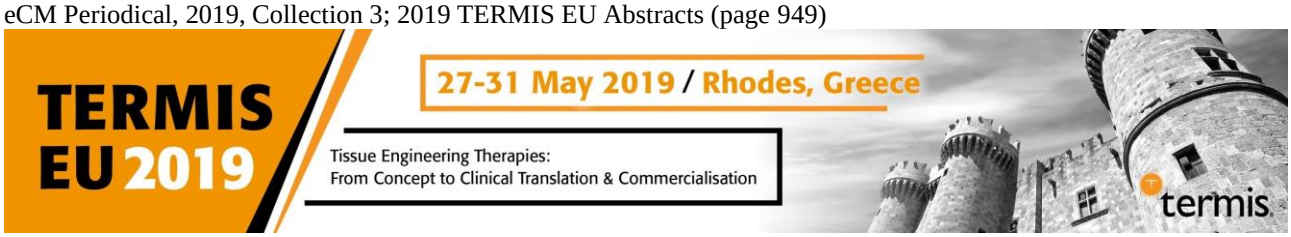
METHODS: Bio-inspired coating made of calcium phosphate/chitosan/hyaluronic acid was built up by simultaneous spray coating of interacting species [1]. Bone-like nodule (morphology/size) was followed by digital microscopy (Keyence, VHX-5000). Structural organization was investigated by histological stainings, field emission gun-scanning electron microscopy (FEG-SEM, JEOL JSM-7900F) and atomic force microscopy (AFM, Brucker). Chemical analysis were carried out on microscale using fourier transform infrared (IR) micro-spectroscopy (Brucker, Vertex 70v) and on nanoscale using an AFM-IR (Anasys, Nano-IR2).

RESULTS & DISCUSSION: After four weeks of culture on biomimetic material, human stem cells started to form nodules (≈ 0.4 mm^2 and 121 µm in area and height). Histological sections examination revealed cohesive tissue with continuous cell layers at the surface with randomly distributed cells embedded within newly formed collagen and proteoglycans. Infrared (IR) micro-spectroscopy at the scale of 40-80 µm in transmission and reflexion modes highlighted heterogeneities in the biochemical composition within the nodule as a function of the region. Major absorption features were attributed to amides bands from proteins, and characteristic bands from phospholipids, polysaccharides and phosphate bands from both organic and mineral origins. IR analysis performed by nanoIR at the scale of 50-100 nm confirmed heterogeneities in identified nodule cell-rich regions and in the organization of collagen fibril structures within nodule collagen-rich regions. Such heterogeneity is to be attributed to the relative contribution of ECM components (orientation, maturation….).

CONCLUSIONS: These findings shed the light of the potential application of spectroscopy as a tool for deciphering the chemical signature of in vitro generated bony tissues.

REFERENCES

[1] Rammal H et al., et al. Acta Biomater. 2017; 49:575-589.
[2] Rammal H et al., ACS Appl Mater Interfaces 2017; 9:12791-12801



Cowries derived aragonite as raw biomaterials for bone regenerative medicine

H. Rammal[1], N. Akilal[2], S. C. Gangloff[1], Y. Khelfaoui[2], H. Kerdjoudj[1]
Presenting Author: Halima Kerdjoudj, Halima.Kerdjoudj@univ-reims.fr
[1]EA 4691 « Biomatériaux et inflammation en site osseux » (BIOS), URCA, Reims, France
[2]Laboratoire de Mécanique, Matériaux & Energétique (L2ME), Faculté de Technologie, Université de Bejaia, 06000 Bejaia, Algeria

INTRODUCTION: Oceans, covering more than 70% of the earth surface, represent an enormous resource for natural products. Marine invertebrate molluscs, the largest animal phylum on earth, were tested for a broad range of biological activities. The extraction of bioactive agents of marine molluscs' metabolites and/or shells is one of the most intensive areas of natural product research. Molluscs derived shells exhibit a remarkably stable organo-mineral biocomposite at the adult stage of the development. Among a large variety of molluscs, tropical Cowries (Mollusca; Gastro- poda; Cypraeidae) are used in many areas of medicine, including treatments of dyspepsia, jaundice, enlarged spleen and liver, asthma and cough. In light of these thoughts, we address, for the first time, the potential use of Cowrie's derived shell as a natural biomaterial for bone regeneration[1].

METHODS: Structural and chemical compositions were provided by scanning transmission electron microscopy (STEM), X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). Cowrie's derived shell bioactivity was performed in culture medium using an induced coupled plasma-optical emission spectroscopy (ICP). Finally, Cowrie's derived shell cytocompatibility was investigated using mesenchymal stem cells (MSCs).

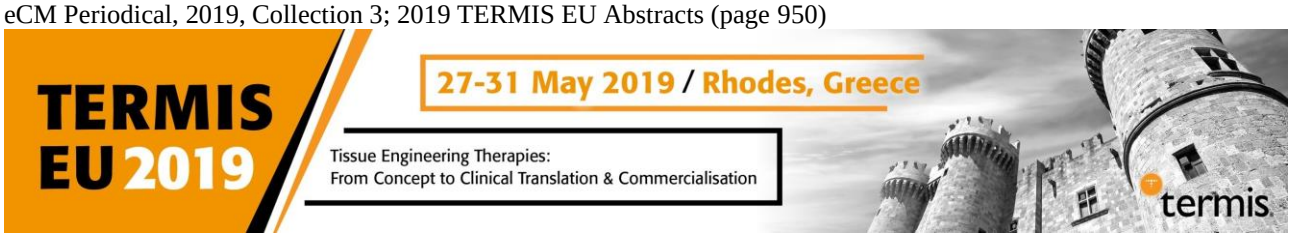
RESULTS & DISCUSSION: STEM micrograph of Cowrie's fracture showed "brick and mortar" structure with ordered lamellae of thin laths (around 300 nm of thickness). Composition, crystallographic and crystal chemical properties investigations revealed the presence of aragonite crystals. FTIR analysis showed that aragonite crystals were converted into poorly crystalline B- type carbonate apatite once soaked, at 37 °C, in DMEM culture media for 7 days, reflecting bioactive features. Cytotoxic assays showed that Cowrie's derived shell powder boosted human MSCs proliferation over the study time compared to nacre, used as positive control. MSCs adopted a flattened morphology and established physical contact with Cowrie's derived shell powder, signature of a good biocompatibility.

CONCLUSIONS: These results suggested that Cowrie's derived shell powder presents a great interest for bone regenerative medicine, and could be a useful and versatile carrier/scaffold for bone tissue engineering or a raw material for 3D printed orthopedic devices.

ACKNOWLEDGEMENTS: This work was financed by the PHC-Tassili 16MDU968/35162XC grant

REFERENCES

[1] Akilal et al., Mater. Sci. Eng. C Mater. Biol. Appl. 2019, 94: 894-900.



Biomimetic hydrogels for human induced pluripotent stem cells

H. Pirmahboub[1], M. Vierthaler[2,3,4], S. Traub[5], E. Ade[5], R. Horlacher[5], J. Utikal[2,3], C. Lee-Thedieck[1]
Presenting Author: Hamidreza Pirmahboub, pirmahboub@cell.uni-hannover.de
[1]Leibniz Universität Hannover, Institute of Cell Biology and Biophysics, Herrenhäuser Str. 2, 30419 Hannover [2]Skin Cancer Unit, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany [3]Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68135 Mannheim, Germany [4]Faculty of Biosciences, Ruprecht-Karl University of Heidelberg, Im Neuenheimer Feld 234, 69120 Heidelberg, Germany [5]Trenzyme GmbH, Byk-Gulden-Str.2, D-78467 Konstanz

INTRODUCTION: Induced pluripotent stem cells (iPSCs) technology was discovered by Shinya Yamanaka's lab in Kyoto, Japan [1]. iPSCs are able to differentiate towards cells from all three germ layers. Due to this capability, they can be used as a cell source for regenerative medicine applications and in vitro disease models. To date, culture substrates coated with Matrigel have been presenting promising results for iPSC culture and differentiation. On the one hand, Matrigel provides an improvement over standard monolayer cultures. On the other hand, it also raises difficulties such as batch-to-batch variation and complexity in composition [2]. To tackle these issues, engineered biomaterials can be fabricated by adjusting biological and biophysical characteristics to maintain iPSCs culture. Cellular behavior is controlled by a complex set of biophysical (e.g. stiffness, porosity, topography) as well as biochemical (e.g. matrix protein composition, growth factors) parameters. The aim of this study is to develop a fully synthetic iPSC-supporting gel that allows investigating the influence of biophysical, chemical and biological parameters on iPSCs.

METHODS: Heparin (Merk) and starPEG (JenKem) molecules with different molar ratios were successfully crosslinked to form biohybrid hydrogels. Stiffness of substrates was tuned by altering the molar ratios (1kPa-10kPa). Surfaces were then functionalized with 2 methods based on NHS ester binding to amine groups of laminins (method A and B). iPSCs were seeded on substrates in 24- well plates. The medium was changed every day (mTeSR, 10µM Rock inhibitor [Y-27632]). Cells were stained after 72 hours for pluripotency markers (OCT4-SSEA4).

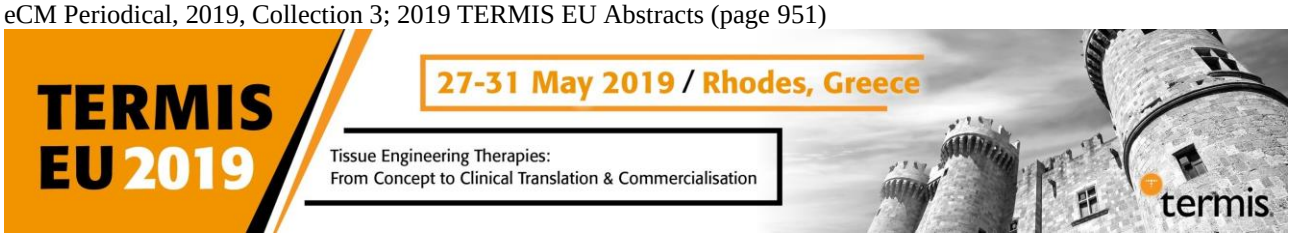
RESULTS & DISCUSSION: No adherent cells were observed on the substrates with a storage modulus of 10kPa and 1kPa. iPSCs formed colonies after 24 hours on the substrates with 4kPa storage modulus. The ratio of different laminin types bound to the hydrogel surfaces also affected iPSCs' adhesion and colony formation. Laminin 521 and 111 functionalized hydrogels could mimic the matrigel as iPSCs maintained their pluripotency after 72 hours.

CONCLUSIONS: Laminin functionalized sPEG-heparin hydrogels clearly illustrated a suitable matrigel-mimetic growth substrate for the iPSC culture and maintainance of their pluripotency.

ACKNOWLEDGEMENTS: The project is funded by Zentrale Innovationsprogramm Mittelstand.

REFERENCES

[1] K. Takahashi et al. Cell, 2006; 126(4): 663–676.
[2] Katt et al., Biotechnoogy, 2016; vol. 4



Coating of hydroxyapatite, aluminium oxide and zirconium oxide by sol-gel method on 3D printed polylactic acid structures: Pilot study

G. Baysan[1], R. B. Husemoglu[1], H. Havitcioglu[1,2]
Presenting Author: Hasan Havitcioglu, hasan.havitcioglu@deu.edu.tr
[1]Biomechanics Department, Dokuz Eylul University, İzmir, Turkey [2]Orthopedics and Traumatology Department, Dokuz Eylul University, İzmir, Turkey

INTRODUCTION: Stem cells are frequently used in regenerative medicine [1]. They can be obtained from many resources such as bone marrow, adipose tissue, periosteum, etc. However, we used adipose tissue derived stem cells (ADSCs) in this study which is relatively easier to obtain and isolate [2]. This study is about comparing the mechanical isolation of ADSCs with a 3D printed Polylactic Acid (PLA) with or without coating. Herein, we used three coatings which are Hydroxyapatite (HAp), Aluminium Oxide (Al) and Zirconium Oxide (Zr).

METHODS: First of all, we prepared filtrates by using a custom-made 3D printer. 1.75 mm diameter thick PLA filament was extruded from a 300 µm nozzle by a speed of 60 mm/sec. Four filtrates with 2.5 mm thickness, 15 mm diameter and different pore sizes which are 530 µm, 365 µm, 310 µm, 165 µm were obtained. 3D printed PLA filtrates were grouped in four. First group was left without any coating. Other groups were coated with Hydroxyapatite(PLA-HAp), Aluminium Oxide (PLA-Al) and Zirconium Oxide (PLA-Zr) by using Sol-gel method. Characterizations were done by XRD and SEM. Also, we tried them with adipose tissue from a 81 years old woman patient. Flow cytometry analyses were performed with two markers of CD45 and CD34.

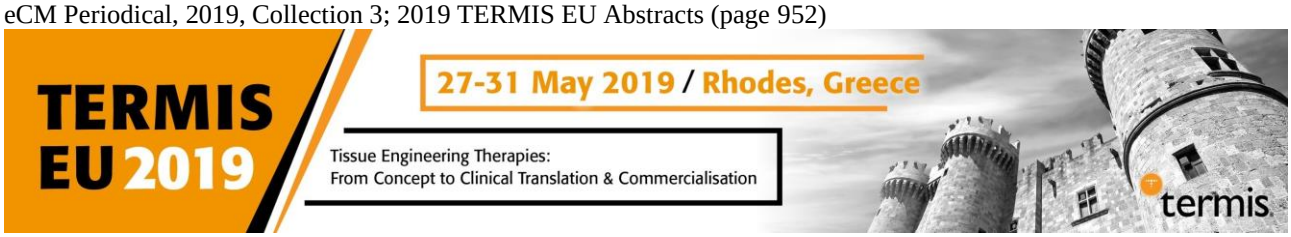
RESULTS & DISCUSSION: XRD and SEM results showed us that, we managed to coat PLA structures with the desired biomaterials. Moreover, flow cytometry results gave us ratio of cells with these markers below %5.

CONCLUSIONS: Flow cytometry analyses were performed for only two markers. They should be performed for more to make sure they are ADSCs. However, as being a pilot study, we aim to improve the coating techniques and perform additional in vitro analysis with more patients. Also, we might compare the results with enzymatic isolation of ADSCs. All in all, these filters give hope for a better mechanical ADSCs isolation technique.

ACKNOWLEDGEMENTS: Financial support was received from Dokuz Eylul University Scientific Research Department.

REFERENCES

[1] Cheng-Han Wu, Fa-Kung Lee, S. Suresh Kumar, Qing-Dong Ling, Yung Chang, et. al. The isolation and differentiation of human adipose-
[2] Akon Higuchia, Chung-Wei Chuang, Qing-Dong Ling, See-Chang Huang, Ling-Mei Wang, et. al. Differentiation ability of adipose-derived stem cells separated from adipose tissue by a membrane filtration method. Journal of Membrane Science 366 (2011) 286–294.



In-vitro and in-vivo evaluation of TCP loaded Hya-gel/BCP bone grafts with hemostatic ability

H.-J. Kang[1], A. R. Padalhin[2], B.-T. Lee[1,2]
Presenting Author: Hoe-Jin Kang, 87jinny@gmail.com
[1]Institute of Tissue Regeneration, College of Medicine, Soonchunhyang University, Cheonan, Republic of Korea [2]Department of Regenerative Medicine, College of Medicine, Soonchunhyang University, Chungnam, Cheonan City, Republic of Korea

INTRODUCTION: Calcium phosphate ceramics such as Hydroxyapatite (HAp), β-tricalcium phosphate (TCP) and Biphasic phosphate (BCP) have been widely investigated due to their intrinsic excellent biocompatibility as well as easy conducting of osteoblast cells. From our previous reports, hyaluronic acid-gelatin hydrogel (Hya-Gel) loaded BCP bone grafts showed active bone regeneration and angiogenesis, thus the defects which was using rabbit model were totally covered with new bones. In this work, we focused on the development of novel bone grafts which showing fast bone formation and hemostatic behavior. In particular, we will discuss the mechanical properties, hemostatic ability, in-vitro biocompatibility as well as bone regeneration capacity by the detailed in-vivo study using TCP loaded Hya-Gel/BCP bone grafts.

METHODS: BCP scaffolds were synthesized by combination of sponge replica, burning-out and sintering processes. As a core part, TCP powder loaded Hya-Gel was combined in BCP scaffolds. On these scaffolds, TCP powder loaded Hya-Gel was loaded again into the silicon molds and finally we could obtain the cone shaped TCP loaded Hya-Gel/BCP bone grafts. The microstructure and EDX analysis were visualized by scanning electron microscopy (SEM). To confirm the biocompatibility, we carried out MTT assay, immunostaining and hemo-compatibility. In addition, bone formation was identified by u-CT and histological analysis using rabbit femur defect model.

RESULTS & DISCUSSION: Outer and core zone of bone grafts showed highly porous morphology of Hya-Gel (A, B) and uniformed spread of TCP powder (C, D). BCP particle confirmed only in the core zone (E).

CONCLUSIONS: TCP loaded Hya-Gel/BCP bone grafts which has highly porous microstructure induced new bone formation by active osteoblast migration and promoted angiogenesis

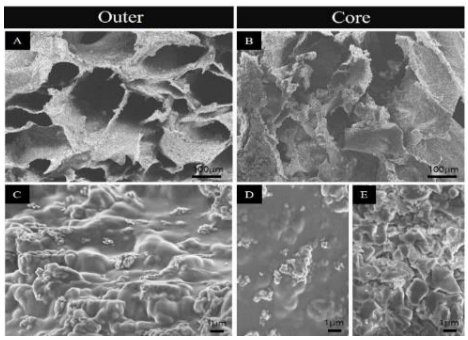
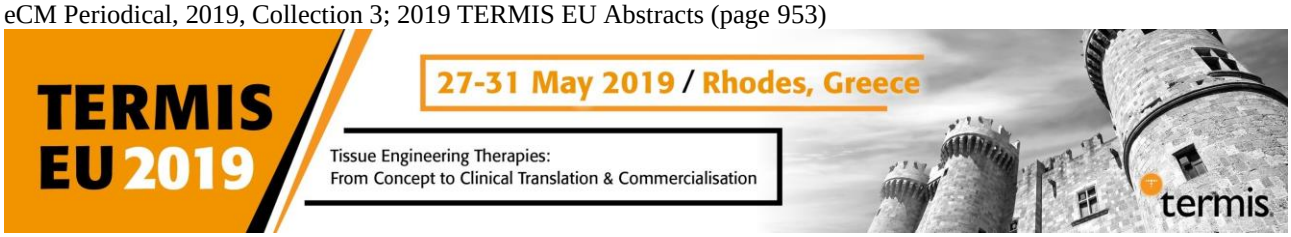


Figure 1: Microstructure for core and outer zone of TCP loaded Hya-Gel/BCP bone grafts was evaluated by SEM (A, B); enlarged image (C~E).

ACKNOWLEDGEMENTS: Basic Science Research Program, National Research Foundation (NRF), Ministry of Education (2015R1A6A1A03032522) and Soonchunhyang University fund.



Gelatin-poly (γ-glutamic acid) scaffolds fabricated by flow focusing microfluidic devices for cartilage tissue engineering

H.-W. Liu[1], C.-Y. Liu[1,2], W.-T. Su[2]
Presenting Author: Hsia-Wei Liu, 079336@mail.fju.edu.tw; 079336@gmail.com
[1]Department of Life Science, Fu Jen Catholic University, New Taipei City, Taiwan [2]Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei City, Taiwan

INTRODUCTION: Flow focusing microfluidic devices have been investigated for the production of monodisperse populations of microbubbles for tissue engineering application. In this study, a newly designed microfluidic device based on flow-focusing geometry was developed to fabricate three-dimensional gelatin-poly (γ-glutamic acid) scaffolds of ordered pores for cartilage tissue engineering in the dynamic bioreactor cultivation.

METHODS: Scaffolds prepared by the flow-focusing microfluidic device. (Figure 1). 7% gelatin and 0.1% poly (γ-glutamic acid) prepared and crosslinked with EDC carbodiimide. The gelatin-poly (γ-glutamic acid) hydrogels were put into a syringe and dropped by the syringe pump. Nitrogen gas and gelatin-poly (γ-glutamic acid) hydrogels with 1% Pluronic® F127 surfactant were pumped through the inner and the outer channels, respectively. The scaffold was then put in the vacuum system overnight for removing air bubbles and synthesizing the interconnecting pore.

RESULTS & DISCUSSION: Scanning electron microscope and confocal microscope examinations showed that the microfluidic scaffold has a regular interconnected porous structure in the scale of 160 μm and high porosity (Figure 1, left). The scaffold is effective in chondrocyte culture; the live/dead cell viability (Figure 1, right), extracellular matrix production (glycosaminoglycans and collagen contents), cell proliferation (DNA contents), and gene expression (real-time PCR) all revealed good results in the dynamic bioreactor cultivation. The chondrocytes can maintain normal phenotypes, highly express aggrecan and type II collagen, and secrete a great deal of extracellular matrix.

CONCLUSIONS: Scaffolds prepared by a flow-focusing microfluidic device of highly ordered and uniform structures are desirable to control chondrocytes cellular behaviors.

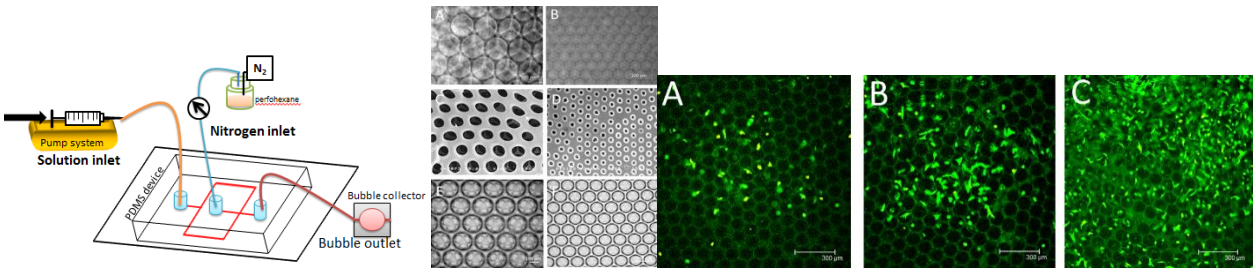
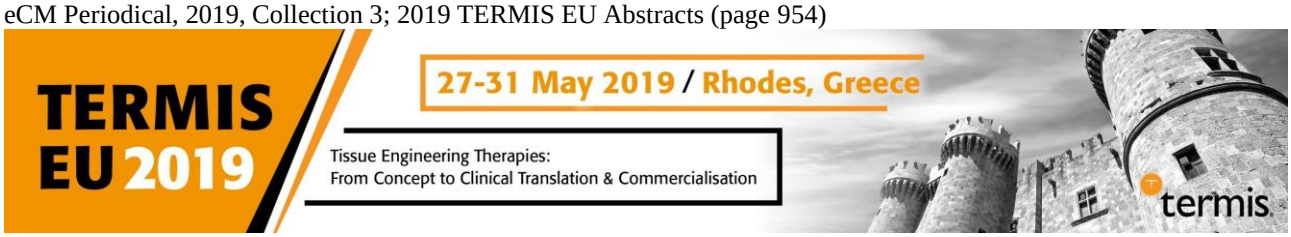


Figure 1: (left) Uniform bubbles were generated and collected. (middle) SEM and (right) confocal microscopy showed the microbubbles self-assembled layer by layer. Chondrocytes were seeded in the microfluidic scaffolds and cultured for 1, 4, and 7 days in bioreactor cultivation.

ACKNOWLEDGEMENTS: Financial and microfluidic device supports were received from Ministry of Science and Technology (MOST) and Institute of Physics, Academic Sinica, Taiwan.



Bevacizumab inhibits choroid-retinal endothelial cells proliferation in three-dimensional modeling of angiogenesis

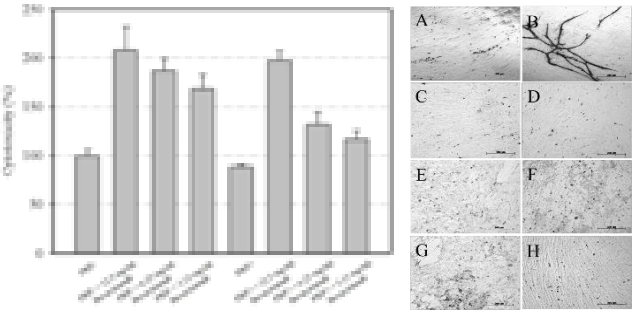
H.-W. Liu[1], W.-C. Hsu[1], C.-C. Hu[2]
Presenting Author: Hsia-Wei Liu, 079336@mail.fju.edu.tw
[1]Department of Life Science, Fu Jen Catholic University, New Taipei City, Taiwan [2]Department of Ophthalmology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei City, Taiwan

INTRODUCTION: Age-related macular degeneration and intraocular neovascular diseases have been treated clinically by anti-VEGF antibody drug bevacizumab. Previously, we have developed an intravitreal drug delivery system for bevacizumab sustained release using temperature-responsive hydrogels. In this study, we examined the anti-angiogenesis and apoptosis effects of bevacizumab on rhesus choroids-retina endothelial cells (RF/6A) in three-dimensional modeling of angiogenesis.

METHODS: Three-dimensional modeling of angiogenesis was using RF/6A cells encapsulated within photocurable hydrogels. Cell-laden poly (ethylene glycol) diacrylate (PEGDA) hydrogels were cultured in DMEM containing and 10% FBS. The cell-laden constructs were treated with platelet rich plasma (PRP) and escalating doses of bevacizumab for 24 hours. The cells proliferation and cytotoxicity were assessed using LDH and live/dead viability assays. Morphological changes of vascular tubes formation on PEGDA hydrogels by endothelial cells were analyzed by bright field cell microscopy. Cell apoptosis in response to bevacizumab was measured by JC-1 staining.

RESULTS & DISCUSSION: Bevacizumab inhibited proliferation of choroids-retina endothelial cells in a dose-dependent fashion (Fig 1). The tube formation of RA/6A cells were significantly inhibited by bevacizumab in a dose-dependent manner (Fig 2). JC-1 staining showed that the % of apoptotic cells were also significantly increased in a dose-dependent treated cells (Fig 3).

CONCLUSIONS: Bevacizumab inhibits proliferation of choroids-retina endothelial cells and arresting the tube formation in a dose-dependent fashion in three-dimensional modeling of angiogenesis.



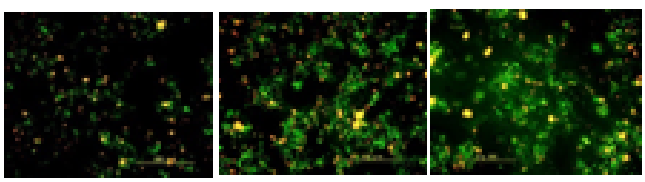
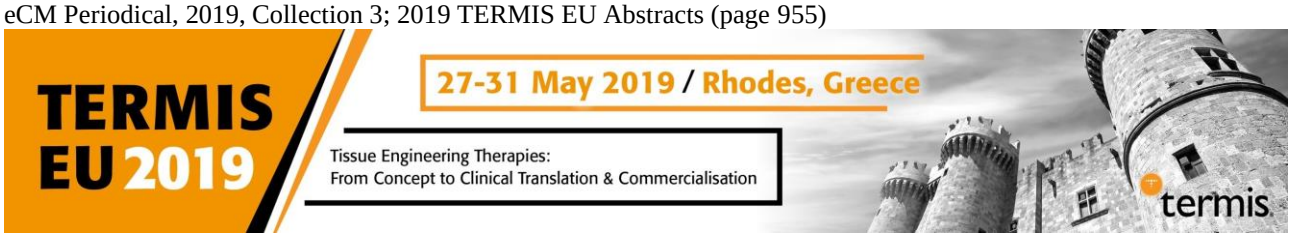


Figure 1: Cells proliferation and cytotoxicity (left). **Figure 2:** Morphological changes of vascular tubes formation on PEGDA hydrogels (middle). **Figure 3:** JC-1 staining of cell apoptosis in a dose-dependent to bevacizumab in three-dimensional cell-laden PEGDA hydrogels (right).

ACKNOWLEDGEMENTS: This research was financially supported by Ministry of Science and Technology (MOST) Foundation and Shin Kong Wu Ho-Su Memorial Hospital, Taiwan.



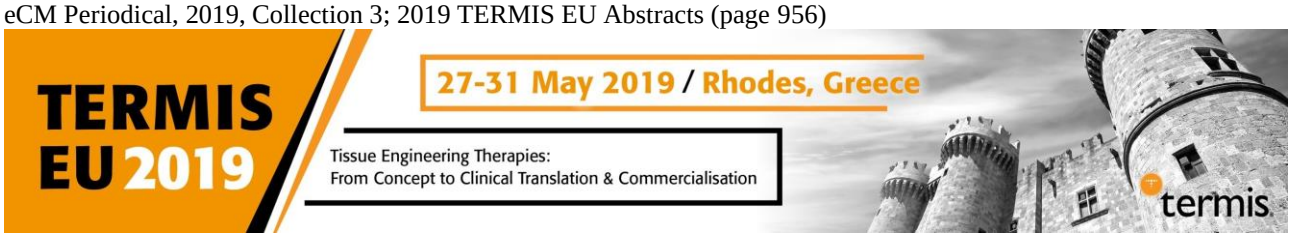
Hybrid photocurable alginate/gelatin macromers bioink toward for cartilage applications

H Mishbak1,2, G Cooper2, P Bartolo2
Presenting Author: Hussein Mishbak, Hussein.mishbak@manchester.ac.uk
[1]School of Engineering, Thi-Qar University, Thi-qar, Iraq [2]School of Mechanical, Aerospace and Civil Engineering, University of Manchester, Manchester, UK

INTRODUCTION: Cartilage has a limited capacity to regenerate due to the avascular composition of the tissue and limited efficacy of current therapeutic interventions. Tissue engineering therapies rely on multiple concepts for scaffold design such surface chemistry, biocompatibility (decreased inflammation and immune response), mechanical properties, swelling, degradation profile and porosity to integrate with the native host tissue. This paper investigates suitable photocurable hybrid hydrogels for cartilage replacements.

METHODS: Natural polymers (Alginate/Gelatin) were chemically-modified with methacrylate anhydride groups to induce photocurable macromers. Resulted bioinks were cured using ultraviolet light (UV) radiation (8 mW/cm^2) for 8 min. hydrogel characrtization through ^{1}HNMR to confirm the functionalisation process was conducted. Morphological properties, swelling and degradation were assessed.

RESULTS & DISCUSSION: Mechanical compression test and rheological characterisation show that prepared bioinks present appropriate mechanical properties 35 kPa approximately, shear thinning behaviour and proper crosslinking structure to support or encapsulate cells during the Bio-printing process, allowing them to growth, proliferate and to produce their own extra cellar matrix (ECM). Furthermore, 3D cell culture shows that hybrid system has overcome some cell compatibility associated to lack of adhering side motifs of alginate with cells as the chondrocyte cell morphology were indicated more spherical shape in 3D compare to 2D.



Enhancing pancreatic islet-like cluster differentiation from hPSC by nanotopographical regulation using a gradient-pattern chip

J.H. Kim[1,3], B.G. Park[2], S.K. Kim[4], D.H. Lee[2], G.G. Lee[1], D.H. Kim[5], H. Kim[1], B.O. Choi[3], K.B. Lee[2], J.H. Kim[1]
Presenting Author: Hyojin Kim, 010hyojin@naver.com
[1]Laboratory of Stem Cells and Tissue Regeneration (LSCTR), Korea University, Seoul, Republic of Korea [2]School of Biomedical Engineering, Korea University, Seoul, Republic of Korea [3]Stem Cell and Regenerative Medicine Institute, Samsung Medical Center, Seoul, Republic of Korea [4]Department of Legal Medicine, Korea University College of Medicine, Seoul, Republic of Korea [5]Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, USA

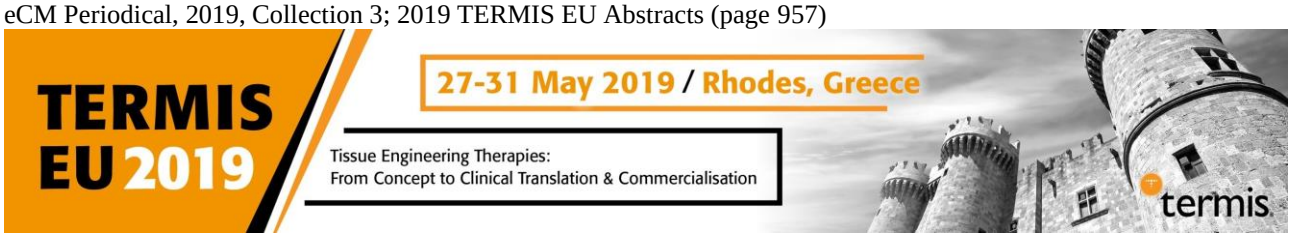
INTRODUCTION: In recent years, manipulating the micro- and nano-scale topography of the stem cell niche has gained considerable interest for the purposes of controlling extrinsic mechanical cues to regulate stem cell fate and behavior in vitro. Here, we established an optimal nanotopographical system to improve 3D differentiation of pancreatic cells from human pluripotent stem cells.

METHODS: H1 hESCs were differentiated into pancreatic islet-like cells under chemical and nanotopographical cues using some cytokines and gradient nano pattern chips with pillar of pore patterns.

RESULTS & DISCUSSION: Single sized nanopatterns have limited utility for testing wide ranges of topographical cues. So we established a gradient nanopattern system which are designed for a diverse range of patterns (pillar or pore) on a single chip. We induced H1 hESCs into pancreatic cells either on 3 different chips of gradient nanopillar patterns (Pi-1,2,3), 3 chips of gradient nanopore patterns (Po-1,2,3) or conventional flat surface dishes. The gradient Po-2 chips has the greatest potential to induce the formation of out-growing pancreatic bud-like structures which express pancreatic marker PDX1 and NGN3. Po-2 pattern-derived endocrine clusters could properly differentiated into functional islet-like 3D spheroids which contain insulin producing-cells and polyhormonal cells.

CONCLUSIONS: Collectively, we have developed gradient-nanopatterned chips to screen various surface nanostructures efficiently and identified that a Po-2 favors pancreatic islet cluster differentiation in the presence of soluble inducers.

ACKNOWLEDGEMENTS: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (Ministry of Science & ICT) (No. 2017M3A9B4042581, 2018M3A9H1019504), School of Life Science and Biotechnology for BK21PLUS for Jong-Hoon Kim.



Direct conversion of fibroblasts into cardiomyocytes using exosomes derived from the process of cardiomyocytes differentiation

H. Kim[1,2], S.J. Park[3], S.W. Choi[3], S.H. Moon[3], S.W. Kang[4], K. Kim[1,2], S.H. Kim[1], I.C. Kwon[1,2]
Presenting Author: Hyosuk Kim, 215346@kist.re.kr
[1]Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology (KIST), Seoul, Republic of Korea [2]KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul, Republic of Korea [3]Department of Stem Cell Biology, School of Medicine, Konkuk University, Seoul, Republic of Korea [4]Predictive Model Research Center, Korea Institute of Toxicology, Daejeon, Republic of Korea

INTRODUCTION: Cell replacement therapy using functional cardiomyocytes is a hot issue in cardiac regeneration. Direct lineage conversion of somatic cell into another functional cell type constitutes an attractive approach for research and clinical use [1]. However, until recently direct conversion has been accomplished by forcing expression of selected transcription factors using viral vectors [2]. Exosomes can mediate phenotypic changes by transferring functional signals and cellular reprogramming factors such as miRNAs between cells involved in various pathophysiological processes [3]. In this study, we present the first demonstration of efficient method for exosome-mediated direct conversion of fibroblasts into cardiomyocyte-like cells.

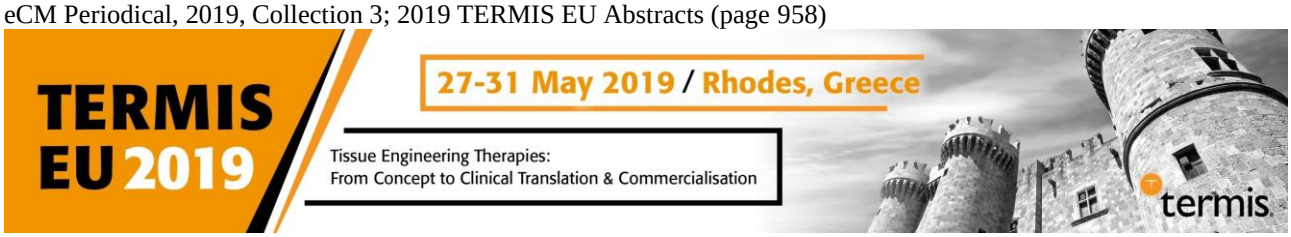
METHODS: To eliminate the effect of feeder layer cells and fetal bovine serum during the isolation of exosomes, we developed a serum and feeder-free two dimensional (2D)-directed differentiation system. Exosomes were isolated from the process of cardiomyocytes differentiation by using differential centrifugation. Exosomes and mouse embryonic fibroblasts (MEF) were co-cultured in defined media for 15 days for transdifferentiation. Induced cardiomyocytes (iCM) were characterized by immunocytochemistry, flow cytometry, quantitative real-time PCR, intra-cellular $Ca2^{+}$ measurement, electrophysiological analysis, and Quantseq 3 'mRNA sequencing.

RESULTS & DISCUSSION: Cell clusters (day20) not only showed a positive response to the cardiac-specific markers: α-actinin, cardiac myosin heavy chain (α-MHC), cardiac troponin T (cTnT), and cardiac troponin I (cTnI), but also displayed clear sarcomere structures. Quantitative RT-PCR also confirmed that increase in the expression of cardiac-specific genes, including Tnnt2, Ryr2, Nkx2.5, and Gata4, compared with control MEF. The ventricular-specific marker, Mlc2v, was more expressed in iCM than Mlc2a, an atrial specific marker. Also, consistent with immuno-staining analysis, the action potentials (AP) were analyzed using the patch clamp technique, and many iCMs showed ventricular-like AP morphology with a mean diastolic potential (MDP) of -73.9 mV.

CONCLUSIONS: In summary, mouse fibroblasts could be converted to cardiomyocytes by a non-viral approach, and these exosome-mediated cardiomyocyte-like cells spontaneously contract, express cardiomyocyte-specific markers, and exhibit typical cardiac calcium flux and electrophysiological features. Our approach to exosome mediated cardiac reprogramming may be a simple and safe method to suggest a pathway that can be a step closer to clinical application.

REFERENCES

[1] Mertens J et al. Nat Rev Neurosci. 2016; 17(7):424-37
[2] Efe JA et al. Nat Cell Biol. 2011;13(3):215-22
[3] Aliotta JM et al. Stem Cells. 2007;25(9):2245-56



A novel hydrogel for tissue engineering: remodeling and neovasculatization

I.O. Roversi[1], M. Henein[2], D.M. Sales[1], D. Mistura[1], M.A. Hausen[1], N.M. Oliveira[1], L. Yahia[2], E.R. Duek[1,3]
Presenting Author: Igor de Oliveira Roversi, iroversi00@gmail.com
[1]Laboratory of Biomaterials, Faculty of Medical Sciences and Health, Pontifical Catholic University of São Paulo, Brazil [2]Laboratoire d'Innovation et d'Analyse de Bioperformance, École Polytechnique, Canada [3]Faculty of Mechanical Engineering, University of Campinas, UNICAMP, Brazil

INTRODUCTION: Hydrogels are three-dimensional network systems composed of polymer chains that exhibit a capacity to absorb and retain water from filling spaces between the chains. The crosslinking of some hydrogels occurs through thermosensitivity applied in the animal tissue, so it is possible to inject with minimally invasive techniques into their gel forms [1-2].

METHODS: 3 ml of injectable and thermosensitive hydrogel of Poly(NIPAAm-co-AAc-co-HEMAPLDLA-co-TMC) (12% m/v) with nankin ink and non-ionic iodinated contrast agent (Iopamiron®300) was implanted by needle on back subcutaneous tissue of 20 Wistar rats divided into 5 groups of 4 animals in periods of 1, 3, 5, 7 and 14 days. High Resolution Computed Tomography (HRCT) was performed on animals immediately after injection of hydrogel and after 24 hours. During all periods the tissue was surgically extracted and processed for histological analysis by Optical Microscopy and Confocal Laser Scanning Microscopy (CLSM).

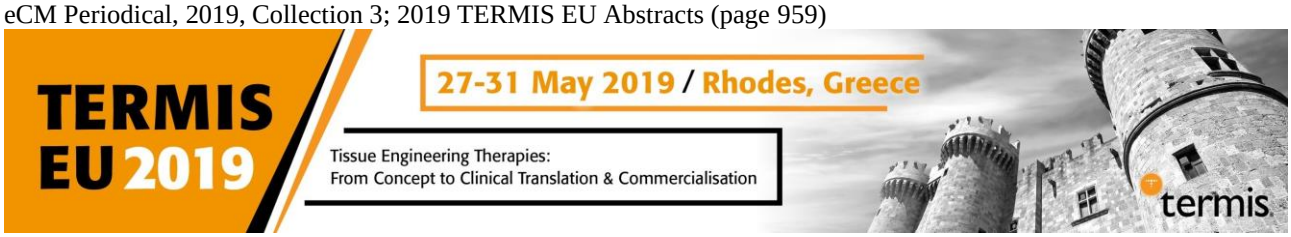
RESULTS & DISCUSSION: HRCT performed after 24 hours of hydrogel injection and visualized subcutaneous cavities with release of water contained in polymer network, distributing in implanted area. Histological analysis by Optical Microscopy demonstrated formation of cavities on subcutaneous tissue and cellular migration to the implanted sites forming inflammatory-cicatricial lamina. Along periods these subcutaneous cavities were dividing in microcavities and mostly after 7 days was observed intense neovascularization surrounding microcavities, not detected apoptosis or necrosis. CLSM analysis was performed by indirect immunohistochemistry for α-SMA (alpha-smooth muscle actin) and observed at 5 days a structural reorganization of the regional vessels and after 14 days an extensive new capillary network in the areas adjacent the tissue where the hydrogel injection occurred. Myofibroblastic cells were not observed in any group.

CONCLUSIONS: Induction of the tissue reorganization after the hydrogel injection direct as a candidate for applications in tissue engineering, storing molecules and cells in microcavities and delivering through local neovascularization network. Cell delivery systems and molecules delivery systems, such as growth factors, cell differentiation factors, drugs or other modulatory molecule can be associated with the three-dimensional polymer network and could be applied promoting directed or local effects with long term delivery.

ACKNOWLEDGEMENTS: CNPq, FAPESP.

REFERENCES

[1] Zhu J et al. Expert Rev Med Devices. 2014; 8(5): 607-26.
[2] Ullm S et al. Biomaterials. 2014; 35(37):9755-66



Comparison of various sources of MSCs in regard to their potential use in cartilage bioprinting

I.V. Vakhrushev[1,2], A.V. Tsvetkova[2], Y.B. Basok[3], A.M. Grigoriev[3], A.A. Gryadunova[1,4], P.A. Karalkin[1], E.V. Koudan[1], E.A. Bulanova[1], Y.G. Hesuani[1], V.A. Mironov[1,4], K.N. Yarygin[2]
Presenting Author: Igor Vakhrushev, vakhrunya@gmail.com
[1]Private Institution Laboratory for Biotechnological Research «3D Bioprinting Solutions», Moscow, Russia [2]V.N. Orekhovich Institute of Biomedical Chemistry, Moscow, Russia [3]V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Ministry of Health of the Russian Federation, Moscow, Russia [4]Institute for Regenerative Medicine, I.M. Sechenov First Moscow State Medical University, Russia

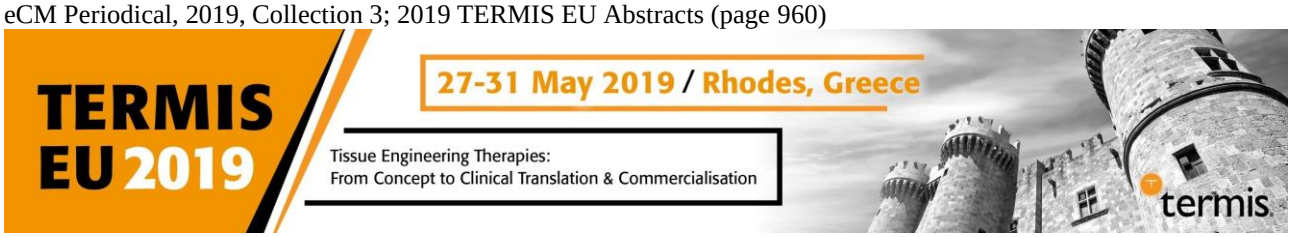
INTRODUCTION: One of the most promising approaches to cartilage tissue engineering is 3D bioprinting using cell spheroids generated from primary chondrocytes. However, obtaining the initial culture of these cells can be difficult. The present study examined spheroids formed by mesenchymal stromal cells (MSCs) of various origin for their suitability for cartilage bioprinting.

METHODS: MSCs were derived from human exfoliated deciduous tooth pulp, umbilical cord stroma and subcutaneous adipose tissue. Surface antigen expression was examined by flow cytometric analysis. Endotheliocytes were isolated from human umbilical cord veins (HUVECs, human umbilical vein endothelial cells). Chondrocytes were isolated from human costal cartilage. Spheroids were generated by aggregation of 8,000 cells in ultra-low attachment plates (Corning). Chondrogenic differentiation of cells comprising spheroids was induced by TGF-β1 (Peprotech). Histological analysis was carried out using staining with hematoxylin and eosin, Alcian blue staining for detection of glycosaminoglycans, and the Masson staining of collagen. Spheroid fusion assay was performed in ultra-low adhesion plates and monitored using the IncuCyte Zoom system (Essen Biosciences).

RESULTS & DISCUSSION: After confirming the presence of MSCs in obtained cultures, the cells were utilized for spheroid formation. The number of cells was chosen so that the diameter of resulting spheroids would be in the range of 200 – 300 μm, which was optimal for the FABION bioprinter (3D Bioprinting Solutions). After 14 days of chondrogenic induction, MSCs from all three sources showed significant signs of differentiation and progressively increased accumulation of cell-produced extracellular matrix containing collagen and glycosaminoglycans. In regard to in vitro chondrogenic potential, MSCs from adipose tissue were most similar to chondroblasts among all the MSC types tested. On the next step, multicellular spheroids combining MSCs and HUVECs were produced. The presence of endotheliocytes in the multicellular spheroids was confirmed by immunohistochemical staining with anti-CD-31 antibody (BD). The ability of spheroids obtained to fuse was successfully demonstrated, thus proving their applicability for bioprinting.

CONCLUSIONS: The data obtained suggest that MSCs from all the sources tested possess a pronounced chondrogenic potential when cultured in a form of spheroids. The ability of MSCs to form mixed MSC/HUVEC spheroids solves the problem of bioprinting of prevascularized tissue constructs. Thus, MSCs can be considered an accessible alternative to primary chondrocytes in the development of cartilage bioprinting techniques.

ACKNOWLEDGEMENTS: This work was supported by the Russian Foundation for Basic Research (grant № 16-29-07322).



Electrospun stimuli-responsive mats for muscle tissue regeneration

I. Apsite[1], S. Salehi[1], L. Ionov[1]
Presenting Author: Indra Apsite, Indra1.Apsite@uni-bayreuth.de
[1]Department of Biomaterials, University of Bayreuth, Bayreuth, Germany

INTRODUCTION: Latest studies have shown great potential of polymeric self-folding films for cell patterning and tissue engineering [1]. Higher porosity ensures easier nutrient and byproduct exchange that can lead to better cell penetration. Until now, there is limited research on porous stimuli-responsive mats [2]. Additionally, porous electrospun mats improved the organization and maturation of engineered muscle tissue [3].

METHODS: Electrospun bilayer was prepared using polycaprolactone (PCL, passive layer) and methacrylated alginate (AA-MA, active layer). To obtain aligned PCL fibers we used two collectors: rotating drum and conductive parallel bars. PCL/AA-MA mat swelling was controlled using Ca^{2+} ion exchange. Mouse myoblast cells (C2C12) were cultured on double layer systems to investigate biocompatibility and formation of functional muscle tissue on fabricated scaffolds. Cell adhesion reagent was used to ensure good cell adhesion on PCL/AA-MA.

RESULTS & DISCUSSION: Evaluation of the cell viability at different time points showed the viability above 90% for all samples. The significant improvement in the cell alignment was seen for the cells, which were grown on bilayer after a week of culture. We recognized that cell growth and proliferation depends on each separate layer thickness. Muscle cells grown and differentiated on fibrous bilayer could be electrically stimulated showing the contraction of cells.

CONCLUSIONS: Fabricated bilayer system possesses positive effect on cell alignment and spreading, leading to more accessible biofabrication method for formation of highly structured tubular tissues like skeletal muscle fiber bundles.

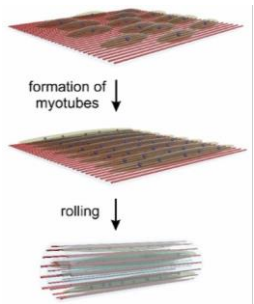
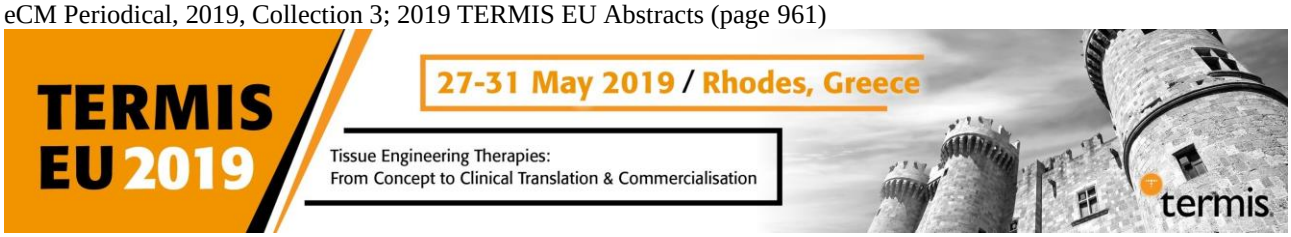


Figure 1: Scheme of skeletal muscle bundle formation.

ACKNOWLEDGEMENTS: Financial support for this research was received from Deutsche Forschungsgemeinschaft, (DFG IO68/1-3).

REFERENCES

[1] Kirillova A et al. Adv. Mater. 2017; 29:1703443
[2] Apsite I et al. Biomacromolecules 2017; 18(10):3178-84
[3] Ostrovidov S et al. Appl. Mater. Interfaces. 2017; 9:42444-58



Generation and optimization of a novel model of human bioartificial cornea using human MSC

I. Garzón[1,2], M.A. Martín-Piedra[1,2], V. Carriel[1,2], J. Chato-Astrain[1,2], O.D. García-García[1,2], D. Durand-Herrera[1,2], F. Campos[1,2], A. Campos[1,2], C. González-Gallardo[3], M. Alaminos[1,2]
Presenting Author: Ingrid J. Garzón, igarzon@ugr.es
[1]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [2]Instituto de Investigación Biosanitaria ibs. Granada, Spain [3]Division of Ophthalmology, University Hospital San Cecilio, Granada, Spain

INTRODUCTION: Several models of bioengineered human artificial corneas have been developed in the laboratory. One of the bioartificial corneas that have efficiently been used clinically is the fibrin-agarose bioartificial human cornea designed and developed by the Tissue Engineering Group of the University of Granada [1,2]. Although results are promising, novel extracorneal cell sources should be investigated. One possible alternative is the use of mesenchymal stem cells (MSC) [3]. The objective of this work is to describe a method to generate bioartificial human corneas using an alternative cell source and to analyze the main structural and histochemical features of these novel organs generated in the laboratory.

METHODS: Human bioartificial corneas were generated using nanostructured fibrin-agarose biomaterials with cornea keratocytes cultured within. For the generation of a cornea epithelium, umbilical cord mesenchymal stem cells (MSC) were isolated from Wharton's jelly biopsies. Then, orthotypical corneas were generated by culturing cornea epithelial cells on top and heterotypical corneas were generated by using MSC as an epithelial substitute. Inductive differentiation media were used. Both corneas were grafted on the cornea of laboratory rabbits by anterior lamellar keratoplasty to determine their usefulness as anterior cornea replacements. Orthotypical and heterotypical bioartificial corneas were analyzed ex vivo and in vivo after 3 and 12 months of grafting.

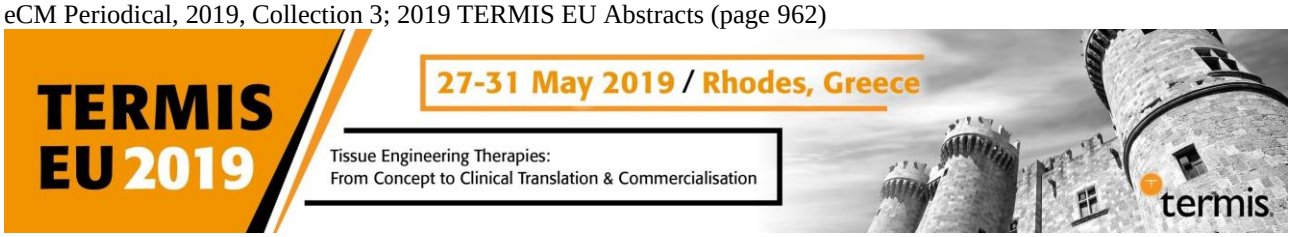
RESULTS & DISCUSSION: Orthotypical and heterotypical bioartificial corneas were safely implanted in rabbit corneas and no mayor side effects were observed after 3 and 12 months. Clinical results as determined by slit lamp and OCT were positive after 12 months, especially in heterotypical corneas. Histology and immunohistochemistry demonstrated that both cornea types had moderate expression of stromal and epithelial cell markers ex vivo. Once grafted in vivo, corneas were highly differentiated, especially in the case of HHC.

CONCLUSIONS: These results suggest that orthotypical and heterotypical bioartificial cornea models could be potentially useful for the treatment of diseases requiring anterior cornea replacement and that heterotypical corneas could be efficiently used as an alternative to the use of orthotypical corneas without the need of generating cornea epithelial cell cultures.

ACKNOWLEDGEMENTS: Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i) from the Spanish Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), Grant FIS PI17/0391 (cofinanced by FEDER funds, European Union).

REFERENCES

[1] Alaminos M et al. Invest Ophthalmol Vis Sci. 2006;47(8):3311-7
[2] González-Andrades M et al. BMJ Open. 2017;7(9):e016487
[3] Garzón I et al. Stem Cells Transl Med. 2013;2(8):625-32



Generation of prevascularized oral mucosa substitutes: A functionalized approach for oral mucosa and palate regeneration

C. Blanco[1], J. Chato-Astrain[1,2], M.A. Martín-Piedra[1,2], V. Carriel[1,2], F. Campos[1,2], D. Durand-Herrera[1,2], A. Campos[1,2], R. Fernández-Valadés[2,3], M.C. Sánchez-Quevedo[1,2], I. Garzón[1,2]
Presenting Author: Ingrid J. Garzón, igarzon@ugr.es
[1]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [2]Instituto de Investigación Biosanitaria ibs. Granada, Spain [3]Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain

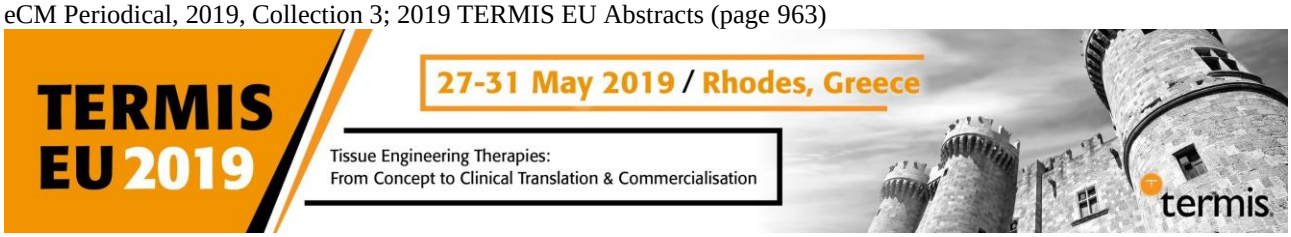
INTRODUCTION: Several strategies have been successfully used to generate artificial oral mucosa substitutes (AOM). However, reconstruction of large oral mucosa defects is often challenging due to the limitations associated to the use of avascular constructs. For this reason, the aim of this study was to evaluate the capability of two mesenchymal stem cell types isolated from bone marrow (BMSC) and dental pulp (DPSC) to increase biocompatibility mediated by rapid blood vessels formation.

METHODS: AOM was developed based on FA hydrogels containing oral mucosa fibroblasts. To evaluate the vasculogenic potential of each cell type, the following study groups were established: 1) only fibroblasts, 2) fibroblasts and human endothelial cells (HUVEC), 3) native DPSC, 4) DPSC differentiated to the endothelial lineage using conditioning media, 5) native BMSC, 6) BMSC differentiated to the endothelial lineage using conditioning media. All these AOM models were grafted in vivo on nude athymic mice. Histological in vitro and in vivo analyses were performed by hematoxylin-eosin staining.

RESULTS & DISCUSSION: Macroscopical analysis showed the presence of a scar at the implant site in all experimental groups. Apart from this, the histological analysis revealed that all AOM kept in vitro showed spindle-like cells inside the stroma. In addition, at in vivo levels, two independent zones were detected between the construct and the host tissue: an interphase zone and a cell-rich zone. The interphase zone was mildly present in negative CTR and in group 4 with differentiated DPSC, being higher in group 6 (differentiated BMSC). The cell-rich zone tended to be moderate in groups 2 (HUVEC) and 3 (DPSC) and mildly present in 4 (differentiated DPSC). Moreover, vascular-like structures were found inside the stroma of the AOM, which were more abundant in group 3 (DPSC). Nevertheless, group 6 (differentiated BMSC) developed a low level of vascular-like structures while 2 (HUVEC), 5 (BMSC) and 4 (differentiated DPSC) showed a moderate vascular pattern.

CONCLUSIONS: These data suggest that the constant interphase zone in differentiated and no-transdifferentiated cells could be related with the intrinsic process of regeneration. Unpredictably, the vascular-like structures are independent of the process of differentiation. However, further experiments are required to determine the potential of MSC to induce neovascularization in AOM tissues.

ACKNOWLEDGEMENTS: Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i) from the Spanish Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), Grants FIS PI18/0331 and FIS PI18/0332 (cofinanced by FEDER funds, European Union).



Bioartificial gelatin coated PLGA membranes for wound healing

I. Carmagnola[1], A. Scalzone[2], P. Gentile[2], V. Chiono[1], G. Ciardelli[1,3]
Presenting Author: Irene Carmagnola, irene.carmagnola@polito.it
[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Italy [2]School of Mechanical and Systems Engineering, Newcastle University, UK [3]Department for Materials and Devices of the National Research Council, Institute for the Chemical and Physical Processes, Italy

INTRODUCTION: Electrospun polymer mats have been widely used as scaffolds for soft tissue engineering due to the similarity to the extracellular matrix morphology [1, 2]. Bioartificial scaffolds based on synthetic and natural polymers lead to combine the processability and high mechanical properties of synthetic polymers with the bioactivity feature of natural materials. The aim of this work was to fabricate bioartificial electrospun membranes obtained through a strategy inspired by the mussel adhesion mechanism wound healing.

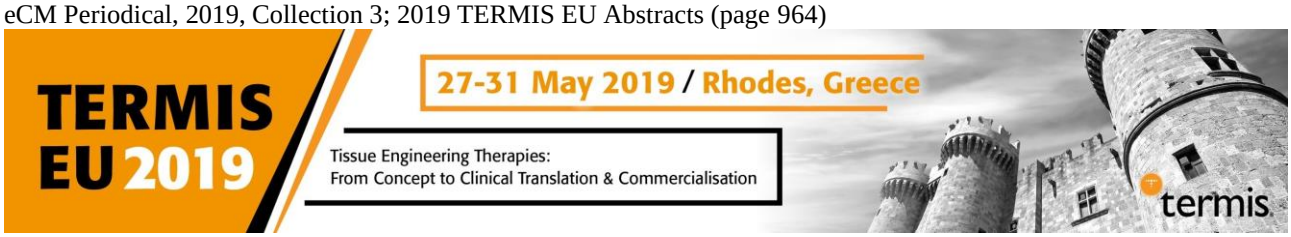
METHODS: Poly(lactic-co-glycolic) acid (lactide:glycolide 75:25, Mw: 66-107 kDa, Sigma-Aldrich, PLGA) was dissolved at 22% wt/v in acetone. Random fibres were produced at room temperature through an electrospinning equipment (Linari Egineering), setting at a flow rate of 1.5 mL/h, an applied voltage of 20 kV and a distance between collector and needle of 20 cm. 3,4-Dihydroxy-DL-phenylalanine (Sigma-Aldrich, DOPA) solution (2 mg/mL) was prepared using Tris/HCl 10 mM at pH 8.5 as buffered solution. After the dissolution of DOPA powder, PLGA membranes were dipping in a DOPA solution for 7 hours at room temperature under continuously stirring and in oxidative condition. Then PLGA fibres were washed and dipped in type A gelatin from porcine skin (Sigma-Aldrich, G) solution (2 mg/mL, Tris/HCl 10 mM, pH 8.5) for 16 hours. Samples were washed and dried at room temperature. Physico-chemical and biological analyses was performed in order to characterized the obtained bioartificial membranes.

RESULTS & DISCUSSION: Morphological analysis demonstrated that PLGA and functionalized PLGA fibres were randomly oriented and presented a porous network. After DOPA treatment the diameter average value increased significantly: 1.37 ± 0.52 µm and 2.94 ± 0.57 µm for PLGA and PLGA- DOPA respectively. The G grafting preserved the fibrous structured of the membranes, causing a further increase of fibre diameter. Infrared spectroscopy were used to highlight the successful addition of gelatin mediated by poly(DOPA) coating, where PLGA-DOPA spectrum showed the appearance of new peaks caused by the DOPA presence. After gelatin functionalization, ATR-FTIR spectrum clearly showed the characteristic peaks of the protein: the bands of the amide I, amide II, and the band attributable to the N-H and O-H stretching vibrations. Cell viability was performed by using Neonatal Normal Human Dermal Fibroblasts cells and gelatin coated membranes improved significantly cell adhesion.

CONCLUSIONS: A bioartificial and biomimetic system for soft tissue engineering was prepared through the immobilization of gelatin on dopamine modified PLGA membranes. PLGA membranes were used as substrates, while dopamine was employed as an intermediary to further graft gelatin. Electrospun matrices show morphological and compositional similarities to the natural ECM, with improved cell adhesion as demonstrated by cell response.

REFERENCES

[1] Wang XY et al. Nano Lett. Nov 2002;2(11):1273-1275
[2] Agarwal S et al. Polymer. 2008;49(26):5603-5621



In vitro study of composite µ-gels for hard tissues regeneration

I. Cruz-Maya[1,2], R. Altobelli [1], V. Guarino[1]*, L. Ambrosio[1], M.A. Alvarez-Perez[3]
Presenting Author: Iriczalli Cruz Maya; cdiriczalli@gmail.com
[1]Institute of Polymers, Composites and Biomaterials, National Research Council (IPCB-CNR), Naples, Italy [2]Department of Chemical, Materials and Industrial Production Engineering, University of Naples Federico II, Naples, Italy [3]Tissue Bioengineering Laboratory, DEPeI-FO, Universidad Nacional Autónoma de México, México

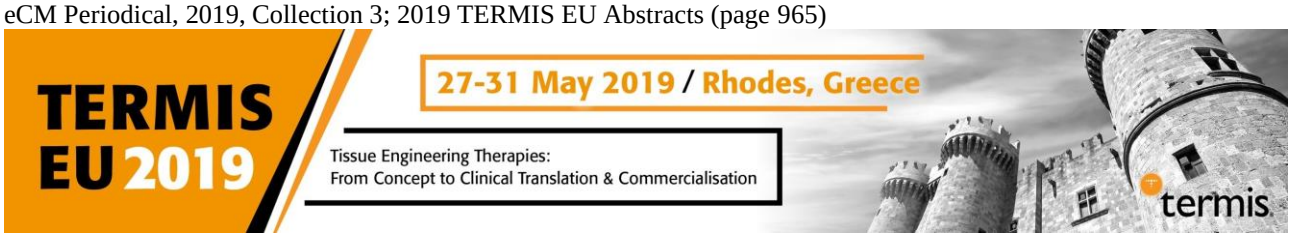
INTRODUCTION: Hydrogels have been considered as suitable materials for mineralized extracellular matrix analogue to be used for innovative tissue engineering therapies, but some lacks still concern their ability to mimic tissue microenvironment to promote cell differentiation into osteogenic way. In the last years, an increasing attention is growing towards micro-scaled hydrogels or µ-gels able to more efficiently support tissue regeneration by the delivery of bioactive signals, thus mimicking cell niche structure and functionalities. In this context, Electro Hydro Dynamic Atomization (EHDA) is emerging as a promising technique to encapsulate different bioactive species (i.e., drugs, proteins, mineral precursors). Herein, the use of composite µ-gels fabricated via EHDA is proposed to support the in vitro regeneration of mineralized tissues into dental cavities.

METHODS: Aqueous alginate solutions - from 0.5 to 2% wt/v -with and without mineral precursor were processed by EHDA. The solution was moved through a steel capillary – 0.2 or 0.8 mm as diameter – by different feed rates from 0.1 to 5 ml/h. A voltage (22kV) was applied to break polymer solution into micrometric droplets, then stabilized into the round shape after deposition in calcium chloride, $CaCl_2$, solution. a mineral precursor (Na_2HPO_4) was added to the polymer solution which was dripped into the calcium containing gelling bath to form mineralized stable µ-gels. These systems were characterized by optical and scanning electrical microscopy and FTIR. In vitro studies were also performed to validate their use as fillers to support the regeneration in dental cavities. Briefly, premolars teeth extracted for orthodontic reasons were cut transversely, washed and sterilized. Microspheres were placed in pulp cavity, and dental pulp cells were seeded.

RESULTS & DISCUSSION: Narrowly dispersed composite µ-gels with sizes ranging from 290 µm to 500 µm and round-like shape were fabricated by optimizing EHDA conditions. Characteristic peaks in FTIR and EDAX spectra confirmed the formation of calcium phosphates into the µ-gels. In vitro cell proliferation showed an increase rate until 14 days, opposite to the control (no microspheres). At 21 days, a decay of proliferation was recorded independently to the mineral amount, due to the starting differentiation state of cells. Accordingly, ALP activity related with osteogenic process increases after 14 and 21 days.

CONCLUSIONS: Composite µ-gels fabricated via EDHA contribute to generate a friendly microenvironment for cells able to recapitulate main activities involved in in vitro ECM mineralization, thus resulting a candidate material for hard dental tissues regeneration.

ACKNOWLEDGEMENTS: H2020, Marie Skłodowska-Curie Actions (Grant n. 665403) and DGAPA-UNAM: PAPIIT IT203618.



Spheroids from human melanocytes as a test-system for drug screening and as a cellular module for tissue engineering

E.V. Dzhussoeva[1], I.N. Saburina[1,2], A.A. Gorkun[1,2,3], I.M. Zurina[1,2,3], N.V. Kosheleva[1,2,4], T.D. Kolokoltsova[1,2], S.G. Morozov[1]
Presenting Author: Irina M. Zurina, izurina@gmail.com
[1]FSBSI Institute of General Pathology and Pathophysiology, Moscow, Russia [2]FSBEI FPE "Russian Medical Academy of Continuous Professional Education" of the Ministry of Healthcare of Russia, Moscow, Russia [3]Sechenov First Moscow State Medical University, Institute for Regenerative Medicine, Moscow, Russia [4]Lomosov Moscow State University, Faculty of Biology, Moscow, Russia

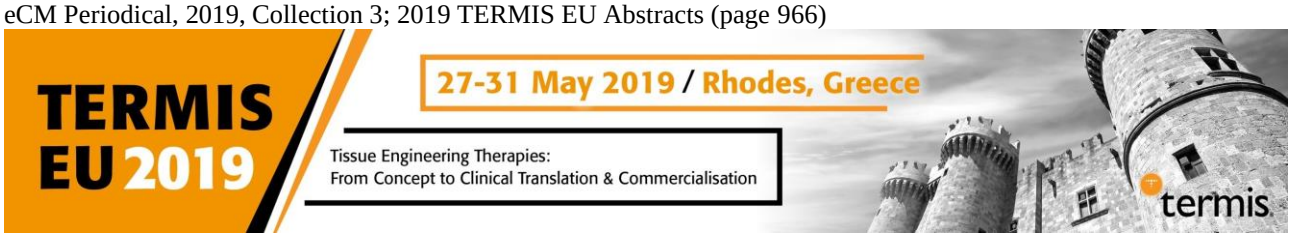
INTRODUCTION: Pigmentation is a result of the synthesis of melanin, which takes place in melanocytes, and its further distribution. Its dysregulation can result in loss of pigmentation, appearance of pigment spots, and melanoma. Development of new skin lightening drugs requires a simple and reproducible in vitro model. Tissue engineering is also a field that needs maintaining functional activity of different components. The aim of the study was to obtain and characterize spheroids from melanocytes and compare them to commercial skin equivalent.

METHODS: The study was conducted on primary culture of human melanocytes (CELL Applications) and skin equivalent Melanoderm (MatTek Corporation). Cells were cultured in monolayer up to the fourth passage in full growth medium (CELL Applications) and then transferred under non-adhesive conditions in agarose plates with microwells (Microtissue, USA). Melanoderm was cultured in specialized culture medium (MatTek Corporation). Spheroids and Melanoderm were cultured either in culture medium or in the presence of fucoxanthin (1:10) for one week and were then characterized using photometry, immunocytochemical staining, and real-time PCR.

RESULTS & DISCUSSION: Cells in primary culture synthesized melanin but its amount gradually reduced. When transferred in non-adhesive agarose plates, melanocytes aggregated and formed spheroids. Within the 7 days the amount of melanin elevated almost two times. Cells in spheroids expressed transcription factors that regulate melanogenesis: gp100, MITF и Sox10. Expression of both gp100 and MITF was up-regulated by day 3 and reached its maximum by day 7 in 3D culture. Real-time PCR revealed the expression of tyrosinase (TYR) – one of the melanogenesis enzyme, and MCR1 – the main receptor regulating melanin synthesis. Their expression was maintained during 3D culturing. Thus, spheroids provide maintenance of melanocytes' functional activity comparable to that in multilayered skin equivalent Melanoderm.
Culturing both spheroids and Melanoderm for 7 days in the presence of the skin-lightening agent fucoxanthin resulted in the lower level of melanin, and in down-regulation of gp100, MITF, and TYR expression, thus inhibiting maturation of melanosomes and synthesis of melanin.

CONCLUSIONS: Collectively, these data illustrate that 3D culture system can better maintain the initial cell function in vitro compared to monolayer culture of melanocytes. The obtained spheroids can be used as a convenient and affordable test-system for drug screening and as building blocks for tissue engineering of skin equivalents.

ACKNOWLEDGEMENTS: This work was supported by the Research project "The study of mechanisms of aging and regeneration and development of conditions for obtaining tissue-engineered constructs using 2D and 3D cell cultures".



Induced pluripotent stem cell-derived mesenchymal stem cells seeded on electrospun PCL nanofibers as matrix for tissue engineering

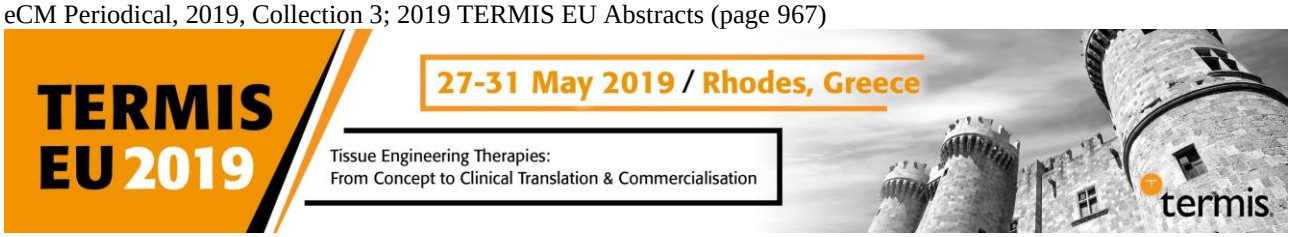
J. Seiler[1], K. Loger[1,2], L. Cyganek[3,4], S.N. Gorb[5], G. Lutter[1,2], I. Haben[1,2]
Presenting Author: Irma Haben, irma.haben@uksh.de
[1]Department of Cardiovascular Surgery, University Hospital Schleswig-Holstein (UKSH), Kiel, Germany [2]DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Hamburg, Germany [3]Stem Cell Unit, Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany [4]DZHK (German Center for Cardiovascular Research), partner site Göttingen, Germany [5]Zoological Institute, Christian-Albrechts-University of Kiel, Kiel, Germany

INTRODUCTION: Induced pluripotent stem cells (iPSCs) have a high medical potential, which can be used to generate autologous mesenchymal stem cells (MSCs) for the individual patient. MSCs have the ability to differentiate into the various cell lines present in native valve leaflets and are therefore ideal candidates for vascular regenerative therapy. The choice of scaffold for cell seeding is crucial in cardiovascular tissue engineering, as it has to provide an appropriate basis for tissue growth and cell proliferation as well as biocompatibility, bioresorbability and excellent mechanical properties. Synthetic biodegradable polymers such as poly(ε-caprolactone) (PCL) combine these properties and 3D scaffolds can be designed by electrospinning. Here, we established and characterized the differentiation of iPSCs to iPSC-MSCs and compared the in vitro seeding capacity of iPSC-MSCs on electrospun PCL leaflets with differently oriented PCL nanofibers.

METHODS: The obtained human iPSCs were generated from the skin biopsy of a clinically silent healthy donor and kindly provided by the Stem Cell Unit, Heart Research Center Göttingen, Germany. iPSCs were cultured on a basement membrane-like matrix in special cell culture medium for stem cells. A two-phase protocol was established for differentiation of iPSC into iPSC-MSCs via the induction of mesoderm cells. Successful differentiation to mesoderm cells and iPSC-MSCs was performed by analysing Brachyury, Oct4 and Sox2 expression. Characterization of iPSC-MSCs was carried out by adherence to plastic, differentiation into bone, cartilage and fat cells, and analysis of surface expression. Cell lines tested positive in all assays were seeded on biodegradable, electrospun polymers with randomly oriented PCL nanofibers and aligned PCL nanofibers (Nanofiber Solutions, OH, USA). Analysis of surface colonization and surface topography quantified the seeding success.

RESULTS & DISCUSSION: Successful differentiation of iPSCs to mesoderm cells and iPSC-MSCs was confirmed by morphology and expression of pluripotency markers. The iPSC-MSCs were capable of differentiating into osteoblasts, adipocytes or chondrocytes and showed $CD34^-$, $CD44^+$, $CD45^-$, $CD90^+$, $CD146^+$ and $CD166^+$ surface expression. Seeding of iPSC-MSCs on electrospun PCL nanofibers resulted in a closed cell layer on the surface of the matrix. Topographic analysis indicated an enhanced adherence on randomly oriented PCL nanofibers.

CONCLUSIONS: We show that iPSC-MSCs can be easily generated by a skin biopsy of the patient. Randomly oriented PCL nanofibers seeded with iPSC-MSCs have a high potential to be used as a matrix for tissue engineering. Additional studies are needed to investigate the ingrowth of cells, formation of an extracellular matrix and the mechanical properties to quantify the functionality of the seeded matrices.



Shortened decellularization protocol for porcine pulmonary heart valves as matrix for seeding with human and ovine progenitor cells

M. Freytag[1], J. Seiler[1], A. Paur[1], S.N. Gorb[2], G. Lutter[1,3], I. Haben[1,3]
Presenting author: Irma Haben, irma.haben@uksh.de
[1]Department of Cardiovascular Surgery, University Hospital Schleswig-Holstein (UKSH), Kiel, Germany [2]Zoological Institute, Christian-Albrechts-University of Kiel, Kiel, Germany [3]DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Hamburg, Germany

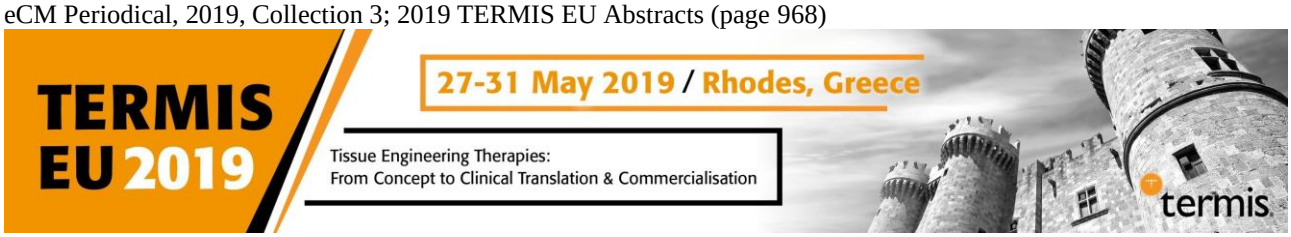
INTRODUCTION: Tissue engineering has the unique opportunity to provide a living valve, capable of growth and biological integration. Biological tissue has great potential to function as bioprostheses in patients for heart valve replacement. The challenge is to reduce immunogenicity without damaging the structure to allow recolonization with autologous cells. For clinical use, it is important that human cells adhere to the decellularized matrix. However, for our large animal model, decellularized heart valves need to be reseeded with ovine cells to generate autologous tissue engineered heart valves. Here, we established a new decellularization protocol for porcine pulmonary heart valves and reseeded the treated leaflets with human and ovine progenitor cells.

METHODS: Our new decellularization protocol is short in time (5 days) and includes a combination of NaOH, Triton X-100, Trypsin and endonucleases for the treatment of porcine pulmonary heart valves. Decellularization was assessed by histological and DNA analysis. Macroscopic structures and cytotoxicity of treated valves were analysed. Isolation of human EPCs from blood was confirmed by surface marker expression and formation of capillary-like structures. Isolation of ovine precursor cells was established and cell types were confirmed by surface marker expression. The decellularized leaflets were reseeded with human and ovine precursor cells with various seeding procedures. Histological and biochemical analysis quantified the reseeding success and morphology of the leaflets.

RESULTS & DISCUSSION: Histological analysis of decellularized leaflets revealed complete removal of all cellular components. The DNA amount was reduced by more than 98.4% compared to native porcine leaflets. The acellular heart valves showed an intact architecture, layer composition and surface topography. In addition, a cytotoxicity of the treated tissues was excluded. Reseeding of decellularized, porcine pulmonary valves with human EPCs resulted in a closed cell layer on the surface of the leaflet tissues. Similarly, decellularized leaflets reseeded with ovine progenitor cells showed cell attachment to the surface.

CONCLUSIONS: Our observations showed conserved leaflet structures after decellularization. Promising results in human and ovine cell reseeding indicated that the decellularized leaflets are suitable as an excellent scaffold for further application in tissue engineering. Additional studies are needed to investigate the mechanical properties to quantify the functionality of the reseeded leaflets before in vivo evaluation of the tissue engineered heart valves in the sheep model can be performed.

ACKNOWLEDGEMENTS: Financial support was received from the German Centre for Cardiovascular Research (DZHK) and the German Heart Research Foundation (DSHF).



Engineering continuous textured composite microfibers for tendon-to-bone mimicry

I. Calejo[1,2], R. Costa-Almeida[1,2], R. L. Reis[1,2,3], M. E. Gomes[1,2,3]
Presenting Author: Isabel Calejo, isabel.calejo@i3bs.uminho.pt
[1]3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [2]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

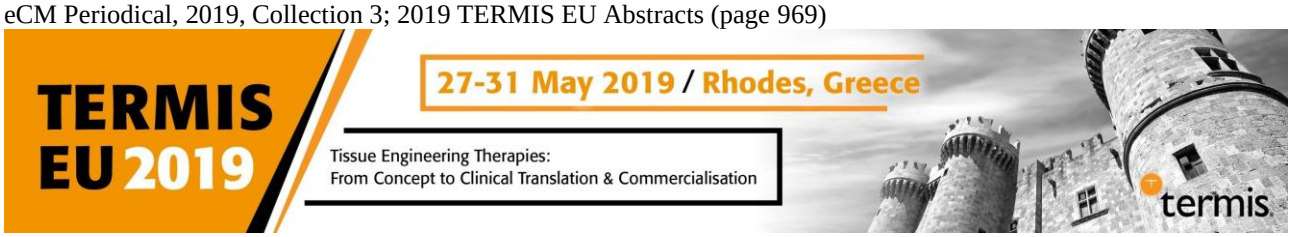
INTRODUCTION: Tendon-to-bone insertion exhibits a continuous structural and compositional gradient. Tendon-to-bone regeneration is limited due to disorganized matrix deposition occurring after injury. To overcome these challenges, we propose the assembly of microfibers with distinct topological and compositional features tailored to guide cell alignment and simultaneously match the mechanical properties of the native tissue.

METHODS: Textured composite microfibers were developed by wet spinning using two polymer blends, namely PCL/Gelatin (16/8, wt%) and PCL/Gelatin/Hydroxyapatite (HAp, 16/8/2.5, wt%). Fiber morphology and mechanical properties were evaluated. Mineral content was determined by alizarin red (AZ) staining and quantification. Human adipose stem cells (hASCs) were seeded to study cytoskeleton alignment and matrix mineralization. Textile techniques were used to create a 3D scaffold and the structure was analyzed by micro-CT.

RESULTS & DISCUSSION: Topographical and mechanical differences were observed between different extrusion flow rates. PCL/Gelatin fibers produced at 1 mL/h extrusion rate exhibited the highest anisotropic alignment, in opposition to PCL/Gelatin/HAp fibers under the same condition. Accordingly, seeded hASCs exhibited higher cytoskeleton alignment and nuclei elongation ($p<0.0001$) in PCL/Gelatin fibers. Significantly higher mineralization was found in PCL/Gelatin/HAp fibers (day 7, $p < 0.04$; day 14, $p<0.0001$) suggesting the induction of an osteogenic-like phenotype. As proof of concept, the two types of microfibers were assembled into a 3D scaffold with a gradual change in composition, resulting in a continuous gradient in HAp content, as well as topographical cues.

CONCLUSIONS: Continuous microfibers with distinct topological and compositional features were developed using a fast and simple wet spinning method. Aligned PCL/Gelatin fibers supported cell alignment replicating tendon cell organization, whereas PCL/Gelatin/HAp fibers triggered mineral deposition and possibly a phenotypic change. Fibers could be combined and assembled using straightforward textile techniques to create 3D scaffolds envisioning tendon-to-bone regeneration.

ACKNOWLEDGEMENTS: Hospital da Prelada (Porto, Portugal) for the lipoaspirate samples; ERC Grant CoG MagTendon nr 772817, Project NORTE-01-0145-FEDER-000021, HORIZON 2020 under the TEAMING Grant agreement No 739572 - The Discoveries CTR and FCT for the PhD grant of IC (PB/DB/128088/2016).



Multi-compartment multifunctional composite living fibers for tendon tissue engineering and regeneration

R. Costa-Almeida[1,2], I. Calejo[1,2], R.M.A. Domingues[1,2], R.L. Reis[1,2,3], M.E. Gomes[1,2,3]
Presenting Author: Isabel Calejo, isabel.calejo@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [2]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

INTRODUCTION: Engineering tendon tissues requires a multi-component, multi-scale strategy. Composite living fibers (CLF) have been developed comprising a mechanically competent core and a cell-laden hydrogel shell [1,2]. Herein, we explored different hydrogel coatings and cell sources to generate artificial living tendon grafts.

METHODS: Commercial sutures were coated using two different strategies. First, an alginate and methacryloyl gelatin (Alg:GelMA) hydrogel was produced by ionic gelation in a $CaCl_2$ bath and subsequent photocrosslinking by UV light. Here, tendon derived cells (TDCs) were encapsulated. Alternatively, bio-functionality was introduced by developing a platelet lysate (PL) hydrogel through thrombin/$CaCl_2$ crosslinking and adipose stem cells (ASCs) were encapsulated. Cell viability (live/dead and alamar blue assays), alignment and expression of tenogenic markers were assessed. Textile techniques were used to generate a 3D construct.

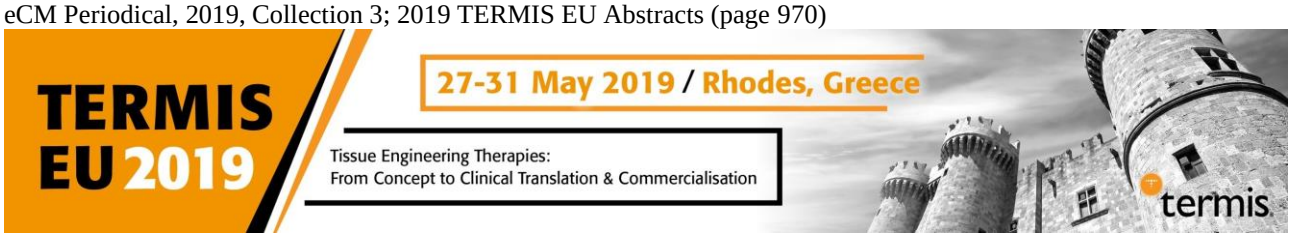
RESULTS & DISCUSSION: The hydrogel layer provided a hydrated microenvironment adequate for cell encapsulation. Both TDCs and ASCs were viable and metabolically active immediately after the coating processes and up to 21 days in culture. In Alg:GelMA hydrogel, TDCs were able to migrate from the outer layer to the surface of the core fiber. In PL hydrogel, the incorporation of this cocktail of biomolecules allowed a fast contraction of the hydrogel by encapsulated ASCs, accelerating the process of migration and microenvironment remodeling by cells. Both cell types were able to align following the direction of the fiber and resembling tendon anisotropic organization. Collagen type I (COL1) expression was assessed (Fig. 1). In both strategies, COL1 could be detected. Strikingly, ASCs in PL hydrogel extensively deposited COL1 in an aligned manner. Due to the demanding mechanical requirements of tendon tissue, the core suture provides a robust mechanical support, which was further improved in textile assembled structures, to sustain tissue level needs.

CONCLUSIONS: As multi-compartment fibers, CLF enable the simultaneous recreation of tissue- and cellular level properties, emulating tendon organization.

ACKNOWLEDGEMENTS: We thank Hospital da Prelada and Serviço de Imuno-Hemoterapia do Centro Hospitalar de S. João, EPE (Porto, Portugal) for providing tissue samples and platelet concentrates; the ERC Grant CoG MagTendon nr 772817, the Project NORTE-01-0145-FEDER-000021, HORIZON 2020 under the TEAMING Grant agreement No 739572 - The Discoveries CTR and FCT for IC and RMAD grants, PB/DB/128088/2016, SFRH/BPD/112459/2015.

REFERENCES

[1] Akbari M et al. Adv Funct Mater. 2014; 24(26):751-66.
[2] Costa-Almeida et al. J Tissue Eng Regen Med. 2018;12(4):1039-48.



Layered scaffolds for nasal cartilages reconstruction fabricated by 3D printing and electrospinning

A. Kurowska[1], E. Menaszek[2], I. Rajzer[1]
Presenting Author: Izabella Rajzer, irajzer@ath.bielsko.pl
[1]University of Bielsko-Biala, Department of Mechanical Engineering Fundamentals, Bielsko-Biała, Poland [2]UJ Jagiellonian University, Collegium Medicum, Department of Cytobiology, Krakow, Poland

INTRODUCTION: Nasal defects that affect tissues such as cartilage or bone can be irreversible and become a challenge not only for the laryngologist but also for plastic surgeons. Even small osteochondral defects may necessitate surgical intervention. Nowadays tissue engineering has appeared as a new approach in treatment of damaged or lost bone and cartilage. In this work, an attempt at combining the advantages of 3D printing and electrospinning was carried out in order to produce scaffolds for TE. It was hypothesized that a composition scaffold combining 3D printed composite porous structure together with nanofibrous nonwovens (containing bioactive and antibacterial additives) can satisfy the biological requirements of scaffolds and provide nasal structural support.

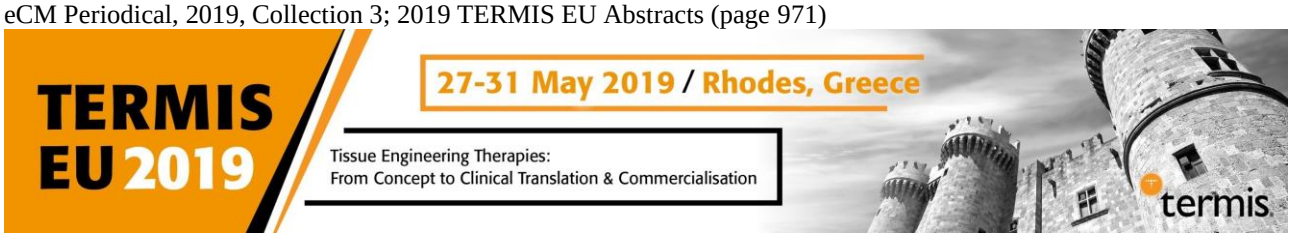
METHODS: Scaffolds were successfully printed using laboratory-made polymeric sticks (modified with bioactive and antibacterial additives) fabricated by injection moulding method. Poly(ε-caprolactone) (PCL) (molecular weight: 80 kDA) and graphene nanoplatelets (powder) were purchase from Sigma Aldrich. Bioactive glass (BG) A2 of the following composition (mol%) 40SiO2–54CaO–6P2O5 was produced at UST-AGH (Kraków, Poland) using the sol–gel method. Osteogenon (Osteo, PIERRE FABRE) is an ossein-hydroxyapatite complex (OHC) also containing osteocalcin and type I collagen. Osteogenon (Osteo) was used as bioactive powder and added to the electrospinning solutions. The composite filament sticks (PCL/graphene; PCL/BG) were used for 3D printing (Fused Deposition Modelling (FDM) technique). In order to prepare fibrous PCL/Osteo or PCL/BG layer on the top of 3D printed scaffold, electrospinning setup (TIC 1092012) was applied. Primary Human Chondrocytes (HCH), Murine fibroblasts L929 (ATCC, USA) and Normal Human Osteoblasts (NHOst, Lonza, USA) were cultured on prepared materials. In order to determine proliferation rate of cells and cytotoxic effect of obtained materials, ToxiLight™ BioAssay Kit and ToxiLight™ 100% Lysis Reagent Set (Lonza, USA) were used according to manufacturer's protocol. Mineralization was assessed by OsteoImage mineralization test (Lonza, USA).

CONCLUSIONS: We expect that the resulting scaffold will have the potential to mimic both the cartilage and subchondral bone tissues needed for nasal defects treatment.

ACKNOWLEDGEMENTS: Financial support was received from the National Science Centre, Poland in the frame of Sonata Bis 5 project: "Layered scaffolds for nasal cartilages reconstruction fabricated by 3D printing and electrospinning" 2015/18/E/ST5/00189.

REFERENCES

[1] Rajzer I et al. Materials and Design 2018;155:297-306



Micro-fibrous multilayered PCL/gelatin scaffold with aligned topological cue using MEHD for skeletal muscle regeneration

J.Y. Kim[1], W.J. Kim[1], M. Kim[1], J. Y. Lee[1], G.H. Kim[1]
Presenting Author: Jae Yoon Lee, dlwodbsdbs@gmail.com
[1]Department of Biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea

INTRODUCTION: Most tissues in human body including muscles, tendons, and nerves show aligned microfibril structures [1]. Especially, skeletal muscle tissue is composed of uniaxially oriented myotubes. For this reason, biomimicking the structure can be a strategy for muscle tissue regeneration [1]. However, the fabrication of aligned microstructures with homogeneously distributed and aligned myoblasts has been remained as the main assignment. In this study, we used melt-electrohydrodynamic (MEHD) to provide topological cues for better cell alignment. We achieved highly aligned PCL/gelatin multilayer structure and the cells were highly aligned after several days of culture.

METHODS: This process uses a melt-electrohydrodynamic (MEHD) process to fabricate PCL bundle with aligned micro fibrous structure. After printing of gelatin (MP bio, Korea) scaffold, melt-electrohydrodynamic was conducted using gelatin as an electrode. As the PCL bundles were stacked on top of the gelatin scaffold by the electric field, the PCL/Gelatin multilayered scaffold is fabricated. Normal printing of PCL on the gelatin structure was used as control group. C2C12 cells were seeded and cultured on the multilayered scaffold in Dulbecco's modified Eagle's medium (DMEM). The effects of aligned microfibrous structure on cells were determined using DAPI/phalloidin staining after 7 days.

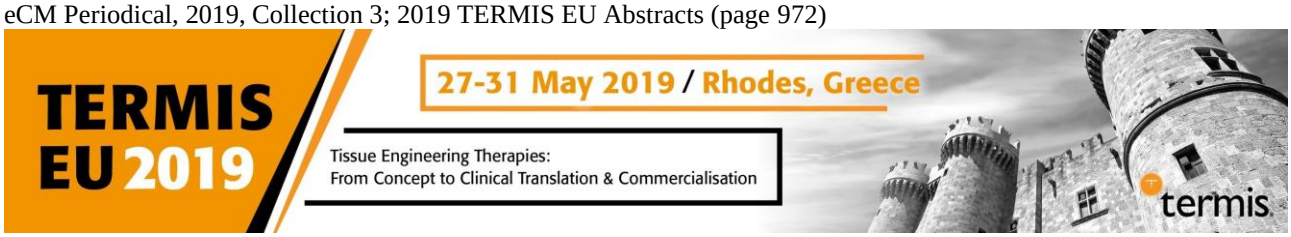
RESULTS & DISCUSSION: We could decrease the diameter of scaffold from 250 μm (nozzle diameter) to 20 μm by adjusting the process parameters. Two groups of multilayer scaffolds (MEHD and normal printing) were examined using DAPI/phalloidin staining. The cytoskeleton and nuclei of cells in aligned scaffold were shown highly aligned to the direction of the aligned microfibrils compared to the control. The micro aligned structure can induce cell orientation and myogenic differentiation more efficiently than normally printed flat PCL/gelatin multilayer scaffold.

CONCLUSIONS: In this study, we developed an innovative printing technique for in situ cell alignment using MEHD. PCL/gelatin multilayer scaffold with MEHD presented a significantly oriented cytoskeleton compared to control. Based on the results, we concluded that the fabrication process of the PCL/gelatin multilayer structure with MEHD can be a promising method for various tissue regeneration.

ACKNOWLEDGEMENTS: This research was supported by the National Research Foundation of Korea (NRF) Grant funded by the Ministry of Science and ICT for Bio-inspired Innovation Technology Development Project (NRF-2018M3C1B7021997) and the National Research Foundation of Korea grant funded by the Ministry of Education, Science, and Technology (MEST) (NRF-2018R1A2B2005263).

REFERENCES

[1] Takagaki Y et al. Int. Rev. Cell Mol. Biol. 2012;296:187-272



Development and validation of a new integrated nanoindentation imaging and incubation mechanobiology platform for the characterization of bio-samples

J. Pyszkowski[1], E. Breel[1]
Presenting Author: Jakob Pyszkowski, jakob.pyszkowski@optics11.com
[1]Optics11, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

INTRODUCTION: Extracellular matrix (ECM) stiffness, one of the principal biomechanical cues of the cellular micro-environment, is known to be critical in regulating cell behavior as well as in directing the development of tissue fibrosis [1, 2]. The examination of very localized micro-mechanical data on cells and its surrounding ECM is challenging due to the structural nature of cells and ECM-like materials [3]. We therefore present a new instrumental method of superimposing micromechanical and imaging data of Fibroblasts inside a collagen-based scaffold by integrated nanoindentation microscopy and incubation.

METHODS: For both the micro-mechanical testing as well as the microscopical analysis a newly developed instrument, the Pavone, is used. The Pavone introduces the semi-automated integration of micro-mechanical and microscopical data, allowing to resolve structure-function relationships of cells and their surroundings. Micromechanical data was collected through nanoindentation experiments, performed in culture medium. The mechanical testing capabilities range from quasi-static experiments to derive elastic moduli, to step-response tests (e.g. creep, stress-relaxation), dynamic mechanical analysis (micro-DMA) and constant strain rate tests to characterize sample viscoelastic behavior, as showed on the cell-matrix construct in this study. For microscopic analysis, the modular inverted microscope, built-in the Pavone, is employed and correlated with the micromechanical dataset. To demonstrate these novel analysis capabilities on cell-ECM-like constructs fibroblast where cultured for 4 weeks in an ECM-like collagen gel inside a 96 well-plate.

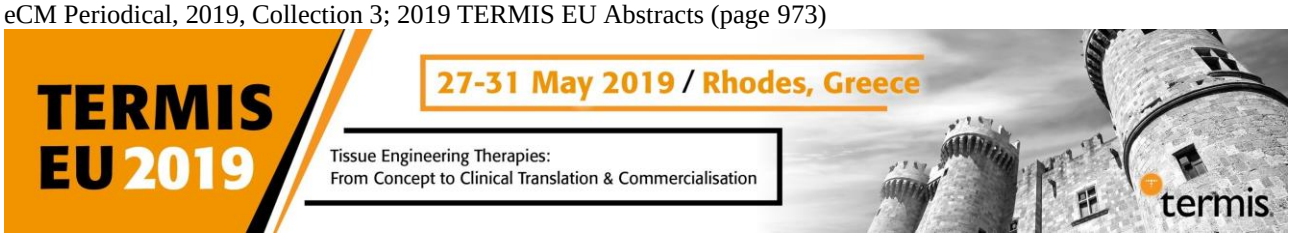
RESULTS & DISCUSSION: For Fibroblast-collagen constructs, this technique can accurately determine moduli in the range between hundreds of Pa up to a few kPa to investigate regional differences in stiffness of cell and non-cell containing regions of the collagen gel.

CONCLUSIONS: The collected preliminary data on Fibroblast-seeded collagen gels demonstrate the ability of the featured automated nanoindentation setup to investigate large quantities of indentation points in short time. This allows, with high spatial and temporal resolution, to obtain insights in structural properties of living bio-samples, along with micromechanical characterization, in a non-destructive way.

ACKNOWLEDGEMENTS: Financial support as well as the Piuma Nanoindenter setup was received and sponsored by Optics11, Amsterdam, NL.

REFERENCES

[1] W. Carver et al, Biomed. Res. Int., 2013 (2013), p. 101979
[2] R.G. Wells et al, Hepatology, 47 (2008), pp. 1394-1400
[3] Mattei et al, Acta Biom 45:60-71, 2016

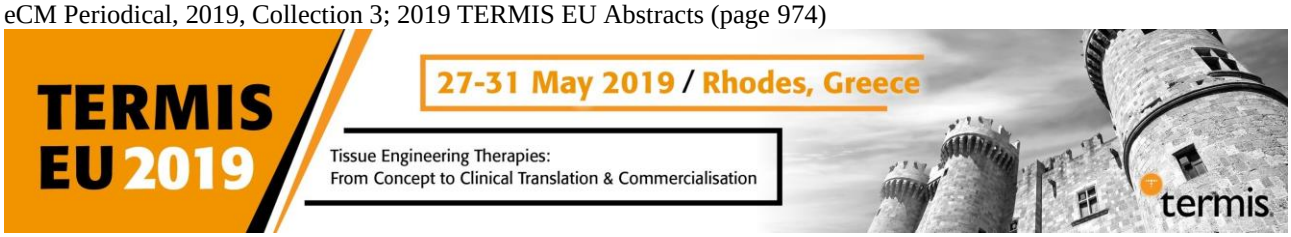


Polysaccharides-based hydrogels for osteochondral defects regeneration

P. Domalik-Pyzik, K. Kosowska, M. Hunger, J. Chlopek
Presenting Author: Jan Chlopek, chlopek@agh.edu.pl
Department of Biomaterials and Composites, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Krakow, Poland

A growing number of patients suffering from obesity and type 2 diabetes, both civilization diseases, combined with other risk factors, means also a growing number of patients suffering from cartilage lesions. The development of osteoarthritis (OA), a degenerative and inflammatory joint disease, has an effect on the cartilage, subchondral bone and joint tissues. With this in mind, there is no surprise that its treatment is complex and at the same time may be hindered by properties of the damaged tissue itself, like lack of vasculature in case of cartilage. Moreover, the chosen therapy should consider different requirements of the gradient structures formed by the cartilage and subchondral bone. Scientists are still in search for an optimal solution within tissue engineering and regenerative medicine fields that will not only give some level of pain relief but also, or in the first place, will prevent further damage and allow regeneration of defected areas. Our research is focused on the development of various types of scaffolds based on polysaccharides (eg. chitosan and sodium hyaluronate) that are combined with hydroxyapatite and graphene family materials (GFM) to form gradient structures. In search for an ideal material solution, we have tested different forms of those, such as injectable hydrogels, porous scaffolds, microparticles that can additionally serve as bioactive molecules delivery agents, etc. Recently, we have obtained hierarchical chitosan/graphene oxide scaffolds modified with electrospun polylactide/hydroxyapatite nanofibers. The detailed physicochemical characterization that consists of structural, microstructural, mechanical, rheological, thermal, chemical stability and surface analysis tests has a crucial importance when such a large variety of materials is considered. We aimed to choose only the most promising ones for further, biological tests. Following biomimetic approach, we are trying to develop materials close to native tissues that will best serve as part of the biomaterials-assisted cell therapy of osteoarthritis. Ideally, they should guide and stimulate differentiation of mesenchymal stem cells and constitute appropriate support for successful tissue formation. This is initially tested in the in vitro conditions. However, one has to remember that only in vivo studies can verify all assumptions and materials choices.

ACKNOWLEDGEMENTS: The authors would like to thank the National Centre for Research and Development, Poland for providing financial support to this project (STRATEGMED3/303570/7/NCBR/2017).



Production of cytokines by injured cornea in in vitro and in vivo experimental models after the treatment with mesenchymal stem cells

J. Kossl[1,2], B. Hermankova[1,2], P. Bohacova[1,2], A. Zajicova[1], V. Holan[1,2]
Presenting Author: Jan Kössl, jan.kossl@iem.cas.cz
[1]Department of Transplantation Immunology, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic [2]Faculty of Science, Charles University, Prague, Czech Republic

INTRODUCTION: Corneal damage is one of the most common causes of impaired vision or even blindness. When the injury is more extensive and the limbal region is involved, the regeneration of cornea is not sufficient. Such damage can lead to limbal stem cell deficiency (LSCD). Nowadays the only option for LSCD treatment is transplantation of the limbal tissue or a transfer of limbal stem cells (LSCs) cultured from the healthy eye. In the case of bilateral LSCD the only option is allogeneic transplantation of the limbus or cultured LSCs with systemic administration of immunosuppressive drugs. As an alternative, mesenchymal stem cells (MSCs) turned out to be a suitable source of autologous stem cells. Despite the large number of experimental works on stem cell therapy of LSCD [1-3], the mechanism of MSC inhibitory properties on the ocular surface is still not well recognized.

METHODS: MSCs were obtained from the murine bone marrow. Flow cytometry was used to characterize the phenotypic markers of such MSCs. For in vitro experiments MSCs were cultured in 48-well plates for 24 hours. Excised corneas were added to cultures alone or in inserts and stimulated by proinflammatory cytokines. After a 48-hour co-cultivation the expression of genes for inflammation cytokines and other molecules in the corneas was detected using qPCR. To elucidate cytokine production by MSCs co-cultured with corneas, inserts were used in to separate MSCs and corneas. In in vivo experiments, MSCs were seeded onto nanofiber scaffolds (allowed to adhere for 24 hours) and transplanted on the injured (0.25M NaOH, 20 seconds) cornea.

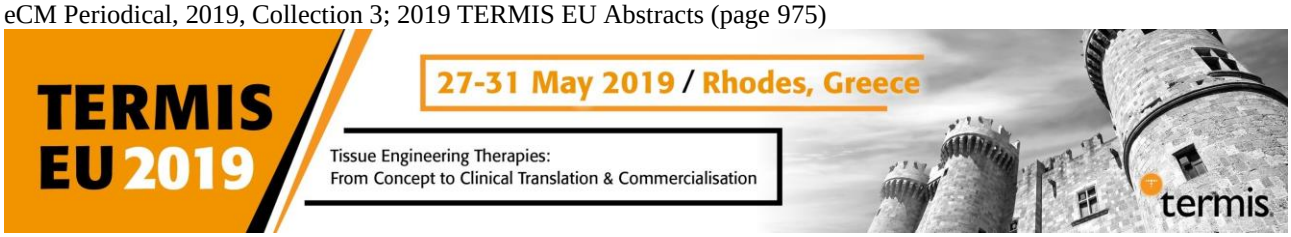
RESULTS & DISCUSSION: Expression of genes for IL-1, IL-6, iNOS and VEGF expression was reduced after the treatment of damaged corneas with MSCs in in vivo experiments. Transparency of corneas treated with MSCs was better than in untreated injured controls or nanofiber scaffold control. In in vitro experiments the expression of IL-1, iNOS, TNF-α and VEGF was reduced in corneas cultured directly with MSCs. IL-6 expression was decreased in corneas cultured in inserts or with MSC supernatant alone.

CONCLUSIONS: Autologous MSCs seeded on nanofiber scaffold represent a suitable source of stem cells for the therapy of LSCD, when there is no possibility to use LSCs. MSCs inhibit a local production of proinflammatory cytokines and help to regenerate the corneal epithelium. Our in vitro model would help us to explore in more details the therapeutic effects of transplanted MSCs.

ACKNOWLEDGEMENTS: This work was supported by grant 1516218 from Grant Agency of Charles University

REFERENCES

[1] Pellegrini G et al. Stem Cells. 2014; 32(1): 26-34
[2] Holan V et al. Stem Cells Transl Med. 2015; 4(9): 1052-63
[3] Mittal SK et al. Stem Cell Reports. 2016; 7(4): 583-90



Decellularized human pericardium crosslinked and coated with bioactive molecular assemblies - Comparison for applications in cardiovascular surgery

Jana Musílková[1], E. Filová[1], J. Pala[2], R. Matějka[1,3], D. Hadraba[1,], D. Vondrášek[1], T. Riedel[4], E. Brynda[4], M. Koňařík[5], J. Pirk[5], L. Bačáková[1]
Presenting author: Jana Musílková, jana.musilkova@fgu.cas.cz
[1]Institute of Physiology of the Czech Academy of Sciences, v.v.i., Videnska 1083, 142 20 Prague 4, Czech Republic [2]National Institute of Mental Health, Topolova 749, 250 67 Klecany, Czech Republic [3]Faculty of Biomedical Engineering, Czech Technical University in Prague, Sitna Sq. 3105, 272 01 Kladno, Czech Republic [4]Institute of Macromolecular Chemistry, the Czech Academy of Sciences, Heyrovsky Sq. 2, 162 06 Praha 6 [5]Institute for Clinical and Experimental Medicine, Videnska 1958/9, 140 21 Prague 4, Czech Republic

INTRODUCTION: Allogenic human pericardium and xenogeneic pericardium are used as biomaterials for applications in cardiovascular surgery. [1]. The pericardia have to be processed prior implantation in order to reduce immunological response of the recipient`s organism. The immunogenicity of the pericardia can be also decreased by decellularizing pericardial tissue which results in cell-free scaffold composed of generally less immunogenic extracellular matrix proteins. Even after the removal of cellular content, the tissue is often treated with glutaraldehyde (GA). The crosslinking blocks immunogenic cellular residues remaining in some cases in the decellularized tissue, improves its mechanical properties, and increases the graft durability by reducing its biodegradability. However, GA-treated tissues are prone to calcification after long-term implantation [1, 2]

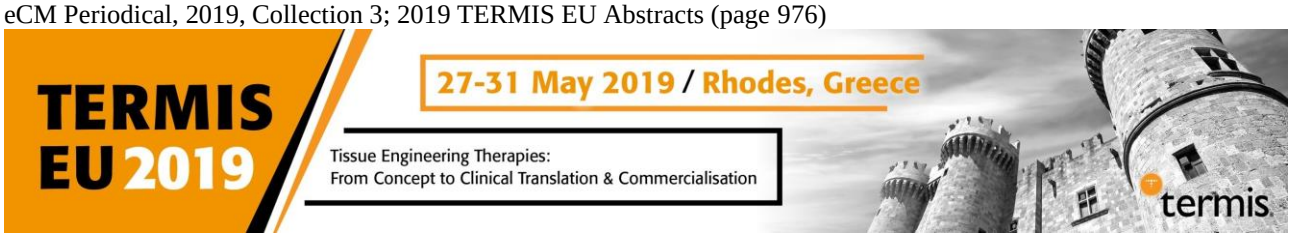
METHODS: In this study, the pericardium was decellularized, crosslinked with GA or genipin and subsequently coated with pure fibrin meshes and meshes modified by the attachment of heparin and/or fibronectin. We focused on testing of mechanical properties, possible toxicity of the crosslinking agent and on the improovement of the endothelial cell growth. Effects of the crosslinking on mechanical properties and collagen structure of the decellularized pericardium were estimated by uniaxial tensile test and multiphoton microscopy, respectively. Viability and metabolic activity of human umbilical vein endothelial cells (HUVEC), seeded on these samples, were then evaluated by a MTS assay in order to find a potential way by which in situ endothelialization of non-autologous pericardium grafts could be encouraged.

RESULTS & DISCUSSION: Viability of the cells measured by their metabolic activity decreased considerably if pericardium was crosslinked with glutaraldehyde. On the other hand the cell viability even increased if pericardium was crosslinked with genipin. The coating of both unmodified and crosslinked pericardium with a fibrin mesh or with the mesh containing attached heparin and/or fibronectin increased significantly the cell viability.

ACKNOWLEDGEMENTS: Supported by Ministery of Health of the Czech Republic (grant No. NV18-02-00422).

REFERENCES

[1] Mai T et al.,Expert Rev Cardiovasc Ther. 2012; 10(8):1039–1049
[2] Cheul L et al., Interactive CardioVascular and Thoracic Surgery, 24(3), 402–406



Novel scaffold for TE/RM based on hyaluronic acid-peptide conjugate

J. Sogorkova[1], S. Karel[1,2], M. Flegel[2], V. Velebny[1]
Presenting Author: Jana Sogorkova, jana.sogorkova@contipro.com
[1]Contipro a. s., Dolni Dobrouc 401, 56102 Dolni Dobrouc [2]The Department of Chemistry of Natural Compounds, University of Chemistry and Technology, Technicka 5, 16628 Prague, Czech Republic

INTRODUCTION: The fibers or non-woven textiles made of native hyaluronic acid (HA) are highly soluble materials with no direct application in tissue engineering or regenerative medicine. By its conjugation with short peptides, we are able to stabilize the structure and furthermore add a functionality to the material using antimicrobial peptide, healing peptide or adhesive peptides supporting cellular adhesion [1].

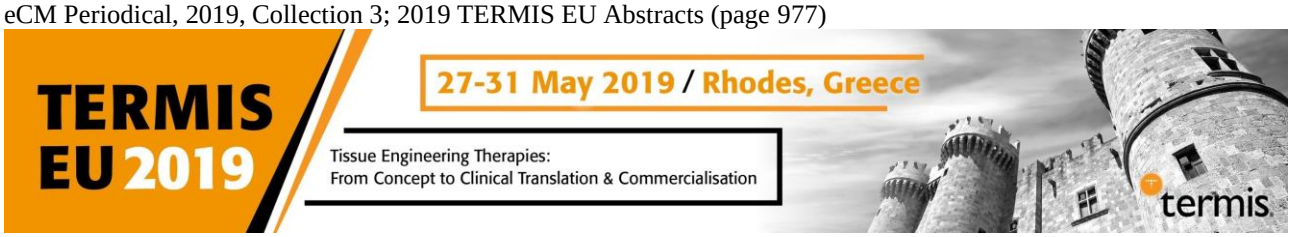
METHODS: HA (Mw 1 MDa, Contipro a. s., Czech Republic) fibers were prepared by wet spinning technology. Cutting fibers by mixer with subsequent compressed filtration create form of non-woven textile. The material was used as a carrier for peptide synthesis using Fmoc/tertBu protection. Peptides supporting cellular adhesion (RGD, IKVAV etc.) were used to confirm the functionality of this conjugate. Dermal fibroblasts (NHDF) and adipose stem cells predifferentiated to cardiomyocytes (ADSC) were stained by DiI, seeded on this material and their adhesion and proliferation was monitored under the fluorescent microscope (Nikon Eclipse-Ti) within 7 days of cultivation. The detail of adhesion as actin filaments and vinculin visualization was captured under the confocal microscope (Leica TCS SP8 X).

RESULTS & DISCUSSION: Double 6-aminohexanoic acid spacer (Ahx) was used with advantage compared to triple glycine, to connect the peptide with HA and made it accessible for cellular binding proteins. The non-woven textile was stable under the cultivation conditions for more than 7 days. The different concentrations of RGD-Ahx peptide was analyzed for NHDF adhesion. The higher the concentration of RGD, the more NHDF adhered. Contrary, using DGR-Ahx peptide was without the effect, as well as adhesion on the Ahx linker alone. ADSC adhered uniformly on GGEGYGEGYIGSR-Ahx-O-HA non-woven textile as YIGSR should support the adhesion of cardiac myocytes [2]. Oppositely, they attached poorly on IKVAV-Ahx peptide, which is primarily used for adhesion of neurons [3].

CONCLUSIONS: The peptide-HA complex is water insoluble, but enzymatically degradable and biocompatible. We are able to control its synthesis by the addition of different peptide equivalents. These peptides are accessible for cellular integrins enabling focal adhesion and their activity is maintained. Furthermore, the prolonged stability (more than 7 days) predetermine applicability of this functionalized non-woven textile in tissue engineering and regenerative medicine

REFERENCES

[1] Karel S et al. Carbohydr. Polym. 2018; 201:300-307
[2] Yu J et al. Tissue Eng Part A. 2014; 20(13-14):1896-1907.
[3] Berns EJ et al. Biomaterials. 2014; 35(1):185-95.



Effects of type, duration and time point of mechanical stimulation in myogenesis

J. Tomasch[1,2], B. Maleiner[1,2], C. Hromada[1,2], A. Teuschl[1,2], D. Rünzler[1,2], C. Fuchs[1,2]
Presenting Author: Janine Tomasch, tomasch@technikum-wien.at
[1]Department of Life Science Engineering, UAS Technikum Wien, Vienna, Austria [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria

INTRODUCTION: Skeletal muscle tissue engineering (SMTE) presents a promising strategy for generating platforms to study muscle development and diseases [1,2]. Herein, we aimed at identifying the effects of various types of physiological and non-physiological mechanical stimulation on the myogenic outcome of a SMTE approach.

METHODS: Murine myoblasts (C2C12) were embedded in ring-shaped fibrin hydrogels and subjected to mechanical stimulation in a custom-made bioreactor system (MagneTissue) [2]. Varying training protocols exerting physiological strain (10% tensile stress, static or cyclic with 0.25 Hz) were applied continuously or with resting phases during early myogenesis. Furthermore, pre-differentiated constructs were exposed to injurious loads of static and cyclic strain (40% static stress, or 10% cyclic stress with 1.2 Hz).

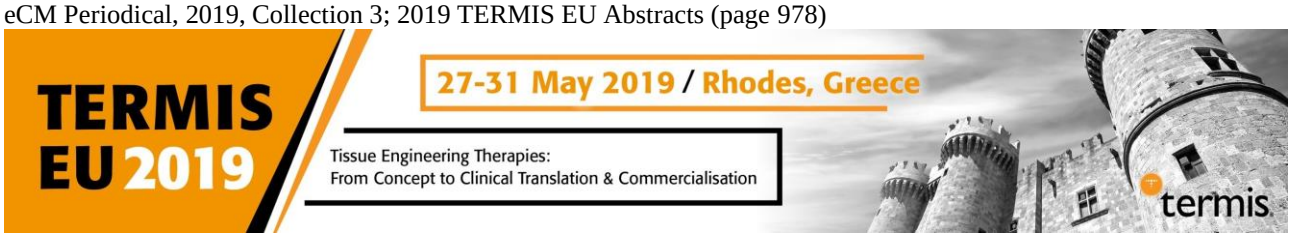
RESULTS & DISCUSSION: Gene expression analysis of early, mid and late-stage myogenic markers (MyoD, myoG, myosin heavy chain (MHC) and troponin T 1) and immunofluorescence stainings for MHC showed that cyclic mechanical stimulation promoted late-stage myogenesis to a higher extent than static stimulation, whereas an opposite trend was observed for early myogenesis (data not shown). Furthermore, it was demonstrated that application of strain with shorter resting phases significantly increased MHC 2b synthesis, an isoform expressed in the last stages of differentiation in C2C12 [3]. Simulation of injuries to mature SMTE constructs revealed that high frequency cyclic stimulation (1.2 Hz) resulted in randomly oriented myotubes with a hypertrophic phenotype, while static tensile stress (40% of initial length) yielded highly-aligned, thicker myotubes.

CONCLUSIONS: It can be concluded that type (cyclic vs. static), and time point of mechanical stimulation, as well as duration of resting phases have a major impact on progress of myogenesis, MHC isoform transition and morphology of myotubes.

ACKNOWLEDGEMENTS: This study was supported by the Signal Tissue (MA23#18-08) and FFG COIN Disease Tissue (FFG#845443) Project.

REFERENCES

[1] Vandenburgh H et al. Am. J. Physiol. 1989;256
[2] Heher P et al. Acta Biomater. 2015;24,251–265
[3] Brown D et al. J. Muscle Res. 2012;32,383–390



Functional correction of hemophilia A phenotype using patient-derived iPSCs and genome editing

J.S. Son, J.H Kim
Presenting Author: Jeongsang Son, sllzzz@naver.com
Laboratory of Stem Cell Biology, Division of Biotechnology, College of Life Science & Biotechnology, Korea University, Seoul 126-713, Korea

INTRODUCTION: Hemophilia A (HA) is a recessive X-chromosome linked bleeding disorder, in which factor VIII (FVIII) is insufficiently synthesized or does not properly function [1]. The current study was carried out to investigate the therapeutic potential of highly purified endothelial cells (ECs) derived from HA patient-IPS cells carrying FVIII corrected by CRISPR/cas9.

METHODS: The original HA patient-derived iPSCs (iPA) and FVIII gene-corrected iPSCs (iCO) were both differentiated into ECs through purification and proliferation steps. The iPSC-derived ECs (ECs from iPA and iCO) were transplanted into HA mouse after two passages. To evaluate the therapeutic ability of grafted iPSC-ECs, total bleeding time after tail vein transection (TVTB) [2] and mice survival rates after tail clipping was measured and compared between the cell types and doses after 2 weeks of transplantation.

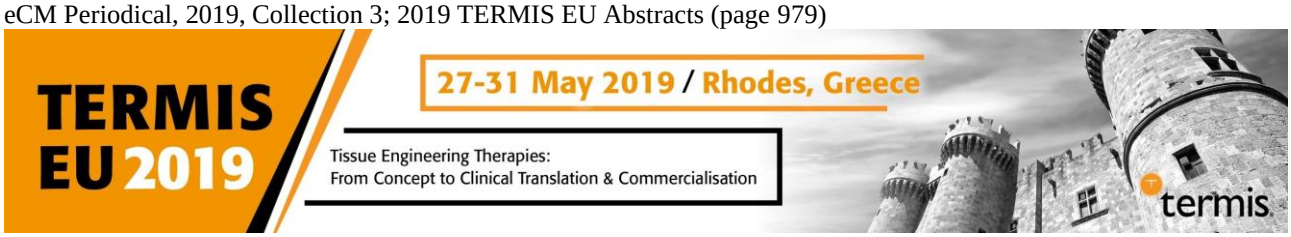
RESULTS & DISCUSSION: qPCR and immunostaining data showed that iPSC-ECs expressed multiple EC markers, including CD31, vWF and VE-cadherin. They were capable of Ac-LDL uptake and formed tube-like structures on solidified Matrigel. The level of FVIII coagulant antigen (FVIIII:Ag) was ~ 5 ng in lysate of 1 x 10^6 iCO-ECs, which corresponds to one-third of that of human liver sinusoid ECs, known to be the main cell type that produces FVIII. After transplantation into HA mice, TVTB data demonstrated that the average of total bleeding time significantly decreased (Sham: 38m 38s, iPA-EC: 41m 53s, iCO-EC: 11m 25s). Tail clipping survival test showed that phenotypic correction was acquired through iCO-EC transplantation: 20% of animal survived after transplantation of 2 x 10^6 cells and 90% survived after 4 x 10^6 cell grafting. Furthermore, FVIII activity in blood plasma of HA mice was increased and reached up to 15% of the normal level.

CONCLUSIONS: HA patient-derived iPSCs could differentiate into ECs that produce functional FVIII after CRISPR/Cas9-mediated gene correction. The genome-corrected HA-iPSC can be used in cell therapy for the treatment of HA.

ACKNOWLEDGEMENTS: We thank Dr. J. S. Kim and Dr. D. W. Kim for providing iPSC lines.

REFERENCES

[1] Escobar M and Sallah S. J Thromb Haemost. 2013;11(8):1449-53
[2] Johansen PB, Tranholm M, Haaning J and Knudsen T. Haemophilia. 2016;22(4);625-31



Production of a highly purified hepatocytes derived from various human pluripotent stem cell lines using a chemically modified compound

J.Y. Park[1], S.J. Uhm[2], J. Han[1], J.S. Son[1], G. Lee[1], H.J. Kim[1], K.J. Park[1], G. Kim[1], G.S. Cho[3], J.S. Kim[2], J.-H. Kim[1]
Presenting Author: Ji Young Park, prssjy@hanmail.net
[1]Laboratory of Stem Cells and Tissue Regeneration, Department of Biotechnology, College of Life Sciences & Biotechnology [2]Department of Chemistry, Korea University [3]NEXEL Co., Ltd3, Seoul, Republic of Korea

INTRODUCTION: Hepatocytes and hepatic progenitors derived from human pluripotent stem cells (hPSCs) are useful sources for both therapeutic and pharmaceutical application. Therefore, identification and purification of these cell types would be important issues after hepatic differentiation of hPSCs.

METHODS: There are very few candidate surface markers that can be used to identify and purify hepatocyte-like cells by using MACS or FACS sorting. In the present study, we tested chemically modified compound for FACS-mediated sorting of hepatocytes after differentiation of various hPSCs established in different institutions.

RESULTS & DISCUSSION: After the final differentiation, differentiated cells and primary hepatocytes (control group) were incubated with a chemically modified compound and were sorted by FACS. Importantly, the chemically modified compound selectively stained cells that expressed albumin. Furthermore, FACS analysis data showed that the purity of hepatocytes that expressed albumin was significantly increased after the purification of chemically modified compound-positive cells.

CONCLUSIONS: Therefore, this compound can be used as a valuable tool to identify and purify stem cell-derived hepatocytes for both in vitro drug screening and in vivo transplantation studies.

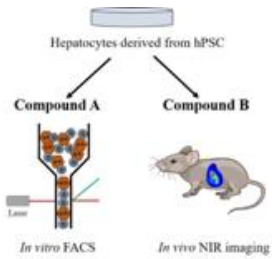
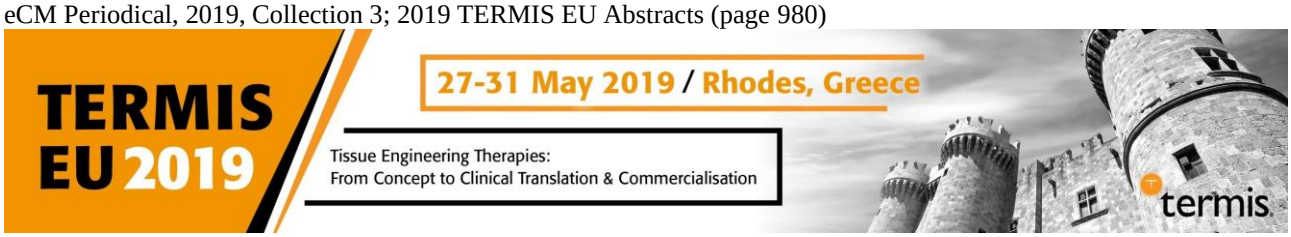


Figure 4: Scheme of purifying hepatocytes using a chemically modified compound

ACKNOWLEDGEMENTS: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. NRF-2017M3A9B4042581 and No. NRF-2018M3A9H1019504).

REFERENCES

[1] Woo DH et al. Gastroenterology. 2012; 142(3):602-11
[2] Kim JH et al. Toxicol Sci. 2015;147(1):190-206
[3] An SY et al. Gastroenterology. 2017;152(5):1174-1186



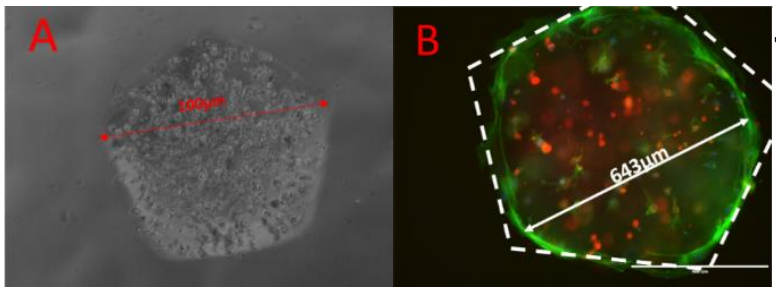
Patterning vasculature within individual tissue building blocks

J. Zhang, P. Padmanaban, J. Rouwkema
Presenting Author: Jiena Zhang, j.zhang-6@utwente.nl
Department of Biomechanical Engineering, Technical Medical Centre, University of Twente Enschede, The Netherlands

INTRODUCTION: Functional vascularization in engineered tissues is essential for its initial survival after implantation. In this project, we are aiming to study the vascular formation within individual tissue building blocks in two aspects: internal forces, such as cell contractile forces; and external forces, such as shear stress. Studies have shown that more vascular structures formation caused by the tissue contractility tends to happen in regions of higher deformations. We fabricated cell-laden GelMA tissue building blocks with different geometries to investigate how the physical forces generated by cells themselves within a hydrogel environment will induce various contractility. A perfusion device is built to apply an interstitial flow to study how shear stress affects cell orientation, alignment and endothelial sprouting. The primary parameters of the device are set based on computational modelling. By combining the effects of geometries and the patterned interstitial flow through individual tissue building blocks, we can guide the vasculature formation to obtain a tissue with desired properties.

METHODS: GelMA of 82% degree of functionalization (DoF) was synthesised according to the predefined protocols. 7.5% (w/v) GelMA solutions was prepared by dissolving in PBS at 80°C. The concentration of photoinitiator (Irgacure 2959) in the solution was 1% (w/v). A mixture of MSCs and HUVECs at the ratio of 1:1 were loaded into GelMA solution at the concentration of 5×10^6 cells/ml. Cell-laden GelMA prepolymer was crosslinked with UV at 365nm wavelength to obtain tissue building blocks. Computational modelling is performed by using COMSOL Multiphysics version 5.3a.

RESULTS & DISSCUSSION: Free standing tissue building blocks deform isotropically and more vascular structures were formed in the regions of higher deformation (figure 1). The primary computational modelling results (figure 2) demonstrated that we can pattern and guide cell alignment hence the vasculature formation by applying interstitial fluid flow through individual building blocks.



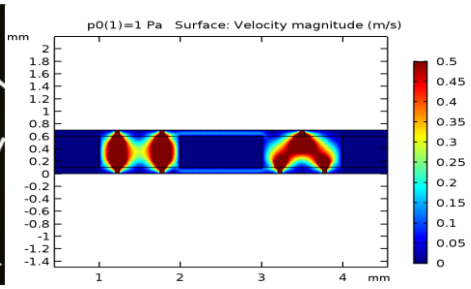
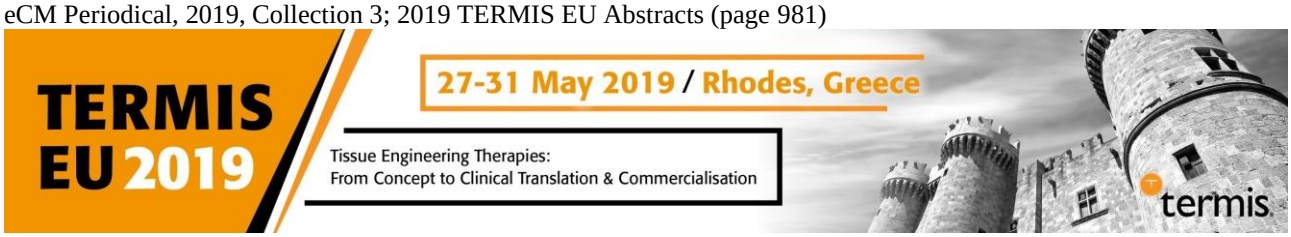


Figure 1: Deformation of the tissue building block. (A) A cell-laden tissue building block with the geometry of a pentagon was fabricated by photo patterning. (B) Deformation of the tissue building block on day 8 (left). **Figure 2:** Velocity profile indicates directed fluid flow patterns in tissue building blocks. Flow speed = 0.11m/s, cell experience 5dyn/cm^2 shear stress (right).

ACKNOWLEDGEMENTS: This work is supported by an ERC Consolidator Grant under grant agreement no 724469.



The effect of secondary crosslinking on material and biological properties of alginate and alginate-based 3D fibrous scaffolds

J. Idaszek[1], M. Volpi[1,2], M. Nguyen Quoc[1], M. Constantini[1,3], N. Contessi[2], S. Farè[2], W. Święszkowski[1]
Presenting Author: Joanna Idaszek, joanna.idaszek@pw.edu.pl
[1]Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland [2]Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy [3]Department of Soft Condensed Matter, Institute of Physical Chemistry of Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Alginate is a natural biopolymer which undergoes rapid gelation in the presence of divalent cations, such as calcium, strontium or barium [1]. This property made it a very popular biomaterial for 3D printing technologies. However, the crosslinked alginate is very unstable in physiological conditions due to substitution of the divalent cations by monovalent sodium. In order to enhance the stability of the printed alginate scaffolds, secondary crosslinking might be performed. Nevertheless, the ion exchange still takes place and leads to release of biologically active cations.
The aim of this study was to examine the effect of the secondary crosslinking of alginate 3D fibrous scaffolds on their material and biological properties.

METHODS: Alginate scaffolds were fabricated using Bioplotter (EnvisionTEC) equipped with a custom-made coaxial needle and microfluidic pumps after thorough optimization of the printing parameters (crosslinker molarity, flow rates, XY speed). After printing, the scaffolds were immersed in 0.03M and 0.3M solutions of $CaCl_2$, $SrCl_2$ and their mixture for 5 and 30 min. Subsequently, shape fidelity, swelling and mechanical properties were evaluated using confocal microscope, gravimetric and compression test, respectively. The scaffolds were also incubated in cell culture media and the obtained extracts used to study bioactivity with L929 fibroblasts, human mesenchymal stem cells (HMSC) and human umbilical vain endothelial cells (HUVEC) with MTS and alkaline phosphatase (ALP) assays.

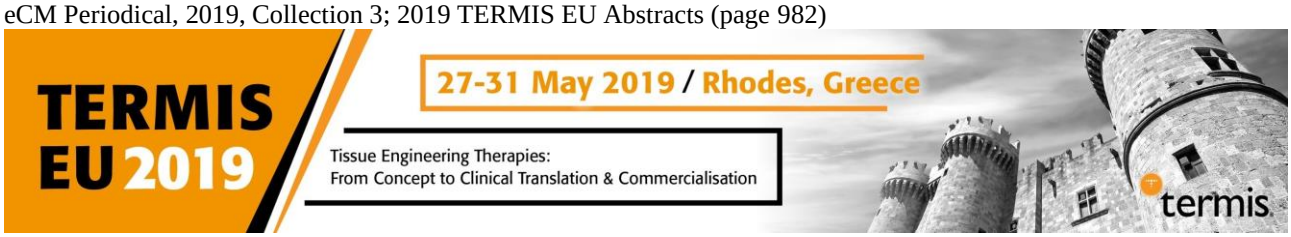
RESULTS & DISCUSSION: Application of the coaxial needle enabled fabrication of hydrogel scaffolds with controlled porosity. The time of the secondary crosslinking and molarity of the crosslinking solution had a significant effect on the fiber diameter, mechanical properties and swelling behavior. The released ions did not exhibit any cytotoxic effect. Interestingly, the ALP activity was increased in the HMSC cultured with the extracts. The most pronounced increase (65% higher than in the control, fig. 1) was measured for cells exposed to extracts obtained from the samples crosslinked with $CaCl_2$/$SrCl_2$ mixture for 30 min.

CONCLUSIONS: The obtained results suggest that the crosslinking agent for alginate scaffolds might exert a biological effect on the encapsulated cells.

ACKNOWLEDGEMENTS: This work was supported the STRATEGMED programme (contract no. STRATEGMED3/305813/2/NCBR/ 2017)

REFERENCES

[1] Lee KY et al. Prog Polym Sci. 2012; 37:106-126



Injectable Hhydrogels with self-healing ability for tissue regeneration

J.M.M. Rodrigues[1], A.M.S. Costa [1], J.F. Mano[1]
Presenting Author: João M. M. Rodrigues, jrodrigues@ua.pt
[1]Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

INTRODUCTION: Hydrogels, as highly hydrated 3D networks, have proved their potential as body tissue mimics [1,2]. However, the current strategies still fail in obtaining materials with all the following properties: cytocompatibility, injectability, self-healing, mechanical robustness and easy and fast processing [3]. Herein, a novel derivative of laminarin (LAM) polysaccharide was prepared and used to produce hydrogels with potential for Tissue Regeneration purposes.

METHODS: LAM from Eisenia Bicyclis (Carbosynth Limited, UK) was chemically modified to yield the LAM-CAT derivative. First, a nucleophilic substitution of the hydroxyl groups on LAM was done using allyl bromide (Sigma Aldrich). Carboxylic acid groups were incorporated in the former derivative using thiolactic acid (Sigma Aldrich). The previous steps were followed to enable the coupling of dopamine hydrochloride (Sigma Aldrich) to the LAM backbone. Afterwards, the resultant LAM-CAT was purified through dialysis and used to form hydrogels upon adding iron ions (Sigma Aldrich).

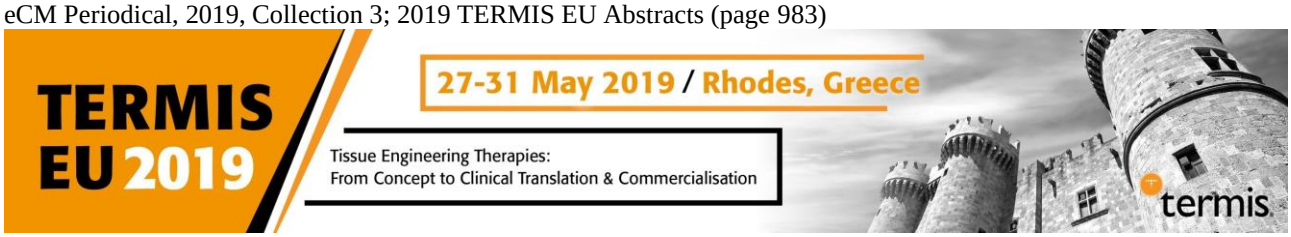
RESULTS & DISCUSSION: $^{1}H/^{13}C$-NMR spectroscopy and FTIR confirmed the successful functionalization of LAM through a: (i) nucleophilic substitution process using allyl bromide; (ii) thiol-ene Michael addition reaction to incorporate carboxylic acid functional groups; and (iii) EDC/NHS coupling chemistry to attach catechol groups to LAM backbone. Importantly, the developed strategy can be used to modify other polysaccharides with a similar chemical structure to LAM. LAM-CAT polymer was further used to form hydrogels through coordination bonds between the catechol groups presented on LAM-CAT derivatives and Fe^{3+} ions (Figure 1). The hydrogel formation was followed by rheology. Interestingly, the obtained robust hydrogels were formed under mild conditions and were able to self-heal upon rupture and to be administered through minimal invasive techniques. LAM-CAT cytocompatibility was proved by producing cell-loaded hydrogels with high cell viability rates. Other cargos such as sensitive growth factors can also be incorporated within the previous 3D networks.

CONCLUSIONS: LAM-CAT was prepared, characterized and employed to fabricate hydrogels with applicability as cell supporters for tissue engineering, as bioadhesives for diverse biomedical applications and/or as cargo devices for drug delivery.

ACKNOWLEDGEMENTS: "ATLAS" (ERC-2014-ADG-669858) funding under Horizon 2020. J.M.M.R. thanks COST ENBA Ca15216 Action and A.M.S.C. to FCT(SFRH/BD/101748/2014).

REFERENCES

[1] Slaughter B V et al. Adv Mater. 2019; 21(33):3307-29
[2] Lee K Y et al. Chem Rev. 2001;101(7):1869-79
[3] Liu M et al. Bone Res. 2017;5:17014-33



Fibrin-heparin coatings of cardiovascular implants serving as a reservoir of growth factor promoting endothelialization

J. Kucerova, Z. Riedelova, E. Brynda, T. Riedel
Presenting Author: Johanka Kucerova, kucerova@imc.cas.cz
Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic

INTODUCTION: Thrombosis of artificial cardiovascular implants remains a critical point that hinders successful medical treatment of cardiovascular diseases. Immediately, after contact with blood any foreign surface induces activation of coagulation and fibrin clot formation. Although, there are some improvements still the main challenge faced by many scientists is to totally suppress the acute as well as the late thrombosis of the artificial surface and to promote endothelialization of the implant. Once possibility how to improve the biocompatibility of cardiovascular implants it is coating with fibrin network [1,2]. Fibrin is formed at the place of vessel injury, accumulates various extracellular matrix proteins and growth factors, and by serving as a provisional matrix for numerous cells invading the wound it takes a crucial part in reendothelialization and wound healing.

METHODS: In this study, we present a method of artificial surface modification with a controlled thin layer of fibrin network polymerized from the artificial surface with covalently attached heparin via Schiff base formation and reductive amination. In addition, fibrin network was used for attachment of FGF (Fibroblast growth factor) and VEGF (Vascular endothelial growth factor) via their heparin-binding domains.

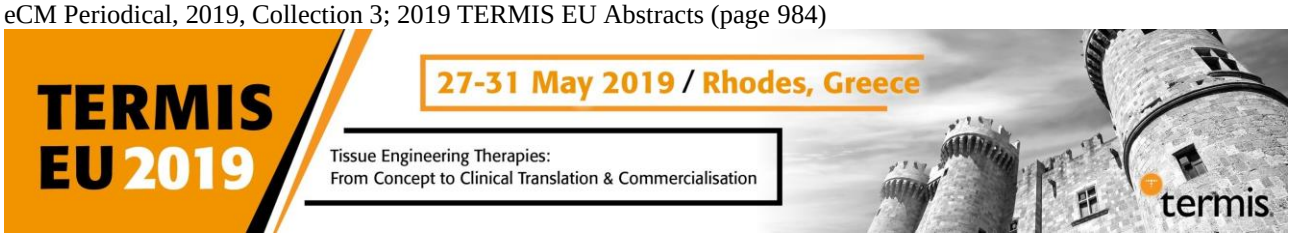
RESULTS & DISCUSSION: The amount of heparin and GF was measured by a chromogenic assay and ELISA. Human umbilical vein endothelial cells (EC) were used to test the effect of the coatings on EC viability, growth and morphology. FGF significantly promoted cell growth, especially in higher concentrations, while the effect of VEGF was less visible. The morphology of EC was influenced by the GF. In case of FGF the shape of EC was elongated after 7 days, while with VEGF the cells had a cobblestone-like structure. Best results were obtained when a combination of both GF was used at the same time confirming their synergic effect on the EC.

CONCLUSIONS: Results & In this work, we show that fibrin network can be considered as one of the most versatile coating for various cardiovascular implants. Fibrin-heparin coating inhibits coagulation and together with GF it promotes EC proliferation and differentiation into mature endothelium.

ACKNOWLEDGMENTS: This work was supported by the Ministry of Education, Youth and Sports of CR within the National Sustainability Program II, Project BIOCEV-FAR LQ1604.

REFERENCES

[1] Riedel T. et al. J. Biomed. Mater. Res. A 2009, 88, 437-447
[2] Riedelova-Reicheltova et al. Physiol Res. 2016, 65, S263-S272



Biomimetic cartilage/bone interface

W. Baumgartner[1], L. Otto[1], S.C. Hess[2], W.J. Stark[2], S. Märsmann[1,3], M. Calcagni[1], P. Cinelli[3], J. Buschmann[1]
Presenting Author: Johanna Buschmann, johanna.buschmann@usz.ch
[1]Plastic Surgery and Hand Surgery, University Hospital Zurich, Zurich, Switzerland [2]Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093 Zurich, Switzerland [3]Trauma Surgery, University Hospital Zurich, Zurich, Switzerland

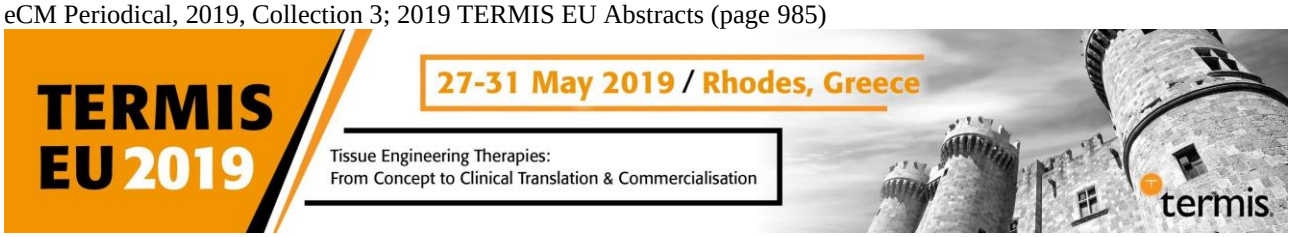
INTRODUCTION: The interface between cartilage and bone is characterized by a gradient of calcium phosphate phases that decline in content from bone towards cartilage. Adipose-derived stem cells are able to differentiate into chondrocytes and osteoblasts, offering the option to use one single cell source for tissue engineering of a biomimetic cartilage/ bone interface.

METHODS: We used an electrospun poly-lactic-co-glycolic acid (PLGA) mesh with incorporated amorphous calcium phosphate nanoparticles in different weight percentages, having a gradient from 30%, 20%, 10% and 0% towards the cartilage mimicking side of the biomimetic interface. The materials were seeded with human adipose-derived stem cells (ASCs) and either cultivated under static conditions or under dynamic conditions in a perfusion bioreactor without any further supplementation of the culture medium. After a total of four weeks, quantitative RT-PCR was performed for eleven relevant genes, including typical marker genes for osteo- and chondrogenesis, but also for adipo- and angiogenesis. In addition, histology and immunohistochemistry was performed to address cell density, proteoglycans and glycosaminoglycans.

RESULTS & DISCUSSION: Under static conditions, the presence of amorphous calcium phosphate nanoparticles did not have any impact on osteo- and chondrogenesis related genes – only CD31 (and endothelial marker gene) was upregulated in the presence of nanoparticles compared to pure PLGA. In contrast, under dynamic conditions, ASCs exhibited an increased expression of chondrogenic marker gene Sox9 towards the cartilage mimicking side. In addition, ASCs showed an increased expression of osteogenic marker gene osteocalcin (OC) towards the bone mimicking side, accompanied by increased OC protein expression. The results found on the gene level were supported by findings on the protein level.

CONCLUSIONS: We conclude that amorphous calcium phosphate nanoparticles incorporated in electrospun PLGA meshes influence the differentiation behavior of human ASCs. Electrospun meshes with gradients of nanoparticles may act as promising cartilage/bone interfaces when cultivated under perfusion in a bioreactor system.

ACKNOWLEDGEMENTS: We thank Andrea Garcete-Bärtschi and Ines Kleiber-Schaaf for Sox9, Alcian Blue and Safranin O staining.



Stem cell-seeded hybrid nanocomposite for chest wall repair in a murine model

J. Buschmann[1], Y. Yamada[2], K. Schulz-Schönhagen[3], S.C. Hess[3], W. J. Stark[3], C. Opelz[4], W. Weder[4], W. Jungraithmayr[4,5]
Presenting Author: Johanna Buschmann, johanna.buschmann@usz.ch
[1]Division of Plastic and Hand Surgery, University Hospital Zurich, Switzerland [2]Department of Thoracic Surgery, Kyoto University Hospital, Kyoto, Japan [3]Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093 Zurich, Switzerland [4]Division of Thoracic Surgery, University Hospital Zurich, Switzerland, [5]Department of Thoracic Surgery, University Hospital Rostock, 18057 Rostock, Germany

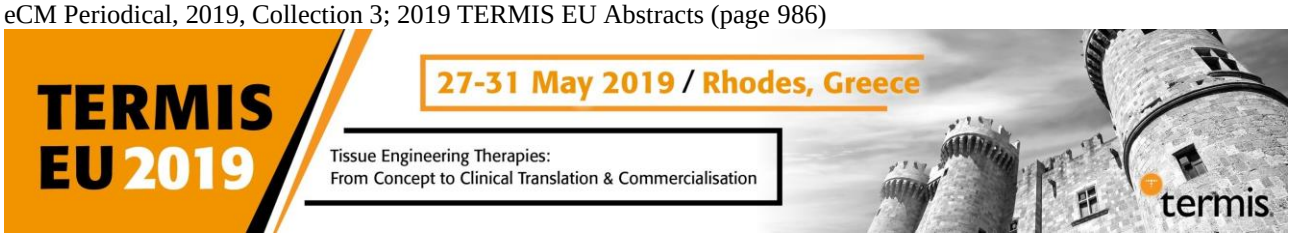
INTRODUCTION: Resection of the thoracic wall is a common procedure to remove malignant tumours. Usually, Gore-Tex® is used to replace the missing tissue; however, it is inert and not degradable. Novel biodegradable materials are nowadays available that stimulate and guide the regeneration process. Furthermore, stem cell seeding may help to achieve a good graft integration.

METHODS: A biodegradable bi-layered hybrid nanocomposite material developed, based on poly-lactic-co-glycolic acid and amorphous calcium phosphate nanoparticles (PLGA/aCaP) and pure PLGA. Mechanical properties under dry (time point 0) and wet conditions (time point 2 weeks) were assessed. Electrospun meshes were seeded with murine adipose-derived stem cells (ASCs of C57BL/6). In a mouse model (C57BL/LY5.1), ASC-seeded hybrid scaffolds were implanted as a chest wall graft in order to study biointegration. After 4 and 8 weeks, implant integration towards the skin and the lung, cell infiltration into the bi-layered material, inflammatory responses, neo-vascularization, fibrosis and ECM components (collagen I and fibronectin) were determined in six different zones of the graft.

RESULTS & DISCUSSION: The bi-layered nanocomposite had an ultimate stress of 6 MPa after 2 weeks in DMEM, which was significantly higher than for the pure PLGA/aCaP where it was approximately 0. In vivo, it was very well integrated and cellular infiltration through the whole material was observed. The layer of pure PLGA had shrunk and was visible as “voids” in the H&E stained sections, while the PLGA/aCaP had partially degraded and the fibers were completely surrounded by newly formed extracellular matrix (ECM). In the ECM, collagen I increased from 4 to 8 weeks, for both, the ASC-seeded and the cell-free scaffolds, while the amount of fibronectin was similar at 4 and 8 weeks for both scaffolds. As for the vascularization, the cell-free scaffolds were more vascularized than the ASC-seeded ones at 8 weeks post-operation.

CONCLUSIONS: Hybrid scaffolds of two layers, one with PLGA and one with PLGA/aCaP are superior in terms of mechanical stability than pure PLGA/aCaP. Murine ASCs from C57BL/6 evoked a mild inflammatory response in C57BL/LY5.1 mice when seeded onto the hybrid scaffold. Despite of this reaction, the stable bi-layered scaffold was very well integrated. Although vascularization was higher in the cell-free hybrid scaffold – probably due to space limitation for invading endothelial cells in the ASC-seeded scaffolds where all pores were filled with ASCs –, it was still vascularized homogenously through the whole material, again showing its superior properties when compared to Goretex® which is not vascularized at all.

ACKNOWLEDGEMENTS: We thank Ines Kleiber-Schaaf and Andrea Garcete-Bärtschi for histological and immunohistochemical staining.



From replicative senescence to cell models, diagnostics and therapies of age-associated diseases: stories of translation

M. Hackl[1], I. Lämmermann[2], O. Kanzler[1,3,4], R. Grillari-Voglauer[2,3,4], J. Grillari[2,3,4,5]
Presenting Author: Johannes Grillari, Johannes.grillari@evercyte.com
[1] TAmiRNA GmbH [2]Department of Biotechnology, BOKU – University of Natural Resources and Life Sciences, Vienna, Austria [3]Evercyte GmbH [4]LBI for Experimental and Clinical Traumatology [5]Austrian Cluster for Tissue Engineering, Austria

INTRODUCTION: Cellular senescence was initially proposed to be an in vitro model of aging, was widely disputed as a mere in vitro artefact but has now been proven to clearly contribute to aging and to the onset and progression of all major age-related diseases including cardiovascular disease, osteoporosis, osteoarthritis, COPD, chronic kidney disease and neurodegenerative disease [1].

METHODS: Our studies aimed at understanding cellular senescence and its contribution to organismal aging as well as counteracting the irreversible growth arrest accompanied by it. During these studies we developed several human, primary cell lines including mesenchymal stem cells immortalized by telomerase [2], a technology that maintains the functions of primary cells, while sufficient cell material for standardized cell based assays or the production of complex biologicals including extracellular vesicles can be produced in standardized conditions. In order to bring these cell lines as well as services including the customer-tailored generation / gene editing of telomerized cells and the establishing of cell based assays to the market we have established Evercyte GmbH in 2011.

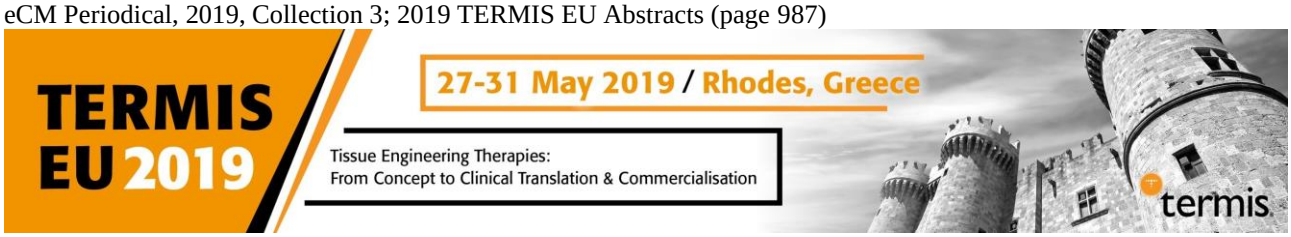
RESULTS & DISCUSSION: Furthermore, we identified miRNAs in extracellular vesicles to be secreted by senescent human endothelial cells and to inhibit osteogenic differentiation in vitro [3]. This led to the idea that specific circulating miRNAs might serve as biomarkers for diagnosis of bone diseases, specifically fracture risk assessment. Promising results in this regard led to foundation of TAmiRNA in 2013, the only company world-wide developing signatures of circulating miRNAs towards certified diagnostics outside the field of oncology, specialising in osteoporosis, liver diseases, senescence as well as drug-induced organ-specific toxicology.
Finally, we identified novel potential senolytic drugs, drug that specifically eliminate senescent cells and which are supposed to have major impact on postponing the onset of age associated diseases. A concept to translate these findings within a company to be founded soon is currently developed.

CONCLUSIONS: Within the session on „Academia to industry: building the bridge“ we will showcase and discuss rationale, approach and potential pitfalls of translating scientific insights into products from which patients and society benefit.

ACKNOWLEDGEMENTS: Financial support was received from FFG, aws, EU-FP7, EU SME instrument, EU-IMI.

REFERENCES

[1] Olivieri F et al. Mediators Inflamm 2018; 2018:9076485
[2] Wolbank S et al. Tissue Eng Part A. 2009;15(7): 1843-54
[3] Weilner S et al. Aging Cell 2016; 15(4):744-54



Isolation of single donor fibrinogen (sDFib) from single donor blood plasma bags

J. Hackethal[1,2], P. Slezak[1,2], C. Gabriel[2,4], A. H. Teuschl[2,3], P. Dungel[1,2], H. Redl[2]
Presenting Author: Johannes Hackethal, johannes.hackethal@trauma.lbg.ac.at
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in AUVA Trauma Research Center, Vienna, Austria [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria [3]Department of Life Science Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria [4]Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria

INTRODUCTION: Fibrinogen is a naturally occurring protein in the blood and precursor to fibrin which is the end product of blood coagulation. As such, it's a perfect base material for clinically used hemostats and tissue glues. Additionally, it may also act as a local reservoir for growth factors, a matrix for cell growth and can foster regenerative processes in wound healing [1,2]. Autologous-derived fibrinogen would have many advantages for clinical applications as it relieves from the risk of potential biologic contamination in hetereologic plasma products.

METHODS: Fibrinogen (sDFib) was extracted from single donor blood plasma bags using 2.2 M glycine and characterized by using BCA assays, polyacrylamide gel electrophoresis, prothrombin-detection and ROTEM thromboelastography.

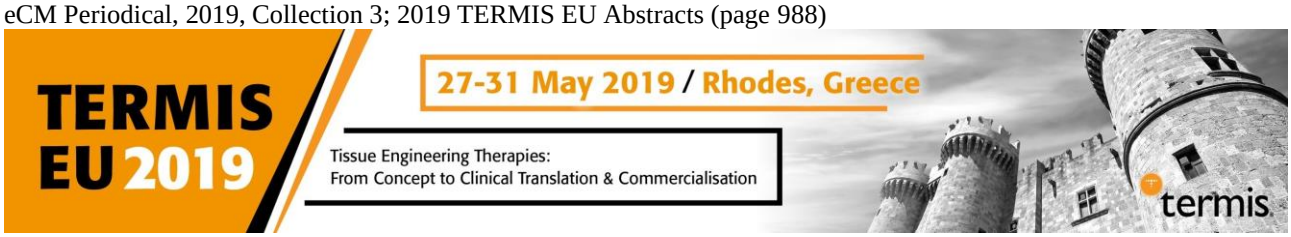
RESULTS & DISCUSSION: We could isolate fibrinogen at concentrations > 20g/L (20.6g/L mean), comparable to commercial fibrinogen concentrates like Haemoclompettan P (CLS Behring). No further impurities (such as prothrombin) were detected in sDFib. No significant differences in clotting time compared to fibrinogen from Tisseel, Baxter were observed.

CONCLUSIONS: We have developed a method to isolate a concentrated fraction of autologous or single donor allogeneic fibrinogen (sDFib). Our isolation procedure would be capable to be manufactured in a closed container system and may be useful for transfusion to become a new alternative to current pooled fibrinogen products for patients.

ACKNOWLEDGEMENTS: The authors like to thank Dr. Thomas Seelich for constant technical inputs for many years. Financial support was received from the City of Vienna Competence Team Project Signal Tissue (MA23, #18-08) and COST Bioadhesives.

REFERENCES

[1] Philipp Heher et al. Expert Rev Med Devices. 2018 Oct;15(10):747-755.
[2] Paul Slezak et al. J Invest Surg. 2018 Mar 21:1-8.



Maxillofacial reconstruction using patient-specific 3D-printed biodegradable scaffold

J.W. Rhie[1], Y.J. Lee, S.H[1]. Moon, J.H. Shim[2], H.H. Han[1], D.W. Cho[3]
Presenting Author: Jong Won Rhie, rhie@catholic.ac.kr
[1]Department of Plastic and Reconstructive Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea,Seoul 06591, Korea [2]Department of Mechanical Engineering, Korea Polytechnic University, 237 Sangidaehakro, Siheung, Gyeongi, 15073, Korea [3]Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheong-Am Ro, Nam-gu, Pohang, Kyungbuk, 37673, Korea

INTRODUCTION: Management of significant facial bone defects or asymmetry are challenging. Conventional options include autogenous bone grafts or alloplastic implants. However, bone grafts are limited by donor morbidity, unpredictable results and long operation time. Alloplastic implants are mainly non-absorbable and may cause foreign body reaction. We present the application of a 3D-printed patient specific absorbable mesh implant for reconstruction of bone defect caused by tumor resection.

METHODS: A customized polycaprolactone (PCL) scaffold was designed and fabricated for each patient. For this purpose, we used computer-aided design and manufacturing combined with 3D printing technology. The patients implanted with the PCL scaffolds were followed up for up to 2 years with careful evaluation of morphological changes in the face. We have experienced 5 more clinical case of craniofacial reconstruction with patient specific 3 D printed implant

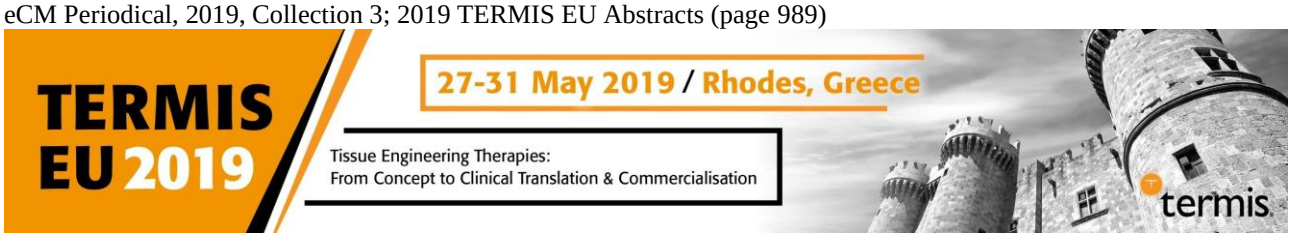
RESULTS & DISCUSSION: We confirmed that the patient-specific 3D-printed PCL scaffold effectively filled the maxillary defect and promoted regeneration of the deficient tissue while remaining stable in the body for a relatively long period of time.

CONCLUSIONS: Employing customized tissue-engineered scaffolds built using the patient's CT data and an extrusion-based 3D printing system is safe and clinically feasible, helping create and maintain improved morphological features of the face, which represents the most important aspect from the perspective of the patients.

ACKNOWLEDGEMENTS: This work was financially supported by the Ministry of Trade Industry and Energy of Korea (10062127 and 10063334) and by the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017M3A9E2060428).

REFERENCES

[1] Shim, J.-H., Yoon, M.-C., Jeong, C.-M., et al. Biomedical Materials 2014;9:065006.
[2] Esses, S. J., et al. American Journal of Roentgenology 2011;196: W683-W688.
[3] Hollister, S. J. Nature Materials 2005;4:518-524.
[4] Shim, J.-H., Huh, J.-B., Park, J. Y., et al. Tissue Engineering Part A 2012;19:317-328.



Effectiveness of microchannel-based nerve conduit for peripheral nerve regeneration of rats

J. Jeon[1,2], M.S. Kim[1,2], J.-W. Kim[1,2,3], J.K. Hyun[1,2,3,4]
Presenting Author: Jooik Jeon, jooikJ@gmail.com
[1]Department of Nanobiomedical Science and BK21 PLUS NBM Global Research Center for Regenerative Medicine [2]Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, Korea [3]Wiregene, Co., Ltd. [4]Department of Rehabilitation Medicine, College of Medicine, Dankook University College of Medicine, Cheonan, Korea

INTRODUCTION: Peripheral nerves can regenerate spontaneously after damage, however, their regeneration ability is limited and functional deficits would be continued following major peripheral nerve injuries [1]. The nerve defects exceeding 5mm have been treated with autologous nerve grafts, allografts or hollow tube type nerve conduits in clinical settings, but complete functional recovery has seldom been reported [2]. Herein, we developed a nerve conduit containing intraluminal structure with unidirectional microchannels to give a physical cue for outgrowing axons of peripheral nerves, and applied the conduit to rats with long defect in the sciatic nerve.

METHODS: To develop microchannels within a nerve conduit, phosphate glass fibers were used as frames, and dissolved poly (lactic-co-glycolic acid) (PLGA) or polycaprolactone (PCL) was filled microchannels. Nerve growth factor (NGF) was also internalized into heparin-treated nerve conduit whether NGF may enhance axonal regeneration in addition to microchannel structure. Lewis rats were used, and 16mm-nerve gap was created after unilateral sciatic nerve transection. Three experimental groups, which received PLGA (PLGA group), PLGA with NGF (PLGA+NGF group), or PCL nerve conduits (PCL group) respectively, and two control groups, which was performed autograft (autograft group) or received hollow tube without intraluminal structure (hollow group), were compared each other until 16 weeks after implantation.

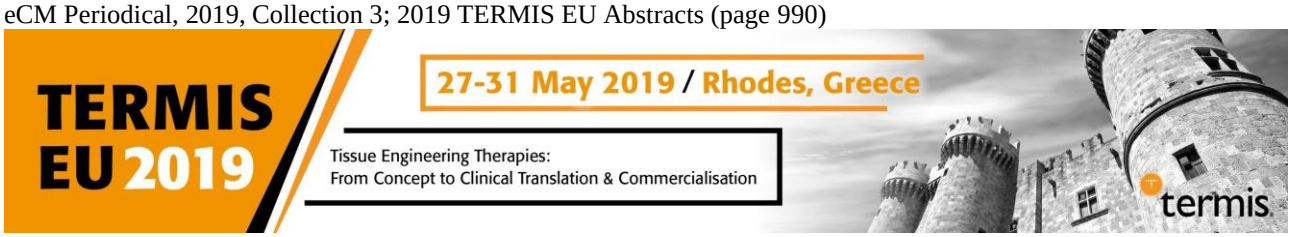
RESULTS & DISCUSSION: All experimental and control groups showed gradual motor and sensory recovery until sacrifice. The number of axons at the proximal end of distal stumps 16 weeks after implantation were higher in autograft and PCL groups than in any other experimental and control groups, and there was no difference between autograft and PCL groups. The sciatic static index, sciatic functional index, thermal sensory test also showed that the motor and sensory recovery was prominent in autograft and PCL groups than in other experimental and control groups from 11 weeks to sacrifice.

CONCLUSIONS: Our new microchannel-based nerve conduit, especially PCL-based, is effective for axonal regeneration after peripheral nerve injury, and nerve growth factor did not show any synergistic effects for nerve regeneration. We conclude that our PCL nerve conduit might substitute for autograft after peripheral nerve transection injury.

ACKNOWLEDGEMENTS: Korea Healthcare Technology R&D Project (HI14C0522), the Commercialization Promotion Agency for R&D Outcomes (COMPA, 2018K000276) and National Research Foundation (2018K1A4A3A01064257, 2018R1D1A1A02047523), Republic of Korea.

REFERENCES

[1] Kim YP et al. J Tissue Eng Regen Med. 2015; 9(3):236-46
[2] Lee SK and Wolfe SW. J Am Acad Orthop Surg 2000;8(4):243-52



A novel, rapid and cost-effective method for separating drug-loaded liposomes

J. Jose[1], V. Muthuvijayan[1]
Presenting Author: Joseph Jose, josephjosekanichai@gmail.com
[1]Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras, Chennai, India

INTRODUCTION: Liposomes are the first nano-scale drug delivery systems that have been transformed into successful clinical products. Over the past few decades, a great deal of work has gone into innovating, improving, and perfecting several aspects of the liposomal drug delivery system. Separating the drug-loaded liposomes from the free drug is an integral part of producing liposomal drug formulations. Conventionally, this separation is achieved using techniques like, ultracentrifugation, chromatography or dialysis. All these methods have their drawbacks, such as cost, scalability and efficiency [1][2][3]. The objective of this work is to develop a simpler, cheaper, and easily scalable technique to perform this separation step.

METHODS: Liposomes were successfully synthesised and precipitated out using different solvents. The efficiency of this separation was compared to other conventional techniques like ultracentrifugation and ultrafiltration. The morphological integrity of the precipitated liposomes was confirmed using dynamic light scattering and electron microscopy. The release profile of doxorubicin from these precipitated liposomes was compared to liposomes separated by the conventional methods.

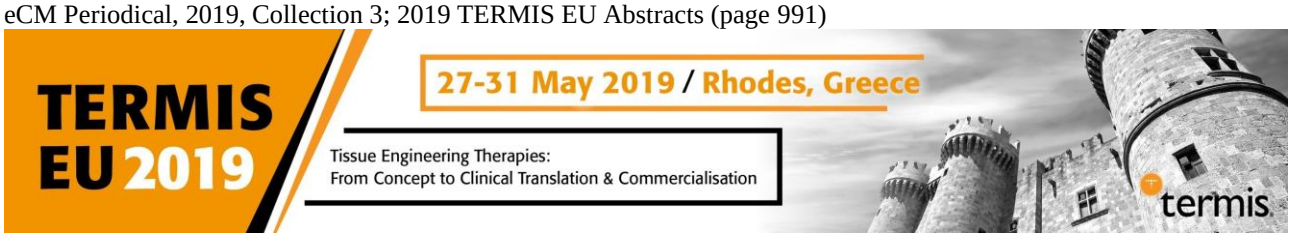
RESULTS & DISCUSSION: This novel precipitation technique was able to separate almost 100% of the liposomes (Figure 1). The structural integrity of the liposomes was found to be preserved, even after precipitation step. The release profile of doxorubicin from the liposomes separated by different techniques (solvent-based precipitation, ultracentrifugation and ultrafiltration) was found to be similar.

CONCLUSIONS: This novel method proves to be an efficient, convenient and a simpler technique to separate liposomes from the free drug compared to conventional techniques.

ACKNOWLEDGEMENTS: Financial support was received from the Project no. BT/PR13880/BBE/117/44/2015, Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, India

REFERENCES

[1] S. R. Dipali, S. B. Kulkarni, and G. V Betageri J. Pharm. Pharmacol., vol. 48, no. 11, pp. 1112–1115, 1996.
[2] J. L. Heeremans et al. J. Drug Target., vol. 3, no. 4, pp. 301–10, 1995.
[3] R. Magin et al. Biotechnol. Tech. vol. 1, no. 3, 185-188, 1987.



A caprine model of intervertebral disc degeneration: a testing platform for an injectable hydrogel

J. Snuggs[1], C. Rustenberg[2], K. Emanuel[2], S. Partridge[1], C. Sammon[1], T. Smit[2], C. Le Maitre[1]
Presenting Author: Joseph Snuggs, J.Snuggs@shu.ac.uk
[1]Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, UK [2]VU University Medical Centre, Amsterdam, The Netherlands

INTRODUCTION: Low back pain affects 80% of the population at some point in their lives with 40% of cases attributed to intervertebral disc (IVD) degeneration. There are many animal models to investigate IVD degeneration, such as murine and porcine models. However due to significant differences in physiology between these types of models and humans, such as the preservation of notochordal cells into adulthood, they are not completely representative of the human condition. Therefore, a more representative large animal model for IVD degeneration is needed to mimic human degeneration. Here we investigate a caprine IVD degeneration model for its use in testing our synthetic, Laponite® cross-linked pNIPAM-co-DMAc, injectable hydrogel (NPgel). NPgel has been shown to induce differentiation of human MSCs (hMSCs) towards a nucleus pulposus (NP) cell phenotype without the need for additional growth factors in vitro [1]. Through utilising the caprine model of IVD degeneration we aim to validate whether the NPgel retains these characteristics in vivo.

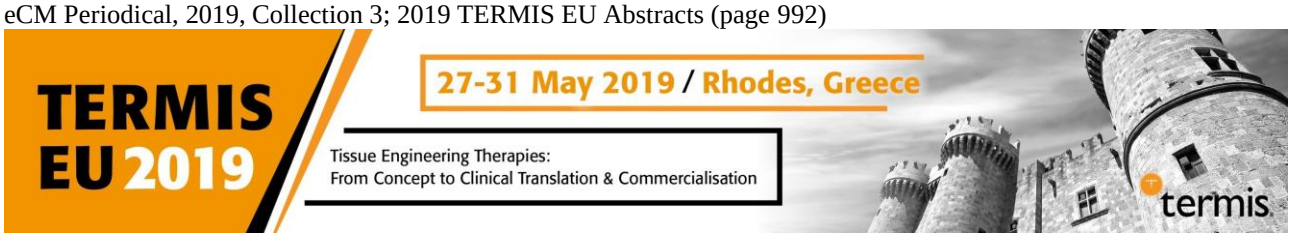
METHODS: After three days of culture in a bioreactor under diurnal, simulated-physiological loading (SPL) conditions, 33 healthy lumbar caprine IVDs were degenerated enzymatically by injecting 50 uL of 1 mg/mL collagenase and 2 U/mL chondroitinase ABC (cABC). After injection, the IVDs were subjected to SPL again after two hours of digestion, for another 10 days. A no-intervention and phosphate buffered saline (PBS) injected group were used as controls. Disc deformation was continuously monitored and changes in disc height recovery behaviour were quantified using stretched-exponential fitting. Histological staining was performed on caprine discs to assess extracellular matrix (ECM) production and immunohistochemistry (IHC) was performed to determine expression of catabolic protein expression. Following establishment of the IVD model, NPgel was injected into goat IVDs and similar tests performed.

RESULTS & DISCUSSION: The injection of collagenase and cABC had severe effects on the mechanical behavior of the IVDs, especially in time constants and creep behaviour (Figure 1). These changes were progressive over time. Histological staining identified a decrease in ECM components such as collagens and glycosaminoglycans (GAGs) in enzyme injected discs. IHC identified an increase in degrative enzymes such as MMP3, MMP13 and ADAMTS4 and a decrease in aggrecan and collagen II also in enzyme treated discs.

CONCLUSIONS: Here we show a novel, reproducible large animal model of IVD degeneration which mimics IVD degeneration. This model allows the testing of biomaterials and other potential treatments of IVD degeneration on a scale more representative of the human disc than more commonly used murine and porcine models. The development of treatments for IVD degeneration is hindered using models which do not closely represent human IVD degeneration. Here we have established a reproducible, large animal model of IVD degeneration that has the potential for use in the screening of biomaterials and other treatments for IVD degeneration.

REFERENCES

[1] Thorpe AA et al., (2016). Acta Biomater. 36:99-111.



Differentiation of mesenchymal stem cells in an injectable hydrogel under the conditions of the degenerate intervertebral disc

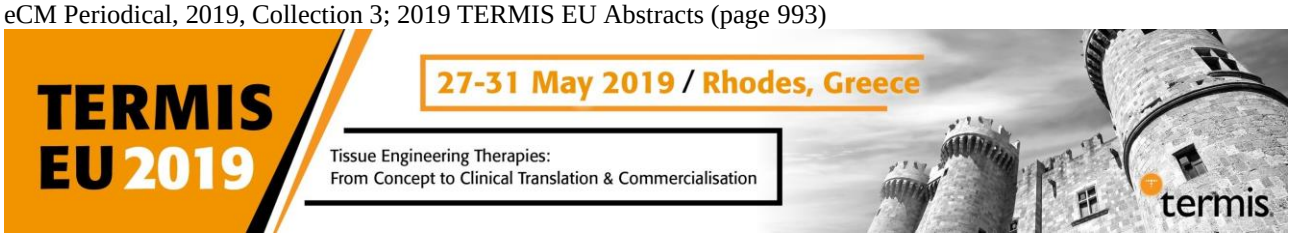
J. Snuggs[1], A. Thorpe[1], C. Hutson[1], S. Partridge[1], C. Sammon[1], C. Le Maitre[1]
Presenting Author: Joseph Snuggs, J.Snuggs@shu.ac.uk
[1]Sheffield Hallam University, Sheffield, UK

INTRODUCTION: Low back pain affects 80% of the population at some point in their lives with 40% of cases attributed to intervertebral disc (IVD) degeneration. IVD degeneration is caused by a cascade of cytokine and catabolic enzyme production leading to the destruction of extracellular matrix (ECM) and irreversible alterations to the disc microenvironment, which all contribute to the loss of IVD function. We have previously reported the development of NPgel, a synthetic, Laponite® cross-linked pNIPAM-co-DMAc, injectable hydrogel. NPgel has been shown to induce differentiation of human MSCs (hMSCs) towards a nucleus pulposus (NP) cell phenotype without the need for additional growth factors in vitro and also fully integrates with NP tissue following injection into the disc. However, the translation of this potential treatment strategy into a clinical application is dependent on the survival and differentiation of hMSCs into the correct cell phenotype within the degenerate IVD. Here, we investigated the viability and the differentiation of hMSCs incorporated into NPgel cultured under conditions mimicking the healthy and degenerate disc.

METHODS: Human MSCs were cultured in monolayer before encapsulation into NPgel, at a density of $4x10^6$, and cultured for up to 4 weeks in 1 of 4 treatment groups (n=3). Each group mimicked conditions from, standard culture (DMEM, pH7.4), healthy disc (DMEM, pH7.1), degenerate disc (low glucose DMEM, pH6) or degenerate disc plus 10ng/mL IL-1β. All culture was performed at 5% oxygen concentration. MSCs were also collected at time-point 0h prior to treatment to serve as controls. Cell viability was assessed by immunohistochemistry for the apoptotic marker, cleaved caspase-3. Histological staining and immunohistochemical analysis investigated ECM synthesis and matrix degrading enzyme expression, specifically MMP3, MMP13 and ADAMTS4. Differentiation capacity of the human MSCs was determined using immunohistochemical analysis for NP extracellular matrix markers collagen type II and aggrecan.

RESULTS & DISCUSSION: Viability of hMSCs was maintained in NPgel; the immunopositivity of caspase-3 was increased in degenerate conditions, but >60% of cells in all treatment groups were viable after 4 weeks of culture. Collagen II and aggrecan immunopositivity increased over the culture period and levels were comparable between healthy and degenerate disc conditions. Expression of degradative enzymes remained low across all treatment groups for the culture duration and masson trichrome and alcian blue staining also confirmed the deposition of matrix within the NPgel scaffolds.

CONCLUSIONS: In agreement with our previous findings [1,2] NPgel was able to induce NP cell differentiation of MSCs, with expression of both aggrecan and collagen type II under standard culture conditions. Here we have shown that viability and NP cell differentiation of MSCs incorporated within NPgel was mostly unaffected by treatment with conditions such as low glucose, low pH and the presence of cytokines, all regarded as key contributors to disc degeneration. In addition, the NPgel was shown to prevent MSCs from displaying a catabolic phenotype with low expression of degradative enzymes, highlighting the potential of NPgel to differentiate hMSCs and protect them from the degenerate disc microenvironment. The NPgel described here not only has the potential to provide mechanical support and deliver MSCs for regeneration of the IVD but also may function to protect delivered hMSCs from the catabolic environment in the degenerate IVD.



ALK5 is essential for tooth germ differentiation during tooth development

W. Guo[1], J. Du[1]
Presenting Author: Juan Du, dujuang16@163.com
[1]Laboratory of Molecular Signaling and Stem Cells Therapy, Molecular Laboratory for Gene Therapy and Tooth Regeneration, Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Capital Medical University School of Stomatology, Beijing 100050, China

INTRODUCTION: The TGFβ superfamily of proteins participates in tooth development. TGFβ1 and TGFβ3 regulate odontoblast differentiation and dentin extracellular matrix synthesis [1]. Although the expression of TGFβ family member ligands is well-characterized during mammalian tooth development, less is known about the TGFβ receptor, which is a heteromeric complex consisting of a type I and type II receptors. The molecular mechanism of ALK5 (TGFβR1) in the dental mesenchyme is not clear.

METHODS: Tooth germ mesenchymal cells (TGMCs) from the lower first molar tooth germs of day 15.5 embryonic mice were harvested. Human recombinant TGFβ3 protein or an ALK5 inhibitor (SD208) was added to the cells. Cell proliferation was investigated by CCK8 assay, and Alizarin red staining and osteogenic and dentinogenic marker genes which were measured by real-time RT-PCR were checked to investigate the cell osteogenesis/dentinogenesis. The expressions of both canonical and noncanonical TGFβ signaling pathways genes were observed by real-time RT-PCR and western blot.

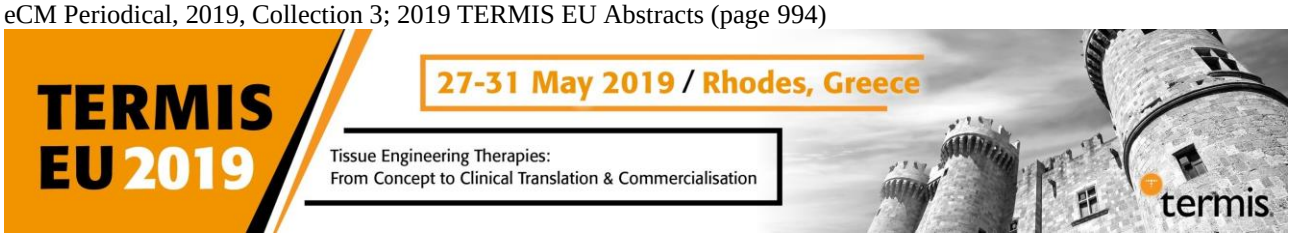
RESULTS & DISCUSSION: The number of cells was reduced significantly in the SD208 group compared to the control group at 1 and 2 days, whereas the TGFβ3 group exhibited an increased number of cells at 1 and 2 days. After 2 days, the cells clearly had differentiated and grew slowly. Alizarin red staining indicated that inhibition of ALK5 markedly decreased mineralization, whereas TGFβ3 increased mineralization of TGMCs. The optical density due to alizarin red in cultured TGMCs increased mineralization in the TGFβ3 group ($p < 0.05$) and decreased it in the SD208 group ($p < 0.05$) compared to the control after cell culture for 14 days. Osteogenic and dentinogenic markers, Dspp, Dmp1, Bsp, Ocn, Opn were increased significantly by TGFβ3 and decreased significantly when ALK5 was inhibited for 7 and 14 days compared to the control group ($p<0.05$). We also found TGFβ3 activated ALK5 and Smad4 and SD208 inhibited ALK5 and Smad4 during TGMCs osteogenesis and dentinogenesis. And inhibition of ALK5 increased the expression of P-TAK1 protein slightly (Figure 3E) and up-regulated P-p38 protein levels in TGMCs after culture for 24 and 48 h.

CONCLUSIONS: We found that TGFβ3/ALK5 signaling regulated the osteogenesis and dentinogenic differentiation potential of TGMCs by both Smad4-dependent and p38 protein kinase signaling pathways. Our findings suggest that both Smad4 and p38 MAPK may be possible targets for optimizing the use of stem cells of dental origin for application to tissue regeneration.

ACKNOWLEDGEMENTS: This work was supported by grants from National Natural Science Foundation of China (81570940 and 81873706 to J Du), and High-Level Talents of the Beijing Health System (2014-3-077 to J Du).

REFERENCES

[1] Derynck R et al. Nature 2003; 425(6958):577-84.



Optimisation of gelatin crosslinking in bicomponent electrospun nanofibres with the use of EDC/NHS

J. Dulnik, P. Sajkiewicz
Presenting Author: Judyta Dulnik, jdulnik@ippt.pan.pl
Laboratory of Polymers and Biomaterials, Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Introducing gelatin into synthetic polymeric materials has unquestionable advantages for tissue engineering. In our previous studies we have demonstrated the feasibility of using nontoxic solvents for electrospinning of polycaprolactone/ gelatin (PCL/Gt) blends [1], as well as a necessity of preserving the biopolymer within the fibre due to its susceptibility to dissolution in hydrous environment [2].
Looking for a compromise between cheap, fast and low toxic, a set of four crosslinking agents were examined during preliminary studies. **EDC/NHS** (1-ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride/N-hydroxy-succinimide) method proved to meet best our requirements.

METHODS: A broad set of varying conditions was established based on the results of EDC/NHS preliminary results. Electrospun materials with different PCL/Gt ratios were subjected to crosslinking with EDC/NHS solutions with varying concentrations for periods ranging from 1 to 9 hours. All samples underwent biodegradation studies, crosslinking degree tests, SEM imaging, as well as wettability and mechanical properties examination.

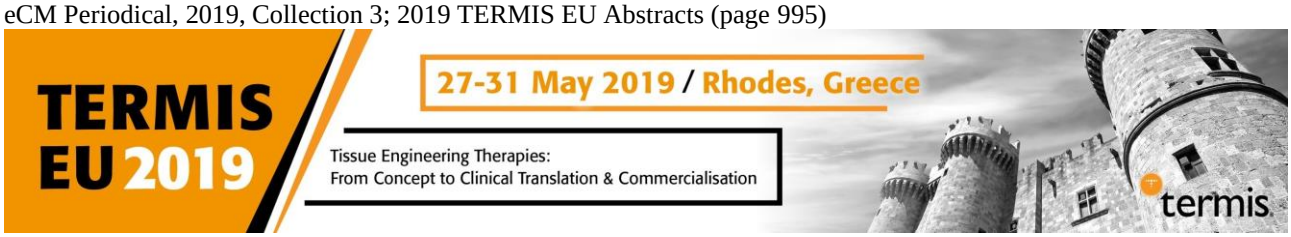
RESULTS & DISCUSSION: Analysis of gelatin crosslinking results, showed that the kinetics of the process is very fast at the beginning followed by slow stage at longer time of the process. The critical time, needed to get 85% of crosslinked gelatin, is a function of EDC/NHS solution concentration, being the shorter the higher is this concentration.

CONCLUSIONS: EDC/NHS method gives an operator the freedom in setting out crosslinking conditions since time and solution concentration are inversely interchangeable. For any studied conditions, this method gives satisfactory crosslinking degree cheaper and faster than any of its competition.

ACKNOWLEDGEMENTS: This work was funded by the Polish National Science Center (NCN) under the Grant No.:
2015/17/N/ST8/02027.

REFERENCES

[1] Denis P et al. Int. J. Polym. Mater. 2015; 64(7):354-364
[2] Dulnik J et al. Polym. Degrad. Stabil. 2016; 130:10-21



Novel electrospun implant ultrasonically coated with nHA particles for tissue engineering applications

J. Higuchi[1,2], B. Woźniak[1], A. Chodara[1], G. Fortunato[3], A. Dommann[3], W. Łojkowski[1]
Presenting Author: Julia Higuchi, juliaRT@labnano.pl
[1]Institute of High Pressure Physics Polish Academy of Sciences, Warsaw, Poland [2]Warsaw University of Technology, Faculty of Materials Science and Engineering, Warsaw, Poland [3] Swiss Federal Laboratories for Materials Science and Technology (EMPA), St. Gallen, Switzerlans

INTRODUCTION: Currently used polymeric resorbable and non-resorbable materials possess many structural, mechanical and bio-functional limitations. The use of biodegradable polymers in a form of fibrous meshes fabricated via electrospinning method are good choice to overcome these limitations due to the flexibility in the use of the components and various morphologies of the fibres [1]. The introduction of components like nanohydroxyapatite (nHA) have been proved to enhance bone tissue regeneration and neutralize material degradation, which would favour the regeneration of lost tissues. Therefore, electrospinning and application of nHA deposition on fibres were proposed to meet challenging clinical criteria [2].

METHODS: Electrospun fibrous meshes prepared from PDLLA and PLGA polymers were used as a base structure material. Coating of the fibrous structure with nHA particles was performed in aqueous solutions as a two stage ultrasonic process according to the patent [3]. We used two types of highly biocompatible hydroxyapatite ceramic nanoparticles (15nm and 45nm) for implant fabrication. It was proved that surface application of ceramic nanoparticles of high specific area can significantly increase water contact angle and cellular activity on the fibrous implants.

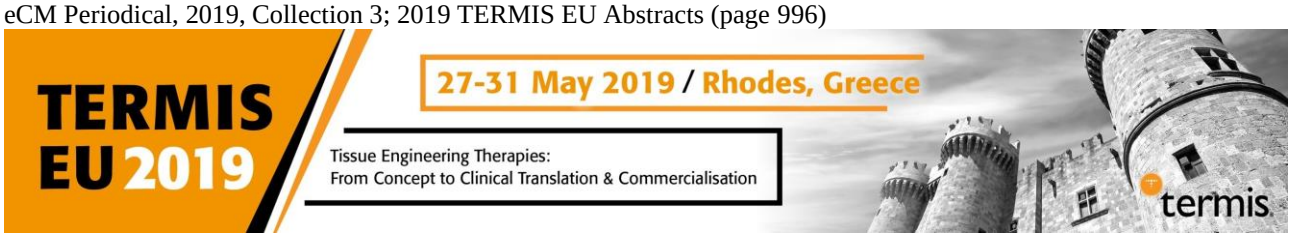
RESULTS & DISCUSSION: As a result of process induced nearby the textile substrate nanohydroxyapatite uniform layers of 150-200nm thickness were obtained on polymeric fibres. Scanning Electron Microscopy imaging revealed that nHA particles cover fibres but do not interfere with the structure of the base material. The porous structure of fibrous mesh was maintained practically unchanged and wettability of the material was highly increased.

CONCLUSIONS: The novel non-destructive method of nHA deposition on the fibres can serve as an alternative for currently used implant modification methods and enhance the bone regeneration process in bone/implant interface region. The method of coating have been proved to be suitable for coating highly porous, thermally and mechanically sensitive materials.

ACKNOWLEDGEMENTS: This work is supported by the polish National Centre for Research and Development (NCBR) project: NANOLIGABOND (POIR.04.01.02-00-0016/16).

REFERENCES

[1] V. Thomas, D.R. Dean, Y.K. Vohra, Current Nanoscience 2006, Vol. 2, 155-177.
[2] Polish Patent PL226891, J. Rogowska-Tylman et al. 2017: Method for producing bone implant and the bone implant.
[3] Polish Patent Application, PL427554, J. Rogowska-Tylman et.al, Biological Barrier Membrane.



3D printing of pluronic sacrificial scaffolds to develop gelatin prevascularized hydrogels

L. Bourguet[1], C. Kuoshe[1], C. Muller[1], N.E. Vrana[1], J. Barthès[1].
Presenting Author: Julien Barthès, j.barthes@protipmedical.com
[1]PROTiP Medical, 8 Place de l'Hôpital, 67000 Strasbourg, France

INTRODUCTION: In tissue engineering and regenerative medicine, one main limitation prevents the development of thick engineered tissue: the lack of vascularization. Indeed, cells located at more than 200µm from blood vessels cannot survive because they do not receive enough nutrients. To overcome this problem, in vitro prevascularization strategies can be considered. In this project, we have used 3D printing strategy to fabricate a Pluronic sacrificial network in the shape of a lattice of 6 layers of material. Then this Pluronic scaffold was filled with gelatin hydrogel and put at 4°C to dissolve Pluronic network. With this strategy, we obtained a gelatin hydrogel with hollow channels that was demonstrated to be colonized with endothelial cells to create an initial vascular network.

METHODS:3D printer has been used to print Pluronic scaffold. Multiple parameters were considered such as extrusion pressure, feed rate, nozzle diameter and Pluronic concentration to define the printability window and to obtain the expected channel dimensions. The diameter of the extruded material was assessed with SEM and numerical microscope. Once printed, Pluronic scaffold was filled with gelatin type A solution and then the hydrogel was enzymatically crosslinked with microbial transglutaminase at 4°C. During this last step, Pluronic scaffold was evacuated from the gelatin structure since it is liquid at this temperature. Endothelial cells were then used to colonize the scaffold and develop a vascular network. Immunostaining (PECAM CD31) and biochemical assay (Alamar Blue) were used to characterize endothelial marker and cell viability within the hydrogel.

RESULTS & DISCUSSION: To keep cells located less than 200µm from blood vessels and to obtain blood vessels of 500µm diameter, it was shown that the best design was a lattice structure composed of extruded materials of 300µm diameter with a horizontal spacing of 1mm between two rods. If the spacing was less than 1mm, the filling with gelatin material was inhomogeneous due to a structure too tight. A new design was also proposed to prevent the fusion between two layers of materials, it was demonstrated that the distance between two horizontal lines should be at least 600 µm to prevent any collapse of the structure. The final design (double rod) is presented in figure 1. The best reproducibility was achieved with a concentration of 30wt% of Pluronic. The final structure was then filled with gelatin hydrogel and successfully colonized with endothelial cells where endothelial cells form dense layers at the inner surface of lattices.

CONCLUSIONS: This project demonstrates the importance of design and the control of printing and material parameters to develop prevascularized hydrogels.

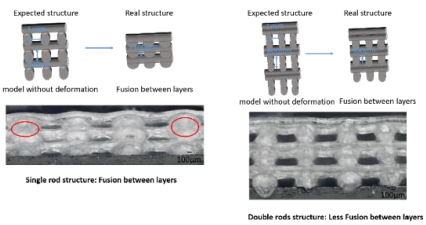
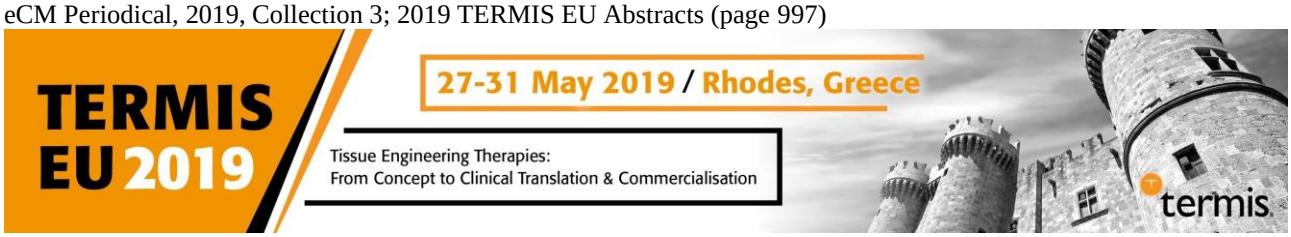


Figure 1: Different Pluronic scaffold designs to obtain the best sacrificial network.

ACKNOWLEDGEMENTS: EU FP7 (IMMODGEL, 602694), H2020 (PANBioRA, 760921).



Enhancement of axonal outgrowth after spinal cord transection of rats using neurotrophic factor-containing microchannel scaffold

J.-H. Choi[1], M.S. Kim[1,2,3], J.-W. Kim[1,2,3], J. K. Hyun[1,2,3,4]
Presenting Author: Jung-ho Choi, wjdgh819@naver.com
[1]Department of Nanobiomedical Science and BK21 PLUS NBM Global Research Center for Regenerative Medicine [2]Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Korea [3]Wiregene, Co., Ltd. [4]Department of Rehabilitation Medicine, Dankook University, Korea

INTRODUCTION: Neurotrophic factors (NTFs) have potential to promote neuronal regeneration and concomitant functional restoration in central and peripheral nervous systems [1-3]. We previously developed microchannel-containing scaffolds for spinal cord regeneration, and found outgrowing axons were observed within the scaffold, however, functional improvement was not detected. Herein, we internalize three types of NTFs into our microchannel scaffold to enhance axonal regeneration after complete transection of spinal cord in rats.

METHODS: Three NTFs; neurotrophin-3 (NT-3), glial cell-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF) with various concentrations (from 0.5 to 10.0 μg/ml) were added respectively into the heparin-immobilized microchannel scaffolds. The spinal cord at T9 level in Sprague-Dawley rats was completely transected and removed, and 5mm-long NTF-coated scaffold was inserted into the gap between the proximal and distal stumps just after transection injury. The spinal cord transected rats which did not receive the scaffold, and those received the scaffold without NTFs were considered as controls.

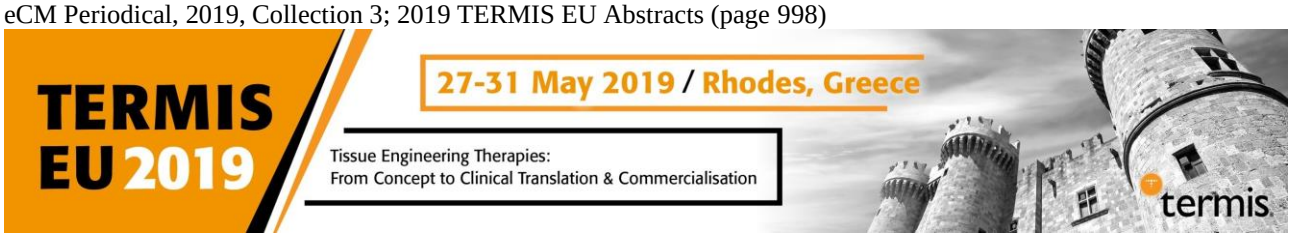
RESULTS & DISCUSSION: According to the immunohistochemical findings, the outgrowing axons were visible in both sides of the scaffold in all experimental groups and control which received scaffold without NTFs 2 weeks after implantation. The maximal length of outgrowing axons from both sides of the stump was longer in 2.5 μg/ml GDNF-containing scaffold received group (2.5 GDNF group) than in other experimental and control groups. Interestingly, the regeneration of afferent pathways was increased more than the efferent pathways when GDNF-containing scaffold was used. In addition, the amount of axons and astrocytes within the scaffold was greater in 2.5 GDNF group than in other experimental groups. The locomotor recovery was prominent in 1.0 and 2.5 GDNF groups more than in other experimental groups and controls 12 weeks after implantation.

CONCLUSIONS: Among three NTFs, GDNF was the most effective to promote axonal outgrowth crossing the microchannel-containing scaffold which was implanted within the transected spinal cord in rats. We concluded the optimal concentration of GDNF might have a synergistic effect to induce axonal regeneration and functional improvements as well as the physical guidance of microchannels within the scaffold following complete transection injury of spinal cord in rats.

ACKNOWLEDGEMENTS: Korea Healthcare Technology R&D Project (HI14C0522), the Commercialization Promotion Agency for R&D Outcomes (COMPA, 2018K000276), and National Research Foundation (2018K1A4A3A01064257, 2018R1D1A1A02047 523), Republic of Korea.

REFERENCES

[1] Harvey AR. et al., Brain Res. 2015;1619:36-71
[2] Gordon T., Neurosurg Focus. 2009;26:E3
[3] Lanfranconi S. et al., J Cell Mol Med. 2011;15:1645-87



Silk fibroin/gelatin film incorporating antibiotic silver sulfadiazine for medical application

P. Umprasert, J. Ratanavaraporn
Presenting Author: Juthamas Ratanavaraporn, juthamas.r@chula.ac.th
Biomedical Engineering Program, Faculty of Engineering, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand

INTRODUCTION: Silk fibroin, a protein derived from cocoons of Bombyx Mori silkworms, has excellent mechanical properties and slow degradation rate while generates minimal inflammatory and immune responses [1]. Gelatin, a denatured collagen, possesses biocompatibility and Arg-Gly-Asp (RGD) sequence to promote cell attachment and proliferation [2]. The blending of silk fibroin/gelatin improved mechanical properties due to the silk fibroin component while enhanced the biological activities by the gelatin component [3]. In this study, the silk fibroin/gelatin mixture was formed into film and incorporated with a cationic antibiotic silver sulfadiazine. Concentration of silver sulfadiazine was optimized to suit the medical application.

METHODS: The regenerated silk fibroin solution was prepared following the method previously reported [3]. The silk fibroin solution was mixed with gelatin solution at a weight ratio of 50/50. Various concentrations of silver sulfadiazine (0, 0.12, 0.62, 1.16, 1.69, and 2.16%wt/wt) were added to the silk fibroin/gelatin solution and stirred for 10 min at 25°C. Then, the mixture was cast in a mold and air-dried at ambient temperature under darkness. Gel fraction, swelling ability, cytotoxicity and anti-bacterial activities of the silk fibroin/gelatin films incorporating silver sulfadiazine were characterized.

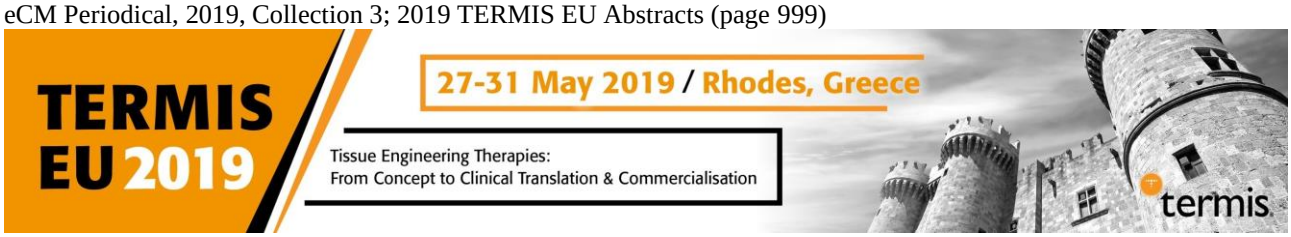
RESULTS & DISCUSSION: The silk fibroin/gelatin films incorporating silver sulfadiazine at every concentration showed a slight reduced swelling ability but higher gel (water-insoluble) fraction when compared to the silk fibroin/gelatin films without silver sulfadiazine incorporation. We also proved that the release of silver sulfadiazine from the films incorporating 1.16 and 2.16% silver sulfadiazine potentially inhibited the growth of both gram-positive and gram-negative bacteria while the toxicity to L929 mouse fibroblast cells was not found.

CONCLUSIONS: The silk fibroin/gelatin films incorporating 1.16% silver sulfadiazine was suit to medical application because it showed potential anti-bacterial activity and non-cytotoxicity to normal cells while the water swelling ability and stability were sufficient.

ACKNOWLEDGEMENTS: Financial support was received from Faculty of Engineering, Chulalongkorn University.

REFERENCES

[1] Thurber AE et al. Biomaterials 2015; 71:145–57.
[2] Vepari C et al. Prog. Polym. Sci. 2007; 32:991–1007.
[3] Jetbumpenkul P et al. Int. J. Biol. Macromol. 2012; 50:7-13.



Are cyclic stretch-induced cell and nucleus deformations mechanical input-energy-dependent?

K.S. Strobel[1], J. Stegemann[2], B. Walters[2], B. Rolauffs[1]
Presenting Author: Kai S. Strobel, reno655@hotmail.com
[1]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Dept. of Orthopedics and Trauma Surgery, Albert-Ludwigs-University of Freiburg, Hugstetter Straße 55, 79106 Freiburg, German; [2]Dept. of Biomedical Engineering, University of Michigan, Michigan, MI 48109, USA

INTRODUCTION: How physical stimuli are perceived by cells is increasingly relevant for controlling cell behavior, and the nucleus is emerging as an important cellular mechanosensor. Since cell morphology and behavior can be influenced using mechanical stretch [1] and this has been shown to be input energy-dependent [2], our hypothesis was that changes in nucleus shape share this dependency, and that it correlates with cell behavior.

METHODS: Human mesenchymal stromal cells (MSCs) isolated from bone marrow were cultured on fibronectin-coated polyacrylamide hydrogels (10%, 0.4% bisacrylamide) for 4 days. The seeded gels were then transferred to an incubator-housed ElectroForce 5210 BioDynamic-Test-System, where sinusoidal stretch experiments with a range of increasing energy inputs were performed. Calcein/DAPI imaging was used with in-house cell segmentation tools to assess morphology (imageJ). mRNA was isolated, transcribed to cDNA and RT-PCR was used to measure expression (ACTA, TGLN, CNN and DES). Correlations were assessed using Pearson Product-Moment Correlation and Partial Least Squares (PLS) analysis.

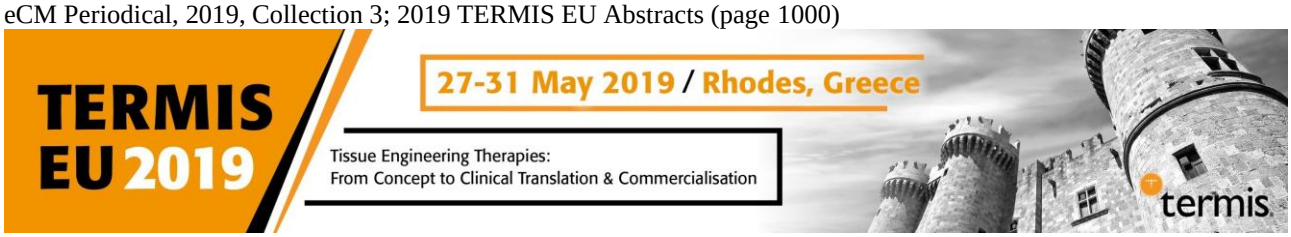
RESULTS & DISCUSSION: Pearson correlation showed nuclear length, area and roundness correlated respectively to that of the cell (ρ = 0.171, 0.393 and 0.283). Nucleus solidity correlated directly with the logarithm of energy input (ρ = -0.01). PLS analysis of nucleus solidity revealed that energy had a low regression coefficient (β = 0.357) when compared to other variables, where the strain amplitude was more strongly correlated (β = 0.830).

CONCLUSIONS: Pearson correlation between energy input and nucleus solidity was small, but mechanical nuclear deformations are generally small (eg [3]), suggesting the small correlation is due to small changes in morphology. While PLS revealed strain amplitude to have higher correlation with solidity than log energy, both parameters positively correlated and amplitude is a contributing variable to the energy input term. In conclusion, small changes in nuclear solidity can be correlated to the log of energy input, with the determining factor being the strain amplitude.

ACKNOWLEDGEMENTS: Financial support was received from the Volkswagenstiftung (92701).

REFERENCES

[1] Rothdiener M, et al. Scientific Reports **6**, 35840 (2016)
[2] Walters B, Roulaffs B In submission (2018) [3] Haase, K et al. Scientific reports **6** (2016): 21300.



Adaptation of tape removal test for sensation measurement in perineal area of rat

K. Neumannova[1,2], L. Machova-Urdzikova[1], J. C. F. Kwok[1,3], J. W. Fawcett[1,4], P. Jendelova[1,2]
Presenting Author: Katerina Neumannova, katerina.neumannova@iem.cas.cz
[1]Institute of Experimental Medicine Czech Academy of Science, Prague, Czech Republic [2]2nd Faculty of Medicine, Charles University, Prague, Czech Republic [3]Faculty of Biological Sciences, University of Leeds, UK [4]John van Geest Centre for Brain Repair, University of Cambridge, UK

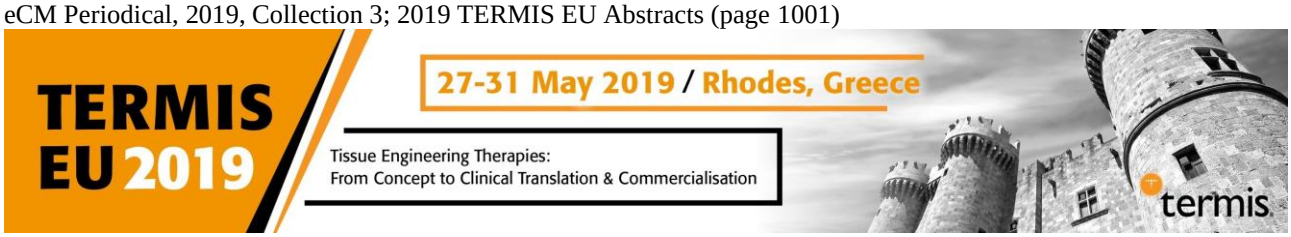
INTRODUCTION: Regeneration after spinal cord injury is a goal of many studies. Though the most obvious target is to recover motor function, sensory regeneration can improve quality life in paraplegic patients. According to a patient's survey, recovery of sensation in the perineal and genital area is one of the highest priorities. Although there are behavioural tests to measure sensitivity, there is no test for measuring sensation in perineal and genital area. The aim of our study was to develop the behavioural test for measuring the sensitivity of perineal and genital area in rats.

METHODS: We have modified the tape removal test used routinely to test sensorimotor deficits after stroke. We adapted the test to perineal area and tested several settings. A small piece of tape (approximately 1 cm^2) was attached to perineal area on the left side. Time to first touch and to remove the tape was measured. If the rat didn't remove the tape until 5 minutes the tape was removed and the time of 5 min was recorded. This was repeated 4 times for each animal with 3 min pause between each trial. Each rat was trained in 5 consecutive days and then tested weekly. We compared different rat strains (Wistar, Sprague-Dawley, Long-Evans and Lewis), both genders, shaving and non-shaving and different types of tapes. All tests were performed on healthy animals and animals with T10 dorsal hemisection. After the spinal cord injury animals tested at day 3, 8, 14 and 21.

RESULTS & DISCUSSION: We found that the test was suitable for all tested strains, however, the Lewis rats achieved the lowest contact times. But this difference was significant only in first few days of learning the task. There were no significant differences between gender and different types of tape or shaving. After the animals underwent dorsal hemisection the test could detect sensory deficit, the time to sense the stimulus increased from 1'32 up to 3'20 in average. This was compared with other behavioural tests (BBB, von Frey, ladder and Plantar test). Animals were divided according to the lesion size to big, medium and small lesion groups. Whilst deficits detected in tape test correlated with the size of the lesion, von Frey and Plantar tests showed only non-significant deficit independent on the lesion size.

CONCLUSIONS: We conclude, tape removal test is suitable for testing perineal and genital sensation in rats and can be used in different strains.

ACKNOWLEDGEMENTS: Supported by Operational Programme Research, Development and Education in the framework of the project "Center of Reconstructive Neuroscience", registration number CZ.02.1.01/0.0./0.0/15_003/0000419



Modelling non-traumatic spinal cord injury in-vitro

K. V. Timms[1,2], J. L. Tipper[3], R. M. Ichiyama[1], J.B. Phillips[4], J.C.F. Kwok[1, 5], L. Urdzikova[5], R.M. Hall[2]

Presenting Author: Katie Timms, bs11kvt@leeds.ac.uk

[1]School of Biomedical Sciences, University of Leeds, UK [2]Faculty of Engineering, University of Leeds, UK [3]Faculty of Engineering, University of Technology Sydney, Australia. [4]UCL School of Pharmacy, University College London, UK [5]Institute for Clinical and Experimental Medicine, Prague, Czech Republic

INTRODUCTION: Non-traumatic spinal cord injury (NT-SCI) involves spinal cord stenosis which increases over months to years; as opposed to the millisecond duration of traumatic spinal cord injuries. Despite affecting up to 150000 people in the UK[1], NT-SCI pathology is poorly understood. This research aims to develop experimental models of NT-SCI in order to better understand the neural cell responses to low-velocity mechanical insults.

METHODS: In-vitro, primary rat astrocytes isolated from P3 pre-weaner rats were seeded into rat collagen I (1.6 $mg.mL^{-1}$) hydrogels at $1x10^6$ $cells.mL^{-1}$ of gel. Gels were compressed to 70% of their height using a BOSE Electroforce 5110 Biodynamic, at 0.1 to 100 $mm.s^{-1}$. Positive control included supplementing with 10 $ng.mL^{-1}$ TGF-β[2,3]. Immuno-labelling for GFAP was carried out over 14 days.

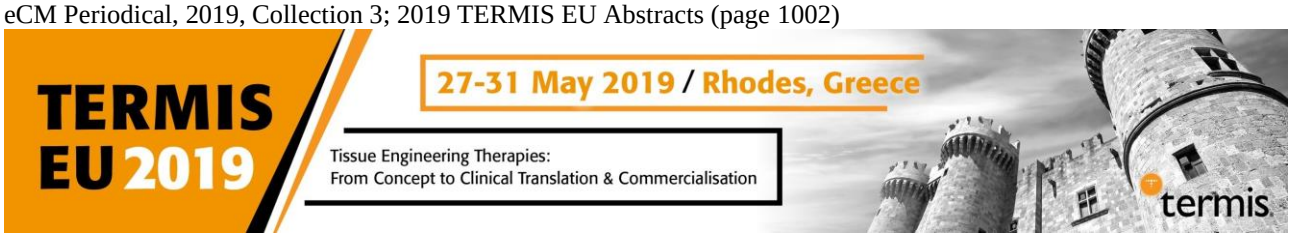
RESULTS & DISCUSSION: On day 1, there was no significant difference in GFAP area or cellular circularity between experimental groups. On day 14, GFAP area was significantly greater in TGF-beta treatment and 100 mm/s velocity groups than in the 0.1 mm/s group ($p < 0.05$). Similarly on day 14, circularity was significantly greater in the 0.1 mm/s group compared to 100 mm/s and TGF-beta treated groups ($p < 0.05$). This reflects the increased ramification of cells compressed at 100 mm/s, and with TGF-beta treatment.

CONCLUSIONS: Preliminary findings indicate methods to model NT-SCI in-vitro have been established. Evaluation of images indicate astrocyte reactivity is increased at 14 days with TGF-beta treatment or compression at a traumatic velocity (100mm/s). However, in NT-SCI (0.1 mm/s) the astrocytes do not behave in the same manner. Further studies will be undertaken to evaluate other markers of astrocyte function, such as ELISA. Better understanding of cellular behavior in NT-SCI will allow improved disease management and therapeutic development

ACKNOWLEDGEMENTS: Funded by EPSRC Centre for Doctoral Training: Tissue engineering & regenerative medicine; grant number EP/L014823/1

REFERENCES

[1] New, P. W., Marshall, R. (2014). Spinal Cord. 52: 123-32
[2] East, E., Golding, J.P., Phillips, J.B. (2009) J Tissue Eng Regen Med. 3:634-46.
[3] Kerstetter, A.E., Miller, R.H. (2012) Methods Mol. Biol. 814:93-104



A novel tissue-engineered 3D tumor model for anti-cancer drug discovery

W. Li[1], X. Li[1], T. Liu[1], K. Song[1*]
Presenting Author: Kedong Song, kedongsong@dlut.edu.cn
[1] State Key Laboratory of Fine Chemicals, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian 116024, China

INTRODUCTION: Decellularized natural matrices provide an alternative platform in tumor tissue engineering for its biomechanical characteristics and tissue-specific ECM composition. Herein, we introduced an organotypic 3D breast cancer model in vitro using decellularized porcine lung and evaluated the 3D cell behaviors and protein expression of breast cancer specific markers.

METHODS: A total of 50 μL cell suspensions at a density of 2×10^6 cells/ml were seeded on both sides of each scaffold in 24-well plates. Immunofluorescence of F-actin was detected to investigate cell distribution in lung scaffolds. Dead/Live staining was conducted to assess cell viability in decellularized lung scaffolds. SEM was conducted to evaluate cell growth. Besides, Immunofluorescence and flow cytometry assays were conducted to evaluate the expressions of hypoxia-inducible factor 1 α (HIF1α), breast cancer susceptibility gene 1 (BRCA1) and human epidermal growth factor receptor 2 (HER2) of cells cultured in 2D or 3D lung scaffolds.

RESULTS & DISCUSSION: F-actin immunofluorescence showed cell closely adhered to the skeleton of lung scaffold, and the porous of scaffold was easily identified. Dead/Live indicated favorable viability of cells, suggesting good cell compatibility of the decellularized lung scaffolds. SEM showed cells gradually developed into multilayers and tumoroids that merged with surrounding cell colonies over time (Figure 1a). The stronger expression of breast cancer-related markers suggested that cells in lung scaffolds displayed higher malignancy as compared to those in 2D cultures (Figure 1b-c).

CONCLUSIONS: Collectively, these data indicate that the organotypic-derived lung scaffold coupled to human cancer cells better simulate the native tumor microenvironment that favors cell progression and higher malignancy.

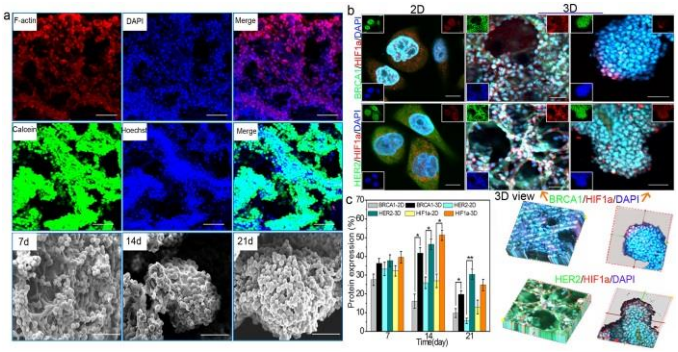
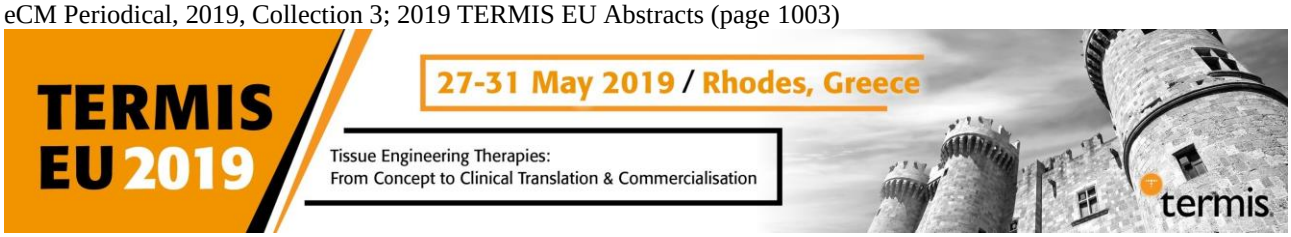


Figure 1: Cell behavior and protein expression of breast cancer markers in 3D lung scaffold. (a) Cell distribution, viability and proliferation in 3D scaffold. (b) Protein expression of BRCA1, HER2 and HIF1α in 2D and 3D lung scaffolds by IF and flow cytometry.

ACKNOWLEDGEMENTS: National Natural Science Foundation of China (31670978/21676041), and the Natural Science Foundation of Liaoning (20180510028).



Pyrogenity evaluation of biodegradable material based on hyaluronan using monocyte activation test

K. Nesporova[1], H. Vagnerova[1], V. Velebny[1]
Presenting Author: Kristina Nesporova, kristina.nesporova@contipro.com
[1]Physiology of Cell, Contipro, Dolni Dobrouc 401, 56102 Dolni Dobrouc, Czech Republic

INTRODUCTION: Monocyte activation test (MAT) is emerging alternative to LAL test and rabbit pyrogen tests [1-2]. Its benefits include in vitro procedure with no need for animals and more relevant response of human immune system comparing to crustacean based LAL test. In this work we have established the procedure of testing slowly hyaluronan (HA) preparations and biodegradable HA-based materials by MAT.

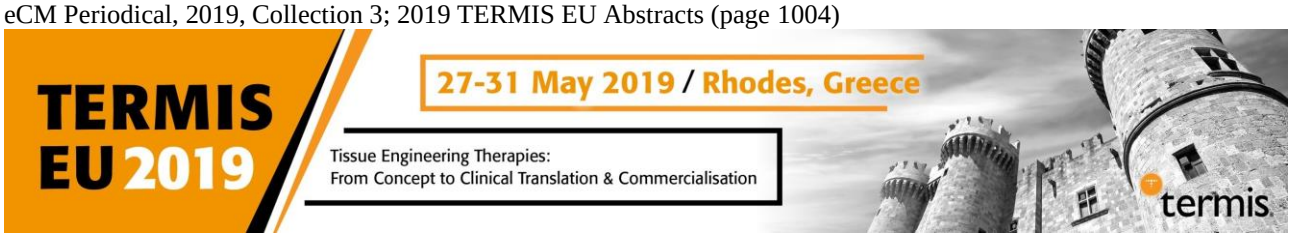
METHODS: Heparinized or citrated human blood was drawn from 4-5 donors (with their informed consent) for every test and assayed within 3 h. Materials both soluble and not-readily soluble were assayed in normal saline at 1-10 mg/ml concentration. Extracts were prepared in saline or media under constant agitation for 24-72 h at 37 °C. Samples were incubated with pooled blood overnight and plasma was then assayed for IL-6 cytokine by ELISA (eBioscience). Samples used for MAT evaluation included: HA of high molecular weight (HMW) - pharmaceutical and cosmetic grade, HA fragments prepared by enzymatic degradation, HA derivatives and HA-based biomaterials.

RESULTS & DISCUSSION: MAT itself was optimized comparing individual donor or pooled blood. The variability among donors proved problematic, thus whole blood pooled from 4-5 donors was used for subsequent experiments. For samples with low or none added Ca^{2+} the heparinized blood was used. The citrated blood necessary for Ca^{2+}-containing biomaterial samples as added $Ca2^{+}$ started to the coagulation spontaneously. The specificity and sensitivity of MAT was tested using endotoxin standard (0.1-10 IU/ml) and non-endotoxin pyrogen (lipoteichoic acid, LTA). The native HMW HA (pharma grade) showed no pyrogenic response in good concordance with standard LAL test. Low-endotoxin cosmetic HA showed increased response to LPS spike suggesting the presence of non-endotoxin contaminants (Fig. 1), such as glucans and LTA. HA-based biomaterials were tested as solutions or extracts and MAT was able to detect pyrogens with the limit of 0.25 IU/mg which is sensitive enough for evaluation of pyrogenity of medical devices.

CONCLUSIONS: MAT using whole human blood is good alternative or addition to classic pyrogen testing methods, providing better insight to pyrogenity of complex biomaterials.

REFERENCES

[1] Borton LK et al. ALTEX. 2018;35(4):453-63.
[2] Stang K et al. J Mater Sci Mater Med. 2014 Apr;25(4):1065-75.



Bioactive Composite Hydrogels Based on Hydroxyapatite/ε-Polylysine/Hyaluronic Acid

K. Salma-Ancane[1,2], A. Sceglovs[1,2], J. Vecstaudza[1,2], D.Loca[1,2]
Presenting Author: Kristine Salma-Ancane, kristine.salma-ancane@rtu.lv
[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU [2]Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia

INTRODUCTION: Repair and healing fractures caused by osteoporosis still remains a major clinical challenge in orthopaedic surgery [1]. In recent years, fabrication of hydrogels mimicking the natural extracellular matrix of the bone has attracted research interest in the field of hydrogel-based bone regeneration [2]. The aim of this study is to develop novel bioactive composite hydrogels based on nanosized hydroxyapatite and natural biopolymers ε-polylysine and hyaluronic acid.

METHODS: Bioactive hydrogel system based on nanosized hydroxyapatite (n-HAp), ε-polylysine (ε-PL) and hyaluronic acid (HA) was prepared using in situ synthesis of n-HAp in ε-PL solution and via chemical cross-linking using the carbodiimide (EDC) and N-hydroxysuccinimide (NHS) (molar ratio of EDC/NHS 1:1; weight ratio of n-HAp and ε-PL/HA 50:50, 70:30 wt.%). The EDC and NHS were added into an in-situ synthesized n-HAp /ε-PL solution and cooled at 4 °C. The HA was added into a synthesized nHAp/ε-PL solution. The n-HAp /ε-PL/HA hydrogel would be produced after rapid stirring within less than 5 min. The composition, purity and microstructure of all synthesized samples will be evaluated using powder X-ray diffractometry (XRD), Fourier transformation infrared spectrometry (FTIR) and scanning electron microscopy (SEM). Investigation of swelling properties in phosphate buffer saline (PBS), the surface and cross-section morphologies before and after immersion tests was performed.

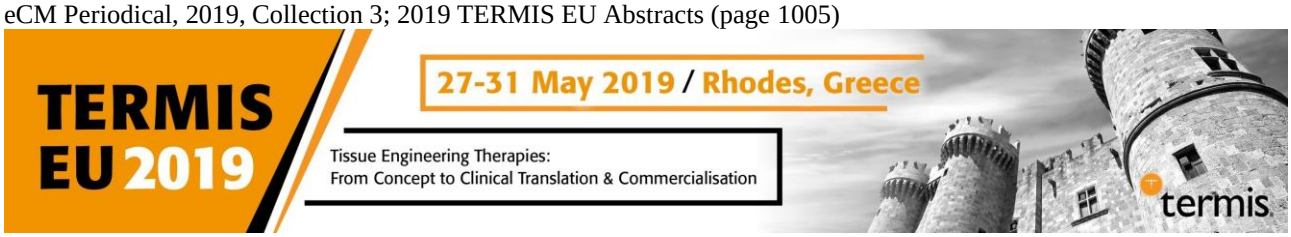
RESULTS & DISCUSSION: SEM micrographs revealed that the freeze-dried n-HAp/ε-PL/HA hydrogels were highly porous and consist of interconnected pores. SEM and XRD results demonstrated formation of n-HAp phase which incorporates into the ε-PL macromolecules. FTIR spectra proved that hydrogels were crosslinked through amide bond linkage. The gelation time in the crosslink process of n-HAp /ε-PL/HA hydrogels was within less than 5 min. The obtained hydrogels were successfully sterilized by autoclave sterilization.

CONCLUSIONS: The development of bioactive composite hydrogel system based on nanosized hydroxyapatite, ε-polylysine and hyaluronic acid was done. Considering the beneficial preparation process and sterilization ability, obtained hydrogels might have high potential for use in bone tissue engineering.

ACKNOWLEDGEMENTS: This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 “Post-doctoral Research Aid” of the Specific Aid Objective 1.1.1 “To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure” of the Operational Programme “Growth and Employment” (No. 1.1.1.2/VIAA/1/16/045

REFERENCES

[1] Bai X et al. Bioact Mater. 2018; 26;3(4):401-417
[2] Hua J et al. Mater. Sci. Eng. C. 2016; 61:879–892



Papillary and reticular fibroblasts differentially regulate angiogenesis in tissue-engineered cell sheets

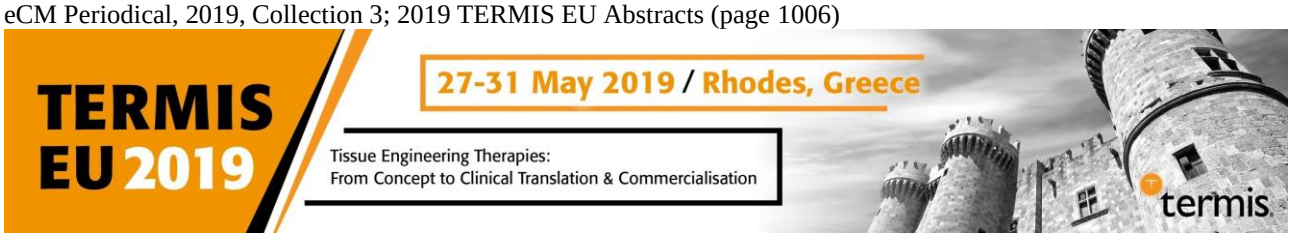
A. Mauroux[1,2,3], P. Joncour[2], B. Closs[3], S. Germain[1], S. Bordes[3], F. Ruggiero[2], L. Muller[1]
Presenting Author: Laurent Muller, laurent.muller@college-de-france.fr
[1]Center for Interdisciplinary Research in Biology (CIRB), College de France, Paris, France [2]IGFL, ENS, University Claude Bernard, Lyon, France [3]SILAB, R&D Department, Brive, France

INTRODUCTION: The dermis is divided into distinct compartments, the superficial papillary dermis and the deeper reticular dermis, which display structural and functional differences especially in terms of vascularization and mechanical properties. Primary human fibroblasts isolated from these compartments differ in morphology, proliferation and patterns of gene expression. To date, we are still lacking models allowing investigation of their microenvironment and ability to support capillary formation, as well as insertion in reconstructed skin. The aim of this research work was thus to study and compare the properties of human papillary and reticular fibroblasts in tissue-engineered cell sheets.

METHODS: Endothelial cells were cultured in fibrin hydrogels in the presence of conditioned medium of human papillary and reticular fibroblasts in order to assess the angiogenic paracrine factors. Both fibroblast subpopulations were also co-cultured with endothelial cells on thermo-sensitive culture dishes under conditions that promote extracellular matrix deposition and support capillary formation. Angiogenic properties were measured, and RNA-seq was performed for gene expression analysis.

RESULTS & DISCUSSION: Using 3D angiogenesis assays based on conditioned medium, we could demonstrate that paracrine factors from papillary fibroblasts induce the formation of dense branched capillary networks. Using cell sheet angiogenesis assays, we could demonstrate that the microenvironment generated by reticular fibroblasts regulates vessel diameter and perivascular cell recruitment. The results from these functional assays were further supported by the identification of genes associated to vascular development and extracellular matrix.

CONCLUSIONS: Our results demonstrate that tissue-engineered cell sheets using subpopulations of dermis fibroblasts are important tools for identification of factors responsible for the specific microenvironment and vascularization of these dermis compartments. Furthermore, our data support the use of such culture technology for the generation of relevant micro-tissues mimicking the complex organization of the dermis.



Plant-derived cellulose hydrogel as a matrix for 3D human stem cell proliferation and differentiation

L. Paasonen[1], J. Spencer-Fry[1]
Presenting Author: Lauri Paasonen, lauri.paasonen@upm.com
[1]UPM-Kymmene, Helsinki, Finland

INTRODUCTION: In the pursuit of in vitro cell models that are biological relevant with improved functionality, new materials and methods for creating three-dimensional (3D) cell culture systems are a key requirement. GrowDex®, nanofibrillar cellulose (NFC) hydrogel which is derived from the Birch tree, has been shown to provide an effective support matrix for culturing cells in 3D.

METHODS: 1) hESC (WA07) and hiPSC (iPS(IMR90)-4): Cell colonies were embedded in 0.5% GrowDex (UPM) in mTeSR1 (STEMCELL Technologies) media and cultured on 96-well plate up to 26 days. hPSCs pluripotency was analysed with OCT4 and SSEA-4 marker expression, in vitro EB-mediated differentiation, and teratoma assay. **2) hESC derived neuronal cells**: Pre-differentiated neuronal cells were embedded in 1.5 and 1.0% GrowDex. Formation of the neuronal networks was evaluated by immunocytochemical staining against neuronal markers MAP-2 and β-Tubulin III and confocal microscopy. **3) Adipose tissue derived hMSCs**: Cells were embedded in 0.2% GrowDex in DMEM media and transferred on 24-well tissue culture inserts. Adiopogenic differentiation and osteogenic differentiation were induced with StemPro™ differentiation kits (ThermoFisher) for 21 days, and analysed with Oil Red O and Alizarin Red S stainings.

RESULTS & DISCUSSION: 1) hESC and hiPSC proliferated in GrowDex without feeder cells, formed spheroids with 100-200 µm diameter (Fig.1A), and the cells remained their pluripotency throughout the 26 day study. **2)** GrowDex supported the 3D growth of hESC derived neuronal cells. The formation of neurospheres and neuronal networks by neurite outgrowth were observed (Fig1B). **3)** Adipose tissue derived hMSCs were able to differentiate to both adipogenic, and osteogenic direction in GrowDex 3D culture (Fig 1C,D).

CONCLUSIONS: GrowDex offers a well-defined, tunable 3D culture matrix for various regenerative medicine applications.

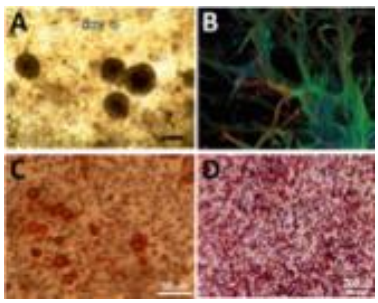
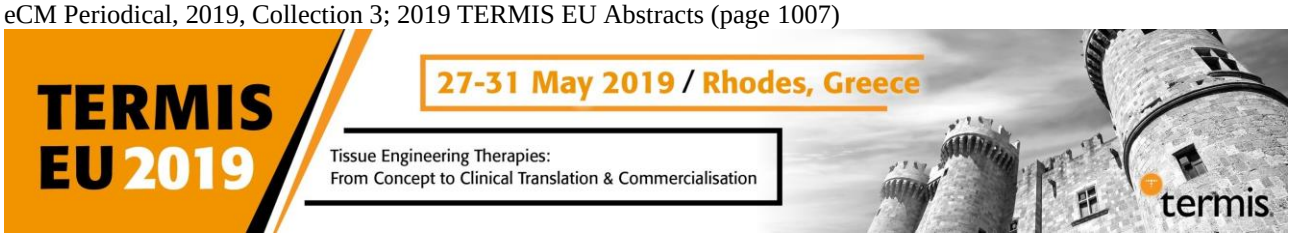


Figure 1: A) iPS(IMR90)-4 spheroids in 0.5% GrowDex. B) Neurite outgrowth from hESC derived neuronal cells in 1.0% GrowDex. C) Adipogenic differentiation and D) osteogenic differentiation of hMSCs in 0.2% GrowDex.

ACKNOWLEDGEMENTS: Authors would like to thank Yan-Ru Lou, Tiina Joki and Jonathan Sheard for performing the experiments.



In situ gelation by UV curing in nasal cavity for multiple sclerosis treatment

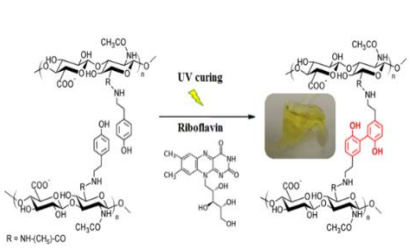
L. Kovarova[1,2], E. Toropitsyn[1,3], I. Scigalkova[1], M. Pravda[1], T. Bobula[1], V. Velebny[1]
Presenting Author: Lenka Kovarova, kovarova@contipro.com
[1]Tissue Engineering Research Group, Contipro a. s., Dolni Dobrouc, Czech Republic [2]Brno University of Technology, Faculty of Chemistry, Purkynova 464/118, Brno, Czech Republic [3]Charles University, First Faculty of Medicine, Katerinska 32, Prague 2, Czech Republic

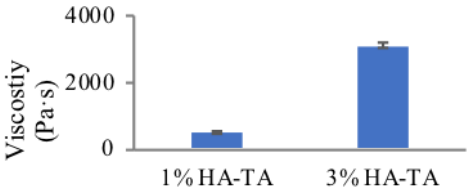
INTRODUCTION: Multiple sclerosis (MS) is one of the leading diseases causing non-traumatic neurological disability in the young adult. One of the treatment approaches is based on delivery of an API via the olfactory region in the nasal cavity. Here we describe development of hyaluronic acid-based hydrogel which can be crosslinked in the nasal cavity and serve as a mounting layer preventing clearance of API from the site of application. Hyaluronan-tyramine derivative (HA-TA)1 is capable of forming hydrogel due to crosslinking by UV light in the presence of photoinitiator riboflavin (Rbf.).

METHODS: Influence of different parameters (HA-TA DS and Mw, Rbf. concentration, UV intensity) on precursor solution viscosity, gelation time and elastic modulus (G´) of hydrogel was evaluated by using rotational rheometer. HA-TA+Rbf. precursor solution cytotoxicity, hydrogel extract cytotoxicity and phototoxicity were tested. 3T3 cells ($1{,}5 \cdot 10^4$/ml/culture well) viability were measured with ATP assay.

RESULTS & DISCUSSION: The viscosity of HA-TA precursor solution is a crucial parameter for material applicability and its spreading in the targeted place of nasal cavity. Kinetics of gelation depends on photoinitiator concentration. Precursor solutions and hydrogel extracts are non-cytotoxic.

CONCLUSIONS: HA-TA hydrogel is possible to crosslinked in situ by UV light. It is non-cytotoxic, stable with tuneable hydrogel properties.





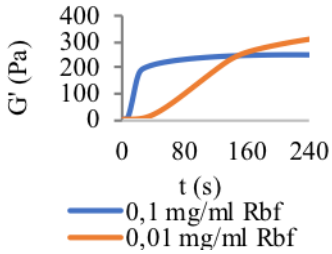
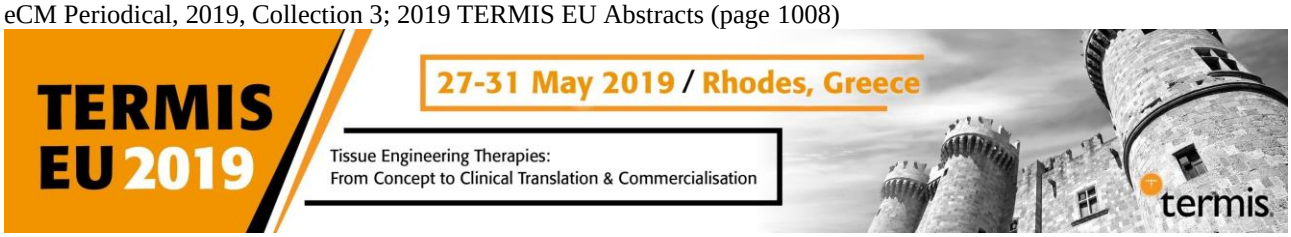


Figure 1: Scheme of hydrogel formation by UV curing of HA-TA+Rbf precursor solution (left). **Figure 2:** Influence of HA-TA concentration on precursor solution viscosity (middle). **Figure 3:** Kinetics of gelation of different concentration of Rbf (right).

ACKNOWLEDGEMENTS: Financial support was received from the European Framework Programme for Research and Innovation Horizon 2020 (Grant No. 721098).

REFERENCES

[1] Wolfova L. et. al., WO/2013/127374, 2013.



A modular joint-on-chip approach to study cellular cross-communication in a simulated osteoarthritic micro-environment

L. Moreira Teixeira[1], C. Paggi[1], S. Piluso[1,2], J. Leijten[1], J. Malda[2], R. van Weeren[2], S. Le Gac[1], M. Karperien[1]
Presenting Author: Liliana Moreira Teixeira, l.s.moreirateixeira@utwente.nl
[1]Department of Developmental BioEngineering, Faculty of Science and Technology, University of Twente, The Netherlands [2]Department of Orthopaedics, University Medical Center Utrecht and the Department of Equine Sciences, University of Utrecht, The Netherlands

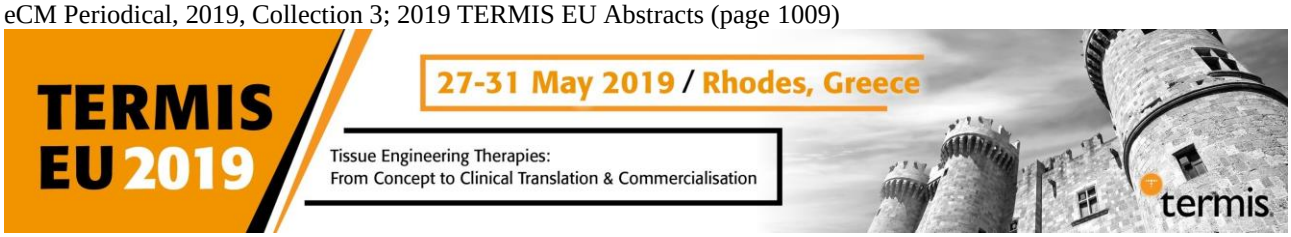
INTRODUCTION: Cartilage degeneration and synovitis are key hallmarks of joint degenerative diseases, such as osteoarthritis (OA). The communication between these two tissues is fundamental to maintain both homeostasis and disease onset. However, studying this communication has remained challenging. Herein, we propose a novel modular precision microfluidic platform, that combines a synovial membrane-on-chip and a cartilage-on-chip platform. This seamless strategy enables the facile and in depth study of cross-communication between these two joint components, specifically via inflammatory mediators, aiming to replicate the onset of OA.

METHODS: Two types of microfluidic PDMS-based chips were designed with actuation chambers to emulate the mechanical forces of the cartilage and synovial membrane. For the cartilage chip, human healthy and OA affected chondrocytes were seeded in an ECM-like hydrogel. The synovial membrane chip was composed of a hydrogel re-enforced by an elastic membrane, which was seeded with synovium fibroblasts. Both chips were connected by a common channel, where synovium mimicking medium (culture medium supplemented with HA) was flowing (60 µl/hr). After both tissues achieved phenotypical maturation, human macrophages were added to the system. The behavior of the immune cells were a key read-out, focusing on their mobility, cytokine and proteinase release profile (ELISA) and polarization ratio between M1 and M2 (qPCR). On-line and end-point analysis were conducted after 1, 3 and 7 days.

RESULTS & DISCUSSION: The unique chip actuator designs allowed for physiologically relevant stimulation of the cartilage and synovial membrane, by cyclic compression and stretching, respectively. The effect of the mechanical load was determined on the release of inflammatory mediators. The proteinases and inflammatory cytokine profiles are the basis of the fingerprint of the influence of the variables: static vs. mechanically stimulated, healthy vs. OA-affected chondrocytes, and, presence vs. absence of macrophages.

CONCLUSIONS: This modular joint-on-chip platform has the potential to provide unprecedented insights in the effects of inflammation of a single joint tissue, the performance of the various joint tissues, and the joint function itself.

ACKNOWLEDGEMENTS: Financial support was provided by the Dutch Arthritis Society and UT/UMC/UU synergy grant.



Repopulated healthy and IPF lung scaffolds show temporal differences in ECM turnover

L. Elowsson Rendin[1], A. Löfdahl[1], C. Müller[1], H. Isaksson[2], L. Bjermer[3], E. Åhrman[4], J. Malmström[4], and G. Westergen-Thorsson[1]
Linda Elowsson Rendin, linda.elowsson_rendin@med.lu.se
[1]Lung Biology, Lund University, Lund, Sweden [2]Biomedical engineering, Lund University, Lund, Sweden [3]Dept. Respiratory Medicine and Allergology, Skåne University Hospital, Lund University, Lund, Sweden [4]Infection Medicine Proteomics, Lund University, Lund, Sweden

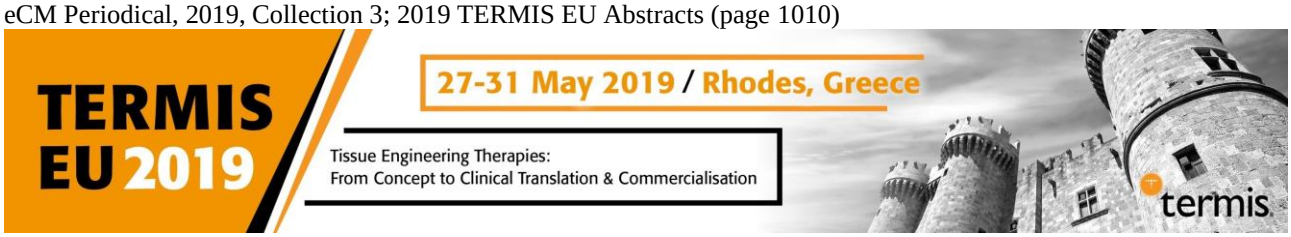
INTRODUCTION: Decellularized tissues offers an excellent approach to study cell-ECM interaction in health and disease. In this study we have used peripheral lung tissue from healthy individuals and patients suffering from idiopathic pulmonary fibrosis (IPF). IPF is a devastating disease characterized by excessive matrix production and stiffening of the tissue. The aim was to examine temporal changes in the original ECM in healthy and diseased scaffolds and how these affect protein production of repopulating lung fibroblasts cultured using heavy isotope labelling (SILAC).

METHODS: Human lung tissue slices (350µm) were decellularized using CHAPS to generate biological scaffolds. The scaffolds were characterized by density, biomechanical properties, histology, and mass spectrometry (MS). Scaffolds were mounted on custom-made holders, to mimic the native setting, seeded with human primary fibroblasts derived from a healthy donor, and cultured using SILAC medium supplemented with $^{13}C_6$ labeled L-Arginine-HCl and $^{13}C_6$ $^{15}N_2$ -labeled L-lysine-2HCl. The repopulated scaffolds were examined as described above to study differences in ECM turnover.

RESULTS & DISCUSSION: Heavy isotope labelling of the cultured cells allowed us to follow up- and downregulation of the newly synthesized proteins in compared to the original scaffold due to a mass shift detectable in MS. Interestingly, healthy fibroblasts cultured on IPF scaffolds had a protein production profile overlapping with the profile of the original scaffold.

CONCLUSIONS: We demonstrated that using SILAC in our biological system allowed us to study temporal differences in protein production in healthy and diseased scaffolds. Furthermore, by culturing under static stretch we created a 3D environment similar to the native situation, strongly influencing the cellular response. Collectively, this opens up for in depth identification of proteins involved in lung disease and tissue regeneration. Our goal is to identify a combination factors that can be used to locally trigger a healthy regeneration of the lung.

ACKNOWLEDGEMENTS: Financial support was received from Swedish foundation for Strategic Research, Swedish Heart-Lung foundation, Swedish Research Council, Crafoord foundation, Royal Physiographical Society of Lund, Österlund Foundation.



A novel bioreactor for studies of lung regeneration

L. Karlsson[1], O. Rosmark[1], A-K. Larsson Callerfelt[1], L. Elowsson Rendin[1], G. Westergen-Thorsson[1]
Presenting author: Lisa Karlsson, lisa.karlsson@med.lu.se
[1]Lung Biology, Lund University, Lund, Sweden

INTRODUCTION: Chronic obstructive pulmonary disease (COPD) is an increasing worldwide health problem characterized by progressive tissue destruction with no cure. In end-stage disease, the ultimate treatment is lung transplantation. Due to lack of compatible donor lungs and the problems with graft rejection, tissue engineering approaches have emerged as an alternative. Knowing that biomechanical loading is fundamental for the optimal cellular response, we have constructed a bioreactor mimicking the in vivo setting. The bioreactor makes it possible to study regeneration of human lung tissue.

METHODS: Lung scaffold slices 350 µm thick and with a surface are of 1 cm2 were produced from both human and porcine tissue using a decellularisation protocol developed in our lab. To introduce static stretch to lung tissue-derived scaffolds, a custom-made scaffold holder was developed (Fig. 1). Scaffolds are placed over a base with an open hole, an O-ring is placed on top and a lid with an open hole secures the scaffolds. The construction of the bioreactor is based on the same principle. Here, scaffolds are mounted on top of an elastic membrane creating a pressurized chamber underneath. Movement of the membrane and the overlying tissue controlled by alternating the pressure. Human fetal lung fibroblasts (HFL-1) were cultured for up to 6 days on the porcine scaffolds. Subsequently, scaffolds were analyzed histologically and assessed for cell viability (resazurin-based assay).

RESULTS & DISCUSSION: In contrast to our previous studies, the tissue-derived lung scaffolds from healthy subjects retained its normal alveolar structure when cultured under static stretch compared to non-mounted scaffolds. In the latter, cells contracted the surface area from approx. 1 cm^2 to 2 mm^2 in 6 days. Cells seeded on scaffolds, cultured under cyclic dynamic loading in the bioreactor, have a higher viability and a more even distribution compared to cells cultured in static stretch. No differences in cell numbers were observed.

CONCLUSIONS: Creating a 3D environment mimicking the in vivo setting of the lung is crucial for the development of tissue engineering approaches in regenerative medicine. We have designed a bioreactor that can be commercialized, and used for studying regeneration of human tissue, as well as an alternative for animal testing in toxicology and/or pharmacology studies.

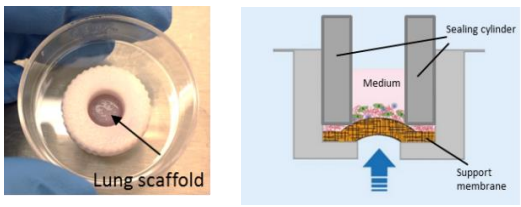
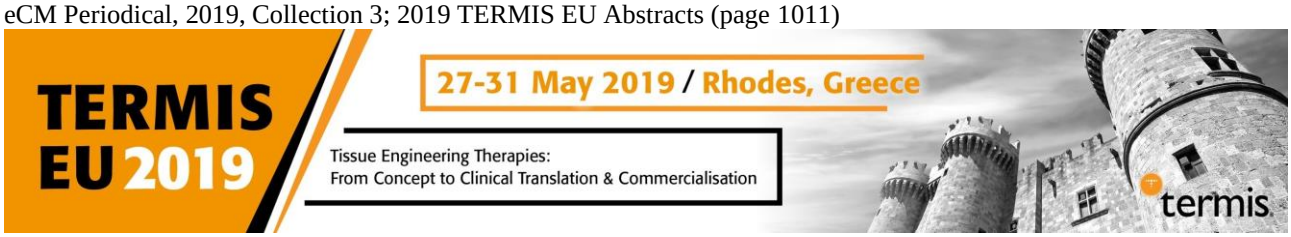


Figure 1: Left: Lung scaffold mounted in custom-made holder. Right: Illustration of the bioreactor construction.

A**CKNOWLEDGEMENT:** Financial support from Swedish foundation for Strategic Research, Swedish Heart-Lung foundation, Swedish Research Council, Crafoord foundation, Royal Physiographical Society of Lund, Österlund Foundation



Bioprinting of (fibro)cartilage microtissues using a photocrosslinkable gelatin

L. De Moor[1], S. Fernandez[1], M. Minne[1], C. Vercruysse[1], L. Tytgat[2], S. Van Vlierberghe[2], P. Dubruel[2], H. Declercq[1]
Presenting Author: Lise De Moor, Lise.DeMoor@UGent.be
[1]Tissue Engineering Group, Department of Human Structure and Repair, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium [2]Polymer Chemistry and Biomaterials Research Group, Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Faculty of Sciences, Ghent University, Ghent, Belgium

INTRODUCTION: To create a (fibro)cartilage construct, a modular strategy can be applied where high quality microtissues are used as building blocks [1]. In this study, 3D spheroids with a (fibro)cartilage phenotype are generated, using porcine articular chondrocytes (pAC) or human bone marrow-derived mesenchymal stem cells (hBM-MSC). For the directed assembly of these spheroids by 3D bioprinting, a modified photo-crosslinkable gelatin (Gel-MOD) was used as a bio-ink [2].

METHODS: Porcine AC were enzymatically isolated from the articular cartilage of the femur. hBM-MSC were purchased from PromoCell. To generate spheroids, a high-throughput agarose microwell system, with 1585 pores of 400 µm diameter, was used. After seeding 0.5 x 10^6 cells per well, uniform spheroids of ± 315 cells were obtained and cultivated at 5% O_2 in chondrogenic medium. To assess the effect of the biopolymer concentration of the hydrogel on the spheroids, encapsulation experiments were performed. For the encapsulation in gelatin methacrylamide, spheroids were mixed in a 10, 15 or 20 w/v% Gel-MOD solution (PBM Group, UGent) with 2 mol% Irgacure 2959 or 20 mol% VA-086 as photo-initiator. Spheroids were bioprinted with a 3D Discovery Instrument (RegenHU). The viability, morphology and extracellular matrix (ECM) of the spheroids were evaluated by live/dead staining, (immuno)histochemistry and RT-qPCR.

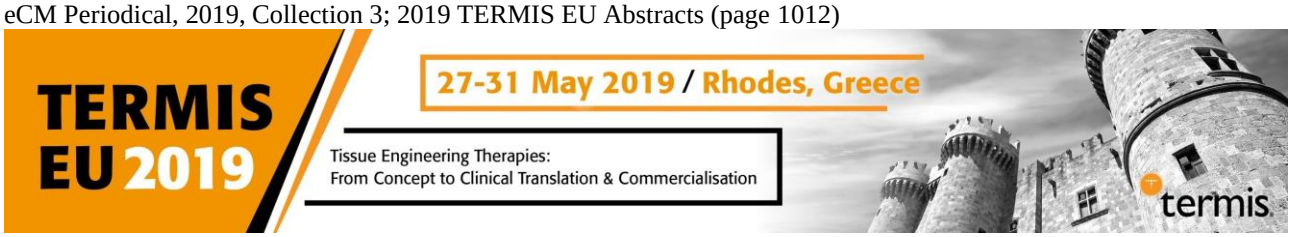
RESULTS AND DISCUSSION: After 14 days of culture, compact and circular pAC or hBM-MSC spheroids were encapsulated in Gel-MOD. Encapsulated spheroids remained viable and were positively stained for GAGs, collagen I and collagen II. Hydrogel properties had an influence on spheroid phenotype. More importantly, using different biopolymer concentrations led to a shift of the spheroid phenotype to a more fibrocartilage or cartilage-like phenotype, demonstrating the impact of biopolymers on the cellular behavior.
(Fibro)cartilage spheroids combined with a 10 w/v% Gel-MOD solution with Irgacure 2959 as photo-initiator, were used to bioprint a larger construct. After printing, spheroid viability and ECM remained stable.

CONCLUSIONS: This study demonstrated that spheroids can be used as modular building blocks for the engineering of a larger-scale (fibro)cartilage construct. Furthermore, Gel-MOD is a suitable bio-ink for 3D bioprinting as it supports the viability and phenotype of the spheroids.

ACKNOWLEDGEMENTS: The authors would like to thank Liesbeth Tytgat, Prof. Dr. Sandra Van Vlierberghe, Prof. Dr. Peter Dubruel and the PBM Group (Faculty of Sciences, Ghent University) for kindly providing the Gel-MOD.

REFERENCES

[1] Berneel E et al. Cells, tissues, organs. 2016; 202(5-6): 369–381
[2] Billiet T et al. Biomaterials. 2014; 35(1): 49–62



Generation of functional corneal endothelial cells from human embryonic stem cells for cornea regeneration medicine

L.W. Hsu[1], W.J. Huang[1], Y.J. Chang[1], W.L. Chen[2], S.M. Hwang[1]
Presenting Author: Li-Wen Hsu, lwh@firdi.org.tw
[1]Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, R.O.C [2]Department of Ophthalmology, National Taiwan University, Taipei, Taiwan, R.O.C

INTRODUCTION: The corneal endothelium is a hexagonal monolayer which plays a crucial function in the maintenance of the dehydration and transparency of cornea. Once corneal endothelial cells (CECs) are damaged or malfunctions, corneal edema and vision loss ensues. However, the CECs are terminally differentiated with barely regenerative capability and can lose by aging or injury. Therefore, the acquisition of proper and sufficient cell resource for CECs transplantation is extremely important in corneal regenerative medicine [1-2]. Herein, we explicate an applicable procedure to access differentiated CECs from human embryonic stem cells (hESCs) by using serum-free and feeder-free culture system.

METHODS: Human embryonic stem cells were seeded on Matrigel™ coated dishes in mTeSR™1 medium for 5-6 days by changing medium daily. When the hESCs reached 60–70% confluence, the medium was changed to hESCs medium supplemented with B-27 (Invitrogen), PDGF-BB (PeproTech) and DKK-2 (PeproTech). Media were changed every other day. Immuno-cytochemistry, and real-time PCR analyses were conducted after 1, 2 or 4 weeks.

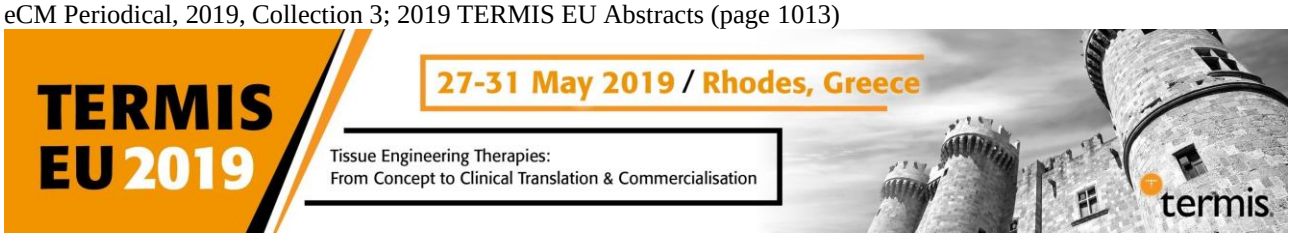
RESULTS & DISCUSSION: After differentiation of hESCs into CEC-like cells, the corneal endothelium marker N-cadherin, tight junctional protein ZO-1, functional pump protein sodium-potassium ATPase, and the main component of the corneal stroma collagenVIIIA1 were detectable. Real-time PCR analyses illustrated significant increase ($p < 0.05$) in AQP1 expression after CECs induction.

CONCLUSIONS: Through this study, we can obtain quantity of hCECs in hope of bringing these cells into tissue engineering and clinical use in the future.

ACKNOWLEDGEMENTS: We thank the financial support by the Ministry pf Science and Technology (MOST), Taiwan (MOST-107-2627-M-080-001-).

REFERENCES

[1] Zhang K et al. Stem Cells Dev. 2014; 23(12):1340-54
[2] McCabe KL et al. PLoS One. 2015; 10(12):e0145266.



Cross-talk between immune cells, ECM and hepatic cells in a bioengineered model of liver fibrosis

C. McQuitty[1,2], D. Natarajan[1,2], W.T. Ma[1,2], L. Sassi[1,2], R.R. Siena[1,2], O. Ajayi[1,2], A. Pellegata[3], P. De Coppi[3], A. Riva[1,2], S. Chokshi[1,2], L. Urbani[1,2]
Presenting Author: Luca Urbani, luca.urbani@researchinliver.org.uk
[1]Institute of Hepatology, Foundation for Liver Research, London, UK [2]Faculty of Life Sciences & Medicine, King's College London, UK [3]Stem Cells and Regenerative Medicine Section, Great Ormond Street Institute of Child Health, UCL, London, UK

INTRODUCTION: Liver fibrosis is accompanied by aberrant changes in the extracellular matrix (ECM) structure and composition that alter cell signaling when interacting with hepatic cells and invading inflammatory cells. Understanding the mechanisms of interaction between these elements in the context of liver injury is key to determine new therapeutic interventions, but current culture and animal models do not recapitulate the complexity of liver fibrosis. Here we used a tissue engineering approach to create a 3D-dynamic, humanized model of liver fibrosis incorporating the liver ECM, native hepatic cells and immune cells circulating in a custom-made perfusion bioreactor.

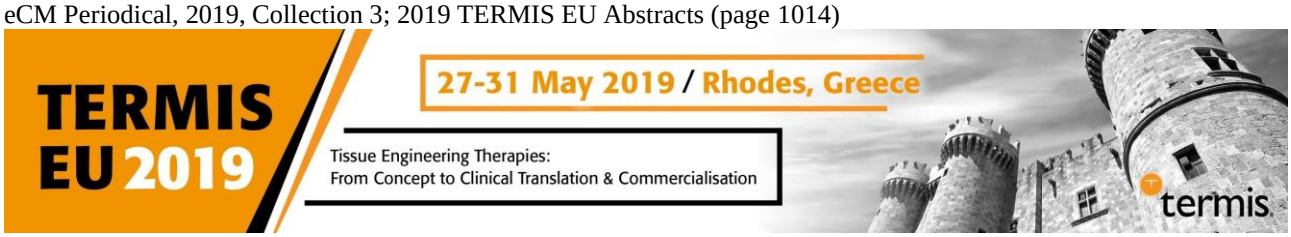
METHODS: Liver scaffolds were produced by decellularization of whole rat liver with established treatment [1]. Hepatic stellate cells (HSCs) were isolated from liver biopsies and peripheral blood mononuclear cells (PBMCs) from matched whole blood or from healthy controls. Decellularised scaffolds were perfused with either PBMCs or HSCs and cultured in static or dynamic conditions for up to 7 days. Scaffolds were analyzed by histology and immunofluorescence.

RESULTS & DISCUSSION: Whole livers were successfully decellularized to remove native cells and DNA, retaining ECM and vasculature. In both static and dynamic culture conditions, scaffolds supported PBMC viability and homing of immune cell subsets. Whole liver decellularized scaffolds perfused with PBMCs showed homing of CD68+ macrophages and CD3+ T cells. The custom-made bioreactor was able to support continuous perfusion of PBMCs and different shear stresses were tested on cell viability by modulating media viscosity and perfusion flow rate. Bioreactor-guided serial seeding of HSCs produced re-populated liver scaffolds where fibrosis was induced by adding TGFβ, activating αSMA expression in HSCs. Cells were also transfected to express the enzyme luciferase allowing non-invasive cell tracking in 3D cultures using bioluminescence imaging for daily quantification/localization analysis of cells.

CONCLUSIONS: These data support the use of decellularized whole liver scaffolds seeded with primary HSCs and perfusion of matched PBMCs in a custom-made bioreactor as an immune-competent 3D model of liver fibrosis.

REFERENCES

[1] Maghsoudlou P et al. PLoS One. 2016; 11(5).



Reconstruction of large bone defect in sheep with customized 3D printed calcium phosphate scaffolds

L. Vidal[1], S. Krissian[2], M.A. Brennan[1,3], Y. Massouz[4], S. Raymond[4], M.P. Ginebra[4], J. De Lima[1], P. Humbert[1], C. Kampleitner[5], P. Rosset[2], P. Layrolle[1]
Presenting Author: Luciano Vidal, lucianovidal75@gmail.com
[1]Inserm UMR 1238, PHY-OS, Faculty of Medicine, University of Nantes, France [2]CHU Tours, Orthopedic surgery Dept., Tours, France [3]Harvard School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts 02138, USA [4]Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgy, Technical University of Catalonia, Barcelona, Spain [5]Department of Pharmacology and Toxicology, University of Vienna

INTRODUCTION: The reconstruction of large bone defects resulting from severe trauma or resection of tumors remains a challenge for orthopedic and plastic surgeons. Today, a vascularized bone (e.g. fibula, floating rib) is taken from the patient and grafted into the defect. However, this transplantation adds morbidity and requires extensive micro surgery to adapt to both the vasculature and the skeleton. We propose an alternative approach consisting of manufacturing patient specific biomaterial scaffolds through 3D printing. This study aims to demonstrate the feasibility of regenerating large bone defects with 3D printed anatomically accurate biomaterial scaffolds in sheep.

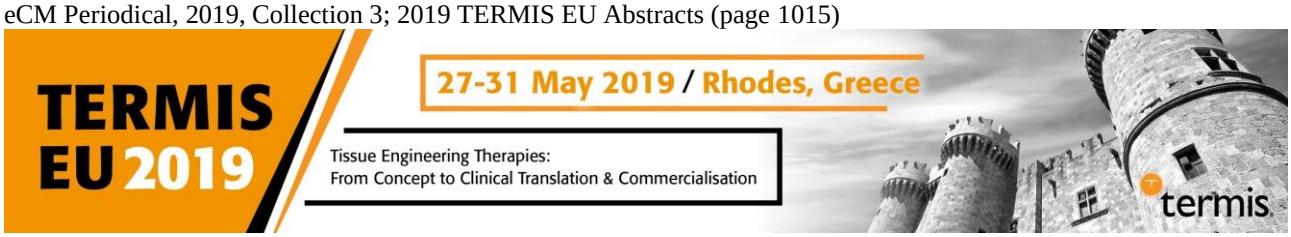
METHODS: The left posterior limb was scanned by computed tomography (CT, Siemens Somatom) and a contrast agent was injected in order to visualize the metatarsus and the vasculature. CT data were imported into medical imaging software and converted to STL files. The metatarsus bone of sheep was 3D printed with PLA filament (Ultimaker 2) from the CT scans. It allowed surgical planning with the placement of an osteosynthesis plate and screws. A cutting guide was also designed and 3D printed in order to create a segmental defect of 35 mm in the metatarsus. A specific biomaterial was produced by 3D printing using a calcium phosphate /pluronic paste that hardens into a porous scaffold. Three groups were considered: empty defects, and defects filled with either a customized biomaterial scaffold, or the biomaterial scaffold with a vascular pedicle running through it. After surgery, bone regeneration and vascularization were followed by CT at 30, 60 and 90 days. Sheep were euthanized and a vasculature contrast agent (Microfil) was injected into the femoral artery. Metatarsus were dissected, fixed in formadehyde 4%, analysed by microCT (Skyscan 1076) and embedded in PMMA for non-decalcified histology.

RESULTS & DISCUSSION: The biomaterial scaffold was made of calcium phosphate apatite with similar composition as bone mineral. Its shape perfectly fitted the metatarsus bone defect. The scaffold had interconnected porosity to favor bone regeneration. CT scans indicated that the empty defect remained non-bridged after 3 months. A limited bone healing was observed with the 3D scaffold. The vascular pedicle going through the scaffold was functional without thrombosis. The vasculature favored bone regeneration of the critical size metatarsus defect. Numerous capillaries were observed inside the 3D scaffold by microCT and histology.

CONCLUSIONS: This pre-clinical study demonstrated the feasibility of 3D printing patient specific biomaterial scaffolds for regeneration of large bone defects resulting from severe trauma or resection of tumor.

REFERENCES

[1] Hoang D et al. Ann Transl Med 2016;4(23):456.



An innovative bioink for 3D bioprinting of mesenchymal stem cells

L. Vidal[1], M. Maillard[1], L. Marchandet[1], B. Brulin[1], J. De Lima[1], P. Humbert[1], G. Louarn[1], E. Courtial[2], L. Pourchet[2], C. Mandon[2], C.A. Marquette[2], P. Layrolle[1]
Presenting Author: Luciano Vidal, lucianovidal75@gmail.com
[1]Inserm, UMR 1238, PHY-OS, Bone Sarcomas and Remodeling of Calcified Tissues, University of Nantes, France [2]CNRS, UMR 5246, ICBMS, INSA, Université Lyon 1, France

INTRODUCTION: 3D bioprinting is an emerging technology which involves additional complexities compared to additive manufacturing, known as three-dimensional (3D) printing, such as hydrogels, cells and growth factors for tissue engineering. 3D bioprinting is ideal for translational medicine and also for basic research applications as it allows tissue engineering based on patient-specific designs. During the bioprinting process, hydrogels are used for printing cells in order to create 3D tissue constructs. This hydrogel, also called bioink, must possess appropriate mechanical, biological and rheological properties for maintaining its shape during cell culture and for allowing cell proliferation and functionality for the tissue development. 3D bioprinting is also an excellent technology for studying drugs and cells interactions in 3D human tissue constructs at the pre-clinical level.

METHODS: In our study, we have developed an innovative bioink, combining gelatin, alginate and fibrinogen (GAF) hydrogels for 3D bioprinting and culturing human mesenchymal stem cells (hMSC). Spherical Hydroxylapatite particles were also loaded in GAF hydrogels containing hMSC and successfully bio-printed into 3D constructs for bone tissue engineering. The rheology of this bioink was first studied in order to optimize the best printability and stability in culture medium as function of composition.

RESULTS & DISCUSSION: Cell viability and proliferation of human mesenchymal stem cells in 3D bioprinted GAF scaffolds were investigated by using fluorescence microscopy and Alamar Blue assay. Results demonstrated a good cell adhesion, spreading and proliferation of hMSC in the GAF-HA bioink.

CONCLUSIONS: In summary, this study showed that this innovative bioink is suitable for bone tissue engineering. This 3D model may be useful for studying bone metastasis behavior of different kinds of cancers like breast and prostate tumors as well as for drugs interactions (ex: chemotherapy).

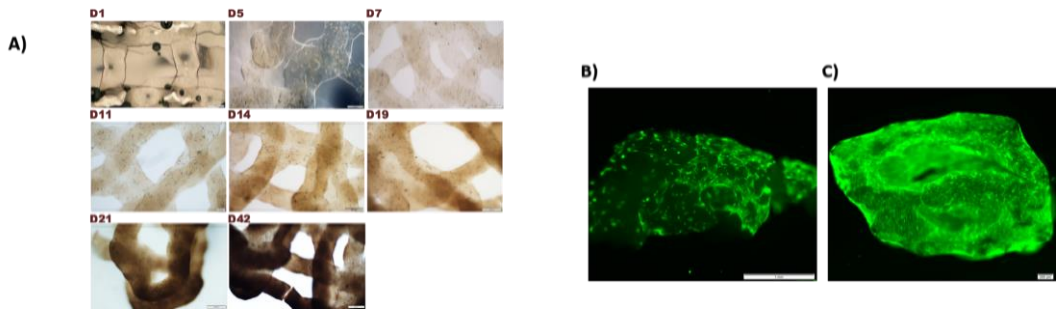
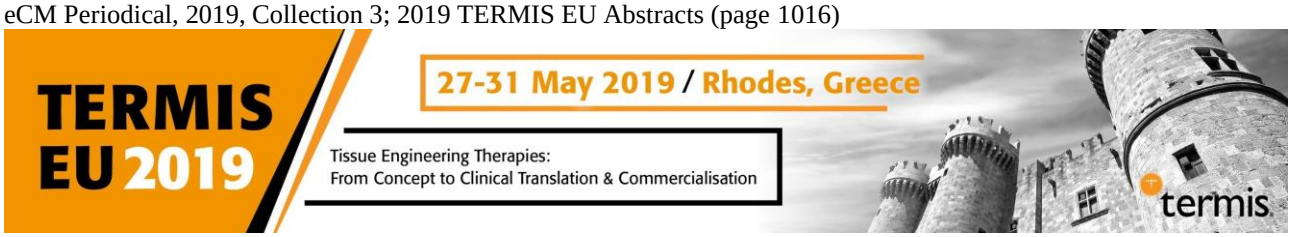


Figure 1: A) Bright field images of GAF bioink scaffolds with NHDF, from D0 till D42. B) C) Fluorescence microscopy images from Live and Dead assay on NHDF at D25 and D29.

REFERENCES

[1] Pourchet L et al, Adv. Healthcare Mater. 20 17, 6, 1601101.



Materials based on human bone extracellular matrix for applications in tissue engineering and regenerative medicine

L. Wolfová[1], V. Lukášová[1], V. Sofková[1], M. Šupová[2], T. Suchý[2]
Presenting author: Lucie Wolfova, lucie.wolfova@iem.cas.cz
[1]Institute of Experimental Medicine CAS, Prague, Czech Republic [2]Institute of Rock Structure and Mechanics CAS, Prague Czech Republic

INTRODUCTION: Worldwide, a large number of surgical procedures to replace or repair tissues damaged by disease or trauma are performed every day. For this purpose, decellularized extracellular matrix (ECM) derived from native human tissues has been recently considered for clinical use and been extensively studied. The advantages of ECM include their full biocompatibility and biodegradability. Importantly, this material contains endogenous bioactive molecules such as cell adhesion proteins, growth factors etc. that support cell adhesion, growth and proper function leading to production and growth of the new tissue.
In this study, we focus on development of biomaterials composed of isolated human bone ECM. Within the study, materials based on inorganic (bioapatite) and organic parts of human bone ECM is developed. The isolated bioapatite is developed as an additive with osteoinductive and osteoconductive properties for enhancement of biological properties of other commonly used materials in clinical practice. The demineralized bone matrix is developed also as an additive (same as the bioapatite) or in the form of a hydrogel scaffold for cell incorporation.
Data obtained from evaluation of their osteoconductive and osteoinductive potential will be presented at the conference.

METHODS: A sample of human bioapatite was isolated from chemically and thermally treated human femoral head. Briefly - bone was cut into slices and treated by 2% NaCl solution at 150°C and 0.2 MPa in an autoclave, followed by degreasing in an acetone-ether mixture for 24 hours and by treatment with 4% NaOH solution at 70°C for 24 hours. The chemically treated bone was calcined overnight at 500°C under atmospheric pressure and dried at 105°C. Organic part was obtained by demineralization of human femoral head by 0,6M HCl for 48 hours followed by neutralization and lyophilization.

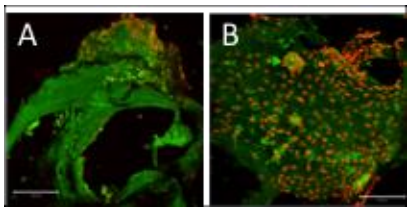
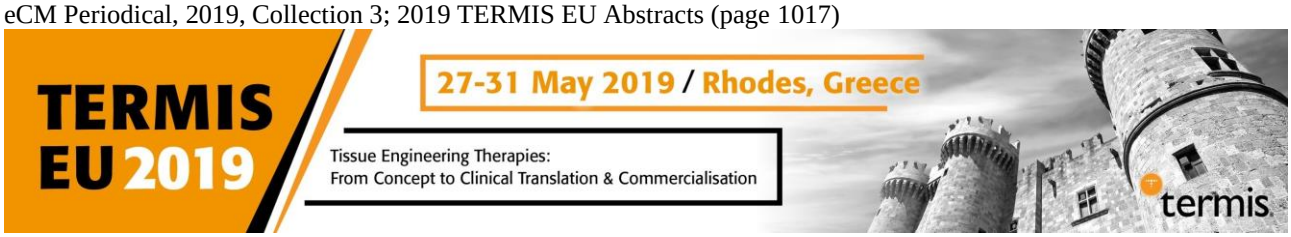


Figure 1: Visualisation of hMSCs cultured on PVA hydrogel film with incorporated isolated human bioapatite (A) and organic part (B) after 10 days of cultivation by confocal microscopy: cell DNA was stained by propidium iodide (red) and membrane structures by DiOC6 (green).

ACKNOWLEDGEMENTS: Financial support was received from Czech Ministry of Industry and Trade (CZ.01.1.02/0.0/0.0/17_107/0012524).

REFERENCES

[1] L. Rosetiet al. / Materials Science and Engineering C 78 (2017) 1246 –1262
[2] M.J. Sawkins et al./Acta Biomaterialia 9 (2013) 7865–7873



Controlled release systems based on increase the conductivity of natural materials

M. Malki[1,2], A. Shapira[2,3], T. Dvir[1,2,3,4]
Presenting Author: Maayan Malki, Maayan.malki1@gmail.com
[1]The Department of Materials Science and Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel [2]The Center for Nanoscience and Nanotechnology, Tel Aviv University, Tel Aviv 69978, Israel [3]The School for Molecular Cell Biology and Biotechnology, Faculty of Life Sciences [4]Sagol Center for Regenerative Biotechnology, Tel Aviv University, Tel Aviv 69978, Israel

INTRODUCTION: Cardiac tissue engineering is envisioned as a regenerative therapy to treat myocardial infarction (MI). In this approach cardiac cells are seeded within three-dimensional (3D) porous scaffolds to create functional cardiac patches. AuNRs are well-known for their biocompatibility and chemical inertness. Recently, it has been shown that gold nanostructures, such as spheres or particles can be incorporated into 3D scaffolds to increase the transfer of the electrical signal between electrogenic cells. In addition, it has been demonstrated that AuNRs are able to significantly increase the conductivity of natural materials without affecting the relevant mechanical properties for cardiac tissue engineering. We sought to utilize these properties to improve the electroactive properties of a chondroitin sulfate hydrogel and develop 3D scaffolds for tissue engineering with an efficient control release system.

METHODS: Chondroitin sulfate (CS) hydrogel was fabricated by dissolving in NaOH and crosslinking with ethylene glycol diglycidyl ether (EGDGE). In order to integrate the AuNRs within the hydrogel, a concentrated solution was added on the last step of gel formation. Using electrical stimulation we released stromal-cell derived factor 1 (SDF-1), which was evaluated by ELISA. To assess the effect of release cell migration towards the released factor was evaluated using a chemotaxis chamber.

RESULTS & DISCUSSION: We have integrated AuNRs in to the CS hydrogel (Figure 1). We have shown that SDF-1 could be released into the scaffold's microenvironment in a voltage-dependent manner. Alternating the electrical stimulation showed a significantly higher release of the protein from the Au-modified CS hydrogel (Figure 2). Moreover, the release was translated into higher number of migrating cells within the modified scaffolds (Figure 3).

CONCLUSIONS: We have shown that the protein could be released into the scaffold's microenvironment in a voltage-dependent manner. The results indicate a high release of SDF-1 from CS with high concentration of AuNRs. This suggests that integrating the particles into 3D electroactive CS hydrogel improved the local electrical conductivity of the hydrogel.

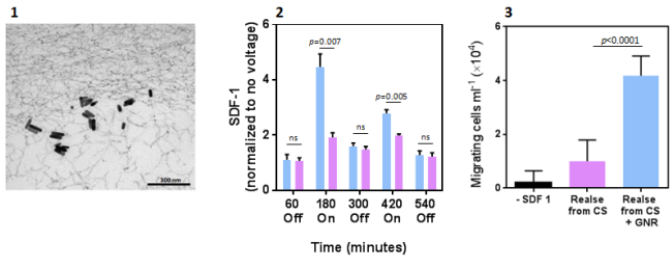
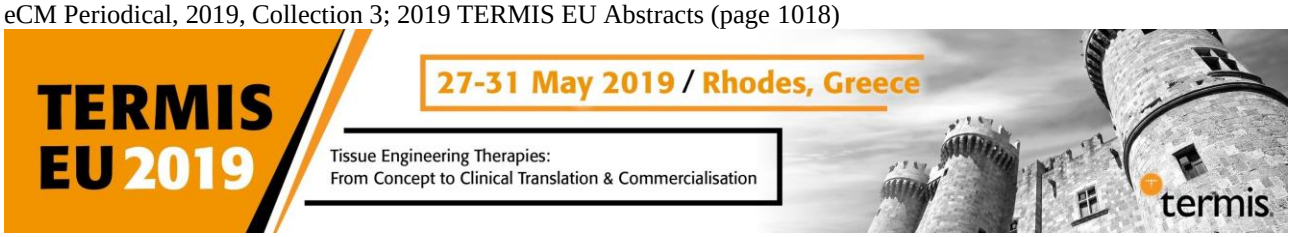


Figure 1: (1) TEM micrographs of a CS-AuNRs composite system. (2) An On–off controlled release profile of SDF-1 from the scaffolds. (3) Cell migration as a result of SDF-1 release.



Alginate/agarose scaffolds induce gene expression of cartilage-related genes in vitro

M. Mata[1,2], L. Milián[1,2], M. Oliver-Ferrandiz[1], M.F. Mínguez[2,3], C. Carda[1,2,4], M. Sancho-Tello[1,2]
Presenting Author: Manuel Mata, manuel.mata@uv.es
[1]Department of Pathology, Universitat de València (Valencia, Spain) [2]INCLIVA, Valencia (Spain)
[3]Department of Surgery, Universitat de València [4]CIBER-BBN, Spain

INTRODUCTION: Hydrogels, used in tissue engineering to stimulate the poor ability of cartilage to regenerate, have demonstrated good chondrogenic properties. We reported that alginate/agarose hydrogels generated a chondrocyte-friendly environment in vivo and in vitro [1], inducing chondrocyte primary cultures and human dental pulp mesenchymal stem cells (hDPSCs) to differentiate into chondrocytes, that presented type II collagen and agreccan. Our main objective was to characterize at the molecular level the expression of different genes in order to explore if both cell types grown in these scaffolds differentiate to a stable or to a hypertrophic chondrocyte phenotype.

METHODS: Low-melting fusion point agarose (1%) was mixed with alginate (3%) and polymerized together. Human primary articular chondrocytes and hDPSCs were isolated and cultured in the 3-D agarose/alginate scaffolds with chondral proliferation or differentiation media [1] for up to 6 weeks. Chondrocytes differentiation markers (type I and II collagens and aggrecan) were studied by immuno-fluorescence. Morphology changes were evaluated by fluorescence microscopy using rhodamine-phalloidin. Total RNA was extracted and real time RT-PCR was used in order to analyze the expression of extracellular matrix (COL1A, COL2A1, ACAN), SOX (SOX5, SOX6, SOX9) and RUNX (RUNX9, COL10A1, MMP1, MMP3 and MMP13) related genes.

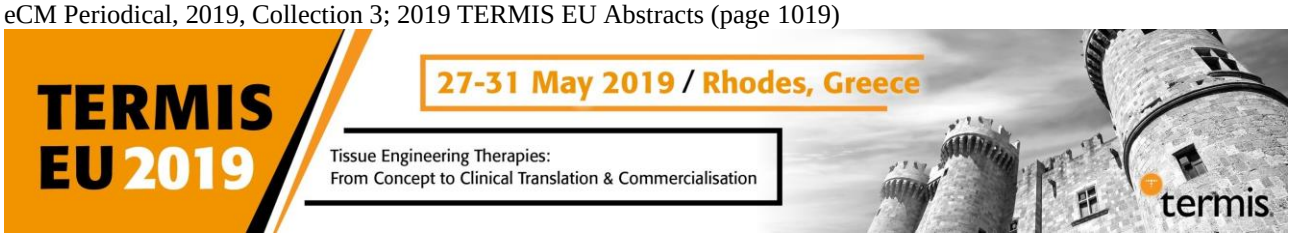
RESULTS & DISCUSSION: Cells grown in alginate/agarose scaffolds developed a chondral-compatible phenotype, with a high presence of type II collagen and aggrecan when differentiation medium was used. They became rounded and formed spheroids, with no stress fibbers observed. Concerning gene expression, the relative expression levels of COL2A1 and ACAN increased, whereas SOX5, SOX6 and SOX9 were significantly upregulated in both cell types. No increase of COL10A but a decrease of COL1A was observed. Nevertheless, a significant increase of the expression levels of MMP13 was found.

CONCLUSIONS: Cells grown in these scaffolds developed a round shape, formed spheroids and expressed high levels of COL2A1 and ACAN, observed by immunofluorescence and real time RT-PCR. The high increase of SOX gene levels, together with a decreased of COL1A1 indicate a stable chondral phenotype, which was enforced by the poor levels of COL10A1, associated to hypertrophic chondrocyte. Nevertheless, the increase of the expression of MMP13 could be indicative of a hypertrophic phenotype development, therefore more studies are necessary in order to clarify this question.

ACKNOWLEDGEMENTS: Supported by MAT2016-76039-C4-2-R (MST and CC) and PI16-01315 (MM) from the Spanish Ministry of Economy and Competitiveness and the Instituto de Salud Carlos III. CIBER-BBN is funded by the VI National R&D&I Plan 2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with the assistance of the European Regional Development Fund.

REFERENCES

[1] Mata M et al. Stem Cells Int. 2017;8309256



Novel potential polymeric nanostructurated scaffolds for guided bone regeneration processes

M. Toledano[1], D. Torres-Lagares[2], J.L. Gutierrez-Perez[2], A. Gutierrez[2], M.A. Serrera[2], M. Toledano-Osorio[1], R. Osorio[1].
Presenting Author: Manuel Toledano, toledano@ugr.es
[1]Department of Stomatology, Dental School, University of Granada, Spain [2]Department of Stomatology, Dental School, University of Seville, Spain

INTRODUCTION: Functionalization of the polymeric membranes through the incorporation of bioactive components may create a specific chemical context, enhancing their osteoconductive properties [1]. The aim of this study was to evaluate the bone-regeneration efficiency of novel polymeric nanostructured membranes and the effect of zinc, calcium, titanium and bone morpho-protein (BMP) loading of the membranes, through in vivo rabbit model.

METHODS: Nanostructurated membranes of methyl-methacrylate were loaded with zinc, calcium, TiO_2 nanoparticles and a recombinant bone-morphogenetic protein (BMP-2). A fifth group of unloaded membrane was tested. These membranes covered the bone defects prepared on the skulls of six rabbits. A sixth bone defect was uncovered (control). Animals were sacrificed six weeks after surgery. Micro computed tomography (µ-CT) was used to evaluate bone architecture trough BoneJ pluging and ImageJ script. Three histological processing of samples, including von Kossa (VK) silver nitrate, toluidine blue (TB) and fluorescence by the deposition of calcein were utilized.

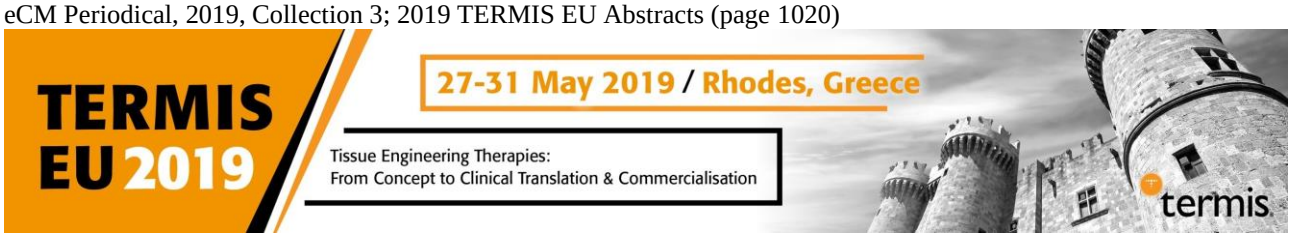
RESULTS & DISCUSSION: Zn-Ms (M-Zn) promoted the highest amount of new bone and higher bone perimeter than both unloaded and Ti-Ms (M-Ti). Ca-Ms (M-Ca) promoted higher osteoid perimeter and bone perimeter than Zn. The skeleton analysis showed that Zn promoted higher branches and junctions of the trabecular bone than BMP-loaded membranes (M-BMP). Euler characteristics and spatial connectivity were higher in samples treated with Zn and BMP, when compared with the control group (CG). Samples treated with Ti showed less bone formation and bony bridging processes. Both Zn and Ca promoted higher number of osteoblasts than CG. BMP and Ca originated higher number of blood vessels than Ti and CG.

CONCLUSIONS: Almost complete union was observed throughout the defects treated with M-Zn showing zones which were occupied by bone-like structures, suggesting that substantial level of new bone was formed in the defect area [2]. In other groups (M-Ti and M-unloaded) the center of the defect was still radiolucent which suggests less bone formation. New bone appeared at both sides of the membrane after using Zn and Ca. M-Ca showed small trabeculae and maximum branch length but low osteogenic potential and scarce angiogenesis [3] than M-Zn. It may be concluded that Zn provided higher regenerative efficiency for bone healing at the created calvarian defects.

ACKNOWLEDGEMENTS: Financial support was obtained by the Ministry of Economy and Competitiveness (MINECO) and European Regional Development Fund (FEDER) [Project MAT2017-85999-P MINECO/AEI/FEDER/UE].

REFERENCES

[1] Castillo-Dalí et al. J Dent. 2014; 47, 71-79.
[2] Fujioka-Kobayashi et al. 2017; 105, 2655-2661.
[3] Turri and Dahlin, 2015; 26, 501-506.



Elastin-like recombinamers-coated surfaces for smart cell harvesting

M. Herrero-Gutiérrez[1], I. González de Torre[2], M. Alonso[1], J. C. Rodríguez-Cabello[1]
Presenting Author: Marcos Herrero-Gutiérrez, mherrero@bioforge.uva.es
[1]BIOFORGE Lab, University of Valladolid − CIBER-BBN, Paseo de Belén 19, 47011 Valladolid, Spain [2]Technical Proteins Nanobiotechnology (TPNBT) S.L., Valladolid, Spain

INTRODUCTION: The harvesting of adherent cells can be carried out by two ways: enzymatically or mechanically. Both procedures can reduce the cell viability, while are difficult to scale up. Although alternative systems have appeared with the temptation of replace the current methods, very little efforts have been exerted employing biosynthetic polymers, such as elastin-like recombinamers (ELRs). Herein has been exploited the versatility of ELRs. Different modules and bioactive sequences can be added, making ELRs promising building blocks for stimuli-responsive materials[1]. On the other hand, the poly(methyl methacrylate) (PMMA) has been widely used in medical applications due to its versatility and biocompatibility, making it a very interesting option as substrate beneath the ELRs.

METHODS: ELRs were obtained by genetic engineering techniques and produced in E. coli. All ELR sequences contain lysine residues in order to graft them covalently onto the surfaces through the amine groups. Including the integrin-recognized RGD[2] sequence, cell adherence is conferred to the recombinamers. The functionalization of the biomolecules was performed following a three-step protocol.

RESULTS & DISCUSSION: The biosynthesized ELRs were characterized by: SDS-PAGE, MALDI-TOF, NMR, FITR-ATR, and DSC. The PMMA control and the resulting coated surfaces were characterized by water contact angle XPS, ESEM and AFM. Studies of adhesion, proliferation and harvesting were accomplished employing HFF-1 cells (figure 1).

CONCLUSIONS: The previous mentioned disadvantages can be overcome by employing surfaces coated by a temperature-responsive bioactive polypeptides showing stimuli-dependent cell adhesion. The values of transition temperature of ELRs were in the range 24.2−32.2°C, indicating that all the recombinamers would be folded at 37°C and unfolded at temperatures below 15°C. The contact angle measurements showed the temperature-responsiveness of the surfaces at different temperatures. Studies of adhesion and proliferation accomplished confirmed the efficacy of the cell harvesting.

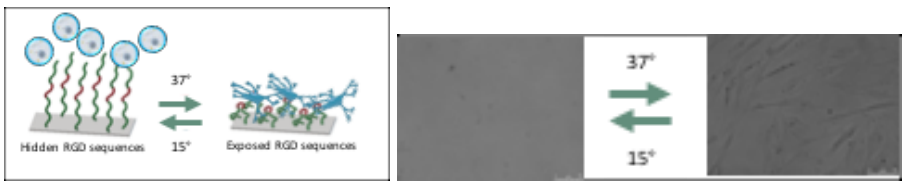
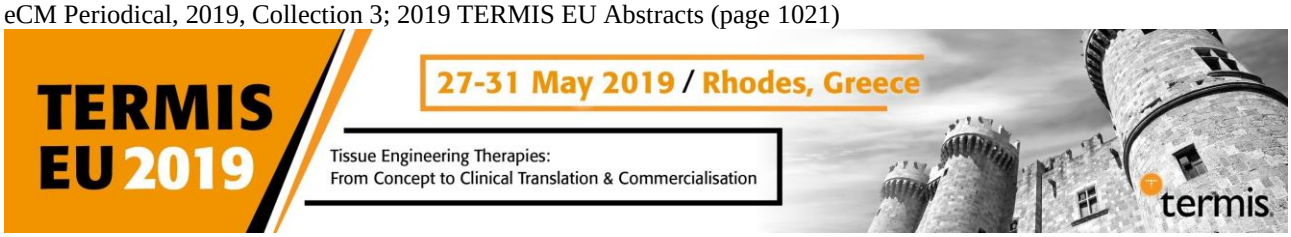


Figure 1: Reversible transition of the ELRs and the RGD motif-cells interaction at cell culture (37°) and harvesting conditions (15°)

ACKNOWLEDGEMENTS: This work was funded by the European Commission (H2020 programme) through the ELASTISLET project (NMP-2014-646075) and Junta de Castilla y León (BOCYL-D-16112016-11).

REFERENCES

[1] Rodríguez-Cabello J C et al. Progress in Polymer Science. 2005; 1119-1145.



Are dynamic culture conditions influencing the detachment of confluent cell sheets from thermoresponsive surfaces?

M. Lindner[1], D. D. Stöbener[1], A. Hoppensack[1], L. Elomaa[1], M. Weinhart[1]
Presenting Author: Marcus Lindner, m.lindner@fu-berlin.de
[1]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Germany

INTRODUCTION: The coating of cell culture substrates with thermoresponsive polymers provides a valuable technique in the field of tissue engineering. In conventional cell harvesting, proteases are employed which degrade the endogenously produced extracellular matrix of cells and cell-cell junctions. In contrast, thermoresponsive coatings allow a gentle harvest of single cells and confluent cell sheets by switching from a less hydrated, cell-adhesive state to a more hydrated, cell-repellent state by simply reducing the temperature (Figure 1). In this work, we focused on the generation of renal and vascular cell sheets to develop an advanced model for studying drug transport and toxicity in the kidney. We hypothesize, that dynamic culture conditions influence the thermoresponsive cell sheet harvest by induction of tight junction formation.

METHODS: We established functional thermoresponsive polyglycidyl ether (PGE) coatings for the detachment of human renal proximal tubule epithelial cells (RPTEC) and human umbilical vein endothelial cells (HUVEC) as mono- and bilayers. Detachment characteristics and functionality of the cell sheets were analysed. Cells were also cultured in a bioreactor system to investigate the impact of flow conditions (time and shear rate) on cell orientation and cell sheet detachment (Figure 2).

RESULTS & DISCUSSION: The PGE coatings permitted the successful detachment of epithelial and endothelial cells. The functionality of the cell sheets regarding polarisation, expression of transport proteins and barrier integrity was demonstrated. Preliminary experiments revealed that the application of shear stress under dynamic cultivation conditions influences the detachment of cell sheets.

CONCLUSIONS: We were able to harvest intact and functional sheets of different cell types via thermoresponsive PGE-coatings. In perspective, the combination of these cell sheets with 3D-printed scaffolds will allow the establishment of an advanced 3D-model of the proximal tubule for further dynamic maturation and evaluation of drug transport and toxicity.

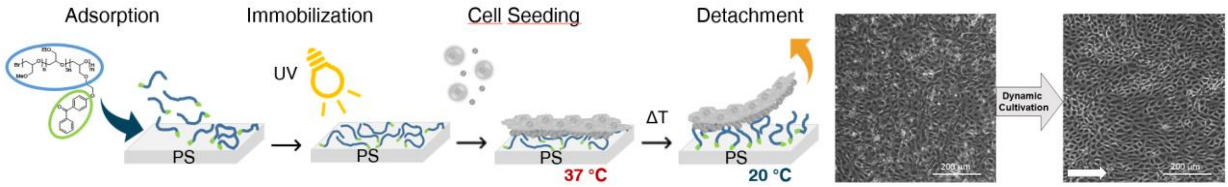
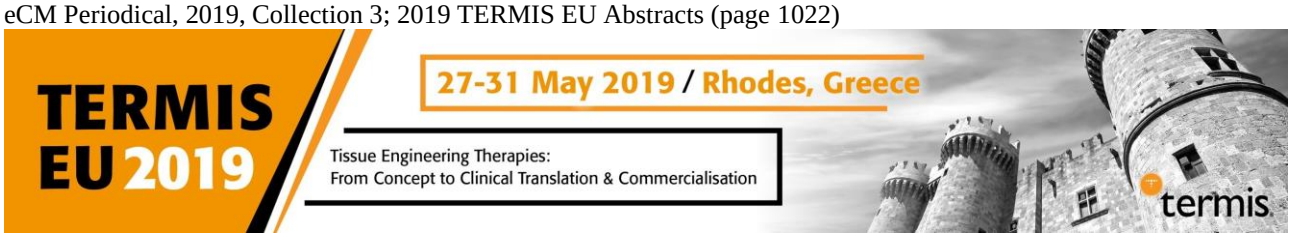


Figure 1: Workflow for the fabrication of thermoresponsive coatings and harvesting of confluent cell sheets for tissue engineering (left). **Figure 2:** Impact of dynamic cultivation on the orientation of HUVECs (13h, 5 dyn/cm²). The arrow indicates the direction of the fluid flow (right).

ACKNOWLEDGEMENTS: M. L. kindly acknowledges the financial support of the Helmholtz Graduate School through a Macromolecular BioScience scholarship; M.W. is grateful to the Federal Ministry of Education and Research through grant FKZ:13N13523



In vitro degradation of collagen-nanohydroxyapatite membranes

M.E.V. Costa[1], J. Baltazar[1], M. Barros[2], M.H.V. Fernandes[1,3]
Presenting Author: Maria Elisabete Costa, elisabete.costa@ua.pt
[1]Department of Materials and Ceramic Engineering and CICECO, University of Aveiro, Portugal [2]Catholic University of Viseu – Regional Center of Beiras, Viseu, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Surgical procedures are nowadays a current practice in dentistry not only for oral rehabilitation (implants) but also for guiding periodontal regeneration. These practices often require the use of membranes to stimulate tissue regeneration, mostly based on natural polymers, such as collagen (COL).
Periodontitis is a common disease of the gums that may develop with severe consequences if an appropriate solution is not timely implemented. Under a weak immune response of the patient periodontitis may progress to periodontal bacteria, allowing infection to propagate from the gum to the roots of the teeth, with serious damages including the destruction of periodontal ligament and the supporting bone and ultimately the loss of teeth [1]. The objective of the present work is to develop composite collagen membranes filled with nanohydroxyapatite to be used in oral rehabilitation and periodontal regeneration.

METHODS: Hydroxyapatite nanoparticles, as fillers, were synthesized by chemical precipitation. COL-based composite membranes were produced by two different techniques including (i) freeze drying and (ii) freeze drying followed by uniaxial pressing under controlled conditions. The produced materials were fully characterized by XRD, SEM/TEM, zeta potential, gas adsorption and FTIR spectroscopy. The in vitro bioactivity of the produced membranes were accessed by immersion tests in synthetic plasma. The degradation profile of the membranes was also examined in PBS.

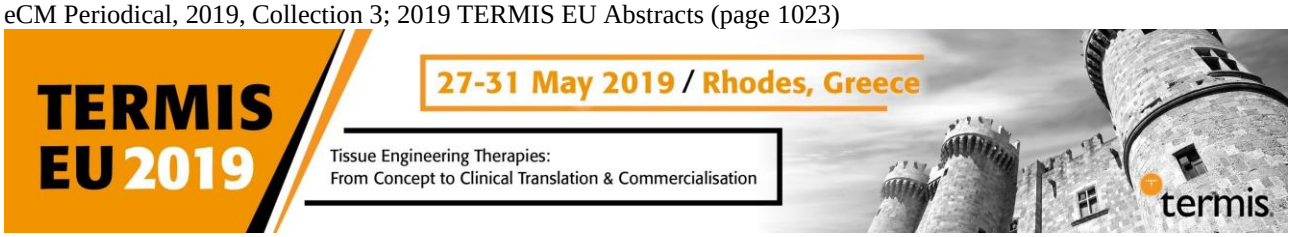
RESULTS & DISCUSSION: The composite membranes exhibited a bioactive behavior suggesting its potential for application in dental tissue regeneration. Furthermore, the degradation rate of the membranes is affected by the technique used for the membrane manufacturing, being higher for the uniaxially pressed membranes.

CONCLUSIONS: The developed COL-hydroxyapatite composites are an interesting alternative to the current barrier periodontal membranes. The performance of the membrane in terms of in vitro surface activity and/or degradation may be triggered by an appropriate selection of the fabrication details.

ACKNOWLEDGEMENTS: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, FCT Ref. UID/CTM/50011/2019, financed by national funds through the FCT/MCTES

REFERENCES

[1] Pihlstrom BL et al, “Periodontal diseases” Lancet (London, England), 2005, 366, no. 9499, 1809–20.



Biopolymer-based, BMP-2 functionalized laser fabricated 3D scaffolds promotes mineralized matrix formation

K. Parkatzidis[1,2], M. Kaliva[1,3], M. Chatzinikolaidou[1,3], M. Farsari[1], M. Vamvakaki[1,3]
Presenting Author: Maria Kaliva, kalivm@iesl.forth.gr
[1]Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas, Heraklion, Greece [2]Department of Chemistry, University of Crete, Heraklion, Greece [3]Department of Materials Science and Technology, University of Crete, Heraklion, Greece

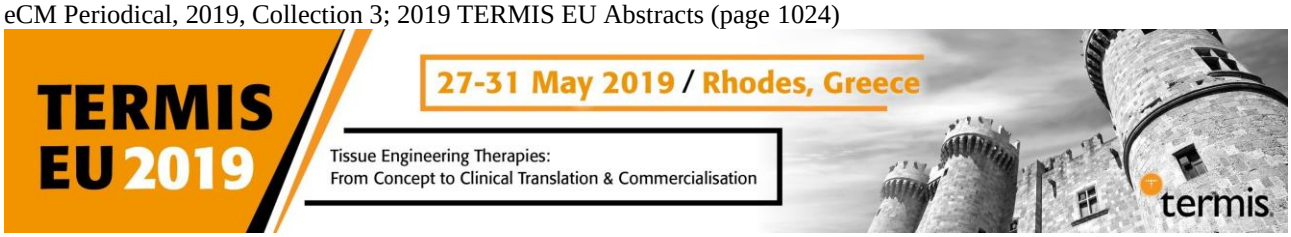
INTRODUCTION: The success of tissue engineering (TE) is based on the choice of the 3D matrix to support the adhesion, proliferation and differentiation of cells. The chemical composition, the physical and mechanical properties, the biodegradability, biocompatibility, and functionality of the 3D scaffolds are crucial characteristics for their use in TE applications [1]. Direct Laser Writing (DLW) is a powerful technique for the fabrication of 3D structures, via a computer-aided design (CAD), for use in optics, metamaterials as well as in the field of biomedical applications [2]. In this study, we have synthesized gelatin methacrylamide (GelMA) and a water-soluble chitosan derivative (chitosan-mod). Hybrid materials of these two biopolymers were employed to fabricate 3D scaffolds via DLW and their ability to support cell growth and mineral matrix was investigated in cell culture.

METHODS: Near IR laser irradiation, operated at 800 nm, was employed to fabricate the 3D scaffolds, in the presence of eosin-Y, as a water soluble, FDA-approved photoinitiator and without any other co-initiators or co-monomers [3]. Cell behavior on the hybrid materials (2D films and 3D scaffolds) was examined using dental pulp stem cells, generously donated by Prof. Athina Bakopoulou, School of Dentistry, Aristotle University of Thessaloniki. Cell adhesion on the 3D scaffolds was visualized by immunocytochemical staining of the actin of the cytoskeleton and the cell nuclei as well as by scanning electron microscopy (SEM). In addition, the 3D scaffolds were functionalized with physically adsorbed BMP-2 and cultured for 21 days.

RESULTS & DISCUSSION: Grid-shaped porous scaffolds with pore size of 70 μm and dimensions 400x400x37 μm^3 were fabricated. We suggest that the free amine groups of GelMA and the chitosan derivative act as co-initiating moieties and support the photopolymerization process. We carried out the cytocompatibility investigations on geometrically well-defined films in order to quantify the cell proliferation results. For the hybrid material we observed a strong initial cell adhesion and a subsequent cell proliferation increase. The results indicate the absence of any cytotoxic effects and suggest that the biopolymer-based hybrid material is biocompatible and exhibits better biocompatibility than the TCPS control. Both immunocytochemistry and SEM characterization showed a strong cell attachment and an increased proliferation over time within the 3D porous scaffolds. The cells cultured for 21 days on the hybrid scaffolds demonstrate an enhanced mineral matrix compared to the control, and this effect is significantly higher on BMP-2 functionalized hybrid structures, as observed by the alizarin red staining.

REFERENCES

[1] Georgopoulou A et al., J Mat Sci Mat Med. 2018; 29: 59.
[2] Parkatzidis K et al., Mac Biom Eng. 2018; 1800458.
[3] Noshadi I et al., Biomater Sci. 2017; 5: 2093



Bio-inspired, biocompatible thymol-functionalized 3D scaffolds with antimicrobial activity

K. Parkatzidis[1,2], M. Kaliva[1,3], M. Chatzinikolaidou[1,3], E. Koufakis[1,3], M. Farsari[1], M. Vamvakaki[1,3]
Presenting Author: Maria Kaliva, kalivm@iesl.forth.gr
[1]Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas, Heraklion, Greece [2]Department of Chemistry, University of Crete, Heraklion, Greece [3]Department of Materials Science and Technology, University of Crete, Heraklion, Greece

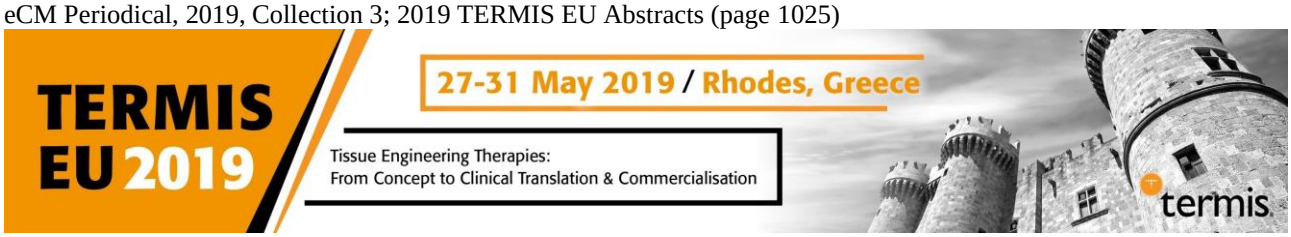
INTRODUCTION: The Direct Laser Writing (DLW) technique is based on the localized polymerization/cross-linking of photosensitive materials, induced by femtosecond laser pulses, and has attracted great interest for the fabrication of 3D scaffolds for use in the fields of tissue engineering and regenerative medicine [1]. In this work, we present the development of a novel photosensitive, hybrid organic-inorganic material which combines excellent cell biocompatibility, calcium matrix deposition, and antibacterial properties conferred by a thymol methacrylate (TM) derivative as a natural antimicrobial agent [2].

METHODS: The material underwent a sol-gel process followed by two-photon polymerization (2PP) to fabricate complex 3D structures of high resolution. The cell viability and proliferation on photopolymerized thin films as well as 3D scaffolds, fabricated by 2PP, were examined by human dental pulp stem cell cultures, generously donated by Prof. Athina Bakopoulou, School of Dentistry, Aristotle University of Thessaloniki. In the current study two controls were used, tissue culture treated polystyrene (TCPS) and the hybrid material without the TM moieties. Cell adhesion on the 3D scaffolds was visualized by immunocytochemical staining of cytoskeleton actin and cell nuclei as well as by scanning electron microscopy (SEM). In addition, 3D scaffolds were further functionalized with bone morphogenetic protein 2 (BMP-2) via physical adsorption and cultured for 21 days [3]. Moreover, the antimicrobial action of the 3D scaffolds was evaluated against E. coli.

RESULTS & DISCUSSION: Grid-shaped porous scaffolds with pore size of 80 μm and dimensions of 555x555x50 μm^3 were fabricated. The cells adhered strongly onto the material surfaces, similarly to the TCPS control. On days 2, 4 and 7 post-seeding, cell viability and proliferation increased. Both immunocytochemistry and SEM characterization showed a strong cell attachment and proliferation within the 3D porous scaffold of both hybrid materials. A large number of cells attached on the 3D scaffolds and attained their characteristic spindle-shaped morphology. The release of physically adsorbed BMP-2 from the scaffolds significantly increased the calcium mineralization deposits onto the 2PP structures after 21 days compared to the controls without BMP-2. Following the biocompatibility testing of the TM-functionalized hybrid material, we examined the antibacterial action of the 3D scaffolds. E. coli were cultured on the scaffolds for 1, 2, 3 and 4 days. The results showed that the bacteria can grow on the 3D scaffolds of the hybrid material that did not contain TM, while a dramatic reduction in the number of attached bacteria on the TM functionalized scaffolds was observed, which underlines the potency of the TM functionalized material for use in the development of biocompatible and antimicrobial 3D scaffolds that support mineralized tissue formation.

REFERENCES

[1] Parkatzidis K et al., Mac Biom Eng. 2018; 1800458.
[2] Bedel S et al., Polym Chem 2015; 53:1975–1985
[3] Chatzinikolaidou M et al., Col Surf B 2016; 149: 233-242



Near infrared light-activated drug delivery

M. Psarrou[1,2], T. Manouras[2], M. Vamvakaki[2,3]
Presenting author: Maria Psarrou, psarroum@iesl.forth.gr
[1]Department of Chemistry, University of Crete, 710 03, Heraklion, Crete, Greece [2]Department of Materials Science and Technology, University of Crete, 710 03, Heraklion, Crete, Greece [3]Institute of Electronic Structure and Laser, FORTH, 711 10, Heraklion, Crete, Greece

INTRODUCTION: Photo-sensitive drug delivery systems have attracted significant attention over the last decades. Near Infrared (NIR) light is a powerful tool for triggering drug release from nanocarriers because it offers deep tissue penetration without damaging the healthy ones. However, the majority of NIR-sensitive nanocarriers include the incorporation of upconverting nanoparticles or two-photon absorption processes [1]. Herein, we present for the first time a novel NIR-photoactivated chemically amplified drug release system by combining acid-degradable nanocarriers based on PEG-b-polyacetal block copolymer micelles with a photosensitization system comprising a photo-acid generator (PAG) that absorbs in the UV region and a photosensitizing molecule that absorbs in the NIR. The degradation of the nanocarriers is based on the acid-catalyzed cleavage of the acetal bonds along the main chain of the polyacetal block. The acid molecules are generated via an electron transfer process from the exited photosensitizer molecules to the PAG molecules, both loaded within the core of the block copolymer micelles.

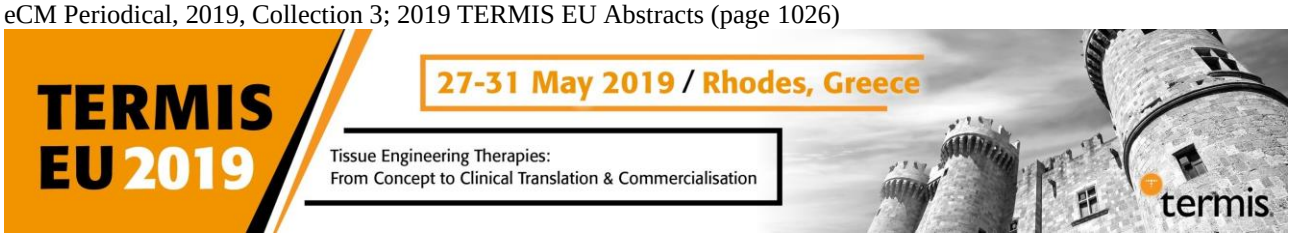
METHODS: The PEG-b-polyacetal copolymers were synthesized via a two-step acid catalyzed polycondensation reaction between 1,4-benzenedimethanol and divinyl ether (first step) and end-capping of the precursor polymer with monohydroxy terminated poly(ethylene glycol) at the vinyl ends (second step) to afford the final block copolymer. Spherical micelles loaded with PAG/photosensitizer were prepared in water. The release of the cargo, following irradiation at 440 nm, 510 nm and 808 nm, was monitored by measuring the absorption of the released photosensitizer molecules as a function of irradiation time.

RESULTS & DISCUSSION: The proposed photoactivation mechanism was verified for three different irradiation wavelengths which correspond to the absorption maxima of the different photosensitizer molecules. In each case, the photosensitizers were chosen to have appropriate HOMO-LUMO energy states in order to achieve an effective electron transfer process. An increase in the degradation rate of the micelles and the release of the cargo was observed when increasing the PAG/photosensitizer mole ratio within the micellar core. Both the PEG-b-polyacetal micelles and the small molecule degradation products are non-toxic, hydrophilic molecules which can be effectively removed from the human body without causing side toxicity.

CONCLUSIONS: In conclusion, we introduce herein a novel NIR-activated system based on polyacetal nanocarriers and organic photosensitizers. Our novel system operates at 808 nm and very low irradiation energies which opens new pathways in the development of photo-activated drug delivery.

REFERENCES

[1] Karimi M. et al. J. Am. Chem. Soc. 2017;139:4584.
[2] Pasparakis G. et al. Nat. Commun. 2014;5:3623



Computational model for the cartilage regeneration process induced by scaffold implantation

K. Márquez-Flórez[1,2,3], D. Garzón-Alvarado[1,2], C. Carda[3,4], M. Sancho-Tello[3,4]
Presenting author: María Sancho-Tello, stello@uv.es
[1]Department of Mechanical and Mechatronic Engineering, Universidad Nacional de Colombia, Bogotá, Colombia [2]Numerical Methods and Modeling Research Group (GNUM), Universidad Nacional de Colombia, Bogotá, Colombia [3]Department of Pathology, Universitat de València, Valencia, Spain [4]INCLIVA Biomedical Research Institute, Valencia, Spain

INTRODUCTION: Articular cartilage has a limited ability to repair itself, therefore, it is common to find pathologies related to cartilage injuries. In previous studies, where scaffolds/cell-free implants were used in an in-vivo experimental model in rabbits, hyaline cartilage is regenerated the upper part of the scaffold three month after the scaffold implantation [1,2]. This suggests that the rapid growth of the superficial layer of the cartilage covers the scaffold and the mechanical conditions due to the presence of the scaffold displaces it to the subchondral bone [1]. Given the above, our interest is to propose and develop a simulation tool to predict the results of polyacrylate polymer implants, their weaknesses and advantages. In this way, to achieve a deeper understanding of the processes involved in the regeneration of articular cartilage.

METHODS: A computational simulation was developed, using finite element methods. The geometry of the cartilage, implant, subchondral compact and trabecular bones were considered (Figure 1). The subchondral compact bone, the trabecular bone, and the implant were modeled as poroelastic materials [3], whereas the cartilage was modeled as a poroelastic, fibril reinforced material [4]. Additionally, for the bone, a remodeling model was applied, so that its Young's modulus changed according to the deformation [5]. Moreover, a model for bone and cartilage regeneration was included.

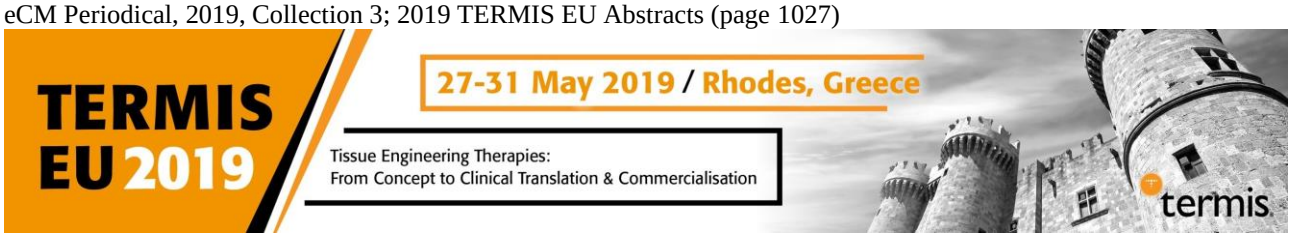
RESULTS & DISCUSSION: It is noticeable that the abnormal mechanical conditions that both bones, subchondral and trabecular, goes under might generate a bone remodeling process under the implant. Meanwhile, the implant gives support to the surrounding cartilage tissue. Also, as the bone under the implant remodels, it allows the implant to move downwards inside the bone. Moreover, as the implant displaces inside the bone, the cartilage tissue experiments ideal conditions to regenerate.

CONCLUSIONS: Computational simulation is suitable to explain the phenomenon of the articular cartilage regeneration. The obtained results are comparable to those observed in the experimental studies.

ACKNOWLEDGEMENTS: Spanish Ministry of Economy, research grant MAT2016-76039-C4-2-R.

REFERENCES

[1] Sancho-Tello M et al. Int J Artif Organs. 2015; 38(4):210-23.
[2] Sancho-Tello M et al. Int J Artif Organs. 2017; 40(7):350-7.
[3] Isaksson H et al. J Theor Biol. 2008; 252(2):230-46.
[4] Wilson W et al. J Biomech. 2004; 37(3):357-66.
[5] Martínez-Reina J et al. PLoS ONE. 2016; 11(12): 615-9.



Chondrocytes maintain their phenotype and viability after exposed to a magnetic field in vitro

M. Sancho-Tello[1], R. Salvador-Clavell[1], L. Milián[1], M. Mata[1], J.J. Martín de Llano[1], C. Carda[1,2]
Presenting Author: María Sancho-Tello, stello@uv.es
[1]Department of Pathology, Universitat de València (Valencia, Spain) and INCLIVA, Conselleria de Sanitat, Valencia (Spain) [2]CIBER-BBN, Spain

INTRODUCTION: Magnetic fields are applied in patients with articular chondral lesions in order to improve cartilage regeneration, but little is known about its direct effect on chondrocyte survival or morphology. In the present study we used chondrocyte cultures differentiated from human dental pulp mesenchymal stem cells (hDPSCs), and exposed them to a controlled magnetic field that reproduce mechanical forces by acting on cells, which could improve articular cartilage regeneration.

METHODS: hDPSCs (Lonza) were centrifuged into 0.5 ml microtubes to obtain 3-D pellets, and cultured with chondral proliferation medium (αMEM with 10% FBS, 1% L-glutamine and antibiotics). Samples were randomly assigned to 3 different groups. Treated cells (group A) were exposed on a daily schedule of 2 cycles (20 min irradiation, 40 min resting) x2, with 90 min resting time between each cycle, for 3 days, by means of a bioreactor [1] that originates a magnetic field (30 T/m, pulse direction changing every 3 s). Control non-irradiated samples were kept outside the cell incubator at RT (group B) in parallel to group A, or were maintained inside the cell incubator (group C). After the 3-day treatment period, viability assay (MTS) was carried out, cell morphology was studied by Harris hematoxylin-eosin staining, and chondral markers (type II collagen and aggrecan) were assessed by immuno-fluorescence, as well as actin content.

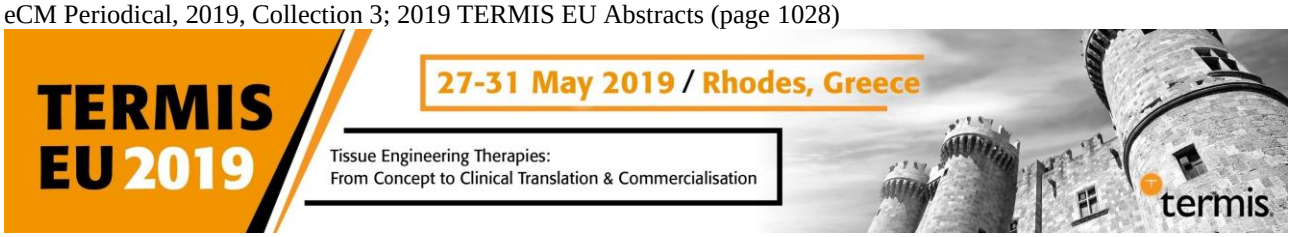
RESULTS & DISCUSSION: MTS viability test showed that irradiated cells (group A) presented an increased absorbance values with respect to control groups B and C. Cellular morphology were similar in all three groups, with cells showing a polygonal morphology with a spherical nucleus, and eventually small vacuoles in the cytoplasm, with similar actin content and distribution. Type II collagen was not observed in any group at this time of culture, whereas aggrecan was first observed in irradiated samples.

CONCLUSIONS: We have previously characterized the proliferation of hDPSCs and their ability to differentiate in chondrocytes [2]. We observe now that 3-day magnetic irradiation does not damage cell survival, on the contrary, an increase in cell viability was observed with respect to controls, as well as signs of early chondral differentiation (presence of aggrecan), suggesting it induces cellular proliferation and differentiation without altering cellular morphology.

ACKNOWLEDGEMENTS: Supported by the Spanish Ministry of Economy, Industry and Competitiveness research grant MAT2016-76039-C4-2-R, and the Instituto de Salud Carlos III. CIBER-BBN is funded by the VI National R&D&I Plan 2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with the assistance of the European Regional Development Fund.

REFERENCES

[1] Salvador-Clavell R et al. Histol Histopathol. 2017;32(Suppl. 1):170
[2] Mata M et al. Stem Cells Int. 2017;8309256



Nanotherapeutic strategy to promote bone regeneration. Effects of ipriflavone-loaded mesoporous nanospheres on in vitro osteogenesis

L. Casarrubios[1], N. Gómez-Cerezo[2,3], M. J. Feito[1], M. Vallet-Regí[2,3], D. Arcos[2,3], M. T. Portolés[1]
Presenting Author: María Teresa Portolés, portoles@quim.ucm.es
[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), 28040 Madrid, Spain [2]Departamento de Química en Ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria Hospital 12 de Octubre i+12, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain [3]CIBER de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain

INTRODUCTION: Nanotherapeutic strategies based in nanoparticles for local delivery of drugs, growth factors and other bioactive molecules inside the bone cells represent a significant advance in bone tissue engineering therapies [1]. Our recent studies evidence that ipriflavone-loaded mesoporous nanospheres induce a significant decrease of osteoclast proliferation and resorption activity after 7 days in coculture with osteoblasts, favouring the macrophage M2 reparative phenotype [2]. In the present study, the incorporation and effects of these nanospheres on undifferentiated MC3T3-E1 preosteoblasts, the most common model of in vitro osteogenesis [3], have been evaluated through specific cell parameters and key differentiation markers.

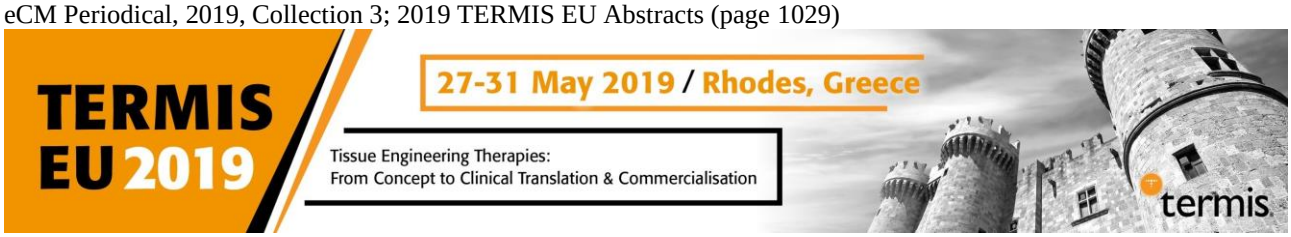
METHODS: Hollow mesoporous SiO_2-CaO nanospheres were labelled with fluorescein isothiocyanate (FITC-NanoMBGs) and loaded with ipriflavone (NanoMBG-IPs) as described previously [2]. MC3T3-E1 preosteoblasts were cultured in the presence of different doses of these nanospheres (10, 30 and 50ug/ml) in Dulbecco's Modified Eagle´s Medium with 10% fetal bovine serum, 50 ug/ml -glycerolphosphate, 10 mM L-ascorbic acid, 1 mM L-glutamine, penicillin and streptomycin. FITC-NanoMBG incorporation by preosteoblasts was evaluated by flow cytometry and confocal microscopy. Cell viability, cell cycle phases, apoptosis, intracellular reactive oxygen species (ROS) and calcium were evaluated by flow cytometry. Alkaline phosphatase (ALP) activity and matrix mineralization were studied as key differentiation markers. Interleukin 6 (IL-6) secretion was quantified by ELISA.

RESULTS & DISCUSSION: FITC-NanoMBGs were incorporated by MC3T3-E1 preosteoblasts in a dose and time dependent manner. NanoMBG-IPs induced a significant increase of S cell cycle phase without inducing apoptosis. Intracellular ROS and calcium content decreased significantly after treatment with 50 μg/ml of NanoMBG-IPs 24 hours. No significant changes of IL-6 secretion were detected. Significant increases of both ALP activity and matrix mineralization were observed after 11 days of treatment with 50 μg/ml of NanoMBG-IPs, thus indicating their positive effects on in vitro osteogenesis.

CONCLUSIONS: These results ensure the potential application of ipriflavone-loaded mesoporous nanospheres as intracellular drug delivery system for bone regeneration by promoting osteogenesis.

REFERENCES

[1] Walmsley GG et al. Nanomedicine 2015; 11:1253-63
[2] Casarrubios L et al. Eur J Pharm Biopharm 2018; 133:258-68
[3] Addison WN et al. Bone 2015; 71:244-56



Highly efficient and biocompatible photoinitiators for multi-photon polymerization

K. Parkatzidis[1,2], G. Noirbent[3], D. Ladika[1,4], M. Chatzinikolaidou[1,5], F. Dumur[3], M. Farsari[1], M. Vamvakaki[1,5]

Presenting Author: Maria Vamvakaki, vamvakak@iesl.forth.gr

[1]Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas, Heraklion, Greece [2]Department of Chemistry, University of Crete, Heraklion, Greece [3]Aix Marseille University, CNRS, ICR, UMR, Marseille, France [4]Department of Physics, University of Crete, Heraklion, Greece [5]Department of Materials Science and Technology, University of Crete, Heraklion, Greece

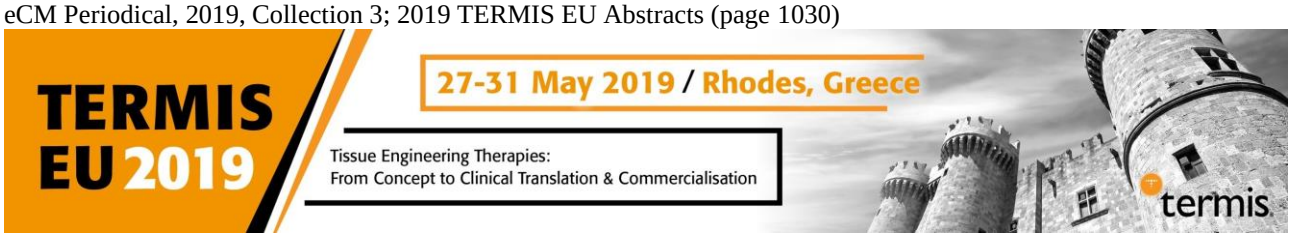
INTRODUCTION: The Direct Laset Writing (DLW) technique is based on the localized polymerization/cross-linking of photosensitive materials induced by femtosecond laser pulses. An essential component of the photosensitive system is the photoinitiator (PI), required to initiate the polymerization/cross-linking process by multi-photon adsorption [1]. While, a large number of organic molecules, possessing high multi-photon absorption cross-sections (σ_2), have been employed as PIs, the vast majority of them suffer from poor solubility in the solvent medium combined with a low yield of radical generation. Furthermore, many of the PIs are toxic and highly fluorescent, which limit their use in biomedical applications. To overcome these challenges, highly soluble, biocompatible and low-fluorescent molecules are essential for use as PIs in this field [2]. In the present study, we report the use of novel PI molecules for multi-photon polymerization.

METHODS: The photophysical properties of the PIs were investigated by UV−Vis spectroscopy. Two beam initiation threshold (2-BIT) was employed in order to determine the order of the effective nonlinear absorption of the new PIs. In addition, 3D structures were fabricated at different laser intensities and writing velocities. Finally, the biocompatibility of the PIs was assessed by investigating the adhesion, viability, and proliferation of human dental pulp stem cells on photopolymerized thin films containing 1 mol% of the employed PIs and comparing them to tissue culture treated polystyrene (TCPS) control surfaces.

RESULTS & DISCUSSION: All the PIs investigated in this study possess an absorption peak at around 370 nm and therefore, are expected to exhibit a two-photon absorption at around 800 nm. In addition, the PIs are transparent at 800 nm and have low fluorescence. Via the 2-BIT measurements the non linear order of absorption was found at around two, suggesting that these PIs have two-photon absorption at 800 nm. The polymerization threshold in all cases was lower than 3 mW, while the polymerization window was higher than 35 mW. The combination of low energy and high speed of polymerization resulted in the fabrication of 3D structures with high resolution (130 nm). Finally, we demonstrated the biocompatibility of the PIs by visualizing the cell morphology of the adhered cells onto the PI thin films, which is the same as on control TCPS surfaces. The cells show a very strong adhesion from the first day in culture and proliferate increasingly up to day 7 in culture. The viability of human dental pulp stem cells cultured on photopolymerized thin films containing 1 mol% of each PI indicate 70-80% of the cell viability on the TCPS control surface on day 1, and 90-100% on days 3 and 7 in culture.

REFERENCES

[1] Nazir R et al. Macromolecules 2015; 48: 2466-2472.
[2] Nazir R et al. Chem Mater. 2014; 26: 3175-3184.



Multi-functional antimicrobial surface coatings

E. Koufakis[1], T. Manouras[1], E. Vasilaki[2], I. Peraki[3,4], M. Vamvakaki[1,5]
Presenting Author: Maria Vamvakaki, vamvakak@materials.uoc.gr
[1]Department of Materials Science and Technology, University of Crete, 700 13 Heraklion, Crete, Greece [2]Department of Chemistry, University of Crete, 700 13 Heraklion, Crete, Greece [3]Department of Medicine, University of Crete, 700 13 Heraklion, Crete, Greece [4]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, 700 13 Heraklion, Crete, Greece [5]Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas, 700 13 Heraklion, Crete, Greece

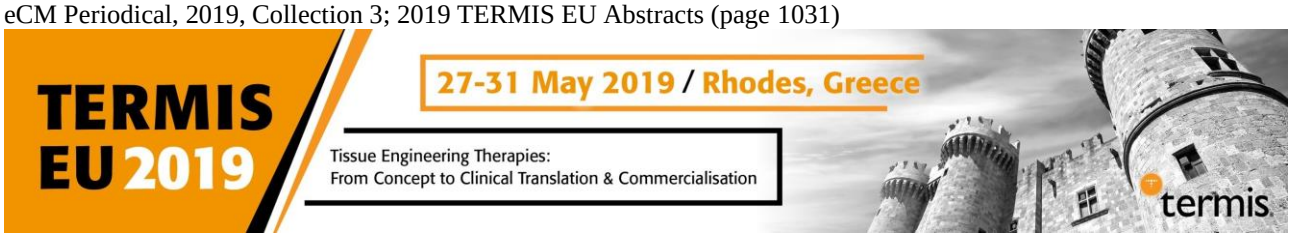
INTRODUCTION: Antimicrobial surfaces that prevent biofouling from any type of microorganism are attractive in inhibiting the spread of microbial infections. Antibacterial surfaces are continuously being developed for a plethora of applications spanning from biomedical tools, packaging, marine technology and navigation.

METHODS: The primary amine groups along the main chain of chitosan were transformed into biocidal, cationic quaternary ammonium salt moieties upon reaction with alkyl halides. The degree of quaternization of chitosan was determined by proton nuclear magnetic resonance spectroscopy. Furthermore, this modification reaction enhanced the solubility of chitosan in water. Modified chitosan and an acid-labile acetal-based bifunctional alkyl halide, to act as a cross-linker of the polymer, were deposited and reacted on glass and silicon substrates. The thickness, wettability and morphology of the polymer films were assessed by ellipsometry, water contact angle measurements and scanning electron microscopy, respectively.

RESULTS & DISCUSSION: In this work, we have developed novel, biodegradable polymeric coatings based on modified chitosan bearing environmentally and toxicologically friendly biocidal groups. These coatings are able to self-polish and regenerate their antimicrobial activity upon repetitive bacterial fouling. The antimicrobial action of the polymer films was evaluated using two representative gram-positive and gram-negative bacteria strains. The controlled self-polishing behavior and the regeneration of the antimicrobial activity of the polymer films were investigated.

CONCLUSIONS: Chitosan was modified by reaction with alkyl halides to introduce cationic, biocidal groups along the polymer chain. The modified chitosan films were cross-linked using an acid-degradable acetal-based cross-linker. The antimicrobial activity of the cross-linked chitosan thin films was tested against both Gram-positive and Gram-negative bacteria and their self-polishing behavior was monitored in aqueous media by ellipsometry.

ACKNOWLEDGEMENTS: The project "Novel hybrid biocidal surfaces with self-renewal properties and direct detection of their antimicrobial activity" is implemented through the Operational Program "Human Resources Development, Education and Lifelong Learning" and is co-financed by the European Union (European Social Fund) and Greek national funds.



Structural and histochemical properties of the attachment cement from Dermacentor marginatus

J. Suppan[1], M. Fürsatz[2], B. Engel[3], M. Marchetti-Deschmann[3,6], L. Maers[4], M. Valtiner[4], O. Andriotis[5], P. Thurner[5,6], S. Nürnberger[1,2,6]
Presenting Author: Marian Fürsatz[2], marian.fuersatz@trauma.lbg.ac.at
[1]Department of Orthopedics and Trauma-Surgery, Medical University of Vienna, Austria [2]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria [3]Institute of Chemical Technologies and Analytics, TU Wien, Vienna, Austria [4]Institute for Applied Physics, TU Wien, Vienna, Austria [5]Institute of Lightweight design and structural biomechanics [6]Austrian Cluster for Tissue Regeneration, Austria

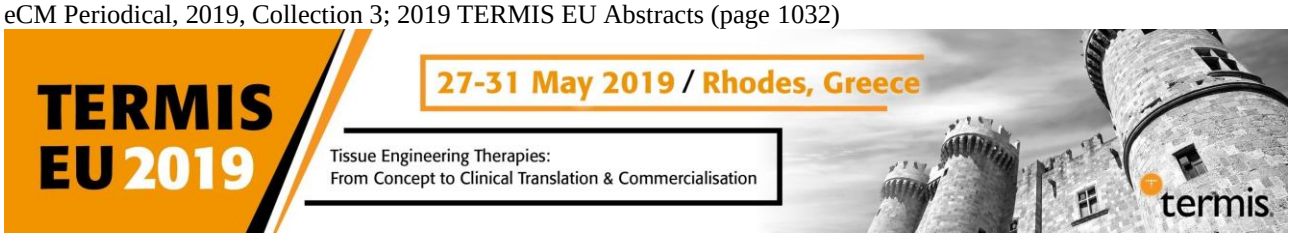
INTRODUCTION: Clinical tissue adhesives are important surgical tools, but currently available products have either weak cohesive bonding forces (e.g. fibrin-based glues) or harmful side effects of toxic components (e.g. cyanoacrylate-based glues). This leads to the need to develop new, biocompatible and strong bonding tissue glues. Ixodid ticks (Arachnida) produce a secretion, the cement, that bonds to tissue naturally. When the animals stitch their mouthparts into the host skin, they produce the cement to support anchoring during blood feeding. Since this adhesive has the potential to bond to humid and even wet tissue and is further suggested to be biocompatible, the material is of interest for medical glue research.

METHODS: Tick cement was harvested from adult Dermacentor marginatus that were fed in artificial feeding units on cattle blood. The material was macroscopically documented with high-resolution light microscopy, the adhesiveness tested with a surface force apparatus (SFA) and biomechanical properties measured with atomic force microscopy (AFM). Oil red staining was performed to detect lipids. For paraffin histology samples were fixed in paraformaldehyde and sections were stained with Arnow staining for catecholes, Biebrich Scarlet (BBS) for basic proteins, Periodic Acid Schiff (PAS) for carbohydrates and Alcian blue for acid muco-polysaccharides. For ultrastructural investigations samples were fixed with OsO_4, resin embedded, sectioned and observed in a transmission electron microscope (TEM). Scanning electron microscopic (SEM) analysis were done with dehydrated, dried samples in high vacuum mode.

RESULTS & DISCUSSION: The overall structure of the cement revealed a droplet-like sub-compartmentation and partial positive staining for lipids with oil red. Histochemically, the most dominant staining of the cement was observed with BBS for basic proteins. Moderate PAS-positive regions indicated carbohydrates at the margins of the cement cones and Alcian blue staining was almost completely absent. Arnow staining was negative. TEM revealed a heterogeneous ultrastructure with locally elongated, electron dense material embedded in homogenous ground substance. In SEM, the surface of the cement droplets appeared rough. AFM revealed viscoelastic properties and pull-off tests the adhesiveness of the material.

CONCLUSIONS: The analysis reveal that tick cement consists of protein and lipid rich components and has viscoelastic properties.

ACKNOWLEDGEMENTS: Financial support was received from the Austrian Science Fund FWF (Project Number P 28962) and the COST Action CA15216 “European Network of BioAdhesives” ENBA



A culturing system for induction of autonomous pellet formation for chondrogenic differentiation

M. Fürsatz[1,2], C. Gahleitner[1], B. Schädl[3], H. Redl[2,4] S. Nürnberger[1,2,4]
Presenting Author: Marian Fürsatz, marian.fuersatz@trauma.lbg.ac.at
[1]Department of Orthopedics and Trauma-Surgery, Division of Trauma-Surgery, Medical University of Vienna, Vienna, Austria [2]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria [3]University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria [4]Austrian Cluster for Tissue Regeneration

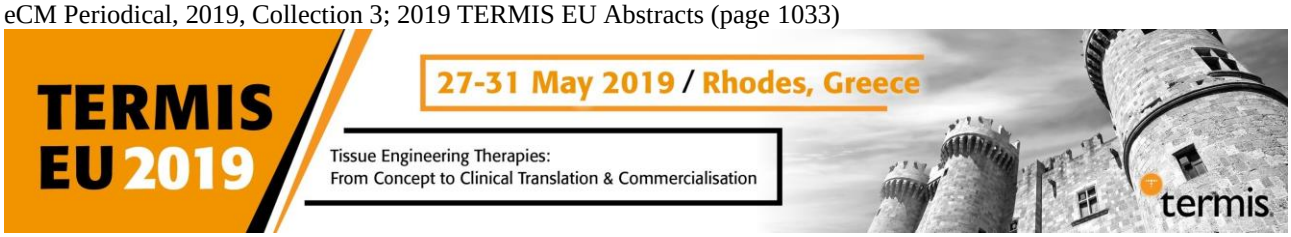
INTRODUCTION: Scaffold free spheroid cultures are the current standard in vitro model for chondrocyte re-differentiation and chondrogenic stem cell differentiation. However currently used systems often have time and reagent intensive maintenance. Herein we present an efficient system for spheroid mass-production, by self-assembling of cell spheroids from monolayer, with promising application in cartilage regeneration.

METHODS: Standard cell culture dishes were compartmentalized using CO_2 laser engraving. Telomerase immortalized adipose derived stromal/ stem cells (ASC/TERT1) and primary human articular chondrocytes (HAC) were seeded on these plates at a total amount of 1 x 10^6 cells either alone or in co-culture (1:4,1:1,4:1 HAC:ASC/TERT1 ratios). Cells were further cultivated in chondrogenic media containing low doses of growth factors (1 ng/ml TGFβ-3 and BMP-6). Spheroid formation kinetics and controllability of size by variation of compartment size were investigated. Spheroids were analyzed using time-laps imaging, light-/electron-microscopy, (immuno-)histology (Alcian blue, collagen type 2 (Col2)). Additionally chondrogenic differentiation over the duration of 3 and 5 weeks was compared to standard pellet culture using (immuno-)histology and qRT-PCR (collagen type 1/2(Col1/2)).

RESULTS & DISCUSSION: Spheroid size was controllable by variation of grid size and yielded reproducible diameters (SD < 10%) with mean values of 340.9 µm and 134.4 µm for 3 mm and 1 mm grids respectively ($p < 0.0001$). Mature spheroids formed within 3 weeks for ASC/TERT1 monoculture and 1-2 weeks for HAC:ASC/TERT1 co-cultures, with significant difference in formation speed for 1:1 and 4:1 HAC:ASC/TERT1 ratio ($p < 0.05$ and $p < 0.01$ respectively). Histological analysis showed differences in matrix distribution between ASC/TERT1-only and co-cultures. When comparing grid plate and standard pellet culture derived cell spheroids histologically, similar or slightly improved matrix deposition in grid plate spheroids could be observed. First results showed that while ASC/TERT1-only spheroids had very low Col2 expression in PCR in both culture systems, co-cultures had similar Col2 expression in both systems, but showed increased Col2/Col1 ratio in grid plate culturing system.

CONCLUSIONS: The data herein presented show an efficient system for cell spheroid self-assembly from monolayer with highly reproducible spheroid size, and similar or improved differentiation capacity compared to standard pellet culture.

ACKNOWLEDGEMENTS: Financial support was received from the "Österreichische Forschungsförderungsgesellschafft" (FFG; Projectnr.: 867720). ASC/TERT1 were kindly provided by Evercyte GmbH.



In vitro Study of Ti-34Nb-6Sn alloy in mesenchymal stem cell culture for biomedical applications

M.C. Rossi[1,2], D.L. Bayerlein[3], J.S. Brandão[1], J.P.H. Pfeifer[1], F.C. Stievani[1], C.A. Houssini[1], M.J. Watanabe[1], M.J. Saeki[2], A.L.G. Alves[1]
Presenting Author: Mariana Rossi, mrncorrea90@gmail.com
[1]Cell therapy and Regenerative Medicine Laboratory, São Paulo State University, Botucatu, São Paulo, Brazil [2]Department of Chemistry and Biochemistry, Bioscience Institute, São Paulo State University, Botucatu, São Paulo, Brazil [3]Institute of Technological Research São Paulo, Brazil

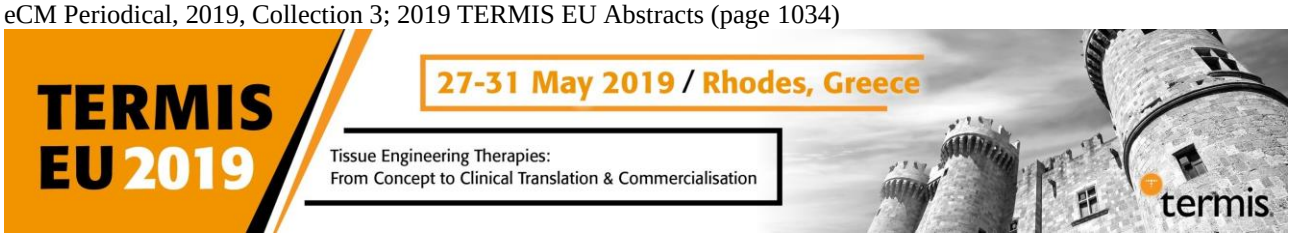
INTRODUCTION: The increase in life expectative of the population and the numerous traffic accidents have motivated the need for the development and improvement of orthopedic devices to replace the functionality of the bone tissue in the long term. Metallic devices are the most widely used, such as Ti-6Al-4V and Co-Cr alloys. However, they have cytotoxic nature over long periods, causing respiratory and neurological conditions. On the other hand, the Ti-Nb-Sn has no harmful elements, and its rigidity and mechanical strength are similar to the bone. In this work we performed a safety test, analyzing the biological effect of conditioned culture medium after the synthesis of the Ti-34Nb-6Sn alloy in stem cells derived from equine bone marrow (BM-MSCs).

METHODS: BM-MSCs were seeded at $1x10^5$ cells/well for the cytotoxicity, osteogenic differentiation and wound healing assays. For these tests we conditioned the culture medium with the alloy and treated the cells at the respective times of each assay to evaluate this medium released particles that would be toxic in the main biological functions. After, we evaluate cell morphology and adhesion on the biomaterial by Scanning Electron Microscopy (SEM).

RESULTS & DISCUSSION: In 24h the conditioned medium did not promote harmful effects in the cells compared to the control ($P > 0.05$). We observed that the medium conditioned differentiation with the material did not influence the osteogenic differentiation since the lineage was confirmed with calcium marking by Alizarin red after 10 days. The treated group showed a significant migration in 24h. The cells seeded on the surface material presented fibroblastoid character in addition to being anchored and spreaded on its surface.

CONCLUSIONS: This material may be a good candidate for the development of orthopedic devices, because during the treatment the cells with material we did not found toxic effects.

ACKNOWLEDGEMENTS: Financial support was received from FAPESP (2017/13876-2), Institute of Technological Research State of São Paulo for materials development.



Mesenchymal stem cell-derived extracellular matrices assembled in the presence of high molecular weight hyaluronic acid exhibit anti-inflammatory properties

M. Assunção[1,2], Anna Blocki[1,2]
Presenting author: Marisa Assunção, marisa.assuncao@link.cuhk.edu.hk
[1]Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR [2]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR

Cell-based therapies are facing many challenges as the hostile inflammatory and ischemic environment in the affected tissue impairs integration, survival and hence therapy efficacy upon direct implantation. Nevertheless, the cellular secretome was shown to augment tissue healing and even regeneration. So far, the major focus for the related mechanism of action lied on the soluble paracrine factors. We hypothesized that the insoluble fraction of the cellular secretome, namely the extracellular matrix (ECM), would also hold a pro-regenerative potential. Hence, we decided to engineer tailor-made ECM in vitro. Mesenchymal stem cells (MSCs) were allowed to assemble their ECM in the presence of exogenously added high molecular weight hyaluronic acid (HMWHA) to harness the anti-inflammatory and pro-regenerative potential of both components. Indeed, supplementation of HMWHA to human bone marrow MSC (bmMSC) cultures resulted in increased expression levels of anti-inflammatory cytokine interleukin 10. Addition of exogenous HMWHA over a time course of 2 to 6 days, enhanced the deposition of a bmMSC-derived ECM in a dose dependent manner. The ECM was enriched in provisional matrix components HA and fibrillar fibronectin (Fig. 1). Next, the engineered matrices were decellularized and investigated for their effect on macrophage polarization. THP-1 cells (human monocytic cell line) were differentiated into macrophages (MØ), seeded on decellularized ECMs and pulsed with pro-inflammatory factors to induce polarization into a pro-inflammatory phenotype (M1). As quantified by ELISA for TNFα, engineered matrices impaired macrophage polarization towards M1. The HMWHA-mediated enhanced deposition of an anti-inflammatory ECM enables us to construct a bio-instructive material to promote healing and regeneration in diseased tissues facing chronic inflammation.

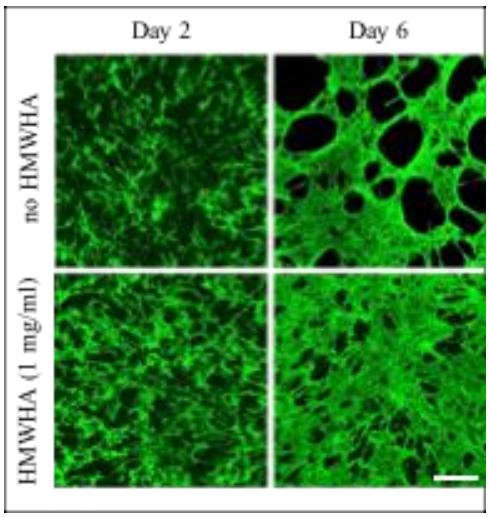
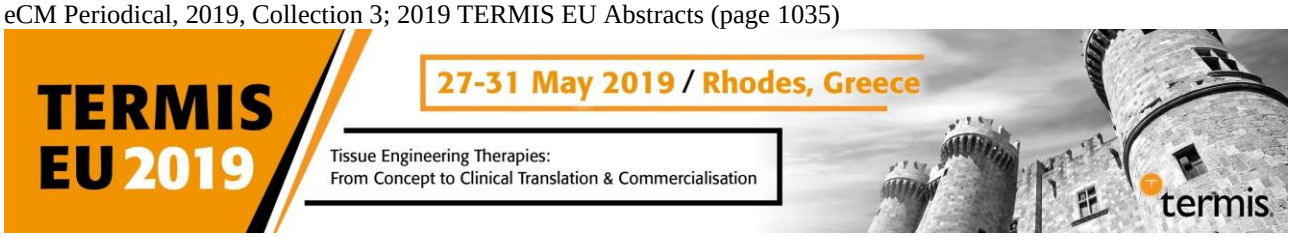


Figure 1: HBMSCs cultures supplemented for 2 to 6 days with HMWHA (1 mg/ml) assemble fibronectin fibrils (green) depending on the HMWHA dose. Scale bar: 500 um.



In situ gellable hyaluronan based hydrogels with antimicrobial activity

M. Pravda[1], L. Kovarova[1,2], I. Scigalkova[1] V. Velebny[1]
Presenting Author: Martin Pravda, pravda@contipro.com
[1]Tissue Engineering Research Group, Contipro a. s., Dolni Dobrouc, Czech Republic [2]Brno University of Technology, Faculty of Chemistry, Purkynova 464/118, Brno, Czech Republic

INTRODUCTION: Hyaluronan derivative-based in situ gellable hydrogels were suggested as suitable materials for prevention of post-operative adhesions [1]. The aim of this study was to develop an injectable hydrogel containing an antibacterial compound which would be easily delivered to the side of application, e.g. through minimally invasive procedures. The hydrogel should cover surrounding tissues in order to prevent organ adhesions. Moreover, the presence of antimicrobial agent should decrease the risk of post-operative infection.

METHODS: Hyaluronan polyaldehyde derivative (HA-CHO; degree of substitution 10 %; 30 mg/ml)-based matrix crosslinked by O,O′-1,3-propanediylbishydroxylamine (POA; 0,4 mg/ml) was designed to provide in situ gellable hydrogels with tunable time of gelation and viscoelastic properties. Triclosan/hydroxypropyl-β-cyclodextrin inclusion complex (TCS/CD) was used as antimicrobial component. Time of gelation and viscoelastic properties of hydrogel were evaluated. Moreover, cytotoxicity (ATP assay) and antimicrobial activity (diffusion plate method) of the developed material were evaluated.

RESULTS & DISCUSSION: TCS is a hydrophobic drug poorly soluble in water. It is well known in the field of coating implantable devices [2]. The TCS/CD inclusion complex was prepared to solubilize the drug in water in order to produce a homogenous distribution of TCS in the hydrogel. Hydrogels containing TCS in concentration 0,1 and 1 mg/ml were prepared. Presence of the inclusion complex did not affect neither gelation time nor viscoelastic properties of hydrogels. Hydrogels containing 1 mg/ml TCS had a significant antimicrobial effect on all tested microbial strains. Viability assay proved that hydrogel extracts did not negatively affect viability of 3T3 mouse fibroblast cells.

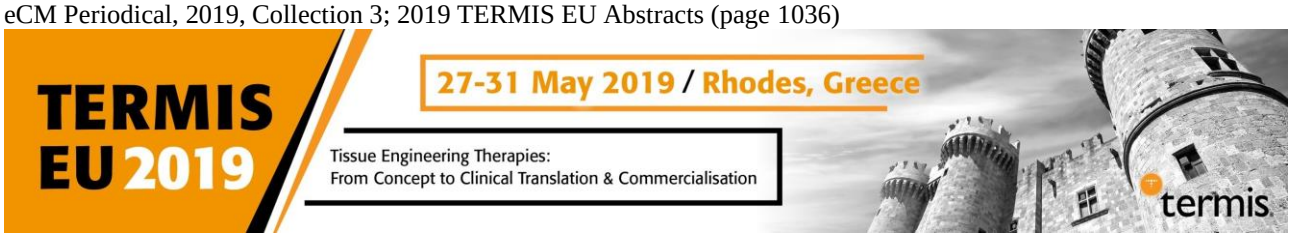
CONCLUSIONS: New in situ gellable hyaluronan-based hydrogel with antimicrobial properties was designed. Further preclinical studies will be needed to evaluate its ability to prevent post-operative adhesions and infections.

Hydrogel	TCS (mg/ml)	E. coli	C. sporogenes	S. aureus	C. albicans
HA-CHO (30 mg/ml); POA 0,4 (mg/ml),	0	N	N	N	N
	0	N	N	Y	N
	0,01	N	N	Y	N
	0,1	Y	N	Y	N
	1	Y	Y	Y	Y

Table 1: Antimicrobial effect of hydrogels. Y indicates presence of the inhibition zone. N – No inhibition zone detected.

REFERENCES

[1] Yang, Y., et al. Acta Biomater. 2017; 62: 199-209.
[2] Wu, X., et al. Eur J Clin Microbiol Infect Dis. 2017; 36:19–32



Chitosan/hyaluronate blends and their modification as gradient materials for osteochondral tissue regeneration

M. Hunger, P. Domalik-Pyzik, J. Chłopek
Presenting Author: Martyna Hunger, hunger@agh.edu.pl
Department of Biomaterials and Composites, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Krakow, Poland

INTRODUCTION: Biomedical applications require specific properties of materials, such as biocompatibility, stability in physiological environment and appropriate mechanical strength. Polysaccharides, e.g. hyaluronates or chitosan are of particular importance here. However, relatively low mechanical properties of natural polymers make their application problematic. It may be overcome by forming blends or composites. Moreover, it is desirable to imitate biomimetic hierarchical structure [1-3].

METHODS: The aim of this research was to fabricate blends based on chitosan (CS, 2% w/v; 5% lactic acid) (Acros Organics, MW=100,000-300,000) and sodium hyaluronate (HA, 0-30% w/v; water) (Acros Organics), as well as to obtain composite systems with graphene oxide (GO, 0-3% w/w of CS) (ITME, Poland) and hydroxyapatite (HAp, 20 % w/w of CS) (MKN-HXAP, 12 μm) as a basis for gradient materials development. Firstly, aqueous solutions of CS and HA were prepared and mixed at different ratios of CS:HA (10:90, 30:70, 50:50 70:30, 90:10). To obtain composites, GO and HAp dispersions were separately prepared by sonication (30 min) and then added to the polymers mixture. Freeze-dried (72h, -80°C) scaffolds and films were fabricated and following properties were evaluated: microstructural (SEM), structural (FTIR-ATR), mechanical (static compression test) and surface (wettability).

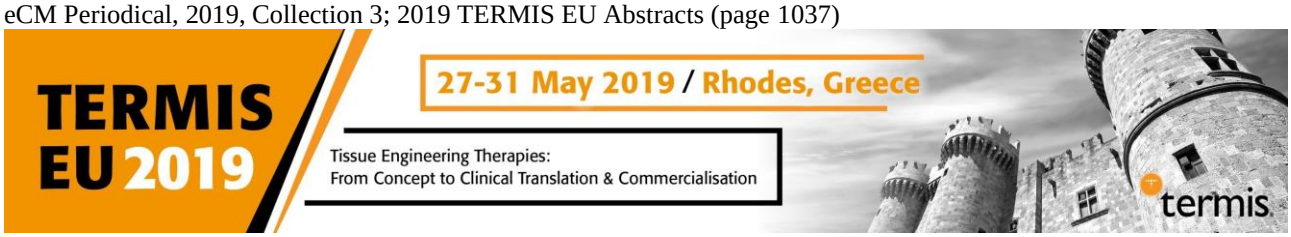
RESULTS & DISCUSSION: Optimal proportion in blend was chosen – CS:HA 30:70. This material had the highest mechanical properties after compression test (Figure 1), as well as the best stability in water and PBS. The 30:70 blend was used to obtain composite systems, and then – hierarchical structures. The addition of GO to the blends improved the compression strength, and HAp made the blends more brittle – with their simultaneous additions it is possible to obtain gradient structure and control the properties. Preliminary in vitro cell culture studies showed promising results, detailed biological examination is planned.

CONCLUSIONS: The biomimetic hierarchical structures based on chitosan, sodium hyaluronate, graphene oxide and hydroxyapatite ware fabricated. It seems that they possess promising properties as potential materials for osteochondral regeneration.

ACKNOWLEDGEMENTS: The authors would like to thank the National Centre for Research and Development, Poland for providing financial support to this project (STRATEGMED3/303570/7/NCBR/2017).

REFERENCES

[1] Neethu M et al. Int J Biol Macromol. 2017; 104, 1936-1945
[2] Sionkowska A et al. Int J Biol Macromol. 2016, 442-448
[3] Unnithan A R et al. J Ind Eng Chem. 2017, 182-191



Novel decellularization techniques for peripheral nerves

M.El Soury[1], S. Geuna [1,2], A.B. Lovati [3] , M. Bottagisio [3], A. Crosio[4], S. Odella[4], P. Tos[4], F. Campos[5,6], V. Carriel [5,6] , S. Raimondo [1,2]
Presenting Author: Marwa El Soury, marwa.elsoury@unito.it
[1]Department of Clinical and Biological Sciences, San Luigi Gonzaga Hospital, University of Turin, Turin, Italy [2]Neuroscience Institute Cavalieri Ottolenghi (NICO) [3]Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy [4]Reconstructive Microsurgery and Hand surgery Unit, ASST Pini-CTO Milano, Italy [5]Department of Histology & Tissue Engineering Group, University of Granada, Granada, Spain and [6]Instituto de Investigacion Biosanitaria, Ibs.GRANADA, Granada, Spain

INTRODUCTION: Over the past years, the decellularization of peripheral nerves has been used to provide a natural substrate composed of nerve extracellular matrix without the resident cells to prevent the host immune response when transplanted in patients. Despite the existence of other protocols, as the Hudson and Sondell, they are either complex to prepare or the nerve extracellular matrix is slightly affected by the chemicals used, our aim is to find a new efficient decellularization protocol that is both easy to prepare and conserves well the nerve extracellular matrix.

METHODS: Rat Sciatic nerves were harvested and divided in 3 groups, each followed a different decellularization protocols, protocol 1 (already described in the literature for nerve decellularization), protocol 2 (described in the literature for tendon decellularization and here applied on nerves), protocol 3, a new protocol proposed by our group. Following decellularization, nerves were fixed and included in resin for light and electron microscopy analysis. Other parameters will be tested in the future such as mechanical testing and ex-vivo cytocompatibility.

RESULTS & DISCUSSION: Preliminary analysis in light and electron microscopy demonstrated that nerves decellularized using protocol 1 showed good tissue organization with intact collagen, but some axons with undamaged intracellular matrix integrates are still present. Nerves decellularized using protocol 2 showed rich matched connective matrix, no integral axons are present.

CONCLUSIONS: The combination of chemicals (several detergents at low concentration) with physical forces (agitation) can be a promising technique for nerve decellularization that is effective in removing cells and preserving ECM in short time nerve processing.

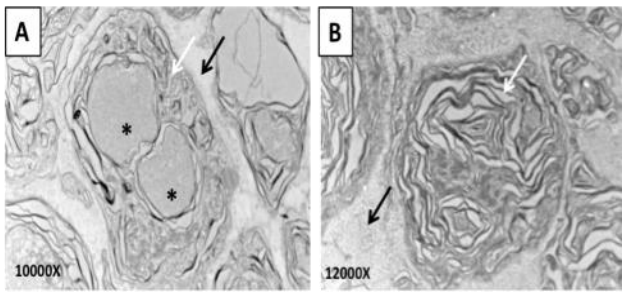
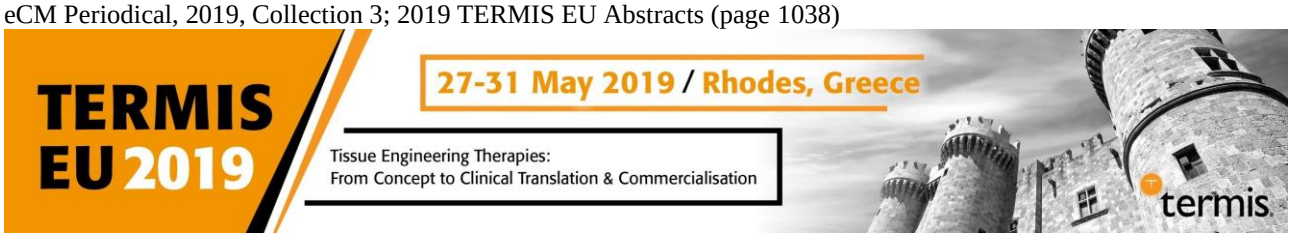


Figure 1: Electron microscopy images of transverse section of decellularized nerve. A: protocol 1, B: protocol 2. Black arrows: collagen, white arrows: myelin debris, asterisks: axons.

ACKNOWLEDGEMENTS: Financial support was received by the Fondazione Cassa di Risparmio di Torino (Turin, Italy), protocol 702 number 2017.AI190.U219, RF: 2016.2388.



Engineering of in vitro vascular anastomosis under vascular endothelial growth factor (VEGF) gradients in a microfluidic device

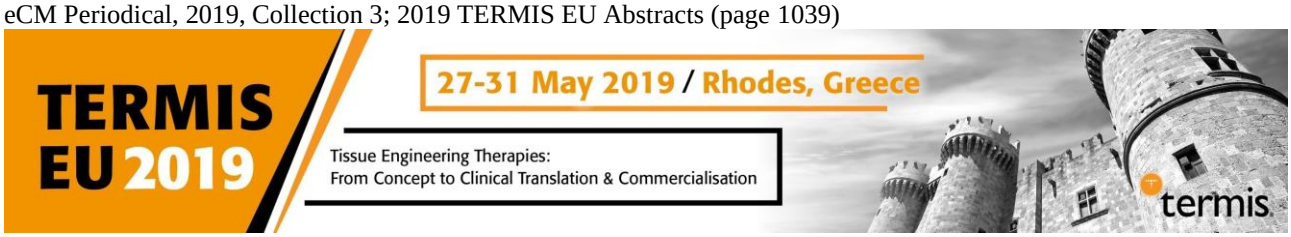
M. Watanabe[1], R. Sudo[1,2]
Presenting Author: Masafumi Watanabe, watanabe@sudo.sd.keio.ac.jp
[1]School of Integrated Design Engineering, Keio University, Yokohama, Japan, [2]Department of System Design Engineering, Keio University, Yokohama, Japan

INTRODUCTION: In vitro engineering of vascular anastomoses is critical for the development of transplantable tissue-engineered grafts, because rapid blood perfusion is required for the maintenance of implanted tissue grafts. However, the process of vascular anastomosis between host and engineered vessels remains unclear due to difficulties in observing vascular anastomosis after transplantation. Therefore, we aimed to establish an in vitro vascular anastomosis model in a microfluidic device using human umbilical vein endothelial cells (HUVECs) mesenchymal stem cells (MSCs).

METHODS: Microfluidic devices were designed and fabricated with polydimethylsiloxane, which comprised two side microchannels sandwiching a gel microchannel. The central gel channel was filled with fibrin/collagen gel. A side channel was filled with HUVECs through an outlet of the channel, while the other channel was filled with HUVECs and MSCs at ratios of 2:8, 5:5, and 8:2 (Figure 1). Constructed vascular anastomoses were observed by confocal microscopy. Chemical gradients of vascular endothelial growth factor (VEGF) in the gel were calculated by Fick's second law using COMSOL Multiphysics. VEGF distributions in the gel at 24 h were calculated.

RESULTS & DISCUSSION: Firstly, HUVECs were cultured in both side channels to confirm the ability of vascular anastomosis formation. Contrary to expectations, vascular sprouts were not formed at least by day 10. In addition, the simulation results indicated that VEGF gradient was not maintained at 24 h when HUVECs were seeded in both microchannels. Next, we added MSCs to the HUVEC monoculture condition to induce angiogenesis. Confocal images showed that vascular geometry was dependent on HUVEC:MSC ratios. At a HUVEC:MSC ratio of 2:8, vascular networks extended from both microchannels formed vascular anastomoses by day 10. Quantitative analysis showed that the number of vascular anastomoses was significantly increased at the HUVEC:MSC ratio of 2:8, which was >3-times greater than the other ratios (5:5 and 8:2). Moreover, the simulation results showed that the steepest VEGF gradient was maintained at the HUVEC:MSC ratio of 2:8. Furthermore, live-cell imaging revealed the process of vascular anastomosis. Vascular anastomoses in our model were formed through a multistep process such as EC migration and vascular contact. Moreover, the detailed structure of vascular anastomosis indicated that vascular anastomosis was constructed with continuous lumens during days 8–10.

CONCLUSIONS: In summary, we successfully established an in vitro vascular anastomosis model in a microfluidic device. Furthermore, our data showed that VEGF gradients played important roles in vascular anastomosis formation. This model will provide insights for both the development of tissue-engineered grafts and for the construction of large tissues by assembling multiple tissue-engineered constructs.



3D extracellular matrix derived model of alveolar Rhabdomyosarcoma

M. Saggioro[1,2], M.G. Muraro[3], S. D'Agostino[1,2], G. Germano [2], E. Poli[1,2], L. Tombolan[1], P. Bonvini[2], G. Bisogno[1], P. Gamba[1], I. Martin[3], M. Pozzobon[1,2]
Presenting Author: Mattia Saggioro, mattia.saggioro@studenti.unipd.it
[1] Department of Women's and Children's Health, University of Padova, Italy [2] Institute of Pediatric Research (IRP) Città della Speranza, Stem Cells and Regenerative Medicine Laboratory, Padova, Italy [3] Department of Biomedicine, University of Basel and University Hospital of Basel, Basel, Switzerland

INTRODUCTION: Alveolar Rhabdomyosarcoma (ARMS) is one of the most aggressive pediatric soft tissue sarcoma with a high tendency to metastasize [1]. The need of new models to study cell-extracellular matrix (ECM) interactions are needed. Integrins are a class of transmembrane adhesion molecules that mediate cell survival, differentiation, migration and differentiation [2]. The aim of this study is to create an engineered 3D ARMS model using a U-Cup bioreactor. We will investigate the protein expression with specific attention on the role of integrins in ARMS metastatic migration.

METHODS: ARMS xenografts were obtained from subcutaneous injection of RH30 (ARMS cell line) cells in immunodeficient mice. ECM composition was determined by proteomic analysis. ARMS cells were seeded in a 3D collagen scaffold cultured in a perfusion bioreactor (U-Cup). Cells before and after 3D culture were analyzed by qPCR, flow cytometry for the expression of a panel of integrins and CXCR4. MMPs expression was evaluated by zymography.

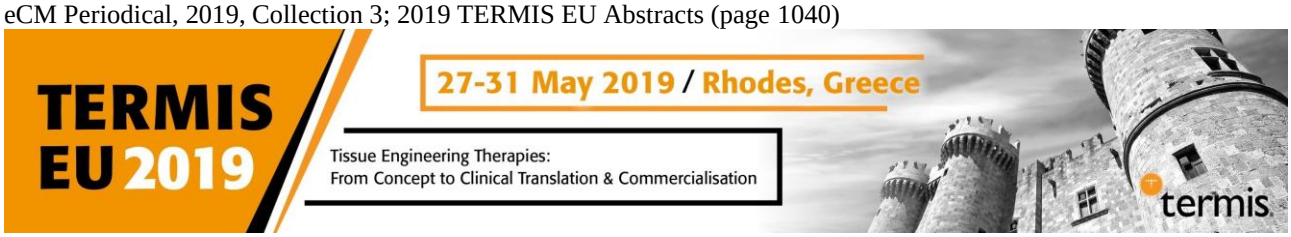
RESULTS & DISCUSSION: After ARMS xenografts characterization, proteomic analysis was performed: the main components of the ECM will be used to enrich the collagen scaffold. Preliminary data culturing RH30 cells in U-Cup bioreactor with collagen scaffold showed upregulation of ITG□5 and CXCR4 receptor compared to 2D condition. Expression of MMP-9 and MMP-2 was assessed by zymography comparing the expression of these MMPs in ARMS cells cultured in 2D versus 3D dynamic culture and isolated from the xenograft samples.

CONCLUSIONS: We demonstrated that our 3D ARMS model better recapitulates the complex in vivo environment than 2D, enhancing the expression of MMPs, ITG□□□and CXCR4. The important interaction between ITG□5 and MMP-2 [3] will be deeper studied in a more representative engineered 3D scaffold to shed light on the complex cross talk between cell-ECM interaction and metastatic progression.

ACKNOWLEDGEMENTS: Financial support was received from University of Padova and Associazione Puzzle, Italy.

REFERENCES

[1] Ognjanovic S et al. Canc 2009. 115(18): 4218-4226
[2] Hamidi H et al. Nat Rev Canc. 2018 18(9):533-548
[3] Kesanakurti D et al. Oncog. 2013. 32: 327-340



Microsphere-mediated delivery of anti-inflammatory cytokines in inflammatory in vitro chondrocyte models

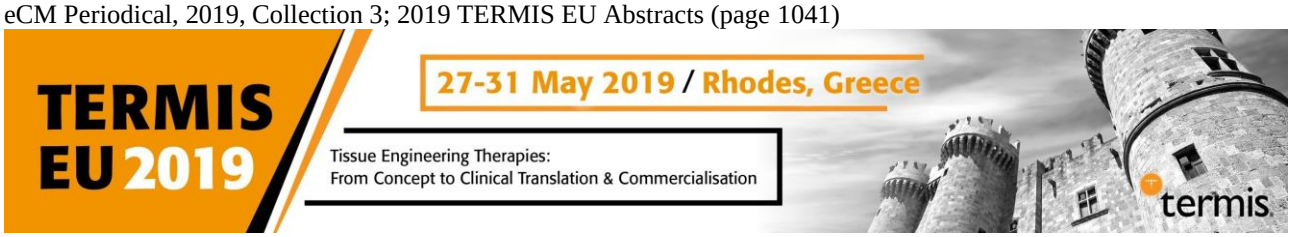
E. Park[1,2], M. L. Hart[1], B. Rolauffs[1], J. P. Stegemann[2], R. T. Annamalai[2]
Presenting Author: Melanie Hart, melanie.lynn.hart@uniklinik-freiburg.de
[1]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis Lab, Dept.of Orthopedics & Trauma Surgery, Medical Center-Albert-Ludwigs University of Freiburg, Germany [2]Dept. of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease. The pathogenesis is not fully understood but studies show that inflammation following joint injury plays a key role in its onset. Microspheres (µSp) releasing anti-inflammatory agents could potentially attenuate the inflammatory response and the inflammatory milieu in the degenerating joint and prevent OA. Herein, negatively charged microspheres (µSP) ionically complexed with positively charged anti-inflammatory cytokines IL-4, or IL-10 or IL-13 were developed and tested in inflammatory in vitro chondrocyte mouse models.

METHODS: Gelatin µSP using non-ionic surfactants 0.1% vol L101 vs. Tween 20 were produced and then cross-linked with genipin. Cytotoxicity of the surfactants was measured using proliferation and metabolic assays. µSP were characterized for average size (ImageJ), zeta potential (Zetasizer), and collagenase-mediated degradation. 200ng of IL-4, IL-10 or IL-13/mg µSp were loaded and release was measured (ELISA). µSp were then tested in murine chondrocyte ATDC-5 inflammatory in vitro models simulated with IL-1β (2 ng/ml) or LPS (200 ng/ml). Loaded, unloaded µSp vs. bolus treatment (10 ng/ml) were added. Nitrite (NO) was measured (Griess assay).

RESULTS & DISCUSSION: Surfactant treatment had no effect on metabolic activity but Tween resulted in a higher cell viability at day 7 ($p<0.001$). There-fore µSp (avg. diameter 15+/-4.2 µm; were produced using Tween. Increasing the density of genipin crosslinking decreased the zeta potential facilitating loading with cytokines. µSp loaded with IL-4, IL-10 or IL-13 resulted in a loading efficiency > 80%. Upon collagenase degradation the cytokines were incrementally released for up to 7 days. To mimic OA, mouse chondrocytes were activated with and without IL-1β or LPS. At day 3, NO chondrocyte production showed that the IL-4 and IL-13 released from the µSp significantly reduced NO production vs. control (unloaded µSp), while IL-10 only showed marginal effects.

CONCLUSIONS: These results demonstrate that cross-linked gelatin µSp can be used to deliver anti-inflammatory cytokines. Moreover, the inflammation-dependent degradation and release of IL-4 or IL-13 by the stimulated cells can be harnessed to minimize unnecessary drug release during periods of low disease activity thus enhancing their therapeutic effect. Hence, µSP could minimize inflammation and immuno-mediated injury of chondrocytes and alleviate post-traumatic inflammation in OA.



Dental pulp stem cells and leukocyte- and platelet-rich fibrin for articular cartilage repair

M. L. Monaco[1,2], P. Gervois[1], J. Beaumont[1], P. Clegg[3], A. Bronckaers[1], J.-M. Vandeweerd[2], I. Lambrichts[1]
Presenting Author: Melissa Lo Monaco, melissa.lomonaco@uhasselt.be
[1]Morphology Research Group, Biomedical Research Institute, UHasselt, Diepenbeek, Belgium [2]Department of Veterinary Medicine, Integrated Veterinary Research Unit-Namur Research Institute for Life Science, University of Namur, Namur, Belgium [3]Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom

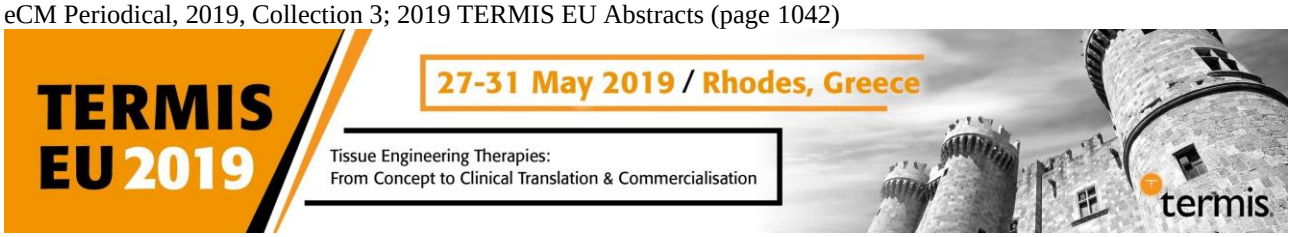
INTRODUCTION: Osteoarthritis (OA) is a degenerative and inflammatory condition of synovial joints with irreversible loss of supportive cartilage matrix. Dental pulp stem cells (DPSCs) can be differentiated into cartilage-producing cells and secrete numerous growth factors associated with tissue repair and immunomodulation. Moreover, leukocyte- and platelet-rich fibrin (L-PRF), a blood-derived and clinically applied biomaterial, has recently emerged as a promising treatment in regenerative medicine due to its growth factor content and supportive fibrin matrix. The main goal of this study is to evaluate whether DPSCs and L-PRF are promising treatment strategies in OA via immunomodulation and stimulation of cartilage regeneration.

METHODS: First, DPSCs were subjected to a chondrogenic differentiation system, with or without L-PRF conditioned medium (CM) (5% and 25%) and exudate (3%), and compared to bone marrow-mesenchymal stem cells (BM-MSCs) via (immuno)histochemistry. Secondly, immature murine articular chondrocytes (iMACs) were isolated and cultured with either DPSC CM, L-PRF CM (5%, 25% and 50%) or exudate (1%, 3% and 5%). Healthy iMAC survival, proliferation and cartilage-specific extracellular matrix production were assessed. Finally, IL-1β- and TNFα-stimulated iMAC viability, cartilage matrix production and inflammatory cytokine secretion were analyzed.

RESULTS & DISCUSSION: Preliminary data suggest that L-PRF does not increase the expression of chondrogenic markers after differentiation. DPSC and L-PRF CM have a significant pro-survival and proliferative effect on iMACs. IL-1β- and TNFα-stimulated iMAC viability follows an increasing trend after exposure to L-PRF and DPSC CM. iMACs cultured in DPSC and L-PRF CM show a more cartilage-like matrix production. DPSC CM appears to prevent and decrease nitrite secretion in IL-1β- and TNFα-stimulated iMACs.

CONCLUSIONS: Our in vitro data show that DPSCs and L-PRF are a promising treatment option for OA via secretome-mediated effects. Our final goal is to test in vivo if DPSCs and L-PRF improve cartilage repair and have immunomodulatory effects in an ovine model of OA.

ACKNOWLEDGEMENTS: Financial support was received from Bijzonder Onderzoeksfonds, Fonds spécial de recherche (BOF16DOCNA02 - FSR-confin UHasselt-UNamur) and Fonds Wetenschappelijk Onderzoek (FWO, grant 12U7718N).



MSC metabolimaging to discern the influence of substrate elasticity and perfusion flow using fluorescent lifetime imaging microscopy with a miniaturised optically accessible bioreactor

S. Perottoni[1,2,3], N. Neto[1,2], L. Izzo[3], M.T. Raimondi[3], M.G. Monaghan[1,2,4]
Presenting Author: Michael Monaghan, monaghmi@tcd.ie
[1]Department of Mechanical and Manufacturing Engineering, Trinity College Dublin [2]Trinity Centre for BioEngineering, Trinity College Dublin, Ireland [3]Department of Chemistry Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, 32, piazza L. da Vinci, 20133 Milano, Italy [4]Advanced Materials and BioEngineering Research (AMBER) Centre at Trinity College Dublin and Royal College of Surgeons in Ireland, Dublin, Ireland

INTRODUCTION: Recapitulating tissue elasticities can direct mesenchymal stem cell (MSC) differentiation through mechano-transduction [1]. However, it is unknown how elasticity affects MSC metabolism which could be an important feature to enhance differentiation and mineralisation. To delineate this, our study investigates real-time MSC metabolism and differentiation on substrates of varying stiffnesses using a miniaturized optically accessible flow bioreactor (MOAB) with Fluorescence Lifetime Imaging Microscopy (FLIM).

METHODS: Polyacrylamide gels of varying stiffnesses were fabricated via UV-crosslinking, seeded with MSCs and placed within MOABs with or without continuous flow of 5 µL/min. Real-time FLIM was performed to quantify free and protein bound nicotinamide adenine dinucleotide (NADH). Computational Fluid Dynamic (CFD) analysis was used to predict the velocity field and shear stress profile within the MOAB. The effect of substrate stiffness and flow, in combination or independently; on MSC metabolism was investigated.

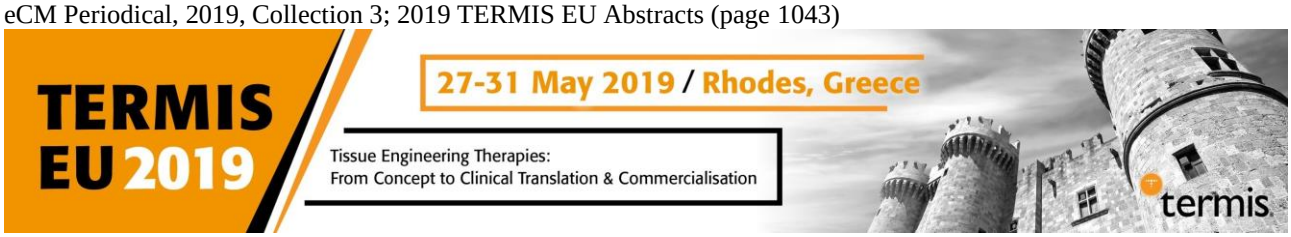
RESULTS & DISCUSSION: MSCs cultured on substrates exhibited distinct metabolic patterns over 14-days, observed as early as day 3. Overall, by day 10, soft substrates induced a more glycolytic response while cells grown on stiffer substrates displayed a transition to oxidative phosphorylation, measured by protein-bound NADH fraction of the fluorescence lifetime decay). CFD results showed uniform shear stress profile within the chamber in the presence of non-porous polyacrylamide scaffolds with values lower than 3 mPa at a flow rate of 5 µL/min.

CONCLUSIONS: Monitoring the effect of stiffness and flow in real-time with FLIM was achieved via the optically transparent miniaturized system. Matrix stiffness induced metabolic perturbations in MSCs up to 14 days.

ACKNOWLEDGEMENTS: This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 825159-MOAB).

REFERENCES

[1] Engler AJ et al. Cell. 2006; 126(4):677-689



A bibliometric evaluation of the tissue engineering cognitive framework from 1991 to 2016

A. Santisteban-Espejo[1], J.A. Moral-Munoz[2], J. Chato-Astrain[3,4], O.D. García-García[3,4], V. Carriel[3,4], F. Campos[3,4], M. Alaminos[3,4], I. Garzón[3,4], A. Campos[3,4], M.A. Martin-Piedra[3,4]
Presenting Author: Miguel Angel Martin-Piedra, mmartin@ugr.es
[1]Division of Hematology and Hemotherapy. Puerta del Mar Hospital, Cadiz, Spain [2]Institute of Research and Innovation in Biomedical Sciences of the Province of Cadiz (INiBICA), Cadiz, Spain [3]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [4]Instituto de Investigación Biosanitaria ibs.GRANADA, Spain

INTRODUCTION: Science mapping analysis (SMA) has been described as "a spatial representation of how disciplines, fields, specialties, and individual papers or authors are related to one another" [1]. This methodology enables the user to explore the underlying relationships between apparently different literature [2].

METHODS: The search strategy ("TISSUE ENGINEER*" or "TISSUE-ENGINEER*") was entered as the topic in the SCI-Expanded collection of Web of Science (WoS) for the period between 1991 and 2016. Reviews, book chapters, proceedings papers and meeting abstracts were excluded. Author's Keywords and Keywords Plus were retrieved to perform a longitudinal SMA with SciMAT software to elucidate the main concepts arising from TE [3]. Clustering of different keywords into themes was performed and a network of different themes was obtained. Callon's centrality and density was measured to setup a 2d layout that could divide each theme in 4 categories: "motor themes", "basic and transversal themes", "highly developed or isolated themes" and "emerging or declining themes".

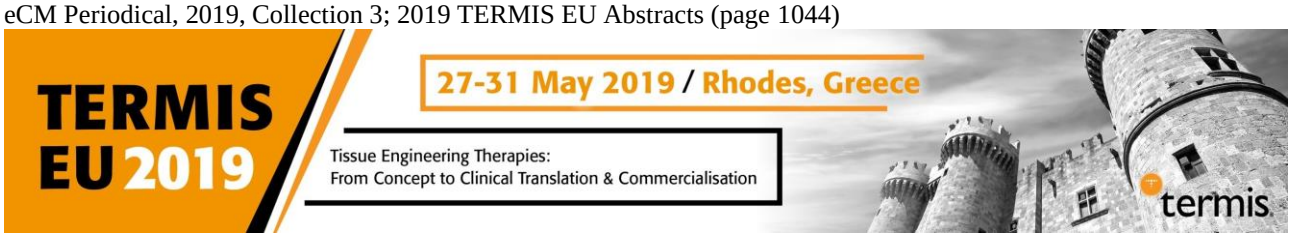
RESULTS & DISCUSSION: The present cognitive analysis allowed us to identify three main vectors: cells, material biodegradation and growth factors. TE has evolved from a focus on differentiated cells such as chondrocytes, toward a specific type of stem cell (MSC) with a high capacity for differentiation. Since the emergence of biomaterials in the first subperiod, "biodegradation" appeared as a motor theme not only in the first but also in the second subperiod. However, the development of new biomaterials with biodegradable properties, such as hydrogels, has displaced biodegradation as a motor theme. The selection of appropriate growth factors to influence new tissue activity was the third vector present in all three subperiods.

CONCLUSIONS: A better understanding of the cognitive structure behavior of tissue engineering can contribute not only to our knowledge of the conceptual evolution, its main historic vectors, and other research foci, but can also help administrative authorities to better plan funding allocations.

ACKNOWLEDGEMENTS: Financial support by CTS-115 (Tissue Engineering Research Group, University of Granada) from Junta de Andalucía, Spain.

REFERENCES

[1] Small H. J AM Soc Inf Sci. 1999;50:799
[2] Borner K et al. Rev Inform Sci Technol. 2003;37:179
[3] Cobo M et al. J Am Soc Inf Sci Technol. 2012;63:1609.



Generation of novel models of artificial human skin functionalized with differentiated mesenchymal stem cells

M.A. Martín-Piedra[1,2], A. Irastorza[1], V. Carriel[1,2], F. Campos[1,2], D. Durand-Herrera[1,2], O.D. García-García[1,2], M.C. Sánchez-Quevedo[1,2], R. Fernández-Valadés[2,3], M. Alaminos[1,2], I. Garzón[1,2]
Presenting Author: Miguel Angel Martin-Piedra, mmartin@ugr.es
[1]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [2]Instituto de Investigación Biosanitaria ibs. Granada, Spain [3]Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain

INTRODUCTION: Mesenchymal stem cells (MSC) previously demonstrated to have epithelial differentiation capability [1]. Therefore, complex artificial tissue models containing epithelial layers could be generated using MSC as alternative cell sources, since human keratinocytes are difficult to isolate and expand under culture conditions. In fact, several previous reports demonstrated that human artificial tissues can be generated without the need of obtaining epithelial cells [2]. Here, we investigated the capability of 4 different MSC sources to generate a functional skin epidermis.

METHODS: Human skin substitutes (HSS) were developed by tissue engineering protocols using alternative epithelial cell sources. Briefly, a stromal substitute was generated using a mixture of human fibrin and 0.1% type VII agarose. These hydrogels contained 500,000 skin fibroblasts. For the generation of a epithelial layer, 250,000 MSC obtained from adipose tissue (ADSC), dental pulp (DPSC), Wharton's jelly (WJSC) or bone marrow (BMSC) were seeded on top of the artificial stroma. After 28 days in culture, HSS were grafted on the back of nude mice for in vivo evaluation during 15 and 30 days. Surface and morphology of MSC differentiated to skin epithelium were assessed using SEM and histological methods.

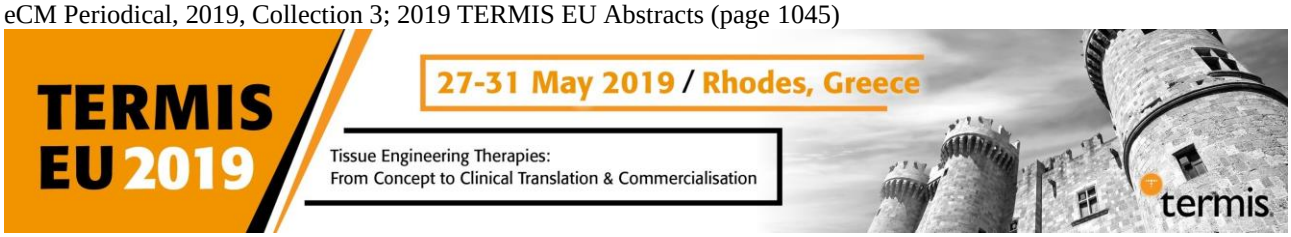
RESULTS & DISCUSSION: The analysis of the epithelial surface of the 4 different HSS demonstrated that all MSC types used as epithelial-like substitutes were able to develop a well-structured epithelium. At the beginning, all cells had the typical spindle-like shape, elongated morphology of native MSC. However, cells tended to partially change their morphology and acquire a more polygonal shape with time in culture, especially in the case of WJSC and BMSC at day 28. Once grafted in vivo, the 4 MSC types showed a clear morphological change, and cells became very flat and polygonal, showing keratinization and desquamation signs, especially after 30 days of in vivo development.

CONCLUSIONS: our results demonstrate the partial epithelial differentiation capability of ADSC, DPSC, WJSC and BMSC, which could be used for the generation of bioengineered human skin substitutes without the need of obtaining keratinocyte cell cultures.

ACKNOWLEDGEMENTS: Financial support was obtained by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) from the Spanish Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), FIS PI15/2048 (co-financed by ERDF-FEDER, European Union), grant AC17/00013 (NanoGSkin) by ISCIII thorough AES 2017 and within the EuroNanoMed framework and grant PE-0393-2018 from FIBAO foundation, Spain.

REFERENCES

[1] Jadalannagari S et al. Biochem Biophys Res Commun. 2016;478(2):831-7
[2] Garzon I et al. Stem Cells Transl Med. 2013 Aug;2(8):625-32



Engineered melt-electrowritten myocardial patches using co-cultured human-derived cardiac cells

M.J. Ainsworth[1,2], C.H.G. Metz[2], A.K. Braat[3], J.P.G. Sluijter[2], J. Malda[1,4], A. van Mil[2]*, M. Castilho[1,5]*

Presenting Author: M.DiasCastilho@umcutrecht.nl

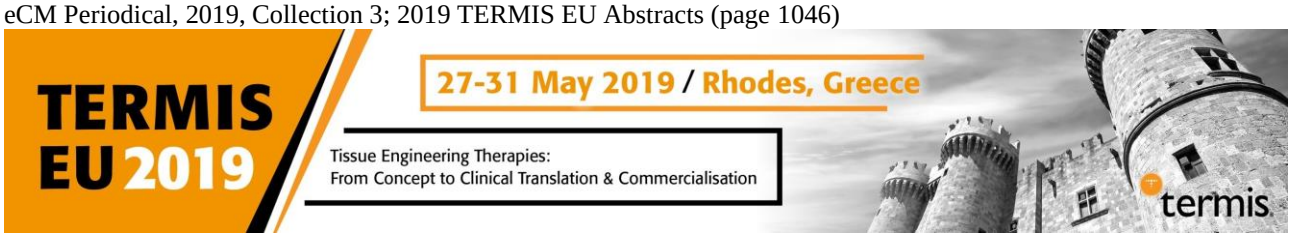
[1]Department of Orthopaedics, University Medical Centre Utrecht, the Netherlands [2]Department of Cardiology, University Medical Centre Utrecht, the Netherlands [3]Center for Molecular Medicine, University Medical Centre Utrecht, the Netherlands [4]Department of Equine Sciences Faculty of Veterinary Sciences, Utrecht University, the Netherlands [5]Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands

INTRODUCTION: Heart failure is a global health epidemic, with myocardial infarction being the most common cause of heart failure. Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) have shown great promise for myocardium repair, however their immaturity and poor engraftment are still challenges. Recently, we showed that well-ordered hexagonal fiber scaffolds obtained by melt electrowriting (MEW) can support contracting human iPSC-CMs and drive their maturation. Here we hypothesized that miniaturisation of the hexagonal microstructures will improve cellular adhesion, and co-culturing of iPSC-CMs with other native cell types, such as cardiac fibroblasts (FBs) and endothelial cells (ECs), will further enhance iPSC-CM maturity and functionality.

METHODS: Hexagonal microarchitectures were fabricated with a custom-built instrument [2]. Miniaturisation was systematically investigated according to key MEW parameters. After fabrication, hexagons were structurally and mechanically characterized. MEW meshes then were combined with a collagen-based hydrogel containing iPSC-CMs (CMs), human foetal cardiac fibroblasts (FBs) and human umbilical vein endothelial cells (ECs) using three combinations (100% iPSC-CMs, 90% iPSC-CMs + 10% FBs or 90% iPSC-CMs + 5% FBs + 5% ECs). Functionality, morphology and maturation were evaluated within a one-week culture period using analysis of beating rate, immunofluorescent staining, anisotropy and gene expression.

RESULTS & DISCUSSION: Hexagonal pores with 200 µm side lengths were successfully manufactured. In vitro culture shows that beating rate is enhanced in the CM + FB coculture, whereas CM + FB + EC coculture causes a decrease in contraction rate over a 7-day culture period. Immunofluorescent staining showed that CM alignment was enhanced in the CM + FB coculture after 4 days, compared with both the CM only and CM + FB + EC groups. Increased cell coupling was observed, resulted in larger tissue-like structures in both the CM + FB and CM + FB + EC groups in comparison to the CM only group. Furthermore, diffusion tensor imaging revealed that the addition of FBs to the patches increased anisotropy and reduced mean diffusivity, closer mimicking values of the native myocardium.

CONCLUSIONS: The present study demonstrated that MEW technology can print unique fibre designs at micrometre resolution. Co-culturing iPSC-CMs with other cardiac nonmyocytes can further enhance maturation and efficacy of myocardial patches, in comparison to CMs alone. In the current research, we are further optimizing our concept through local and controlled deposition of different cell types to drive this technology towards preclinical studies.



Multi-scale fabrication of an osteochondral implant with hierarchy in fiber orientation and cell distribution

M. de Ruijter[1], P. Diloksumpan[2], I. Dokter[1], H. Brommer[2], F. M. Braganca[2], R. Levato[1], P. R. van Weeren[2], M. Castilho[1,3], J. Malda[1,2]
Presenting Author: Miguel Dias Castilho, M.DiasCastilho@umcutrecht.nl
[1]Department of Orthopaedics, RMCU Utrecht, UMC Utrecht, University of Utrecht, The Netherlands [2]Department of Equine Sciences, Utrecht University, The Netherlands [3]Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands

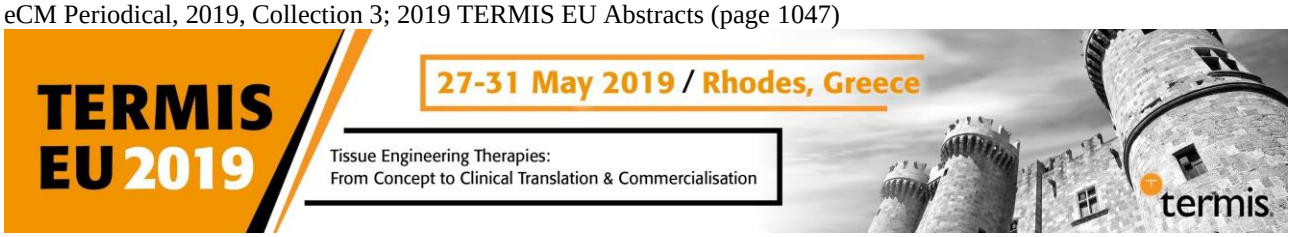
INTRODUCTION: Osteochondral defects are hard to heal due to limited regenerative capacity of cartilage tissue. Recently, we showed to successfully include melt electrowriting (MEW) in the bioprinting process with a single-step approach, which allowed for control over cell deposition and MEW-fiber deposition. However, convergence of biofabrication technologies is still in its infancy. In this study, we increased complexity, and fabricated a zonal osteochondral plug with a bi-layered cartilage layer, which was compared to an osteochondral plug with a uni-layered cartilage layer. The performance of the zonal plug was evaluated in vivo.

METHODS: To recreate the zonal cartilage architecture in both cells and fibers, the zonal osteochondral plug (diameter = 6 mm, h = 7.5 mm) consists of printable calcium-phosphate (pCaP), two architectures of MEW-fibers, (polycaprolactone), and different densities of articular chondroprogenitor cells (ACPCs), embedded in 10% gelatin-methacryloyl (gelMA). The non-zonal osteochondral plug consists of pCaP, one architecture of MEW-fibers, and one ACPC density. After fabrication, osteochondral plugs were cultured for 4 weeks, the compressive Young's modulus and complex shear modulus were measured, and cartilage like tissue formation was quantified (GAG/DNA, Dimethylmethylene blue (DMMB)/Picogreen) and confirmed with histology. Zonal plugs were implanted in Shetland ponies (n = 8), gait analysis (over time) and X-rays (after 3 months) were performed, and tissue formation was evaluated after 6 months of implantation.

RESULTS & DISCUSSION: Fabrication of a multi-phasic osteochondral plug in a single step approach was successful. An increase in GAG/DNA was found after 4 weeks of culture, and newly formed cartilage-like tissue was evenly dispersed throughout the sample. The cartilage layer of the fabricated zonal plugs showed an increase in compressive Young's modulus from 300 to 700 kPa and an increase in complex shear modulus from 40 to 80 kPa compared to the non-zonal cartilage layer. Implantation of the osteochondral plugs was successful, no discomfort in gait analysis was shown, and bone tissue infiltration was found 3 months after implantation.

CONCLUSIONS: Incorporation of MEW in the extrusion based bioprinting process allows for control over fiber architecture and cell distribution. Fabrication of a cartilage layer with hierarchy in cell density and fiber orientation increases the mechanical properties of the cartilage layer, while not limiting cartilage-like tissue formation. Although bone infiltration in the plugs is found after 3 months and the ponies do not show discomfort, end stage in vivo evaluation (March 2019) will reveal if the zonal implants are successful for osteochondral repair.

ACKNOWLEDGEMENTS: European Research Council, grant agreement 647426 (3D-JOINT), Dutch Arthritis Foundation (LLP-12), Eurostars (E11312 BioArchitect).



Enhanced chondrogenic activity of 3D Bioprinted poly(ester urea) scaffolds

M. J. S. Ferreira[1,2], S. Moxon[1], S. J. Kimber[1], A. Gloria[3], A.C. Fonseca[4], J. F. J. Coelho[4], N.M. Hooper[1], M. Domingos[2]
Presenting Author: Miguel Ferreira, miguel.ferreira@postgrad.manchester.ac.uk
[1]Faculty of Biology, Medicine and Health, The University of Manchester, UK [2]School of Mechanical, Aerospace and Civil Engineering, The University of Manchester, UK [3]Institute of Composite and Biomedical Materials, National Research Council, Italy [4]Department of Chemical Engineering, University of Coimbra, Portugal

INTRODUCTION: Poly(ester urea)s (PEUs) are a new class of biodegradable and bioabsorbable α-amino acid-based polymers for tissue engineering [1,2]. This work evaluates the chondrogenic potential of 3D printed PEU scaffolds for the first time and compares it to poly(caprolactone) (PCL).

METHODS: PEU and PCL scaffolds were fabricated using a 3D bioprinter (3D Discovery, regenHU) equipped with a screw-assisted extrusion head and a 330 µm diameter nozzle, with a lay-down pattern of 0/90°. Scaffolds were characterized by scanning electron microscopy (SEM), micro computed tomography (micro-CT), nanoindentation, compression tests, and water contact angle (WCA). The response of seeded cells of the TC28a2 human chondrocyte line was evaluated in terms of cell morphology, metabolic activity and gene expression for 7, 14 and 21 days using the resazurin reduction assay and qPCR.

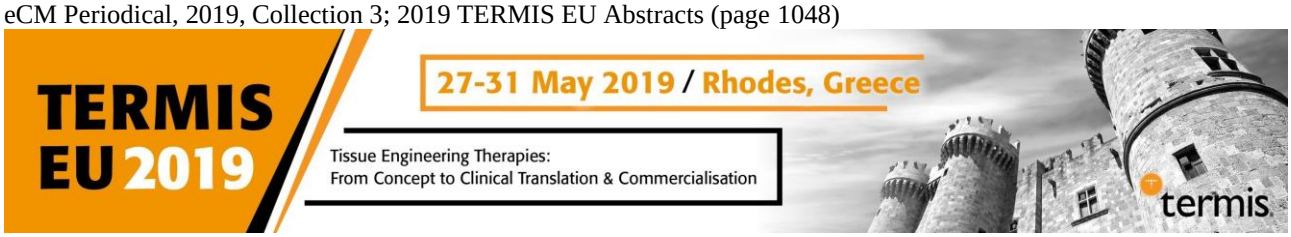
RESULTS & DISCUSSION: SEM and micro-CT revealed PEU and PCL scaffolds with filament diameter of approximately 320 µm, interconnected pores of quadrangular shape, homogeneous pore distribution and similar porosity (63.1% and 62.4% respectively). PEU scaffolds were more hydrophilic (WCA: 52.7°) and had lower nanoindentation hardness (0.20-0.33 GPa) and compressive modulus (60.2 MPa) than PCL (86.9°, 0.27-0.47 GPa, 79.8 MPa). Both materials supported human chondrocyte adhesion. Cells on PEU had higher metabolic activity, expression of chondrogenic genes ACAN and COL2A1 and lower expression of COL1A1, suggesting a more chondrogenic phenotype.

CONCLUSIONS: Taken together, these data suggest that PEU is a promising material for cartilage tissue engineering probably due to its lower hardness and compressive modulus, and higher hydrophilicity compared to PCL.

ACKNOWLEDGEMENTS: Financial support was received from the EPSRC/MRC Centre for Doctoral Training in Regenerative Medicine (The University of Manchester, UK).

REFERENCES

[1] Fonseca et al. Prog. Polym. Sci. 2014; 39(7): 1291–1311.
[2] Rodriguez-Galan et al. Polymers. 2010; 3(1):65–99.



Green processing of SAIB-chitin scaffolds for tissue engineering applications

C. Gonçalves [1,2,3*], S. S. Silva [1,2], J.M. Gomes [1,2], I. M. Oliveira [1,2], F.R. Maia [1,2,3], H. Radhouani [1,2,3], R. L. Reis [1,2,3], J. M. Oliveira [1,2,3]
Presenting Author: Miguel Oliveira, miguel.oliveira@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Sucrose acetate isobutyrate (SAIB), a synthetic disaccharide with extraordinary rheological characteristics. Herein, it is proposed to explore the potential of SAIB in tissue engineering scaffolding applications, by green processing SAIB with chitin using ionic liquids (ILs).

METHODS: SAIB and chitin were dissolved individually in IL, and then mixed by adding the SAIB/IL solution to the chitin/IL solution. Then the solution was transferred to silicone molds (d=8 mm, h=2 mm), followed by gelation, while immerging in solvents - water (A) or water:isopropanol (1:1, B), to obtain the chitin/SAIB gels (SC-A and SC-B, respectively). Chitin/IL scaffolds (C-A and C-B) were used as controls. These were placed in caped flasks at 150 rpm with daily solvent change, and aliquots were collected to measure the conductivity and follow the removal of the ILs. SAIB/Chitin gels were freeze-dried. The physicochemical characterization performed comprised rheology, FTIR, XRD, SEM, microCT and swelling. The in vitro studies performed covered cytotoxicity, cell damage (ISO10993) and cell morphology.

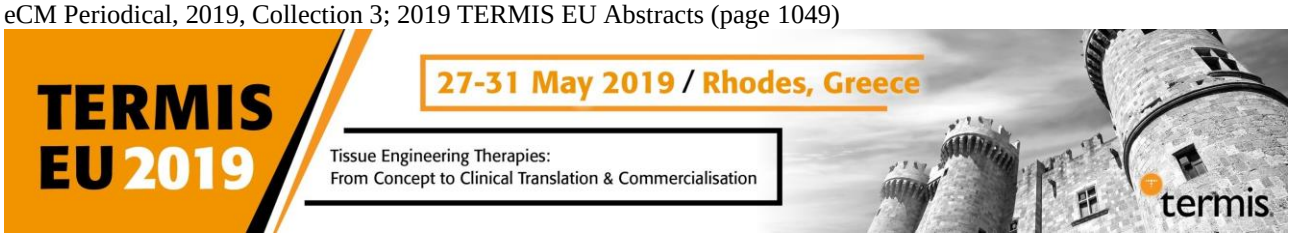
RESULTS & DISCUSSION: The presence of the molecules and the absence of ILs, was confirmed by FTIR and XRD techniques. By SEM and microCT was possible to observe the morphological features of the produced scaffolds, that resembles the typical morphology of aerogels (SC-B, C-A, C-B) but also compact structures (SC-A). The presence of SAIB increased about 4 times the adhesivity (N.s) of the scaffolds and, at the same time, has halved the strength of the structure (G'/Pa).

CONCLUSIONS: It is shown the potential of the used approach, to apply these scaffolds in tissue engineering.

ACKNOWLEDGEMENTS: Financial support was received from FCT (SFRH/BPD/94277/2013, SFRH/BPD/100957/2014 and IF/01285/2015) and NORTE-08-5369-FSE-000044.

REFERENCES

[1] Reynolds, R.C., Food and Chemical Toxicology, 1998. 36(2):95-99
[2] Cheng, T., et al. Eur Cell Mater, 2013. 26:208-221
[3] Silva, S.S., J.F. Mano, and R.L. Reis. Critical reviews in biotechnology, 2010. 30(3): 200-221



Manganese-based methacrylated gellan gum hydrogels as injectable system for image-guided cell delivery

S. Vieira[1,2], P. Strymecka[3], L. Stanaszek[3], J. Silva-Correia[1,2,4], B. Lukomska[3], R. L. Reis[1,2,4], P. Walczak[5,6], J.M. Oliveira[1,2,4]
Presenting Author: Miguel Oliveira, miguel.oliveira@i3bs.uminho.pt
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal [3]NeuroRepair Department, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland [4]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal [5]Department of Neurology and Neurosurgery, School of Medicine, Collegium Medicum, University of Warmia and Mazury, Olsztyn, Poland [6]Russel H, Morgan Department of Radiology and Radiological Science, Johns Hopkins University, Baltimore, MD, USA

INTRODUCTION: Hydrogels hold a great potential on the scope of cell-based therapies and can be used to unravel several bottlenecks of these approaches [1]. Herein, we intend to develop injectable manganese-based methacrylated gellan gum hydrogels that can be monitored via MRI imaging.

METHODS: 0.75% (w/v) methacrylated gellan gum (GG-MA) solutions were supplemented with 0.1 and 1 mM of $MnCl_2$ (Sigma-Aldrich) and further crosslinked with artificial cerebrospinal fluid (aCSF). The resulting hydrogels were characterized regarding their injectability, stability and MRI signal. As proof-of-concept, human-derived adipose stem cells (hASC) were encapsulated in 0.1 mM $MnCl_2$ GG-MA hydrogels and cultured for 7 days. During the culture period, cell viability was assessed using Live/Dead staining kit (Thermo Fisher).

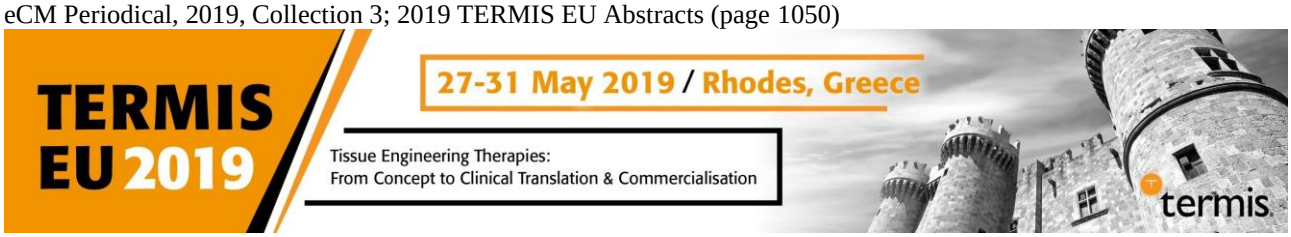
RESULTS & DISCUSSION: All the prepared formulations are injectable, with the force needed to inject gel solution being lower or similar to the force needed to inject water. After the 7 days of incubation in aCSF, hydrogel degradation was approximately 20% for all the formulations tested. T1-weighted MRI scans showed that formulation with 0.1mM $MnCl_2$ exhibits a higher MRI signal. Therefore, in vitro studies were performed using this condition. hASCs encapsulated into hydrogel fibers remained alive up to 7 days of culture.

CONCLUSIONS: Manganese-based GG-MA hydrogels were successfully prepared. These hydrogels hold great potential for image-guided cell delivery approaches, when minimally invasive procedures are envisaged.

ACKNOWLEDGEMENTS: Sílvia Vieira acknowledges FCT PhD scholarship (SFRH/BD/102710/2014). The FCT distinction attributed to J. Miguel Oliveira under the Investigator FCT program (IF/01285/2015) and J. Silva-Correia (IF/00115/2015) are also greatly acknowledged. The authors also acknowledge the funds provided under the project NanoTech4ALS, funded under the EU FP7 M-ERA.NET program

REFERENCES

[1] J.M. Oliveira et al. Npj Regen Med, 3 (2018).



Mechanical suitability of a decellularized extracellular matrix (dECM) hydrogel for vocal fold tissue engineering

M. Brown[1], N.Y.K. Li-Jessen.[1,2,3]
Presenting Author: Mika Brown, mika.brown@mail.mcgill.ca
[1]Biological and Biomedical Engineering, McGill University, Montréal, Canada [2]School of Communication Sciences and Disorders, McGill University, Montréal, Canada [3]Otolaryngology – Head and Neck Surgery, McGill University, Montréal, Canada

INTRODUCTION: Existing biomaterials aimed toward vocal fold (VF) regeneration have failed to augment VF function long-term. These materials degraded rapidly and did not provide sufficient mechanical strength for regeneration of functional VF [1]. In this work, the mechanical properties of a decellularized extracellular matrix (dECM) hydrogel were compared to a collagen and hyaluronic acid (CHA) hydrogel.

METHODS: Porcine VF were dissected, decellularized, homogenized, solubilized with pepsin, and incubated to fabricate 1.5% dECM hydrogels [2]. Collagen and HA solutions were mixed 1:1 and gelled at 37 °C to prepare control CHA hydrogels. To analyze gelation kinetics, frequency dependence, and the deformation limit, each hydrogel underwent 2 h time sweeps, frequency sweeps from 1-10 Hz, and amplitude sweeps from 1-100% strain using a rheometer. Swelling tests were performed over a 21-day period by incubation in PBS. Enzymatic degradation kinetics were determined from residual mass by incubation in 0.05% collagenase.

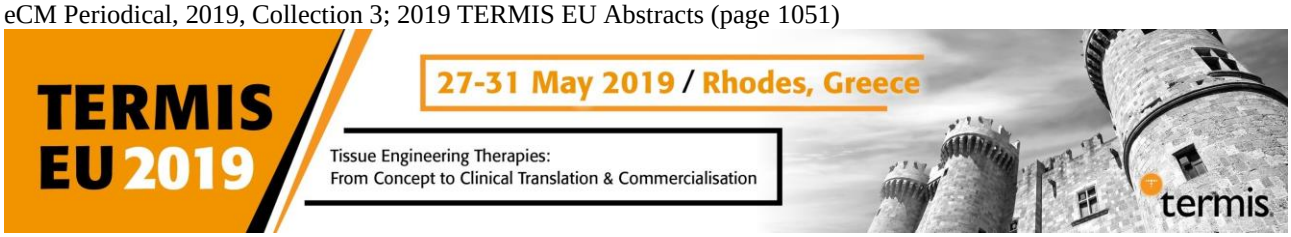
RESULTS & DISCUSSION: The 1.5% dECM hydrogel gelled in 1 h, and the CHA hydrogel in 20 min. Both gels demonstrated storage moduli (G') greater than loss moduli (G") and their G' were above 100 Pa, within the lower limit of human VF G' [3]. Linear frequency dependence was found for both gels, similar to human VF. The deformation limit of the dECM hydrogel was 44.97 ± 7.29%, above the 30% limit of human VF and significantly greater than that of CHA, 20.31 ± 4.65% ($p < 0.05$). Both dECM and CHA gels reached their maximum swelling ratios after 1 day, 4.37 ± 0.14 and 2.81 ± 0.33 times their dry mass, respectively ($p < 0.005$). On day 21, the 1.5% dECM gel retracted by 78% ($p < .001$) and the CHA gel by 68% ($p < .05$) from day 1. When incubated with 0.05% collagenase, both hydrogels degraded in 3.5 days.

CONCLUSIONS: The 1.5% dECM hydrogel had mechanical properties comparable to native VF tissue. However, their rapid gel retraction and degradation might not be favorable for long-term VF tissue reconstruction.

ACKNOWLEDGEMENTS: Financial support from the Natural Sciences and Engineering Research Council of Canada RGPIN-2018-03843 and the National Institute on Deafness and Other Communication Disorders R01 DC005788.

REFERENCES

[1] Li L et al. Biomaterials. 2016;108:91-110
[2] Miri AK et al. J. Voice. 2014;28(6): 657-667
[3] Ling C et al. Science Translational Medicine. 2015;7(314):314ra187



The effect of Wharton jelly-derived mesenchymal stem cells and their conditioned media in the treatment of rat spinal cord injury

M. Chudickova, I. Vackova, L. Urdzikova-Machova, S. Kubinova
Presenting Author: Milada Chudickova, milada.chudickova@iem.cas.cz
Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic

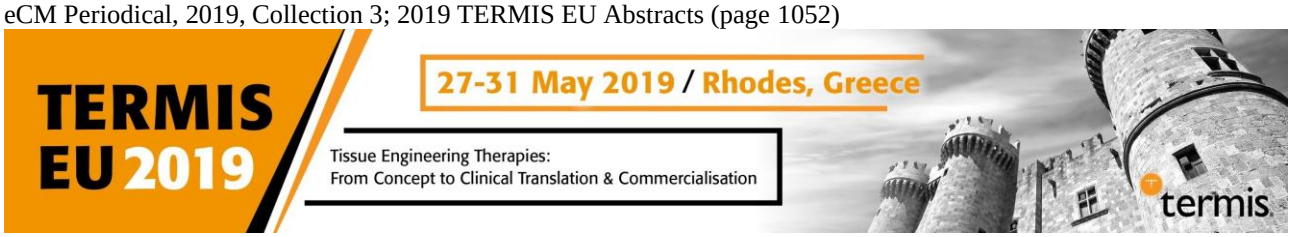
INTRODUCTION: Spinal cord injury (SCI) repair is a great challenge in tissue engineering and regenerative medicine. One of the treatment approaches represents transplantation of mesenchymal stem cells (MSCs), which possess anti-inflammatory, anti-apoptotic and generally supportive impact in wounded tissues by the paracrine effect. However, survival rate of transplanted MSCs is limited. Application of conditioned media (CM) derived of MSCs is an admitted approach how to bypass these limitations. Here we compared the effect of application of MSCs and their CM in the treatment of SCI in rats.

METHODS: We used MSCs derived from Wharton's jelly of umbilical cord (WJ-MSCs), which display high proliferative potential and produce large amounts of neurotrophic and growth factors. 1.5 million of WJ-MSCs and their CM, pooled from three different donors, were intrathecally transplanted in three doses 1st, 2nd and 3rd week after the induction of balloon compression lesion.

RESULTS & DISCUSSION: The behavioural tests (Beam walk, BBB-test, plantar test) showed significant improvement after all three treatments (CM, WJ-MSCs and non-conditioned medium with ITS), in comparison with non-treated controls. Significant improvement in all behavioural tests was found after the treatment with WJ-MSCs, CM as well as control non-conditioned medium in comparison with the saline controls. CM application resulted in significant improvement in Beam walk time and score and plantar test in comparison with WJ-MSCs and control medium. Immunohistochemical stainings (GAP43, GFAP) and Luxol-fast blue are evaluated in order to determine tissue sparing, axonal growth and glial scarring. qPCR analysis will determine changes in expression of genes related to inflammatory reaction, apoptosis, axonal growth, growth factors and astrogliosis.

CONCLUSIONS: We propose that both, hWJ-MSCs and their CM have considerable effect in the functional improvement of motor and sensory functions after SCI and that the paracrine effect of direct cell transplantation can be replaced by the CM derived from these cells.

ACKNOWLEDGEMENTS: Supported by MEYS of the Czech Republic LO1309 and GACR 17-03765S.



Viscous finger as pattern of vasculogenesis

M.-C. Tsai, N.-H. Tai, Y.-C. Chen*
Presenting Author: Min-Chun Tsai, j85111121@gmail.com
Department of Materials Science and Engineering, National Tsing-Hua University,
Hsinchu, Taiwan, R. O. C

INTRODUCTION: Many studies have successfully replicated vascular pattern by 3D fiber printing, microscale continuous optical bioprinting (μCOB), and the use of PDMS molds etc. [1-2]. Even though these approaches had generated well-organized capillary-like structure in vitro, it took 4 to 7 days of implantation and demonstrated poor functional anastomoses between branches and host to form vascular network in vivo. In our study, we can create thick viscous finger pattern similar to native vascular network by Hele-Shaw cell within 5-10 seconds with the capability of controllable resolution in width of branches and size of capillary network. Also, this technique is easy to apply to create functional lumens with core-shell structure [3]. Thus, we adopt this technique into vascular tissue engineering to create the three-dimensional (3D) large and thick vascularized tissue construct by precisely controlling the cell spreading, growth direction, and further to engineer adequate vascular network in vitro rapidly.

METHODS: We successfully created vascular-like structures that obey Murray's law by viscous fingers using the Hele-Shaw cell. It refers to a high-viscosity fluid driven by a low-viscosity fluid between parallel plates, and a finger-like type is formed between the fluid interfaces within 10 seconds.

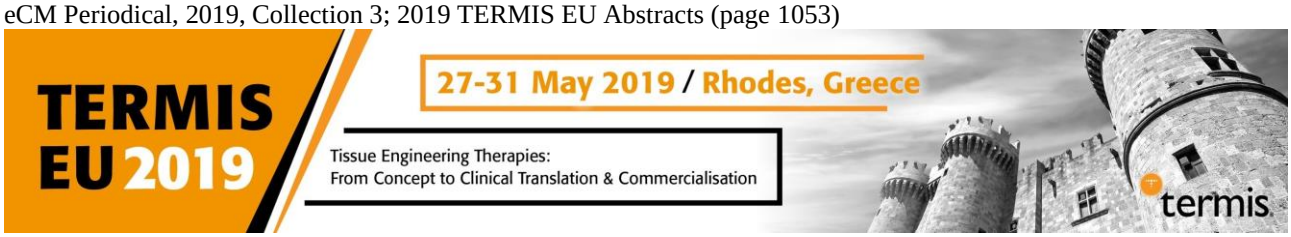
RESULTS & DISCUSSION: By controlling properties of viscous solutions, pressure gradient (∇p) and surface tension coefficient (σ) in Hele-Shaw cell, various size of network structures (5-20 cm^2), with controllable length (0.6-1 cm) and the width (100-400 μm) of fingers in a short time (5-10 s) were demonstrated. Mesenchymal stem cells (MSCs) were successfully encapsulated and patterned into vascular-like gelatin methacrylate hydrogel (GelMA) fingers for 3D cell patterning within 5-10 seconds. Further, we generate lumens surrounded by multiple hydrogel layers for dynamic microfluidics culturing, which extends the potential for modeling functionality of blood vessel in vivo.

CONCLUSIONS: Vascular-like fingers with controllable size, thickness and width of branches created by Hele-Shaw cell can be generated within 10 seconds. Additionally, this new developed technique can create core-shell structure by two different liquids in couple minutes. Our 3D cell patterning technique opens new avenues for the fabricating and investigating thick vascularized tissues for both ex vivo and in vivo applications.

ACKNOWLEDGEMENTS: Financial support was received from Ministry of Science and Technology. (MOST106-2221-E-007-055, MOST 107-2221-E-007 -067 -MY3)

REFERENCES

[1] Moroni, L. et al. Biomaterials, 2006. 27, 5918–5926
[2] Zhu, W., et al. Biomaterials, 2017. **124**: p. 106-115.
[3] Bischel, L.L., S.H. Lee, and D.J. Beebe, Jala, 2012. **17**(2): p. 96-103.



Endothelization in the recellularized rat kidney

M.-J. Choi[1], B.-T. Lee[1,2]
Presenting Author: Min-ji, Choi, mj.c8158@gmail.com
[1]Department of regenerative Medicine, College of Medicine Soonchunhyang University, Cheonan, Chungnam, Republic of Korea [2]Institue of Tissue Regeneration, Soonchunhyang University, Chungnam, Republic of Korea

INTRODUCTION: Although regenerative medicine and tissue engineering technologies have been developed dramatically, there are still many difficulties for the organ regeneration, especially, in the field of regenerative kidneys. Large numbers of chronic kidney disease (CKD) patients are still waiting for the organ transplantation using brain dead and living donor kidneys. To solve this problem, the whole organ decellularization and recellularization techniques have been actively investigated using various kinds of animals due to the existence of perfect vascular net-work systems. In this case, for the realization of renal function, endothelialization on the wall of renal ECM vessels is one of key factors and also it should be considered forming blood vessels of organ tissue for delivery nutrients and oxygen to the other type of cell. In this study, we used endothelial cell for regenerate blood vessel in the recellularized kidneys and investigated the stability of renal vessels by in-vitro approaches.

METHODS: Rat kidneys were extracted from over 8 weeks old rats and cannulated for the decellularization. For the decellularization, we used 1% Sodium dodecyl sulfate (SDS) and 1% Triton X-100 agents. Decelluarized kidneys were pre-incubated in culture medium for 1 hour. Endothelial cells were detached by trypsin treatment and seeded on vascular ECM in acellular scaffold through renal artery. Culture medium was perfused with 1.5ml/min flow rate for 3 days, 1 week and 2 weeks. To confirm the attachment of endothelial cells on the vascular ECM wall, we used optical microscope and H & E staining.

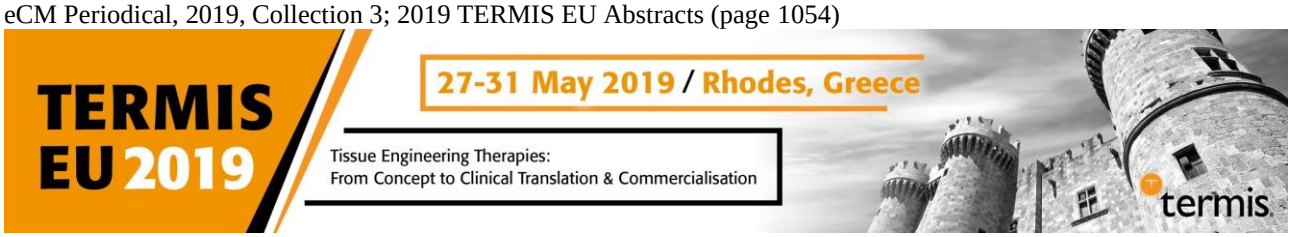
RESULTS & DISCUSSION: Rat kidneys were decellularized successfully with 1% SDS and 1% triton X-100. Endothelial cells were attached well on the vessel walls in ECM kidney scaffolds and cultured for 3 days, 1week and 2weeks. Distribution of endothelial cells and lining view in recellularized kidney were observed by H&E staining. Endothelial cells and angiogenesis markers like Tie-2, Tie-1, PECAM-1 were analyzed by FACS after detach endothelial cells by recellularized ECM kidneys.

CONCLUSIONS: The study of recellularized ECM kidney scaffold with endothelial cell is preliminary study for protecting immune rejection response or blood clot by the thrombosis. Researches about endothelium regeneration and vessel formation mechanism of recellularized kidney in in-vivo need to be performed.

ACKNOWLEDGEMENTS: Financial support was received from National Research Foundation of Korea(NRF)(No. 2015R1A6A1A03032522)

REFERENCES

[1] Andrew R. Padalhin et al. Tissue Eng Part C 2017; 24 (1):42-55



Exosomes in diagnosis and therapy of cardiovascular diseases. Hype or hope?

K. Amirzadeh gougheri[1], M.H. Farjoo[2], H. Niknejad[2]
Presenting Author: Mohammad Hadi Farjoo, m.farjoo@sbmu.ac.ir
[1]School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

INTRODUCTION: Exosomes are small extracellular vesicles released by different kinds of cells. These vesicles involve in intercellular communication by transferring bioactive molecules which are supposed to be useful in cardiovascular diseases (CVDs). We investigated the role of exosomes in different types of CVDs. In addition to pathogenesis, therapeutic, diagnostic, and prognostic roles of exosomes, we discussed a variety of progenitor stem cells and their exosomes. Routes of exosome delivery to target tissue was also mentioned.

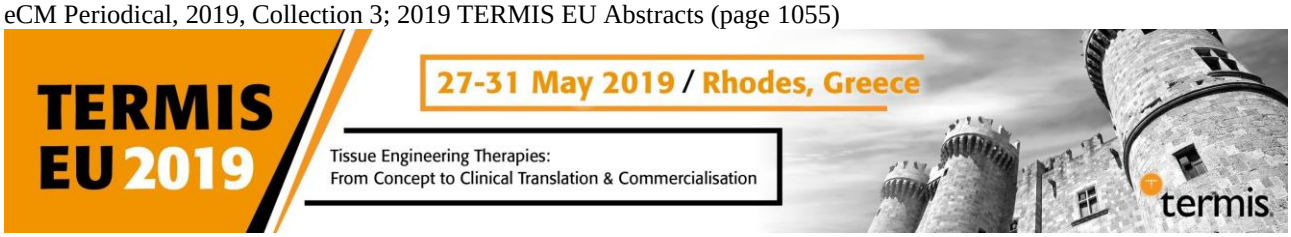
METHOD: The search keywords were "exosome, myocardial infarction, pulmonary hypertension, cardiomyopathy, valvular heart disease, and arrhythmia" in Google scholar and PubMed databases. Most articles are dated after 2012.

RESULTS & DISCUSSION: Exosomes are protective particles in atherosclerosis and therapeutic agents in 1950Wharton's jelly mesenchymal stem cells, and induced pluripotent cells, have shown to be potential bio-factory for therapeutic exosomes which can be delivered by different routes including intramyocardial, intraperitoneal, and intravenous. It seems exosome quantity or content are valuable criteria for adding to traditional risk assessment table of cardiovascular events which makes it more precise for predicting MI prognosis. Some of exosome benefits compared to cell or liposome therapy are less immune provocative, longer lasting, and able to be stored at −70°C for several months. Ischemic heart disease [1]. In pathological states, they induce disease in healthy models [2] and provoke cardiomyopathy in diabetic patients. Exosomes may act as microRNA carriers which gives them the ability to work as biomarkers or targeted therapy [3]. Human cardiac progenitor cells, bone marrow and

CONCLUSIONS: Exosomes are new potential diagnostic and therapeutic agents in CVDs. They have the capability to be a new candidate as prognostic markers. It seems that exosome therapy may have a very important position in cardiovascular disease in the future.

REFERENCES

[1] El Harane N et al. Eur Heart J. 2018; **39**(20):1835-1847
[2] Aliotta J M et al. Cardiovasc Res. 2016; **110**(3): 319-30
[3] Wu T et al. Cellular Physiology and Biochemistry. 2018; **46**(5): 1939-



Recent advances in placenta-derived mesenchymal stem cells and their exosomes in cancer therapy

E. Jamshidi[1,2†], P. Soltani[2,3†], M. Abbasi-Kangevari[2,3†], M. H. Farjoo[3], H. Niknejad[3]
Presenting Author: Mohammad Hadi Farjoo, m.farjoo@sbmu.ac.ir
[1]School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran [3]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[†]Co-first author

INTRODUCTION: This research focused on therapeutic effects of placenta-derived mesenchymal stem cells (MSCs), their conditioned media and their exosomes on tumors, to understand whether these features of MSCs in cancer therapy are capable of being combined in order to achieve an efficient anti-tumor strategy.

METHODS: The relevant articles published from 2007 until 2019 were selected by using "exosome", "mesenchymal stem cell", "cancer" and their MESH terms as keywords for searching in PubMed, Scopus and Google scholar databases.

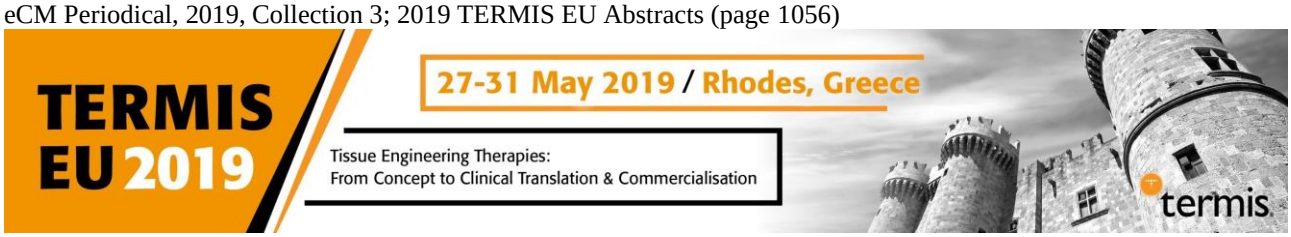
RESULTS & DISCUSSION: Using MSCs in treatment of cancer has benefits including tumor tropism, and delivery of antitumor biomolecules. Multidrug efflux pumps, which are highly expressed in MSCs seem to be useful in modulating adverse effects of drugs [1]. These cells can also produce large number of exosomes which are able to selectively detect their target tissues and bypass immune barriers. They can both suppress/promote tumor growth by different mechanisms which depend on several factors including cell source and content of exosome, target cells and interaction of exosome surface molecules with tumor cells. The bioengineering of stem cells changes gene expression pattern of placenta-derived MSCs and improves their antitumor effects. Both viral and non-viral vectors could potentially be used which are still under investigation for their efficacy on gene transduction and transmission of viral infections [2, 3].

CONCLUSIONS: Overall, it seems that incorporation of antitumor drugs with placenta-derived MSCs may be of potential therapeutic value for cancer which exert both selectivity and efficacy in tumor suppression.

ACKNOWLEDGEMENTS: Research reported in this publication was supported by Elite Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Bosco DB,et al. PLoS One. 2015;10(6):e0128511.
[2] Niknejad H, et al. Cell Tissue Res. 2016;363(3):599-608.
[3] Pinheiro A, et al. J Control Release. 2018;289:56-69.



How ex vivo vascular human-based models can give new insights in the study of vascular diseases

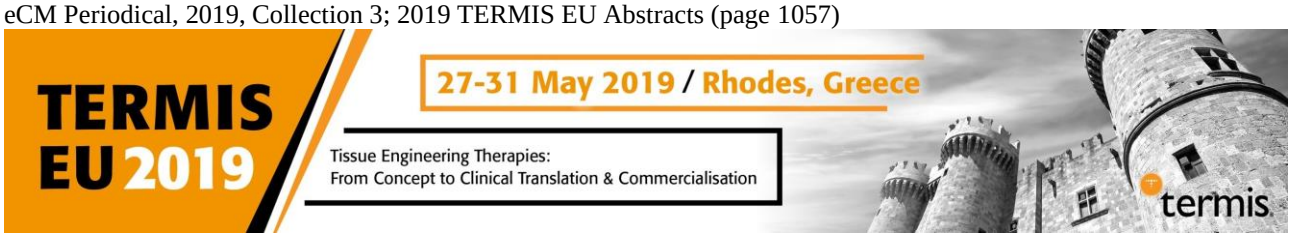
S. Seminati[1], L.P. Coppadoro[1], E. Pederzani[1], M. Piola[1], G.B. Fiore[1], M. Soncini[1]
Presenting Author: Monica Soncini, monica.soncini@polimi.it
[1]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy

Current methodologies to study vascular diseases at a supra-cellular level mainly involve animal models. In line with the 3Rs spirit, we developed several ex vivo culture systems hosting human-derived vascular tissue. We conceived simple and easy-to-use culture chambers - consisting of ad-hoc-developed chassis integrated into conventional lab equipment - for hosting native or engineered vessels coupled with control and fluidic systems enabling realistic and dynamic culture conditions. This approach allows the investigation of human tissue maladaptation in a tightly controlled environment. CYCLIC PRESSURE CULTURE SYSTEM: This model is designed to apply a cyclic pressure stimulus (e.g., 80-120 mmHg), within a controlled environment. We used this system to investigate the effects of strain wall mechanical stimulus on native vessels such as human saphenous veins (hSV) [1], [2], and to enhance the maturation of engineered constructs [3], [4]. CORONARY HEMODYNAMIC SYSTEM: A compact, modular, and low-priming-volume pulsatile simulator was designed enabling stimulating hSVs under realistic coronary artery bypass graft (CABG, Q_{mean} 150-170ml/min, P 80-120 mmHg) and venous perfusion (VP, 5 mmHg; 3 ml/min) conditions. Our results [5] revealed i) decrease of the intima and media thickness in CABG, ii) presence of endothelial cells (ECs, CD31 and vWF markers) in the luminal side with partial endothelial denudation, and iii) cell apoptosis in CABG. Recently, this system is being used to study the role of trombospondin-1 (TPS-1) in CABG-stimulated hSV, investigating the possible strain-dependent activation of adventitial resident progenitors by TPS-1. DOUBLE-COMPARTMENT CULURE SYSTEMS: They are designed to reproduce differential environments in luminal and adventitial sites. We developed two different culture chambers: i) a falcon-tube layout, that we used to investigate oxygen gradients effects on hSV [6] and ii) a new low-priming Petri-like 3D-printed system that we use to reproduce a reliable vascular thrombus ex vivo model. Our experience shows that using bioengineering approaches facilitates the understanding of vascular physio-pathological mechanisms and, in perspective, will speed up the development of new-lifesaving treatments.

ACKNOWLEDGEMENTS: Work supported by Italian Ministry of Health (RF-2011-02346867).

REFERENCES

[1] Piola et al. JTERM 2016; E204-E215
[2] Prandi et al. PLosOne 2015; e0117409
[3] Bono et al. Ann Biomed Eng 2017;45:1496-510
[4] Strobel et al. Tissue Eng A 2018;24:1492-1501
[5] Piola et al. Ann Biomed Eng 2017;45:884-97
[6] Piola et al. Ann Biomed Eng 2016;44:1449-61



Multi-compartment hydrogel capsules for topological 3D co-culture studies

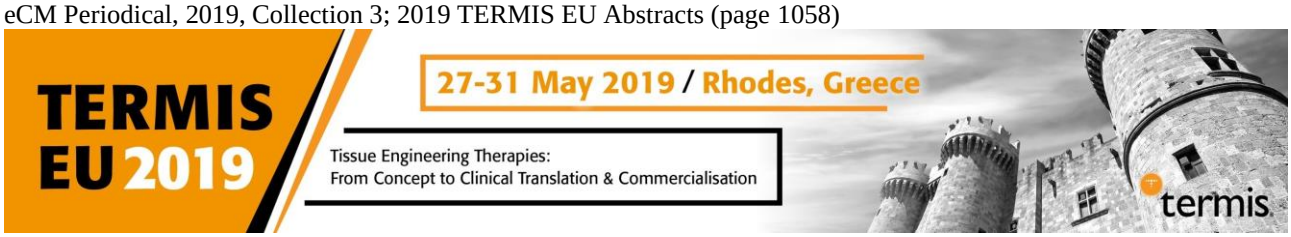
M. Ćwiklińska[1], M. Costantini[1], R. Buda[1], J. Guzowski[1]
Presenting Author: Monika Ćwiklińska, mcwiklinska@ichf.edu.pl
[1]Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: In this study, we demonstrate new microfluidic methods of precise, reproducible production of compact 3D structures from hydrogel microbeads. We use microfluidics both to formulate hydrogel microbeads as well as to reaggregate them into small close-packed agglomerates of well-defined topology. We propose that such advanced topological structures could be used as new 3D culture scaffolds in which cells get segregated in separate microcompartments to reproduce the morphology and functionality of actual tissues.

METHODS: To prepare the microbeads, the hydrogel solution and a cross-linking initiating enzyme were injected at two independent inlets into a PDMS-based microfluidic chip. The two streams merged before entering a T-junction where the solution was turned into droplets carried by fluorinated oil with surfactant as the external phase. After washing out the surfactant, the beads agglomerated and could be easily translated into buffer. After filtering, the suspension was reinjected onto microfluidic chip with gradually narrowing and shallowing channel, designed in order to avoid clogging, terminating at a T-junction for re-encapsulation of the beads into hydrogel shells.

RESULTS & DISCUSSION: Depending on the applied flow rates, we were able to encapsulate from N=2 up to N=12 gelatin beads-cores in a single gelatin shell. The structures had reproducible, predictable topology following the topologies observed earlier for colloidal clusters or in double-emulsion droplets with multiple cores. We also managed to reproducibly encapsulate given numbers of different core types (labelled with TRITC vs FITC). In order to demonstrate suitability for cell encapsulation, we generated simple cell-laden beads (C2C12, fibroblasts, HUVEC) as well as co-encapsulated beads loaded with cancer cells, HUVECs and/or fibroblasts into agglomerates for topological 3D co-culture studies. We found that the re-encapsulation method is suitable to any kind of enzymatically cross-linkable hydrogel, i.e., also to fibrin, typically applied as extracellular matrix substitute, e.g., in angiogenic assays.

CONCLUSIONS: This study provides new type of hydrogel microparticles built of several compartments arranged in well-defined close-packed topology. Such particles allow for formation of microtissues with direct cell-cell communication and predesigned spatial arrangement. We are currently investigating suitability of such platform to co-culturing tumor cells with ECs and fibroblasts with the ultimate goal of mimicking cancer microenvironment for studying drug efficacy and tumor development.



PHLDA3 overexpression in astrocytes causes endoplasmic reticulum stress

M. Řehořová[1,2], J. Turečková[1], I. Vargová[1,2], J. C. F. Kwok[1,3], J. W. Fawcett[1,4], P. Jendelova[1,2]
Presenting Author: Monika Řehořová, monika.seneklova@iem.cas.cz
[1]Institute of Experimental Medicine Czech Academy of Science, Prague, Czech Republic [2]2nd Faculty of Medicine, Charles University, Prague, Czech Republic [3]Faculty of Biological Sciences, University of Leeds, UK [4]John van Geest Centre for Brain Repair, University of Cambridge, UK

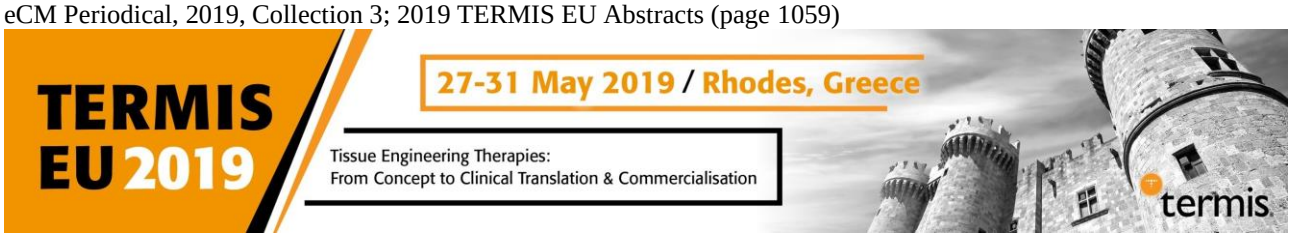
INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with a fatal outcome characterized by the loss of spinal cord and brain motor neurons (MNs), which control voluntary muscles. Previous studies have shown that astrocytes (ACs) contribute to MNs degeneration and death in ALS. However, the mechanisms underlying the behavioral change of ACs during the pathogenesis of ALS remain unclear. Recent evidence suggests that pleckstrin homology-like domain, family A, member-3 (PHLDA3) could participate in astrocyte-specific degeneration process in ALS. PHLDA3 was shown to be significantly upregulated in the ACs in ALS. In additional, PHLDA3 was recently discovered as a molecule implicated in p53-dependent signaling and is activated in endoplasmic reticulum stress (ER stress). ER stress is one of the mechanisms implicated in the pathogenesis of ALS. The aim of this study is investigating the role of PHLDA3 in the interaction of MNs and ACs during activation of ER stress in ALS in vitro model.

METHODS: In this study, we used an optimized isolation protocols suitable for ACs isolation from the cortex of mouse pups of postnatal day 1-3 (P1-P3) and MNs derived from embryonic day 14 mouse spinal cord. The functional effect of PHLDA3 on primary ACs cultures was demonstrated using plasmid delivery of PHLDA3 gene fused with a green fluorescent protein (GFP). The expression of PHLDA3, marker of ER stress (p-eIF2alpha) and p53 were evaluated using Western blot and fluorescence microscopy. To test that PHLDA3 overexpression in ACs may cause astrocyte-mediated toxicity to MNs, we cultivated MNs with conditioned medium collected from ACs overexpressing PHLDA3.

RESULTS & DISCUSSION: We demonstrated that overexpression of PHLDA3 resulted in an increased level of p-eIF2 alpha and decreased survival of transfected ACs, while overexpression of PHLDA3 directly in motor neurons had no effect on activation of ER stress.

CONCLUSIONS: Our results suggest that overexpression of PHLDA3 in astrocytes causes their death by ER stress and the modulation of PHLDA3 expression may be exploited in designing new strategies to control astrocytic death in ALS disease.

ACKNOWLEDGMENTS: Supported by Operational Programme Research, Development, and Education in the framework of the project “Center of Reconstructive Neuroscience”, registration number CZ.02.1.01/0.0./0.0/15_003/0000419



STARSTEM: NanoSTARs imaging for stem cell therapy

N. Duffy[1], G. Shaw[1], V. Raghavan[2], M. Leahy[2], M. Murphy[1]
Presenting Author: Niamh Duffy, nduffy@nuigalway.ie
[1]REMEDI, National University of Ireland Galway (NUI Galway), Galway, Ireland [2]TOMI, National University of Ireland Galway (NUI Galway), Galway, Ireland

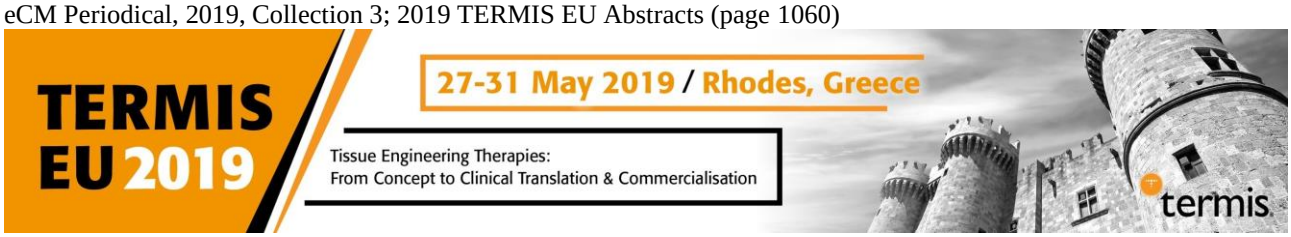
INTRODUCTION: Mesenchymal stem/stromal cells (MSCs) present tremendous potential for cell-mediated therapy in many disease processes such as osteoarthritis (OA). However, one of the major challenges to clinical translation is a lack of understanding about engraftment of delivered cells in a tissue and their biological activity in situ. STARSTEM is an EU horizon 2020 project that aims to develop a novel nanotechnology-enhanced optoacoustic imaging (OAI) platform. The core novelty of this approach is a gold nanoparticle, shaped like a star (the nanostar), which amplifies signal response in OAI. The STARSTEM platform will be capable of tracking MSCs and MSC-derived exosomes, labelled with nanostars, at unprecedented depth and sensitivity.

METHODS: Development of the STARSTEM technology begins with optimization of the nanostar production process to allow generation of high-quality, nanostars capable of being produced under GMP conditions. MSC and MSC-derived exosomes will then be tagged with nanostars and assessed for viability. Nanostar-loaded MSCs will then be deployed in a mouse model of OA and tracked over time using OAI. This will then be extended to a large animal (ovine) model of OA as the selected pre-clinical model. Data generated will support an application for a phase-1 clinical trial of nanostar-enhanced OAI in humans. The development of the STARSTEM technology has included quality control analysis of nanostars and their effect on MSCs. Transmission electron microscopy has provided morphometric analysis of the nanostars. Nanostar-loaded MSCs have been assessed in terms of cell viability, tri-lineage differentiation capacity and cell surface marker expression.

RESULTS & DISCUSSION: Dark field imaging has shown the uptake of nanostars by MSCs. These nanostar loaded MSCs showed no reduction in cell viability or metabolic activity. Their cell surface marker profile was compliant with ISCT standards. Differences in the differentiation potential between control and nanostar loaded cells was observed, however. Nanostar-loaded MSCs showed a promotion of osteogenesis and chondrogenesis, the possible basis for which is currently being investigated.

CONCLUSIONS: In conclusion, the STARSTEM technology will lead to improved tracking of MSCs, and thus a better understanding of their mechanism of action, engraftment and biodistribution in disease models.

ACKNOWLEDGEMENTS: STARSTEM has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 761214. The material presented, and views expressed here are the responsibility of the authors only. The EU Commission takes no responsibility for any use made of the information set out.



Animal moldels for investigation of gold storage solution in organ transplantation

N. Halvaei[1,2], P. Parhizgar[1,2], A. Ahmadi[1,2], R. Mazloomnejad-Meybodi[1,2], H. Niknejad[1]
Presenting Author: Nika Halvaei, nikadvn@yahoo.com
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran

INTRODUCTION: Organ preservation plays an important role in a successful transplantation. Various surgical methods for organ transplantation have been explored throughout the years, each of which possesses its own advantages and disadvantages. To investigate the excellent methods, we made a comparison of different effects in more widely utilized methods of organ preservation and transplantation surgery in a variety of animal models. To do so, we assessed the function of the organ by specific enzymes, necrotizing factors and ischemia after hours of preservation in the transplanted organ.

METHODS: The searched keywords were “organ transplantation”, “animal models”, “Large animal” and “small animal”. Main databases used included “PubMed” and “Google scholar”.

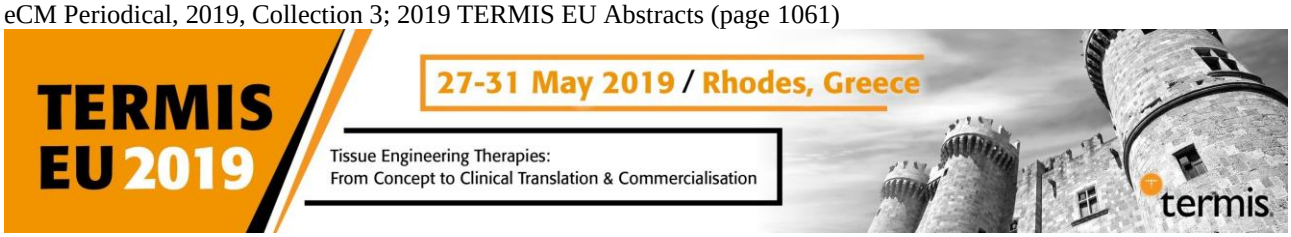
RESULTS & DISCUSSION: The results depend on the organ used in transplantation and size of the animal model. Each organ has its own characteristics that require distinct methods to evaluate cold storage solution in the transplantation process. Temperature changes, whether hypothermia or normothermia affect the reperfusion of organ grafts [1]. Moreover, cytotopic anticoagulants for preventing microvascular thrombosis can reduce reperfusion-related ischemia.[2] Biliary complications are almost inescapable specially in small animals such as rats, mice and rabbits.[3] This results in a vast preference towards using larger animal models for transplantation surgery. These animals include Canine, Porcine and Primate models.

CONCLUSIONS: It is important to find the optimal way for organ preservation and methods of surgery in animal models to reduce the injury and to maximize the function of the transplanted organ. We presented different methods with a variety of functional and mechanical changes in the transplanted organ after a long duration of preservative process.

ACKNOWLEDGEMENTS: Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran. We would also like to thank Dr. Nooshin and Dr. Tayebi for their kindly assists.

REFERENCES

[1] Westerkamp et al, Transplantation 2016;100: 825-835
[2] Hamoui K et al, Transplantation 2016;100: e128-e139
[3] Li et al, World J Gasrtroenterol. 2011 July 14;17(26): 3140-3144



The current approaches on angiogenesis in decellularized scaffolds

R. Mazloomnejad-Meybodi[1,2,†], N. Halvaei[1,2†] , M. Malekpour[1,2], A. Ahmadi[1,2], H. Niknejad[1]
Presenting Author: Nika Halvaei, nikadvn@yahoo.com
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. †Co-first author

INTRODUCTION: There are a lot of studies on decellularized tissues and organs due to their great capacity to use as a substitute for organ donation and transplantation; However, angiogenesis and how to supply oxygen and nutrients for cells remain the decisive challenge in acellularized tissue application. We presented here the recent advances regarding the factors impacting angiogenesis in decellularized tissue and organs.

METHODS: Literature search was done by searching “prevascularization”, ”Re-endothelialization”, ”endothelial differentiation”, ”angiogenesis inducer”, ”vasculogenesis” and ”optimized acellularization” keywords in Pubmed and Google scholar published within the last ten years.

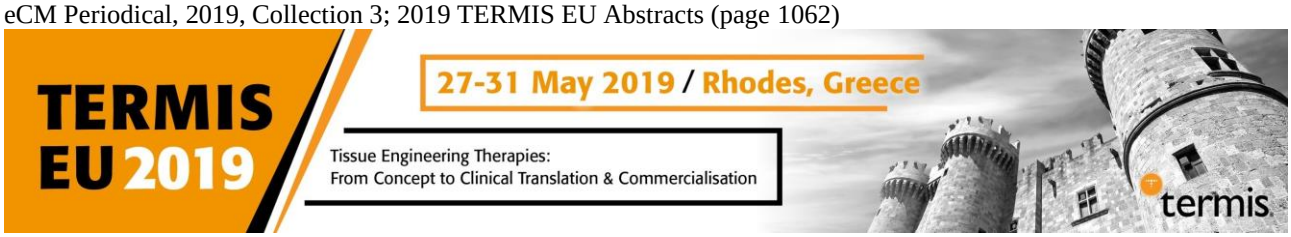
RESULTS & DISCUSSION: Several strategies have been hired to induce in-vivo and in-vitro angiogenesis including re-endothelialization, prevascularization, endothelial differentiation , endothelial culture and cell coating [2]. Scaffold specification is another key matter that influences induction of angiogenesis [3], but what these specifications are and how to optimize them [1]is yet some essential questions that need to be answered in the future studies.

CONCLUSIONS: The current angiogenesis inducing protocols have provided evidence of notable progresses, while there are important challenges needed to deal with.

ACKNOWLEDGEMENTS: Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran. We would like to thank Dr. T. Tayebi and Dr. B. Nooshin for their many assists.

REFERENCES

[1] Modaresifar K, et al. Artif Cells Nanomed Biotechnol. 2018 Dec;46(8):1799-1808
[2] Sasaki K, et al. Biomaterials. 2017 Jul;133:263-274
[3] Peloso A, et al. Transplantation. 2015 Sep;99(9):1807-16



A proof of concept for scaffold testing: Ex ovo chorioallantoic membrane assay for pre-screening scaffolds intended for clinical application

N. Kohli[1], P. Sawadkar[1], S. Ho[1], V. Sharma[1], M. Snow[2], S. Powell[3], M. Woodruff[3], L. Hook[4], E. García-Gareta[1]
Presenting Author: Nupur Kohli, kohliN@raft.ac.uk
[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood, UK [2]Royal Orthopaedic Hospital NHS Foundation Trust, Birmingham,UK [3]Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, 60 Musk Avenue, Kelvin Grove, Australia [4]Smart Matrix Ltd., Leopold Muller Building, Mount Vernon Hospital, Northwood, UK

INTRODUCTION: The scaffold component of tissue engineering is key to achieve tissue regeneration as it provides a 3D environment for cells to build new functional tissue. A vital part of the regenerative process in various tissues such as skin or bone is formation of a vascular network for delivery of oxygen/nutrients and removal of metabolites. Chorioallantoic membrane (CAM) of chicken embryos have been used as a model to study angiogenesis in ovo for nearly 20 years[1]. Whether or not it could be used as proof of concept for scaffold testing is currently unknown. We used the ex ovo CAM assay to substantiate whether structure or composition of a scaffold could influence its angiogenic properties and therefore, determine the feasibility and accuracy of this method for scaffold testing.

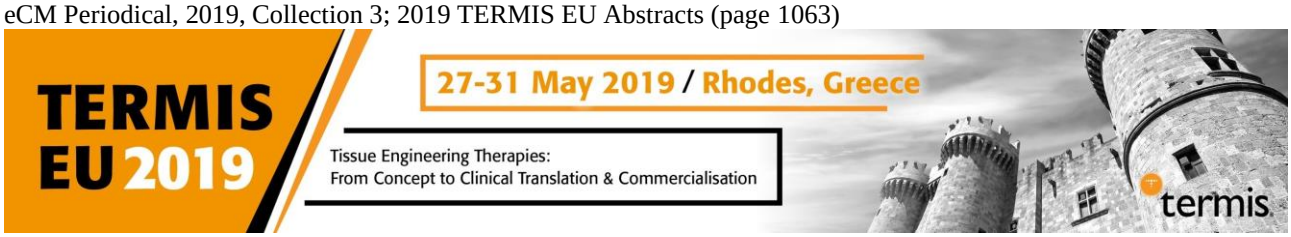
METHODS: Fertile chicken eggs were incubated for 3 days after which, embryos were transferred to shell-less culture system under sterile conditions. A range of commercially available and pre-clinical scaffolds for skin and bone were applied to the developing CAMs at day 6 post ex ovo. The scaffolds tested included but were not limited to Integra®, Matriderm®, demineralized bone matrix (DBM) and polycaprolactone (PCL). Controls were filter discs soaked in VEGF and PBS. From day 9 to day 12, angiogenesis was examined macroscopically. At day 12, embryos were sacrificed ethically and angiogenesis in stereomicroscope images of different scaffolds was quantified using ImageJ software. Histological processing and staining was also carried out.

RESULTS & DISCUSSION: There was a clear trend between porosity and the degree of angiogenesis, where more porous scaffolds showed a higher percentage of vascular area relative to the size of the scaffold. This was also dependent on the composition of the scaffold for e.g., a more porous scaffold made of less angiogenic material like PCL was not as angiogenic as a similarly porous scaffold made of fibrin, which is pro-angiogenic. The data suggests that there is a fine balance between structure and composition that can positively or negatively influence angiogenesis

CONCLUSIONS: Our data suggests that CAM assays can be used as a proof of concept for scaffold testing prior to in vivo animal testing. This ex ovo method is safe and inexpensive compared to the currently used methods such as Matrigel assays or ELISAs which are complex and are far from mimicking the in vivo situation. In the light of the principles of reducing animals for research (NC3Rs), we share the views of the authors who recently published a review in tissue engineering[2] suggesting that researchers should adopt this technique for scaffold testing.#

REFERENCES

[1] Schlatter P, et al. Microvasc Res.1997;54(1):65-73.
[2] Moreno-Jiménez I, et al. Tissue Eng Part C Methods. 2017;23(12):938-952.



Intravenously injected human mesenchymal stem cells restore brain damage and memory impairment in mice

O. Deryabina[1], M. Skok[2], O. Lykhmus [2], N. Shuvalova[1], O. Toporova [1,3], V. Kordium[1,3]
Presenting Author: Olena Deryabina, oderyabina@gmail.com
[1]State Institute of Genetic and Regenerative, Department of Gene Technologies, NAMS Ukraine, Kyiv [2]Palladin Institute of Biochemistry, Laboratory of Cell Receptors Immunology, NAS Ukraine, Kyiv [3]Institute of Molecular Biology and Genetics, Department of Cell Regulatory Mechanisms, NAS Ukraine, Kyiv

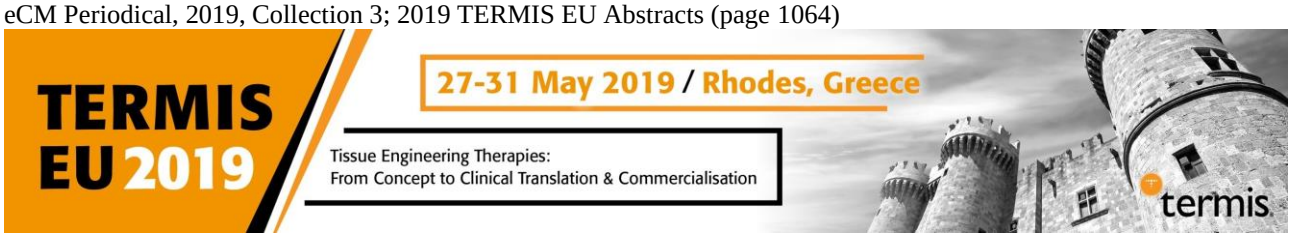
INTRODUCTION: Alzheimer disease (AD) is an age-dependent neurodegenerative disorder resulting in impairment of memory, speaking and practical habits. The AD is usually accompanied by neuroinflammation and the brains of AD patients are characterized by cholinergic deficiency and accumulation of extracellular senile plaques formed by oligomerized amyloid beta (Aβ) peptides. Mesenchymal stem cells (MSCs) are known to be the restoring agent for various damages including neurological. The aim of the present work was to study, whether inflammation-induced effect on the brain and behavior of mice can be reversed by human umbilical cord MSCs (hUC-MSCs).

METHODS: MSCs were obtained from Wharton jelly using the explant method. The cells of the second passage were intravenously transplanted into C57Bl/6 mice (10^6 cells per mouse) three weeks after intraperitoneal LPS injection when memory decline was already observed. Mice were examined in memory test every week thereafter, then sacrificed and their brains and brain mitochondria were studied for the level of nicotinic acetylcholine receptors, Aβ(1-42), micro- and astroglia markers by ELISA and immunohistochemistry.Mitochondria sustainabi-lity to apoptogenic effect of Ca^{2+} was assessed in cytochrome c release assay.

RESULTS & DISCUSSION: hUC-MCS satisfied minimal criteria for defining multipotent mesenchymal stromal cells according to CD34, CD44, CD45, CD90, CD73, CD105 surface markers expression. MSCs restored memory of mice in 1 to 3 weeks after injection and significantly improved their brain mitochondria sustainability to Ca^{2+}. This was accompanied by up-regulation of nicotinic acetylcholine receptors α4, α7, α9, β2 and β4 subunits and decrease of Aβ(1-42) in the brain and brain mitochondria. Surprisingly, MSCs additionally activated astrocytes of LPS-treated mice.

CONCLUSIONS: Positive MSCs effect when pathological symptoms have already developed (MSCs injection three weeks after LPS) allows suggesting that MSCs, or their soluble factors, directly or indirectly (by activating glial cells) restore the activity of damaged brain neurons. Further experiments are needed to reveal whether the recovery observed under the MSCs effect is long-lasting or just temporal

ACKNOWLEDGEMENTS: Financial support was received from the Program on Molecular Biotechnology of National Academy of Sciences of Ukraine (project 34/2015-2019 0115V003646)



Mucoadhesive nanofibers for brinzolamide delivery

O. Cegielska[1], P. Sajkiewicz[1]
Presenting Author: Olga Cegielska, ocegiels@ippt.pan.pl
[1]Laboratory of Polymers and Biomaterials, Institute of Fundamental Technological Research Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: Nanofibrous polymeric materials are well-known for providing local, sustained and prolonged delivery of therapeutic agents. Therefore, compared to conventional therapies, they prolong the therapeutic effect, reduce side effects and increase the drug compliance [1]. In ophthalmic applications they constitute a very promising and yet not deeply studied structure [2]. Nanofibers made of hydroxypropyl cellulose (HPC) with β-cyclodextrin (β-CD) complexes with anti-glaucoma hydrophobic active ingredient brinzolamide were formed using the electrospinning technique. They will be stabilized on the cornea by HPC's mucoadhesive properties [3]. β-CD/brinzolamide complexes should be released from them as a whole, freeing the drug only in contact with the hydrophobic cornea. Here we present basic physicochemical properties of these nanofibers.

METHODS: Formation of β-CD/brinzolamide complexes was done and the complexation effectiveness was determined in a form of apparent stability constant (KS) and complexation efficiency (CE). Solutions of HPC, β-CD and brinzolamide in hexafluoroisopropanol (HFIP) of 3-5% w/w were electrospun. The process parameters were optimized. Morphology of the electrospun nanofibers was observed using scanning electron microscope (JEOL JSM-6010PLUS/LV). Wettability tests were carried out using Dataphysics OCA 15 goniometer. Water solubility test was performed. Photographs were made and videos were recorded, and weight loss over time was measured.

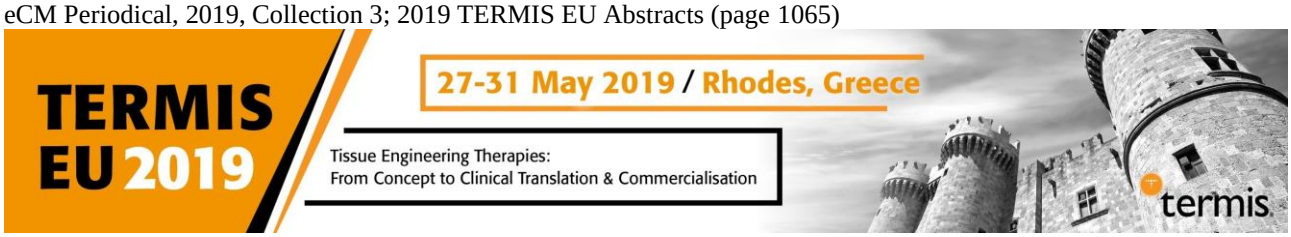
RESULTS & DISCUSSION: Complexation of β-CD with brinzolamide was effective. In all of the three-component nonwovens, nanofibers were randomly oriented and showed adequate morphology. Small beads on thin nanofibers and local thickening were present in some of the nonwovens. The nonwovens were hydrophilic, what should enhance their mucoadhesion. Weight loss studies have shown the possibility of progressive biodegradation of the carrier in the tear film environment.

CONCLUSIONS: Obtained data are highly favorable and show a great potential of HPC/β-CD/brinzolamide nanofibers in ophthalmic applications. Approaches that were made will serve as a base for further research on brinzolamide release from the obtained nanofibers. Fiber modification is planned by replacing HPC with HPC modified by thiolation. Research will be complemented by mucoadhesion studies.

ACKNOWLEDGEMENTS: Research was supported by the funds of the project CePT POIG.02.02.00-14-024/08/ and POWER Och!Dok.

REFERENCES

[1] Wen H., Huijeong Jung and Xuhong Li, The AAPS Journal 2015; 17(6): 1327-1340,
[2] Gagandeep et al., Eur J Pharm Sci 2014; 53: 10-16
[3] Grabova V., Guggi D. and Bernkop-Schnurch A., Adv Drug Deliv Rev. 2005; 57: 1713-1723



Expression and activity of TRPA1 and TRPV1 in the intervertebral disc: association with inflammation and matrix remodeling

T. Kameda[1,2], J. Zvick[1], M. Vuk[1], A. Sadowska[1], K. Tam Wai[3], V. Leung[3], K. Bolcskei[4], Z. Helyes[4], O. Hausmann[5], J. Klasen[6], O. Krupkova[*1], K. Wuertz-Kozak[*1]
Presenting Author: Olga Krupkova, okrupkova@ethz.ch
[1]Institute for Biomechanics, ETH Zurich, Switzerland [2]Department of Orthopaedic Surgery, Fukushima Medical University, Japan [3]Department of Orthopaedics and Traumatology, University of Hong Kong, China [4]Department of Pharmacology and Pharmacotherapy, University of Pecz, Hungary [5]St. Anna Hospital, Lucerne, Switzerland [6]Prodorso, Zurich, Switzerland, [*]contributed equally

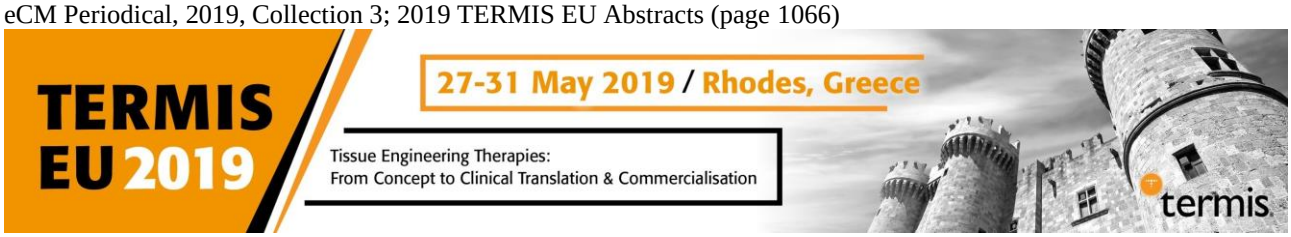
INTRODUCTION: Transient receptor potential (TRP) channels have emerged as sensors and transducers of inflammatory pain, with a possible relevance in degenerative disc disease (DDD). The aim of this study was to test the expression and function of TRPA1 and TRPV1 in intervertebral disc (IVD) inflammation and extracellular matrix (ECM) homeostasis.

METHODS: Human lumbar degenerated discs were collected from patients undergoing spinal surgeries and either analyzed directly by RT-qPCR (n = 25) or used for primary cell cultures (n = 30). Cells were stimulated with cytokines (IL-1β, TNF-α) to mimic the inflammatory/catabolic shift in DDD. Gene expression of TRP channels, inflammation mediators and ECM genes was measured by RT-qPCR, protein release by ELISA. The agonist allylisothiocyanate (AITC) was used to activate these channels (confirmed by calcium flux assay). ECM structure of caudal motion segments from TRPA1 (n = 20) and TRPV1 (n = 15) wild-type (wt) and knock-out (KO) mice was visualized by FAST staining. Statistical analysis was performed in GraphPad Prism.

RESULTS & DISCUSSION: TRPA1 and TRPV1 were expressed in 16% and 100% of degenerated lumbar discs, respectively. Cytokine treatment significantly induced gene expression of TRPA1 and reduced TRPV1 in disc cells. Experiments with AITC revealed that activation of TRPA1 (not TRPV1) reduced gene expression of ADAMTS5 and Collagen I. When compared with wt mice, mature TRPA1 KO mice showed signs of degeneration in the nucleus pulposus and the vertebral growth plate, while these tissues were unaffected in TRPV1 KO mice.

CONCLUSIONS: Our results indicate that TRPA1 may be involved in matrix remodeling and homeostasis during maturation and inflammation of the IVD. Follow-up studies are needed to fully elucidate the role of TRPA1 and TRPV1 in DDD.

ACKNOWLEDGEMENTS: Financial support was received from SNF, Eurospine and University of Pecs (EFOP-3.6.2-16-2017-0006 and GINOP-2.3.2-15-2016-00048).



Hyperacute serum therapy: the evaluation of regenerative potential in osteoarthritis treatment - In-vitro and in-vivo study

O. Kuten-Pella[1,2], D. Kardos[3], A. Hinsenkamp[3], B. Marschall[3], I. Hornyák[1,3], K. Magos[4], T. Bárdos[4], T. M. Paukovits[4], G. Béres[4], S.Nehrer[2], Z.Lacza[1,2,3]
Presenting Author: Olga Kuten-Pella, olga.kuten@orthosera.com
[1]OrthoSera GmbH, Krems an der Donau, Austria [2]Center for Regenerative Medicine, Danube University Krems, Austria [3]Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary, [4]Kastélypark Klinika, Tata, Hungary

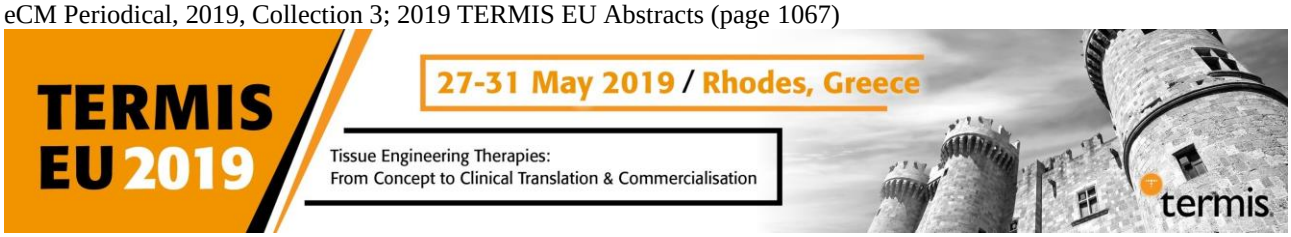
INTRODUCTION: Blood derivatives are becoming the first choice for injectables in the treatment of osteoarthritis (OA). The cytokine milieu of the joint is affected by the injection and may reflect the stage of the disease and any potential improvements. To identify the most beneficial treatment strategy for OA with the use of blood products, we established a 3D cell culture model of osteoarthritic chondrocytes. We investigated the interplay in cytokine composition between hyperacute serum and the osteoarthritic knee in human joint tissue explants and in a clinical investigation.

METHODS: PRP was generated by two centrifugation steps whereas hyperacute serum was produced using the hypACT auto device according to the manufacturer´s instructions. We analyzed the growth factor and inflammatory cytokine content of PRP and hyperacute serum. We established a 3D pellet culture model with OA chondrocytes, where we evaluated the therapeutic efficacy of PRP and hyperacute serum in osteoarthritis by histology, histochemistry and specific gene expression.
The role of hyperacute serum in an inflamed environment was assessed with the co-culture explants model and a first-in man clinical study. Surgically excised knee tissues were activated by IL-1β and co-cultured with or without hyperacute serum. Patients with knee osteoarthritis (n=26) received 3 weekly injections of autologous hyperacute serum. Synovial fluid was harvested before each injection and clinical monitoring was followed up at 3 and 6 months. Forty OA-related cytokines were quantified by multiplex immunoassays in the culture supernatants and the synovial fluid.

RESULTS & DISCUSSION: The results from 3D cell culture model with the use of PRP and hyperacute serum in OA treatment revealed that hyperacute serum is a more effective blood product with a potential use in OA therapy. Hyperacute serum contains less inflammatory cytokines than PRP and enhances the expression of specific chondrogenic markers. Therefore we performed more thorough analysis of the hyperacute serum role on the cytokine milieu of the osteoarthritic joint. We identified that hyperacute serum decreased osteo- and rheumatoid arthritis markers in vitro and provided symptomatic relief in vivo. The cytokine pattern revealed that there are at least three different types of OA knees which probably have different underlying pathologies.

CONCLUSIONS: Hyperacute serum preserved viability of bone, synovium and cartilage and decreased the pro-inflammatory cytokine levels in vitro.

ACKNOWLEDGEMENTS: The authors are thankful for OrthoSera GmbH for the research support to Semmelweis and Danube Universities.



Surface functionalization of polyesters nanofibers via aminolysis and gelatin immobilization

O. Jeznach[1], D. Kolbuk[1], P. Sajkiewicz[1]
Presenting Author: Oliwia Jeznach, oliwia.jeznach@gmail.com
[1]Laboratory of Polymers and Biomaterials, Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION: The main limitation of polymer nanofiber scaffolds is associated with their hydrophobicity and the lack of biological cues on the surface, which hinder their effective interactions with cells [1]. Aminolysis combined with protein immobilization is one of the ways to enhance cellular response to polymer scaffold [2]. In this study, effectiveness of surface functionalization was compared for three types (polycaprolactone (PCL), polylactide (PLA) and poly(lactide-co-caprolactone) (PLCL)) of electrospun nanofibers.

METHODS: Three types of electrospun nanofibers were subjected to surface functionalization at the same conditions to compare effectiveness of process in the case of different polyesters. Firstly, samples were immersed in 6% w/v ethylenediamine /isopropanol solution at 30°C for 5 and 15 minutes to introduce free amino groups on the surface. Then, samples were activated with glutaraldehyde (1% w/v, RT, 2.5h). The last step was immobilization of gelatin on the surface using 0.2% w/v gelatin/water solution at 37°C for 20h. Samples were characterized by SEM observation, water contact angle measurements, quantification of amino groups and immobilized gelatin on the surface and mechanical testing. Also, L929 cells were cultured on the samples to evaluate biological response to modified scaffolds.

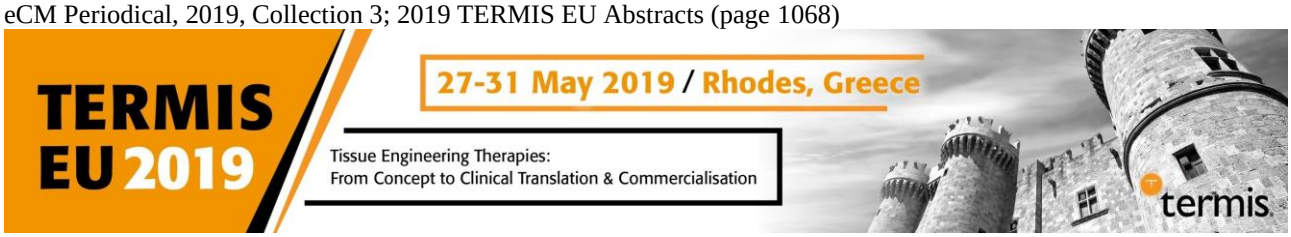
RESULTS & DISCUSSION: The applied process conditions did not change morphology of nanofibers. However, a decrease of Young modulus was observed for all samples, being the most significant for PLA samples. Prolongation of aminolysis time caused drop of stress at break in the case of PLCL and PLA samples. For all samples after gelatin immobilization surface became completely hydrophilic. After 5 days of culturing we observed well spreaded cells on the surface of modified samples in contrast to reference samples. The positive effect was visible even after first step of functionalization - aminolysis reaction, and increased with the time of aminolysis for PCL and PLCL nanofibers.

CONCLUSIONS: This study confirms that aminolysis combined with gelatin immobilization is effective method to enhance cellular response to polymer nanofiber scaffolds. Our study shows that effectiveness of process is different in the case of each type of polyester (the highest for PLA, and the lowest for PCL nanofibers), which has impact on amount of amino groups and gelatin on the surface as well as mechanical properties, but for all samples positive effect on cellular response was achieved.

ACKNOWLEDGEMENTS: This work was supported by OPUS Project (UMO-2016/23/B/ST8/03409) operated by the National Science Centre and project no. POWER.03.02.00-00-I028/17-00 realized as part of the Operational Program Knowledge Education Development 2014-2020 co-financed from the European Social Fund.

REFERENCES

[1] Vasita R et al. Curr Top Med Chem. 2008; 8:341-353.
[2] Krithica N et al. AdvEng Mater. 2012;14(4):149-154.



The recent advances on the ingredients of cold storage solutions for liver transplantation

P. Parhizgar[1], T. Tayebi[1], A. Jafari[1], N. Halvaei[1], H. Niknejad[1]
Presenting Author: Parinaz Parhizgar, parhizgar.p@sbmu.ac.ir
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

INTRODUCTION: The advancement of surgical techniques has strengthened hope in the liver transplantation as the final treatment of liver failure. However, there are obstacles in organ transplantation including the limitations in the number of transplant donors along with the occasional long distances between the transplant donor and receiver. This, in turn, lays the emphasis on the organ preservation methods so that the transplant tissue can be preserved with the least damage for a longer period before transplantation. In this study, we focused on the recent advances in static storage solutions and the effect of temperature on preserved tissue and organs.

METHODS: Articles in English were extracted through a comprehensive search in PubMed, Science Direct, and Google Scholar databases by the keywords “liver preservation”, “preservation solution”, “static cold preservation”, “ischemia-reperfusion”, and a variety of combinations of these words. The recent issue was defined as articles published from 2010 to the end of 2018.

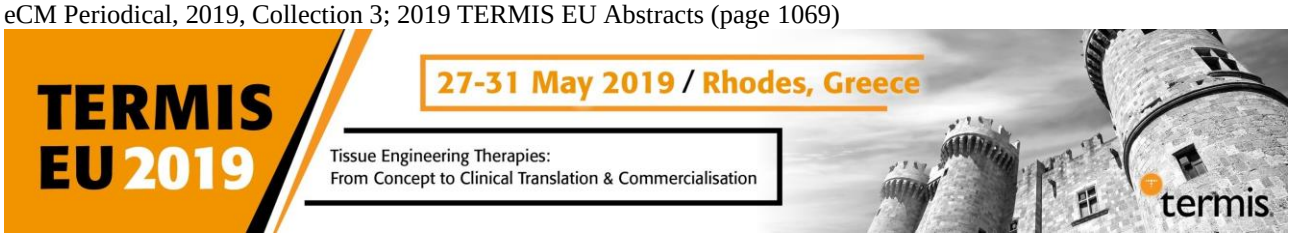
RESULTS & DISCUSSION: The common current methods of organ preservation rely on the basis of preserving the tissue in storage solutions in a low-temperature media to minimize the damage to the tissue as well as to provide the tissue with energy supply [1]. Of the essential compartments of preservation solutions are electrolytes, free radical scavengers, and saccharides. At the time being, scientists are about to find ways to maximize the efficiency of preservation solutions for instance by the use of cell death inhibitors like Caspase-3/ Caspase-8 siRNA at a genetic and transcriptional level [2]. Furthermore, efforts are being made to reduce the adverse effects of temperature change on ischemia-reperfusion injuries.

CONCLUSIONS: Despite the efforts in optimizing the storage solutions, they are still not effective in preserving the liver for a longer period of time. Thus, further alterations must be implemented regarding cold storage solutions.

ACKNOWLEDGEMENTS: Hereby, t**he authors would like to thank Prof. H. Peirovi and the operation room personnel of Taleghani hospital for their assistance in this research.** Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Cavaillé-Coll M, et al. American journal of transplantation. 2013;13(5):1134-48
[2] Yang C, et al. Journal of translational medicine. 2013;11(1):210



Recent advances on application of hydrogels for microfluidic-based stem cell encapsulation

F. Yousefimoghaddam[1†], M. Nourbakhsh[1†], S. Bideri[1†], T. Tayebi[1†], P. Parhizgar[1†], H. Niknejad[1]
Presenting author: Parinaz Parhizgar, parhizgar.p@sbmu.ac.ir
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [†]Co-first author

INTRODUCTION: Microfluidic technology is a powerful tool in biological and medical research because of offering the possibility of controlling samples in the microenvironment by down scaling. The traditional methods of cell culturing need high quantities of reagents and ingredient but these are reduced in this system [1]. Using microfluidic technology monodisperse cell laden microgel with tunable size can be generated making them useful building blocks that can be assembled into tissue constructs with spatially controlled physicochemical properties [2]. In this study we investigate recent advances in cell encapsulation hydrogels.

METHODS: The authors searched PubMed, Google Scholar, Scopus and other databases for keywords including “cell encapsulation”, “hydrogel”, “stem cells”, “ microfluidic” and “Extracellular matrix” from 2012 to the end of 2018.

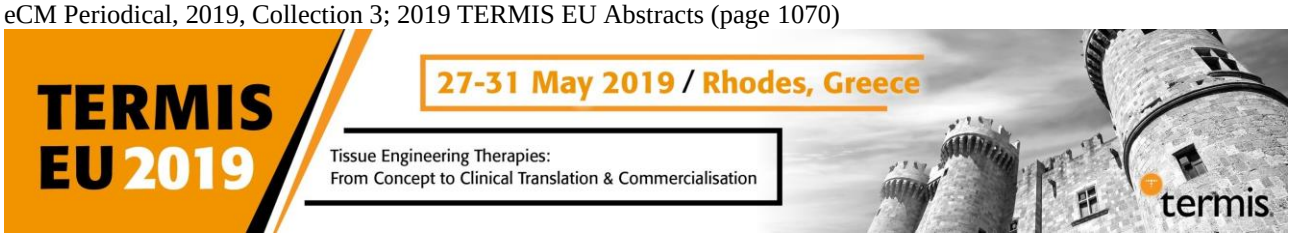
RESULTS & DISCUSSION: Processing capability and bioactivity are two important features of biomaterials used for cell microencapsulation. Only hydrogel materials have especial groups for crosslinking can be used. Alginate and alginate-based hydrogel have been used as main materials in cell encapsulation. However, there are some aother appropriate materials that can be used for specific purpose. For instance, PEG is used for inducing secretory function in endocrine cells like beta pancreas cells with increasing survival rate of cells. Hyaluronic acid revealed specific properties related to angiogenesis and anti-inflammatory functions [3]. Chitosan is used for improving ionic gelation and it improves drug delivery. The crosslinking of hydrogels is a main determinant in cell encapsulation by microfluidic approach.

CONCLUSIONS: Hydrogels are of a very wide use in tissue engineering applications such as space filling agents or delivery vehicles for bioactive molecules. The most important characteristic of hydrogels is to have appropriate physiochemical properties to fit biological variants inherit to each application, for example having enough elasticity when working on uterus or having electrical conductance when working on neurons or myocardial cells or hypothermic preservation when working on adipose tissue.

ACKNOWLEDGEMENTS: The authors thank A. Ebadi for his assistance in this research. Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Torino S, et al. Inventions. 2018;3(3):65.
[2] Jiang W, et al. Lab on a Chip. 2016;16(23):4482-506.
[3] Choe G, et al. Polymers. 2018;10(9):997.



Human dental pulp stem cell-derived extracellular vesicles stimulate the expression of microglial TREM2

P. Gervois[1], M. Lo Monaco[1], Y. Dillen[1], T. Vangansewinkel[1], R.B. Driesen[1], G. Merckx[1], H. Kemps[1], A. Bronckaers[1], E. Wolfs[1] and I. Lambrichts[1]
Presenting Author: Pascal Gervois, pascal.gervois@uhasselt.be
[1]Morphology Research Group, Biomedical Research Institute, UHasselt, Diepenbeek, Belgium

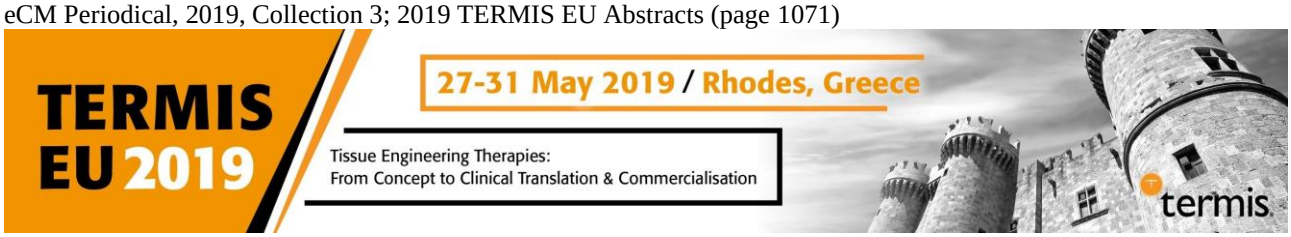
INTRODUCTION: Stroke is the second most common cause of death and is a major cause of permanent disability. Novel therapeutics aim to exploit the regenerative effect of stem cell-based therapies using for example dental pulp stem cells (DPSC). These therapies are believed to be mediated by paracrine factors including extracellular vesicles (EVs) that are produced by the transplanted cells and contain growth factors and (micro)RNAs. Microglia play an important role in balancing the inflammatory post-stroke microenvironment by obtaining a broadly defined pro-inflammatory M1 or pro-regenerative M2 phenotype thereby contributing to the exacerbation or alleviation of ischemic damage. One mechanism that is often overlooked in cell-based therapy is immunomodulation. In this study, the paracrine effect of DPSC factors on microglial polarization was evaluated. Moreover, triggering receptor expressed on myeloid cells 2 (TREM2) expression upon DPSC stimulation was assessed. TREM2 emerged as a novel target in stroke that is involved in phagocytosis and microglial function as TREM2 deficiency was detrimental for ischemic injury.

METHODS: DPSC-EVs were isolated by differential ultracentrifugation and evaluated with nanoparticle tracking analysis. In parallel, complete conditioned medium (CM) and EV-free CM of DPSCs was collected. Afterwards, volume-corrected EVs, full CM and EV-free CM was added to the mouse microglial BV-2 cell line. As controls for the pro- and anti-inflammatory M1 and M2 microglia phenotype, LPS and IL-4 were added to the cells respectively. Subsequently, the BV-2 phenotype was determined using NO measurements, qPCR and immunocytochemistry for TREM2. In another experiment, 24h after LPS stimulation, LPS, IL-4 and the CM fractions were added to the stimulated BV-2 cells in order to evaluate the ability of the DPSC-secretome fractions to counteract the M1 polarizing effect of LPS-induced NO production.

RESULTS & DISCUSSION: DPSC-EVs had an average particle size of 112.95 ± 9.75 nm (n=6). Neither fraction of the DPSC secretome induced an M1 response (n=6), based on NO production. However, no LPS-counteracting effect could be observed. No effect of DPSC-secretome components on Arg-1 and CD206 expression, both M2 markers, could be observed using qPCR. Remarkably, BV-2 cells significantly upregulated TREM2 expression following exposure to DPSC-EVs, but not when exposed to other stimulants (n=5, p-value < 0.01).

CONCLUSIONS: Exposure of BV-2 cells to DPSC-derived stimuli did not enhance their M2 phenotype. Interestingly, as TREM2 in stroke is associated with increased phagocytosis, the influence of DPSC-EV stimulation on the phagocytic capacity of BV-2 cells will be evaluated in future experiments.

ACKNOWLEDGEMENTS: Financial support was received from FWO Flanders grants 12U7718N, G029112N, 1134717N, 1508015N and G026112N. BOF grants BOF16DOC06 and BOF16DOCNA02. And FSR-confin UHasselt-UNamur.



A new role for the WNK1 kinase in corneal wound healing

P. Desjardins[1, 2], C. Couture[1, 2], L. Germain[1, 2], S. Guérin[1]
Presenting author: Pascale Desjardins, pascale.desjardins.1@ulaval.ca
[1]Département d'ophtalmologie, Université Laval, CUO-recherche/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada [2]Département de chirurgie, Université Laval, Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada

INTRODUCTION: Damage to the corneal epithelium triggers important changes in the composition of the extracellular matrix (ECM) to which the basal human corneal epithelial cells (hCECs) attach. These changes are perceived by integrins, a family of trans-membrane receptors that activate different intracellular signalling pathways, ultimately leading to re-epithelialization of the injured epithelium [1]. Our goal is to study the impact of the pharmacological inhibition/activation of specific signal transduction mediators on corneal wound healing using both monolayers of hCECs and human tissue-engineered corneas (hTECs) as in vitro models.

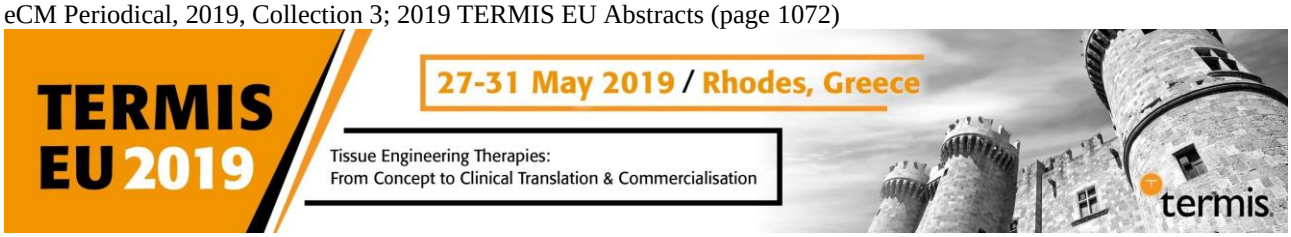
METHODS: hTECs were produced by the self-assembly approach and wounded with a 8-mm biopsy punch whereas hCECs grown as monolayers were wounded using scratch assays (n=4). Total RNA and proteins were isolated from the wounded and unwounded tissues to conduct gene profiling analyses and protein kinase arrays. The wounded tissues were then incubated with the WNK1 inhibitor WNK463, the WNK1 agonist AM1241 [2], or with the vehicle alone (DMSO; negative control) and wound healing was monitored for up to 6 days (n=4).

RESULTS & DISCUSSION: Gene profiling analyses and protein kinases arrays revealed that expression and activity of several mediators from the integrin-dependent signalling pathways were altered in response to the ECM changes taking place during corneal wound healing. Phosphorylation of the WNK1 kinase turned out to be the most striking activation event occurring during wound healing. The pharmacological inhibition of WNK1 by WNK463 significantly reduced the rate of corneal wound closure in our hTECs ($p=1.93E-07$) and hCECs monolayers ($p=2.58E-05$) compared to their respective negative controls. It also reduced phosphorylation of the WNK1 downstream targets SPAK/OSR1 in wounded hTECs. Based on these results, we believe that the pharmacological activation of WNK1 could turn out to be an interesting avenue in order to speed up corneal wound closure, thereby reducing the risks of infection and their associated complications.

CONCLUSIONS: These results will contribute to a better understanding of the cellular and molecular mechanisms involved in corneal wound healing. Furthermore, they also identified a new function for the WNK1 kinase in corneal wound healing and might lead to the identification of a new therapeutic target in the field of corneal wounds.

REFERENCES

[1] Agrawal, V. B., & Tsai, R. J. (2003). 51(1), 5-15.
[2] Buchheim, J. I. et al. (2018) Int Immunol, 30(2), 79-89



The effect of simulated microgravity on 3D Bioprinted vascular construct

L. Somasekhar[1], P. Kharel[1], K. Nunes[1], P. Gatenholm[2,3], U. Reidt[4], C. Bruderrek[5], K. Mitra[1]
Presenting Author: P. Gatenholm, paul.gatenholm@chalmers.se
[1]Florida Institute of Technology, USA [2]Chalmers University of Technology, Sweden [3]CELLHEAL AS, Norway, [4]AIRBUS, Germany [5]kiwi, Germany

INTRODUCTION: 3D Bioprinting technology has already made tremendous impact in the field of tissue engineering with bioprinted complex functional tissues with multiple cells. The translation of this technology to clinic requires however vascularization. Space programs in USA and EU have selected 3D Bioprinting as a future technology for "repair" of astronauts on long flights which are scheduled for example to Mars [1]. Microgravity and irradiation are two factors which might have big influence on success of 3D Bioprinting in Space [2]. Endothelial cells (ECs) which are major component of vascular system are for example highly sensitive to gravitational changes [3]. This collaborative study aims to investigate the effect of simulated microgravity on model 3D Bioprinted vascular tissue.

METHODS: Human Umbilical Vein Endothelial cells (HUVEC) from Promocell (cell density 8.3 x 10^6 cells /ml) were mixed with universal bioink from CELLINK supplemented with fibrinogen at 5M cells/ml and then 3D bioprinted using a custom build extrusion based 3D bioprinter. Typical size of the bioprinted tissue constructs were 13.7 mm x 24 mm x 4 mm.

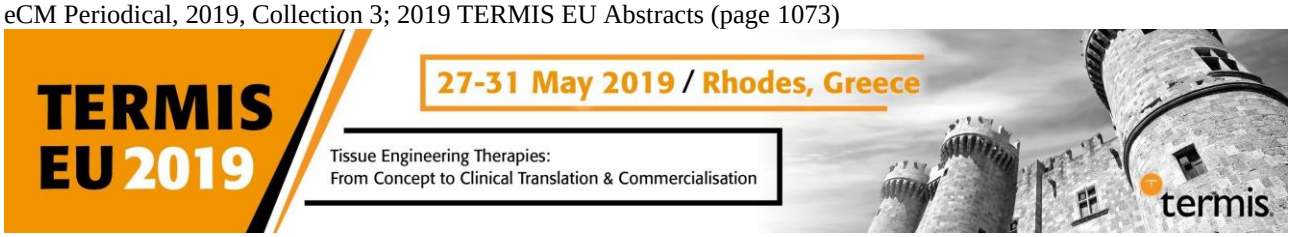
RESULTS & DISCUSSION: Constructs were printed with high fidelity. They were fitted in bioreactor from kiwi, AIRBUS and then exposed to microgravity simulator Gravite system at NASA KSC. Randomness is achieved when the rotational angle differs between the two axes and changes over time. There is a significant decrease in fluorescence intensity for nitric oxide (NO) from bioprinted samples kept in the microgravity simulator for 24 hours compared to control, suggesting vascular relaxation impairment. Results clearly demonstrated that the bioprinting process is not affecting the cellular production and consumption of NO, which supports the suitability of our model for researching pathophysiological imbalances in space.

CONCLUSIONS: This study demonstrates that 3D Bioprinting is suitable for biofabrication of viable large vascular constructs and simulated gravity had measurable effect on functionality.

ACKNOWLEDGEMENTS: The authors acknowledge NASA at KST, CELLINK AB, Sweden and AIRBUS for experimental support.

REFERENCES

[1] Ghidini T, J Thorac Dis 2018;10 (Suppl 20):S2363-S2375
[2] Ulbrich C et al. Journal of Cellular Biochemistry 104:1324–1341 (2008)
[3] Sangha et al., J Appl Physiol 91:789–796 (2001)



3D Bioprinting of autologous adipose tissue for wound healing

A. Back[1], A. Norberg[1], L. Orrhult Strid[1], N. Katz[2], P. Gatenholm[1,3]
Presenting Author: Paul Gatenholm, paul.gatenholm@chalmers.se
[1]Chalmers University of Technology, Sweden [2]JoinTechLab, USA [3]CELLHEAL

INTRODUCTION: Critical skin wounds are a major cost for the health sector and lead to immense suffering for the patients [1]. One way to facilitate healing of these wounds is through autologous adipose tissue transplants [2]. However, the implanted tissue needs to be vascularized or it will suffer from necrosis. To combat this, stromal vascular fraction (SVF), isolated from fat, could possibly be utilized. SVF has been shown to promote both vascularization and the wound healing process. In this project we evaluate a method which combines SVF with 3D Bioprinting to create thick fat grafts for in situ vascularization [3].

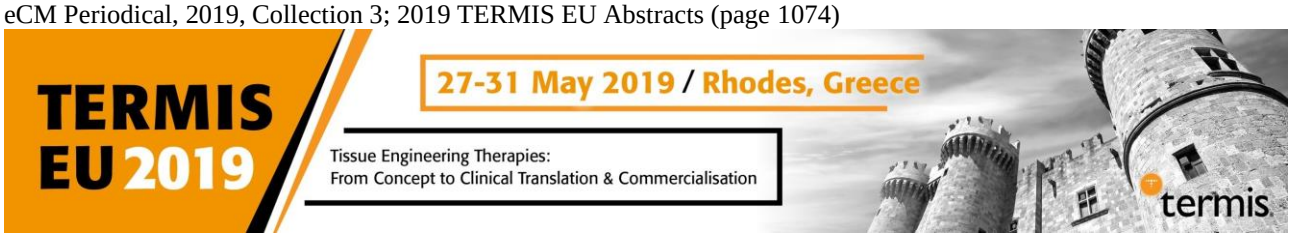
METHODS: Lipoaspirate was taken with consent from patients undergoing plastic surgery and adipose tissue was isolated with MiniStem devices, from JoinTechLabs, USA). Two MiniStem protocols were run: (i) fat was fractured and collected (ii) fat was enzymatically treated with collagenase. After the enzymatic treatment the SVF was collected and analyzed with FACS. Both fractions were mixed with a hydrogel composed of nanocellulose and alginate [4]. The resulting cell laden hydrogels (bioinks) were 3D bioprinted as gridded constructs (3D Bioprinter INKREDIBLE+, CELLINK, Sweden). The properties of the bioinks and final constructs were evaluated from a rheological perspective using a rheometer and by printing constructs with various geometries.

RESULTS & DISCUSSION: FACS of the extracted SVF fractions showed the presence of adipose derived stem cells, pericytes, endothelial cells, and fibroblasts. Rheology testing revealed that the bioinks displayed shear thinning properties. The printing experiments showed that the mixed hydrogels were homogenous and printable. The printing fidelity was improved by adding more nanocellulose and alginate.

CONCLUSIONS: We have shown that we can isolate both fat and SVF from the same liposuction procedure. These results also show that fat and SVF isolated with the MiniStem and mixed with biopolymers contains relevant cells and possesses good printability.

REFERENCES

[1] Pazyar, N., et al. Skin Pharmacology and Physiology, 2014. **27**(6):p.303-310.
[2] Oberbauer, E., et al. Cell regeneration (London, England), 2015. 4: p. 7-7.
[3] Piras, C.C. et al. Biomaterials Science, 2017. 5(10): p. 1988-1992.
[4] Markstedt, K., Mantas, A., Tournier, I., Martínez Ávila, H., Hägg, D., & Gatenholm, P. Biomacromolecules, 2015. 16(5), 1489–1496.



Auxetic and composite scaffolds show potential for use in tissue engineering

P. Mardling[1], P. Godbole[2], N. Jordan-Mahy[1], A. Alderson[3], C. Sammon[3], C. L. Le Maitre[1].
Presenting Author: Paul Mardling, p.mardling@shu.ac.uk
[1]Biomolecular Sciences Research Centre, Sheffield Hallam University, United Kingdom [2]Sheffield Children's Hospital NHS Foundation Trust and Pioneer Healthcare, Sheffield, United Kingdom [3]Materials and Engineering Research Institute, Sheffield Hallam University, United Kingdom

INTRODUCTION: Some natural biological tissues display auxetic properties where upon stretching uniaxially they also expand laterally, becoming thicker which corresponds to a negative Poisson's ratio. A porous auxetic scaffold has the potential to mimic the properties of natural tissue. Here we investigate the mechanical properties of fabricated auxetic and composite scaffolds, and biological tissue using 3D digital image correlation (DIC).

METHODS: Unconverted scaffolds were fabricated from polyurethane using a previously developed technique using thermomechanical triaxial compression (Figure 1). Composite scaffolds were made using converted scaffolds which were filled with a thermoresponsive pNIPAM-Laponite ® hydrogel ($1C_{10}$) (HG). The mechanical properties of the scaffolds were assessed by uniaxial tensile testing on an Instron 3367 and 3D DIC (LaVision Strainmaster). Biological tissue samples were also tested. Smooth muscle cells were suspended in HG at $4x10^6$ cells/ml at 37ºC. The hydrogel was absorbed into the scaffold and set by lowering the temperature below 32ºC. Scaffolds were cultured under standard conditions for up to 6 weeks.

RESULTS & DISCUSSION: Auxetic scaffolds (converted) were successfully fabricated demonstrated by the gaining of a negative Poisson's ratio when compared to the unconverted control. Composite scaffolds were not auxetic. Smooth muscle cells cultured within the composite scaffold were suspended in the pores, supported by the hydrogel. Biological tissue samples showed comparable strain distributions to that of the composite scaffold.

CONCLUSIONS: 3D DIC has shown that the composite scaffold maintains a similar Poisson's ratio and strain values to that of the biological sample over the tested strain range. Therefore this scaffold could be used to recapitulate the natural physical environment of cells from this tissue when under load. Histology revealed smooth muscle cells dispersed throughout the entirety of composite scaffolds when cultured for 6 weeks

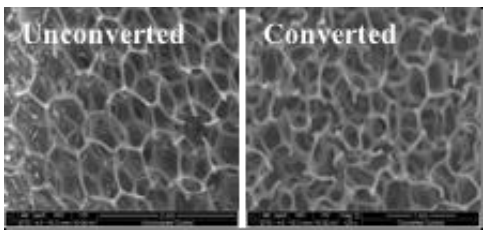
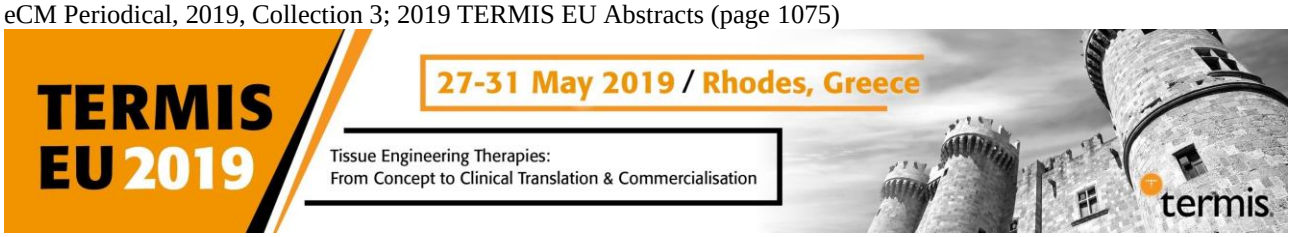


Figure 1: SEM of unconverted and converted polyurethane scaffolds, demonstrating the re-entrant structure of the scaffold after conversion.

ACKNOWLEDGEMENTS: This work was supported by a grant from Sheffield Hallam University, Pioneer Healthcare and Sheffield Children's NHS Foundation Trust



Tecoflex-based electrospun vascular grafts: in vitro and in vivo study

V.S. Chernonosova[1,2], A.A. Gostev[1], I.S. Murashov[1], A.A. Karpenko[1], A.M. Karaskov[1], P.P. Laktionov[1,2]
Presenting Author: Pavel Laktionov, lakt@niboch.nsc.ru
[1]Meshalkin National Medical Research Center, Ministry of Health of the Russian Federation, [2]Institute of Chemical Biology and Fundamental Medicine, SB RAS, Novosibirsk, Russia

INTRODUCTION: Small diameter vascular grafts (VGs) require improved short- and long-term resistance to stenosis [1]. In this study we have investigated VGs produced from blends of polyurethane Tecoflex (PU-Tec) with gelatin (Gl) and bivalirudin (Bv) by electrospinning.

METHODS: Tubular VGs (2 mm diameter, 100 ± 20 um wall thickness) were produced from solution of 3% PU-Tec (w/v %) with 15% Gl and 1.5% Bv (w/w % to PU) in HFIP using the NF-103 electrospinning system. Scaffolds (~150 um) produced from the full composition and scaffolds with only one of the additives were used as controls. VGs were cross-linked by glutaraldehyde as described [2]. Platelet adhesion on the surface of VGs after contact with the platelet-rich blood plasma was investigated by SEM on JSM-6460 LV (Jeol). Cell viability and proliferation of HUVEC on the surface matrices were evaluated after 2 and 4 days of cell seeding using Alamar Blue® reagent. HUVEC cultivated on culture plastic were used as control. VGs (13-15 mm) were implanted in infrarenal position of rats abdominal aorta, their patency was studied by ultrasound tomography, followed by post explantational histological and immunohistochemical examination (1, 12 and 24 weeks). Expanded-PTFE grafts (2 mm diameter, Ecoflon) were used as control.

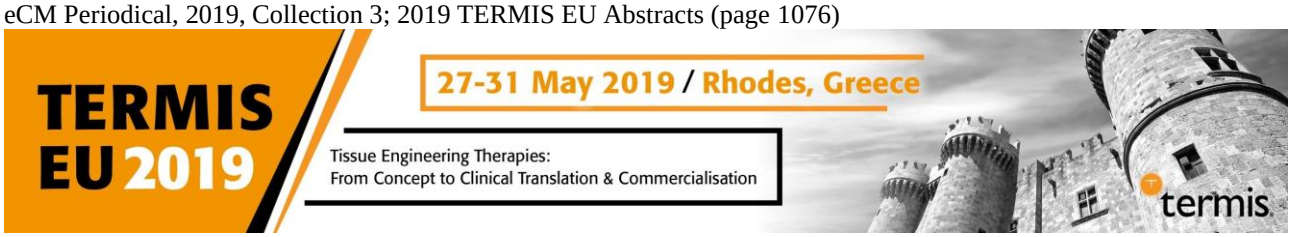
RESULTS & DISCUSSION: SEM demonstrated that presence of Bv in the electrospinning solution and cross-linking of the proteins by glutaraldehyde decreased platelet adhesion to the surface of VGs. HUVEC cells preferentially adhered to and proliferated on VGs containing both Gl and Bv as compared with VGs that contained only one of the additives. Treatment of VGs with glutaraldehyde further promoted proliferation of HUVEC cells on the scaffolds as seen on the 2 and 4 day of incubation as compared with the untreated VGs of the same composition. Data showed that VGs from PU-Tec+Gl+Bv demonstrate 94.5% patency with no signs of aneurysmal dilatation in a long-term study (e-PTFE VGs demonstrate only 66.6%). Intimal hyperplasia of PU-Tec-based VGs was lower than in e-PTFE VGs (31 and 59%, respectively). Histological examination demonstrated that after 3 and 6 months both types VGs were covered with a neointimal layer, which corresponded to the normal structure of the intima of blood vessels. Immunohistochemical study showed weak expression of collagen 4 and factor VIII in the newly formed intimal layer on the surface of electrospun VGs, 3 and 6 months later expression of these markers were detected in both types of VGs.

CONCLUSIONS: Tecoflex-based electrospun VGs with Gl and Bv showed higher patency in a short and long-term perspective as compared to e-PTFE VGs with less pronounced intimal hyperplasia and better ingrowth of host cells.

ACKNOWLEDGEMENTS: The study was supported by a grant from the Russian Science Foundation №17-75-30009.

REFERENCES

[1] Pashneh-Tala S et al. Tis Eng Part B Rev. 2016; 22(1): 68–100
[2] Chernonosova V et al. Biomed Res Int. 2018:1380606



Diclofenac and paclitaxel release from electrospun 3D matrices

K. Kuznetsov [1,2], A. Stepanova [1,2], R. Kvon [3], N. Kuznetsov [1], V. Chernonosova [1,2], M. Kharkova [1], I. Romanova [1], A. Karpenko [2], P. Laktionov [1,2]
Presenting Author: Pavel Laktionov, lakt@niboch.nsc.ru
[1]Institute of Chemical Biology and Fundamental Medicine, SB RAS [2]Meshalkin National Medical Research Center, Ministry of Health RF [3]Boreskov Institute of Catalysis, SB RAS; Russia

INTRODUCTION: Drug-eluting 3D matrices allow controlling the state of surrounding cells [1], however to establish delicate management of drug effects a strict control of release kinetics in required. Herein, we studied drug release from 3D matrices produced by electrospinning (ES) and destined for covering of vascular stents.

METHODS: Tritium-labeled paclitaxel (^{3}H-PTX) and diclofenac (^{3}H-DF) were synthesized by thermoactivated tritium exchange. Matrices were obtained from solutions in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) containing PCL as basic polymer, PTX or DF as drug, human serum albumin (HSA) and DMSO as additives. Electrospun matrices (150–180 μm) were characterized by tensile strength, SEM, XPS, contact angle, water absorption, weight loss etc. Drug release was evaluated by radioactivity of supernatants after incubation of matrices for different time intervals (up to 27 days) in PBS or in human blood plasma (BP). To mimic medium exchange at each time point the supernatant was removed from the matrices and the incubation was continued in fresh PBS or BP until the next time point, when the same procedure was repeated. Radioactivity was measured by β-counting in "ULTIMA GOLD LTT" scintillator.

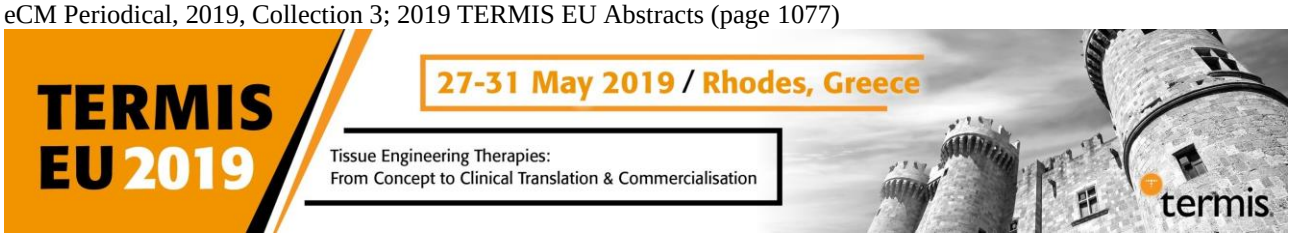
RESULTS & DISCUSSION: Preparations of ^{3}H-DF and ^{3}H-PTX were obtained with a specific radioactivity of ~0.9 and ~0.3 Ci/mM, correspondingly. Radiolabeled drugs were combined with unlabeled to reach doses ~89 and ~0.46 μg/cm^2 of DF and PTX. All matrices consisted of fibers varying in diameter from 0.13 to 0.56 μm with a tensile strength similar to previously published data and a hydrophobic surface (except from matrices with HSA). Release of DF and PTX in PBS was very close to previously published data, suggesting diffusion dependent release. Introduction of HSA in ES solution increased the DF release from 20 to 75% in PBS and from 50 to almost 80% in BP. Incubation of the matrices with BP increased the efficacy of drug release presumably by binding of the drug with serum biomolecules. Similar character of drug release was observed for PTX. Exchange of PBS or serum after every time point increased DF and PTX release; in BP drugs were fully eluted within 3-9 days. It should be noted that SEM did not demonstrate any changes in the matrix structure in these time intervals. So far as XPS demonstrated overrepresentation of PTX in the surface layer of the fibers, DMSO was introduced in the ES solution to facilitate drug deposition in the fibers. It was shown that matrices produced from ES solution with DMSO and HSA released no more than 60% of PTX after 27 days even under medium exchange conditions.

CONCLUSIONS: The data demonstrate the necessity to take into account the medium exchange conditions in living systems as well as properties of drug distribution in the fibers.

ACKNOWLEDGEMENTS: The study was supported by a grant from the Russian Science Foundation no. 18-15-00080.

REFERENCES

[1] Dangas G. et al. J Am Coll Cardiol. 2010; 56: 1897-1907.



Effect of pore microarchitecture in extracellular matrix derived scaffolds on chondrogenesis of bone marrow cells

P.J. Díaz-Payno[1], D.C. Browe[1], D.J. Kelly[1]
Presenting Author: Pedro J. Díaz-Payno, diazpap@tcd.ie
[1]Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

INTRODUCTION: Articular cartilage (AC) has an arcade-like collagen fibre organisation, enabling it to withstand the high loads passing through joints [1]. Despite previous attempts to repair AC defects, recapitulation of the complex tissue architecture remains a clinical challenge. It has been shown that the use of aligned pores improves cell infiltration, differentiation and zonally-organised matrix deposition [2]. The objective of this study is to determine the effect of pore microarchitecture (orientation and size) in AC scaffolds for TE.

METHODS: Porcine AC was pepsin-solubilised and freeze-dried in different conditions to produce scaffolds (Φ5xh3mm) containing random or aligned pores. Pore size was tailored by an annealing step during freeze-drying. Pore morphology was characterised by electron microscopy (SEM). Compression testing (20% ε) was used to characterise stiffness. Scaffolds, seeded with bone marrow cells, were cultured for 28 days in chondro- (CM), osteogenic (OM) or expansion media (EM). Biochemical and histological analysis were used to determined DNA, glycosaminoglycan (alcian blue staining), collagen and calcium levels.

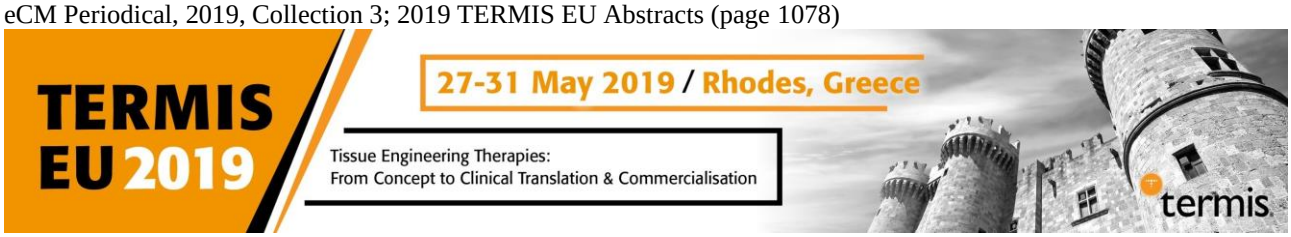
RESULTS & DISCUSSION: Scaffolds with aligned pores showed higher stiffness than random pores. In CM, the highest levels of sGAG and collagen deposition occurred in scaffolds with aligned pores. Conversely, the highest level of calcium deposition occurred in scaffolds with random pores. Increasing pore size did not affect scaffold stiffness but was found to accelerate cellular ingrowth and homogenous tissue deposition.

CONCLUSIONS: The study identified that pore structure can be used to augment the capacity of solubilised AC ECM to support or supress chondrogenesis. Aligned pores enable higher cell/nutrient infiltration which may support condensation prior to chondrogenesis of BMSCs. The higher stiffness associated with the aligned structure did not supress chondrogenesis, but rather enhanced it as evident from higher sGAG and lower mineralization levels, demonstrating its potential for AC tissue engineering.

ACKNOWLEDGEMENTS: IRC.GOIPG/2015/3156, SFI.12/IA/1554 and Enterprise Ireland.

REFERENCES

[1] Jeffery AK et al. J Bone Joint Surg. 1991;73-B(5):795-801.
[2] Petersen A et al. Nat Commun. 2018;9:4430.



Assessing the behavior of steatotic livers under normothermic preservation with a porcine model

G. Pola-Bandrés[1], V.M. Borrego-Estella[1], E. Delgado-Blanco[1], A.G. Gonzales-Sejas[1], J. Martínez-Guillén[1], P. Royo-Dachary[1], F.A. García-Gil[1], V. Alastrué[2], P. Moreo[2]
Presenting Author: Pedro Moreo, pmoreo@ebersmedical.com
[1]HCU Lozano Blesa, Zaragoza, Spain [2]EBERS Medical Technology SL, Zaragoza, Spain

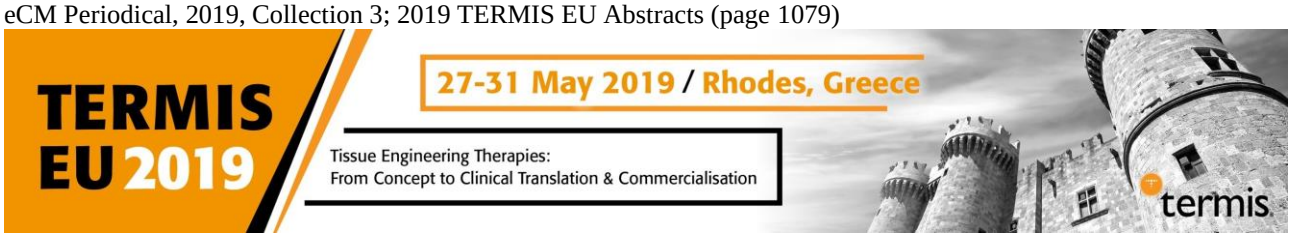
INTRODUCTION: Steatotic livers are more susceptible to ischemia-reperfusion injury than healthy livers, resulting in a higher rate of primary nonfunction, early allograft dysfunction and post-transplant vascular and biliary complications. The goal of our study was to show that normothermic preservation (NP) can minimize preservation-related injury and maintain the organ function and viability.

METHODS: Experimental study with 10 Large-White pigs divided in a control group (healthy livers, n=5) and a steatotic group (animals were subjected to a steatotic diet for 5 weeks, n=5). The existence of pathologic steatosis was confirmed by biopsies performed in the first and second month after the introduction of steatotic diet. Following hepatectomy, livers were connected to the ARK ex vivo NP system developed by EBERS. The ARK system is formed by a portable preservation unit, which features peristaltic and infusion pumps, heating and oxygenation systems, sensors and a control unit; and a disposable closed circuit, where the organ and the perfusate are contained in sterile conditions. The organ was perfused through the portal vein (PV) and hepatic artery (HA) at physiological hemodynamic conditions with a perfusion solution composed by autologous blood of the donor animal, Ringer's Lactate solution, cefotaxime, methylprednisolone, heparin, insulin, a parenteral nutrition solution, epoprosterenol and sodium bicarbonate. Secreted bile was collected for analysis. 0h, 4h and 8h after connection of the organ to the NP system more than 30 variables of different types were monitored and compared: perfusion parameters (PV and HA flow rate and pressure, pH, bile production), biochemistry (AST, ALT, FA, GGT, urea, albumin, total bilirubin, Na+, K+, Cl-, glucose, lipemic/icteric/hemolytic index), histopathology (macro/microsteatosis, fibrosis, portal inflammatory infiltrate, neutrophils infiltrate, isolated hepatic necrosis, central confluent necrosis, subcapsular necrosis, cholestasis) and other (initial/final liver weight, warm ischemia time).

RESULTS & DISCUSSION: Compared to healthy livers, steatotic livers were perfused in an equally satisfactory manner (HA pressure, 95.3±18.7 vs 86.6±9.3 mmHg, p=NS; HS flow rate 15.9±1.5 vs 14.9±4.8 ml/min/100g, p=NS; PV pressure 20.5±5.2 vs 10.8±6.1, $p<0.05$; PV flow rate 54.3±8.7 vs 50.9±12.9, p=NS), did not show worse values of hepatic damage (AST 365.6±165.1 vs 437.2±299.8 UI/l, p=NS; ALT 21.4±8.3 vs 32±7.7 UI/l; p=NS), had a larger production of bile (146.6±77.7 vs 71.7±60.8 g; $p<0.05$) and the histopathological parameters did not show any significant alteration concerning fibrosis, infiltrate, necrosis and cholestasis.

CONCLUSIONS: Collectively, these data illustrate the capacity of the ARK system to preserve healthy and steatotic livers in NP conditions. Steatotic livers maintained a viable hepatic function during NP and their behavior under NP was not inferior to healthy livers. NP can contribute to decrease the number of steatotic livers discarded for transplantation, thus increasing the donor pool.

ACKNOWLEDGEMENTS: Financial support was received from the Spanish Ministry of Economy and Industry (RTC-2016-5108-1), the Spanish Ministry of Energy, Tourism and Digital Agenda (TSI-100700-2015-13) and H2020 SME Instrument Ph.2 (804252).



Assessing the effect of vasodilator in preservation and resuscitation experiments in an ex-vivo normothermic kidney perfusion porcine model

Á. Borque-Fernando[1], Á. Gutiérrez-Dalmau[1], Á. García de Jalón[1], L.M. Esteban[2], V. Alastrué[3], P. Moreo[3]
Presenting Author: Pedro Moreo, pmoreo@ebersmedical.com
[1]IIS Aragón, HU Miguel Servet, Zaragoza, Spain [2]EUPLA, University of Zaragoza, La Almunia, Spain [3]EBERS Medical Technology SL, Zaragoza, Spain

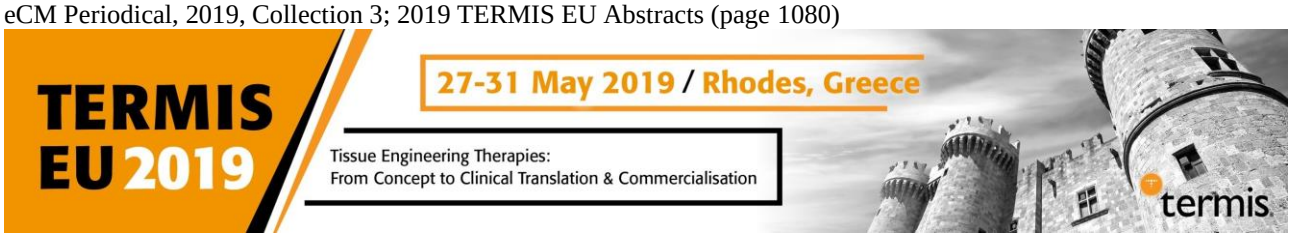
INTRODUCTION: A short period of isolated normothermic perfusion (NP) can be used to improve the condition of the kidney after periods of warm and cold ischemic injury. However, the restoration of the organ function during NP is conditioned by the composition of the perfusate. Herein, we have assessed the influence of the vasodilator selection in preservation and resuscitation experiments.

METHODS: Female pigs were terminated and kidneys were exposed and subjected to 30 min of warm ischemia before being flushed with Ringer's lactate at 4°C. Kidneys were then either directly reperfused for 3 hours on the ARK ex vivo NP system developed by EBERS (preservation) or stored in ice for 24 hours with Custodiol HTK before the 3-hour NP (resuscitation). The ARK system is formed by a portable preservation unit, which features peristaltic and infusion pumps, heating and oxygenation systems, sensors and a control unit; and a disposable closed circuit, where the organ and the perfusate are contained in sterile conditions. Kidneys were perfused with a perfusion solution composed by Ringer lactate, 20% human albumin, red blood cells as oxygen carriers, creatinine and sodium bicarbonate. The perfusate was supplemented continuously with a nutrients solution, insulin and a vasodilator (180 ng/h alprostadil or 0.25 mg/h verapamil). Secreted urine was collected for analysis and the corresponding lost volume of perfusate was replaced in the circuit. Renal flow rate, mean arterial pressure, urine output, temperature, glucose concentration, hematocrit and oxygen saturation were recorded continuously. A blood gas analyzer was used to record parameters for acid–base homeostasis. Serum and urine samples were obtained hourly for biochemical analysis and wedge biopsies were taken after 3 h of reperfusion for histological analysis.

RESULTS & DISCUSSION: The ARK NP system was able to maintain physiological levels of temperature, mean arterial pressure and arterial oxygen saturation in all cases at during the 3-hour NP. Kidneys preserved with alprostadil exhibited stable flow rates during NP, whereas organs preserved with verapamil experienced increasing flow rates in time, which were significantly higher ($p < 0.001$) than those obtained ($p > 0.05$) with alprostadil. No significant difference ($p > 0.05$) was found between the preservation and resuscitation groups. Urine output was increased ($p < 0.001$) in the presence of alprostadil. Tissue damage parameters (GGT, LDH) showed stable parameters during NP perfusion and no significant difference ($p > 0.05$) was found between groups. Histological analysis showed no significant tissue damage in all groups.

CONCLUSIONS: Collectively, these data illustrate the capacity of the ARK system to preserve kidneys in NP conditions. Verapamil led to lower values of intrarenal resistance, which indicates a better renal function.

ACKNOWLEDGEMENTS: Financial support was received from the Spanish Ministry of Economy and Industry (RTC-2016-5108-1), the Spanish Ministry of Energy, Tourism and Digital Agenda (TSI-100700-2015-13) and H2020 SME Instrument Ph.2 (804252).



Cellular self-assembly into 3D microtissues enhances the angiogenic activity and functional neovascularization capacity of human cardiopoietic stem cells

P. Wolint[1*], A. Bopp[1,2*], A. Woloszyk[1,2], Y. Tian[1], O. Evrova[1,3,4], M. Hilbe[5], P. Giovanoli[1,4], M. Calcagni[1,4], S. P. Hoerstrup[2,6], J. Buschmann[1,4**], M. Y. Emmert[2,6**]
Presenting Author: Petra Wolint, petra.wolint@usz.ch
[1]Division of Surgical Research, University Hospital of Zurich, Switzerland [2]Institute for Regenerative Medicine (IREM), University of Zurich, Switzerland [3]Laboratory of Applied Mechanobiology, ETH Zurich, Switzerland [4]Plastic Surgery and Hand Surgery, University Hospital of Zurich, Switzerland [5]Institute of Veterinary Pathology, University of Zurich, Switzerland [6]Wyss Translational Center Zurich, University Zurich & ETH, Switzerland *shared first authorship, **shared last authorship

INTRODUCTION: While cell therapy has been proposed as next-generation therapy to treat the diseased heart, current strategies display only limited clinical efficacy. To improve cellular retention, cellular self-assembly into 3D-microtissues (MTs) prior to transplantation has emerged as an encouraging alternative [1]. Importantly, 3D-MTs have also been reported to enhance the angiogenic activity and neovascularization potential of stem cells. Therefore, here using the chorioallantoic membrane (CAM) assay [2] we comprehensively evaluate the impact of cell format (single cells (SCs) versus 3D-MTs) on the angiogenic potential of human cardiopoietic stem cells [3], a promising second-generation cell type for cardiac repair.

METHODS: A commercially available biodegradable collagen scaffold (Optimaix™, Matricel) was seeded with either 3D-MTs or SCs of human cardiopoietic cells and placed onto the CAM of living chicken embryos. The scaffolds were analyzed for their perfusion capacity in vivo using magnetic resonance imaging (MRI) assessment which was then linked to a longitudinal histomorphometric ex vivo analysis comprising blood vessel density and characteristics such as shape and size.

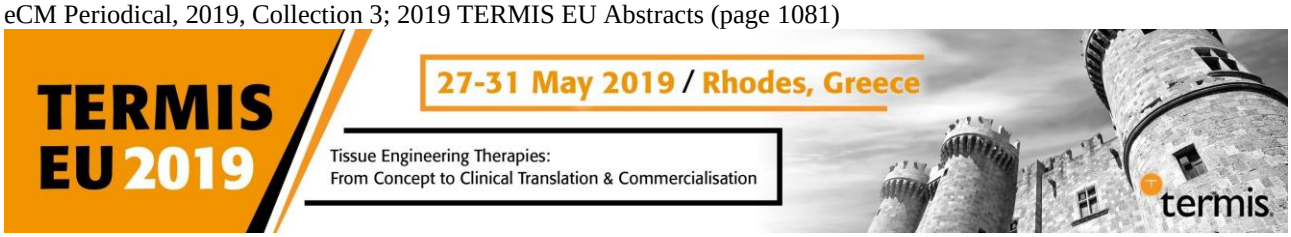
RESULTS & DISCUSSION: Cellular self-assembly into 3D-MTs led to a significant increase ($p < 0.001$) of vessel density mainly driven by a higher number of neo-capillary formation. In contrast, SC-seeded scaffolds displayed a higher frequency of larger neo-vessels resulting in an overall 1.76-fold higher total vessel area (TVA). Importantly, despite that larger TVA in SC-seeded group, the mean perfusion capacity (MPC) was comparable between groups, therefore suggesting functional superiority together with an enhanced perfusion efficacy of the neo-vessels in 3D-MT-seeded scaffolds. This was further underlined by a 1.64-fold higher perfusion ratio when relating MPC to TVA.

CONCLUSIONS: Our study shows that cellular self-assembly of human cardiopoietic stem cells into 3D-MTs substantially enhances their overall angiogenic potential and their functional neovascularization capacity. Hence, the concept of 3D-MTs may be considered to increase the therapeutic efficacy of future cell therapy concepts.

ACKNOWLEDGEMENTS: Financial support was received from Hartmann Müller-Foundation and Swiss Heart Foundation.

REFERENCES

[1] Emmert M. Y. et al. Biomaterials. 2013; 34:6339-54
[2] Kivrak Pfiffner F. et al. Tissue Eng C. 2015; 21:339-46
[3] Behfar A. et al. J Am Coll Cardiol. 2010; 56:721-34



Calcification capacity and angiogenic potential of human mesenchymal stem cell-based 3D microtissues seeded on a collagen Optimaix™ scaffold: Advanced therapy for bone regeneration

P. Wolint[1*], L. Otto[1*], A. Woloszyk[1,2,3], A. Becker[4], A. Boss[4], R. Böni[5], P. Giovanoli[1,6], M. Calcagni[1,6], S. P. Hoerstrup[2,7], M. Y. Emmert[2,7**], J. Buschmann[1,6**]
Presenting Author: Petra Wolint, petra.wolint@usz.ch
[1]Division of Surgical Research, University Hospital of Zurich, Zurich, Switzerland [2]Institute for Regenerative Medicine (IREM), University of Zurich, Zurich, Switzerland [3]Department of Orthopaedics, University of Texas Health Science Center San Antonio, San Antonio, TX, USA [4]Institute for Diagnostic and Interventional Radiology, University Hospital of Zurich, Zurich, Switzerland [5]White House Center for Liposuction, Zurich, Switzerland [6]Plastic Surgery and Hand Surgery, University Hospital of Zurich, Zurich, Switzerland [7]Wyss Translational Center Zurich, University Zurich & ETH Zurich, Zurich, Switzerland *shared first authorship, **shared last authorship

INTRODUCTION: Bone tissue engineering demands for rapidly calcifying scaffolds, for example by adult stem cells differentiating towards the osteoblastic phenotype [1]. Moreover, they should be well vascularized in order to provide sufficient nutrients and oxygen, especially in critical size grafts. Natural bone is mainly composed of calcified collagen where the microenvironment affects stem cells' fate and differentiation. The calcification capacity as well as the vascularization of collagen seeded with stem cells may depend on the cell format the cells are applied.

METHODS: Human adipose-derived stem cells (ASCs) were seeded on commercially available collagen scaffold (Optimaix™, Matricel) to study the calcification. The cells were either seeded as single cells (SCs) or as 3D-microtissues (MTs). Moreover, the scaffold was also soaked with the secretome of 3D-MTs and SCs, respectively, before on-plantation on the chorioallantoic membrane of the chicken embryo (CAM assay) for one week. MicroCT analysis of ex vivo samples was performed to assess micro-calcifications. Histological assessment included Van Kossa staining (extent of calcification), H&E (vascularization), among others.

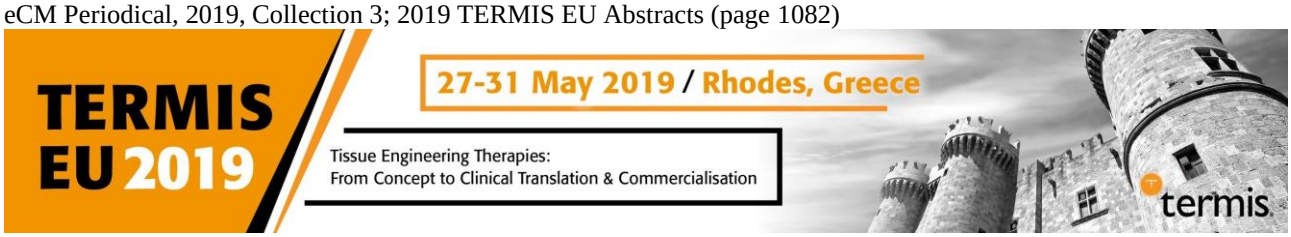
RESULTS & DISCUSSION: There was not only a higher calcification in the case 3D-MTs were seeded onto Optimaix™ scaffolds compared to SCs, but also a higher tissue infiltration from the CAM surface into the collagen scaffold. In addition, vascularization was increased, with more vessels in 3D-MT-seeded scaffolds compared to SC-seeded ones.

CONCLUSIONS: Calcification capacity as well as vascularization are influenced by the cell format the seeded stem cells are applied. Three-dimensional MTs favor calcification and lead to a higher vessel density in collagen scaffolds planted onto the CAM assay. It may be worthwhile to apply stem cells as 3D-MTs in bone tissue engineering to promote two basic and important processes for functionally adequate bone grafts: calcification and vascularization.

ACKNOWLEDGEMENTS: Financial support was received from Hartmann Müller-Foundation.

REFERENCES

[1] Hess S. C. et al. Eur Cell Mater. 2017; 34:232-48



Poly(glycerol sebacate) – Poly(L-Lactide) nonwovens. Towards attractive electrospun material for tissue engineering

P. Denis[1], P. Sajkiewicz[1], M. Wrzecionek[2], A. Gadomska-Gajadhur[2]
Presenting Author: Piotr Denis, pdenis@ippt.pan.pl
[1]Laboratory of Polymers and Biomaterials, Institute of Fundamental Technological Research, Polish Academy of Sciences; Pawińskiego 5B Street, 02-106 Warsaw, Poland
[2]Laboratory of Technological Processes of Faculty of Chemistry of Warsaw University of Technology; Noakowskiego 3 Street, 00-664 Warsaw, Poland

INTRODUCTION: Bicomponent nonwovens consisting of two biodegradable polyesters were electrospun and subjected to investigations.

METHODS: Poly(glycerol sebacate) (PGS) was synthesized using equimolar ratio of glycerol and sebacic acid monomers. Two types of prepolymers were synthesized – one with relatively low degree of esterification (DE) (prepolymeric, Prep), and the other one with higher DE (semi-crosslinked, S-C). Next it was blended with poly(lactic acid) (PLA) in three ratios, and electrospun using hexafluoroisopropanole (HFIP) solvent. Subsequently nonwovens were cured at high temperature (135^{o}C) within 3h - 48h, under vacuum in order to crosslink PGS. Electrospinning process was optimized at preliminary stage. PGS crosslinking conditions were selected on the basis of two meaningful publications about optimizing PGS properties [1, 2]. Materials were characterized and analyzed using various methods: scanning electron microscopy (SEM), differential scanning callorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR).

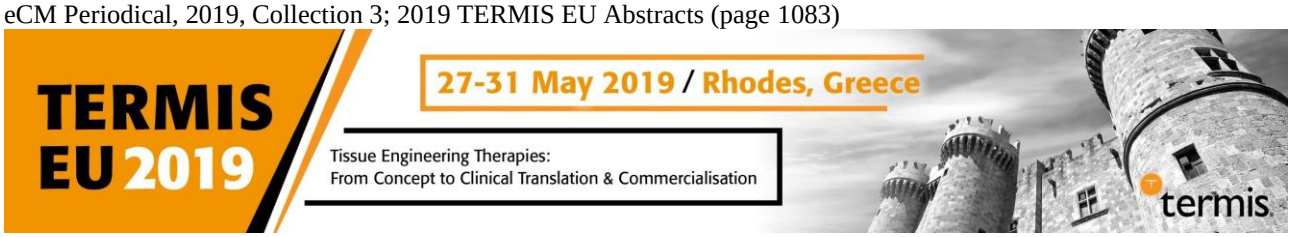
RESULTS & DISCUSSION: Both morphology and structure differ much when comparing samples with various types of PGS, and with various content of PGS within nonwoven. Higher crosslinking degree should preferably enhance PGS elastic properties, however it does not seem to occur in case of bicomponent nonwovens. In addition, nonwovens with various content of PGS are not equally susceptible to crosslinking. The higher content of PGS, the longer time is required to achieve the same crosslinking degree.

CONCLUSIONS: Bicomponent, electrospun nonwovens may be promising but in the case of PLA-PGS system it seems to be difficult to take the full advantage of PGS elasticity.

ACKNOWLEDGEMENTS: That research is supported by Polish National Science Centre (NCN), “Preludium” grant no. 2016/23/N/ST8/03692.

REFERENCES

[1] Li X, T-L Hong A, Naskar N, and Chung H-J, Criteria for quick and consistent synthesis of poly(glycerol sebacate) for tailored mechanical properties, Biomacromolecules, 2015;16 (5):1525–1533
[2] Conejero - Garcia A, Rivero Gimeno H, Moreno Saez Y, Vilarino - Feltrer G, Ortuno - Lizaran I, Valles – Lluch A, Correlating synthesis parameters with physicochemical properties of poly(glycerol sebacate), Eur Polym J, 2017;87:406-419



Development of a natural polymer-based novel tissue engineered breast scaffolds

P. Sawadkar[1], N. Mandakhbayar[2], P. Rajasekar[3], JH. Lee[2], HW. Kim[2], J. Knowles[4], E. García-Gareta[1]
Presenting Author: Prasad Sawadkar, sawadkarp@raft.ac.uk
[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood, UK [2]Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, South Korea [3]School of Medicine, University of Nottingham, UK [4]Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, London, UK

INTRODUCTION: The reconstruction of breast after lumpectomy and radical mastectomy remains a challenge to reconstructive surgeons. Post mastectomy reconstruction of the breast has two major routes, one with synthetic implants and another one with autologous surgical reconstruction but, both strategies bid a substantial overture for reconstruction of the breast (1). However, a tissue engineering approach can serve as a clinical impetus to restore and replace the breast. The key question is whether post-implantation of a tissue-engineered scaffold with/without cells can regenerate the breast.

METHODS: We fabricated 3 different prototypes of breast scaffolds using natural polymers such as collagen, elastin and fibrin as these polymers are an integral part of the human breast's ECM. Scaffolds were fabricated with custom developed methods. Material and material-cell characterisation were performed to understand physical and biological properties of the scaffolds. To study toxicology and biological response of the scaffolds, they were seeded with rat adipose derived stem cells (ADSC) and implanted in 14 SD rats. Output was measured by haematology, microscopy, µCT and immuno-histological analysis.

RESULTS & DISCUSSION: Scaffolds were able to mature over time with increased mechanical properties from day 1 (0.24kPa) to day 28 (9.25 kPa). Expression of adipogenic genes (CEBP and PPARG) had a 7.04-fold increase on day 7. Haematological results indicated that host immune reaction was elevated by day 7 but normalised by day 21. Gross observation showed that scaffolds were able to integrate with the native tissue, vascularise, and presence of the fat depot by day 28 was observed. Histological analysis showed expression of adipose markers PPARG and Caveolin-1.

CONCLUSIONS: developed breast scaffolds had an ideal pore size pattern for angiogenesis, flow of nutrients, cellular migration and microstructure which reflects on dynamic interaction between cells and ECM with integration with the native tissue without eliciting any toxicity. This addresses issues encountered by existing breast scaffolds and implants. Hence, these scaffolds demonstrate translation.

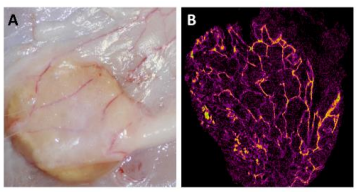
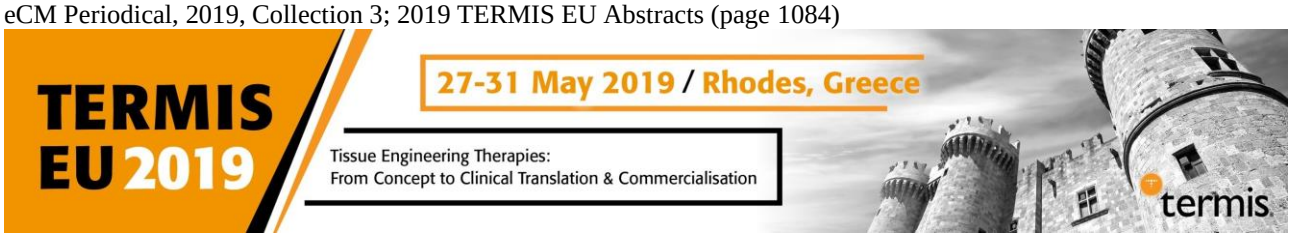


Figure 1: Integration of scaffold with host fat tissue (A), vascularisation and scaffold structure in vivo under µCT (B).

REFERENCES

[1] Yoshimura, K et al. Aesthetic Plastic Surgery, 32**,** 48-55.



A novel scaffold based multicellular model for pancreatic ductal adenocarcinoma – towards a better mimicry of the in vivo niche

P. Gupta[1], P. Campagnolo[2], A. Nisbet[3,4], R. Webb[5], G. Schettino[3,6], E.G. Velliou[1]
Presenting Author: Priyanka Gupta, m10749@surrey.ac.uk
[1]Bioprocess and Biochemical Engineering group, Department of Chemical and Process Engineering, University of Surrey, Guildford UK, [2] School of Biosciences & Medicine, University of Surrey, Guildford, UK [3]Department of Physics, University of Surrey, Guildford, UK [4]Royal Surrey County Hospital, NHS Foundation Trust, Guildford, Surrey, UK [5]Advanced Technology Institute, Ion Beam Centre, Physics, University of Surrey, Guildford, UK [6]National Physical Laboratory, Teddington, London, UK

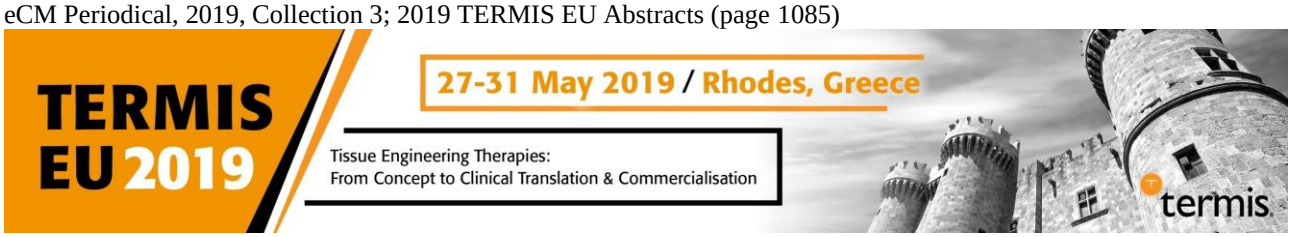
INTRODUCTION: Scaffold based 3D tumour models are considered to have better niche mimicking capability in comparison to 2D systems. This is attributed to their tunable mechanical properties, ability to provide structural integrity along with better cell- cell and cell-ECM interaction. Our lab has previously reported that poly urethane (PU) based scaffolds can be used as a robust 3D structure to develop a pancreatic cancer model using a single cell type. However, the tumour microenvironment is heterogeneous in nature and consists of different cell types along with matrix proteins, all contributing to tumour formation, cancer metastasis as well as its response and resistance to treatment. As a result, recent studies have been focused on the generation of multicellular tumour spheroids; however, no studies have been reported till date for scaffold-based model. The aim of our work is to develop, for the first time, a PU scaffold based, multi cellular, robust 3D pancreatic tumour model using pancreatic cancer, stellate and endothelial cells.

METHODS: PU scaffolds were prepared using Thermal Induced Phase Separation (TIPS) method. Absorption based surface modification of the scaffolds enabled coating with ECM proteins for enhancement of ECM mimicry [2]. Cells were seeded on to the scaffolds at a seeding density of $0.5x10^6$ cells/ scaffold for mono cultures and $0.25x10^6$ cells/scaffold, per cell type for the multi-culture systems. Long term culture was carried out within the scaffolds. Various in situ assays including cell viability, SEM, immunofluorescence assay etc. were carried out at specific time points throughout the culture.

RESULTS & DISCUSSION: Our data show that endothelial cells and stellate cells can proliferate on both coated and uncoated PU scaffolds for 4 weeks. Cell viability analysis also highlighted that endothelial cells preferred ECM protein coated scaffolds in comparison to uncoated ones, similar to the cancer cells. We also show the feasibility of long term co-culturing of pancreatic cancer, stellate and endothelial cells on both uncoated and coated PU scaffolds.

CONCLUSIONS: Our data show, for the first time, the feasibility of PU scaffolds to support a multicellular tumour growth along with the possibility for a robust ECM mimicry. We highlight the development of a robust, long term niche mimicking model system for relatively high throughput, personalized study and treatment of pancreatic cancer.

ACKNOWLEDGEMENTS: Financial support were received from Chemical and Process Engg. Dept., Impact Acceleration Grant (IAA-KN9149C), University of Surrey, IAA–EPSRC Grant (RN0281J) and the Royal Society. P.G is supported by Commonwealth Rutherford Fellowship.



Characteristic of the bacteria population involved in infection of periodontal guided tissue regeneration in dogs

Q.B. Yang, X.N. Sun, R.J. Liu, Y. Liu, Z.L. Zhang
Presenting Author: Qiu-Bo YANG, Email: qiuboyang2003@163.com
Beijing Institute for Dental Research, Capital Medical University School of Stomatology, Beijing, China

INTRODUCTION: Bacterial contamination of expanded polytetrafluoroethylene (ePTFE) membrane may be an indicator of the long-term success or failure of periodontal guided tissue regeneration (GTR) [1-2]. The purpose of this study was to investigate characteristic of the bacteria population involved in infection of periodontal GTR.

METHODS: Class II furcation defects were created in the first and second premolars of 2 beagle dogs. Space-providing ePTFE membranes were implanted to provide for GTR. The ePTFE membranes were retrieved 8 weeks after GTR for detecting the species and population of bacteria on alveolar bone by selective and non-selective culture.

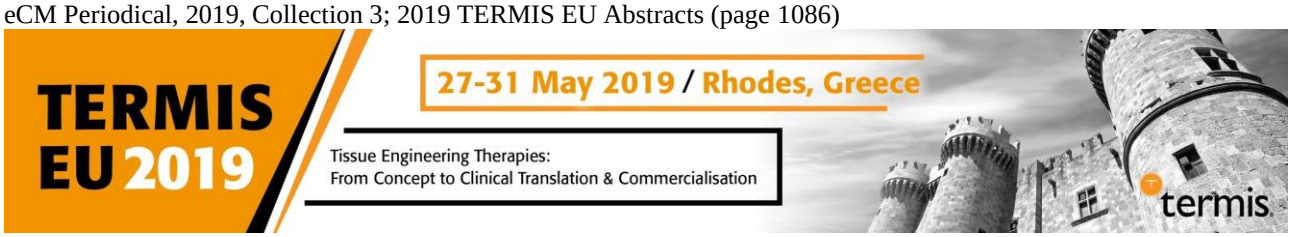
RESULTS & DISCUSSION: Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans, Black-pigmented bacterium, Actinomycete, and Streptococcus mutans were detected from alveolar bone covered with ePTFE membranes or alveolar bone where ePTFE membranes were lost. Alveolar bone covered with the ePTFE membranes showed higher population of Fusobacterium nucleatum and black-pigmented bacterium compared with alveolar bone where the ePTFE membranes were lost.

CONCLUSIONS: Our findings implied that implantation of ePTFE membrane may increase the risk of infection of periodontal pathogens.

ACKNOWLEDGEMENTS: This work was supported by grants from the National NaturalScience Foundation of China (Grant 81470753) and the Beijing Natural Science Foundation (Grant 7182061).

REFERENCES

[1] Selvig KA et al. J Periodontol. 1992;63(12):974-8
[2] Nowzari H et al. J Clin Periodontol. 1994;21(3):203-10



3D-printed, tough and biocompatible photopolymers for in vivo bone tissue regeneration

R. Reichsöllner,[1,5] S. Orman,[1,5] C. Hofstetter,[2,5] M. Schwentenwein,[3,5] G. Russmüller,[4] J. Stampfl,[2,5] R. Liska,[1,5] S. Baudis[1,5]
Presenting Author: Raffael Reichsöllner, raffael.reichsoellner@tuwien.ac.at
[1]Institute of Applied Synthetic Chemistry, TU Wien [2]Institute of Material Science and Technology, TU Wien [3]Lithoz GmbH [4]Department of Oral and Maxillofacial Surgery, Medical University of Vienna [5]Austrian Cluster for Tissue Regeneration, AUstria

INTRODUCTION: Additive manufacturing technologies (AMTs) like digital light processing stereolithography (DLP-SLA) are emerging methods for scaffold preparation in patient-specific bone tissue engineering approaches. These methods are traditionally based on (meth)acrylate resins resulting in highly crosslinked materials, which often suffer from poor mechanical properties. Moreover, (meth)acrylates exhibit considerable cytotoxicity and resulting polymer networks degrade to unfavorable polyacids. We established novel vinyl ester (VE) monomer systems with considerably lower cytotoxicity and polyvinyl alcohol as favorable degradation product. Tailoring of these material systems promises a novel class of DLP-SLA-processable biomaterials with potential applications as scaffolds for in vivo bone tissue regeneration.

METHODS: VE-based systems were explored and compared to (meth)acrylate benchmark systems. Polymer network regulation by established thiol-ene chemistry approaches were employed to increase reactivity and resulting mechanical properties, especially toughness.[1] Further toughness improvement was attained by including newly synthesized VEs with spacers of variable molecular structures [2], and macromolecular reactive and non-reactive resin additives.[3] Monomer cytocompatibility was tested by in vitro tests with murine fibroblasts. Photoreactivity was examined via RT-NIR photorheology. (Thermo-)mechanical properties of the crosslinked materials were determined via dynamic mechanical thermal analysis (DMTA), tensile testing, and impact testing.

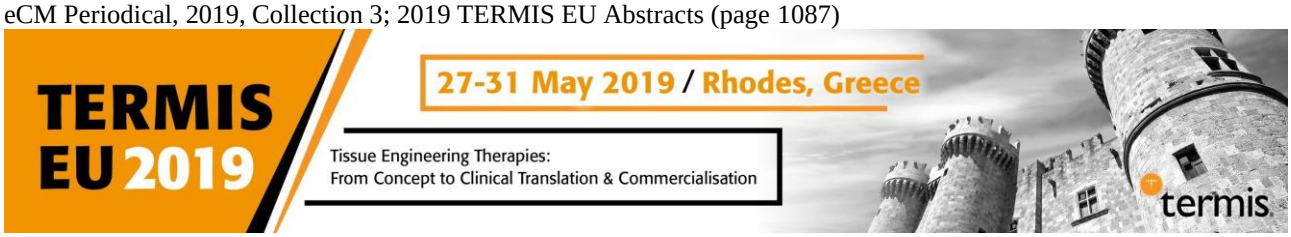
RESULTS & DISCUSSION: Thiol-ene chemistry proved to be a valuable tool to increase resin reactivity and to improve the mechanical performance of the photopolymers due to its inherent network-regulation capabilities. Alternative network-modification strategies further increased the toughness of the materials while still maintaining their 3D printability.

CONCLUSIONS: VE-based resin systems can be tailored towards tough 3D-printable scaffolds as screwable implants for bone tissue regeneration approaches with superior biocompatibility, and biodegradation behavior.

ACKNOWLEDGEMENTS: We would like to thank Synthes and the KLS Martin group for financial support.

REFERENCES

[1] Mautner A et al. J Polym Sci A. 2013; 51:203-12
[2] Mautner A et al. J Polym Sci A. 2016; 54:1987-97
[3] Orman S et al. J Polym Sci A. 2018;



Changes in gene expression of discs from diffuse idiopathic skeletal hyperostosis (DISH) patients compared to traumatic/degenerative discs

R.D. May[1], D.A. Frauchiger[1], C.E. Albers[2] L.M. Benneker[2], W. Hofstetter[1], B. Gantenbein[1,2]
Presenting Author: Rahel May, rahel.may@dbmr.unibe.ch
[1]Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland, [2] Department of Orthopaedic Surgery & Traumatology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

INTRODUCTION: Diffuse idiopathic skeletal hyperostosis (DISH) affects mostly mid-aged and elderly people. DISH is characterized by the formation of bone along the anterior spine. Further ossification of the outer intervertebral discs (IVD) can be observed [1]. However, the nucleus pulposus (NP) remains unaffected. We have recently shown, that the IVD cells are expressing BMP antagonists, which could lead to inhibition of bone formation for spinal fusion. In this study, we investigate the transcriptome of discs of the BMP pathway from DISH patients with traumatic or degenerative discs.

METHODS: Fresh DISH-IVDs and trauma or degenerated IVDs were obtained from patients undergoing spinal surgery (approved by the Ethics Committee of the Canton of Bern, CH). TGFβ BMP signaling pathway genes were compared by qPCR. IVDs of three DISH patients were tested against three control patients (same disc level and similar age). IVD of two donors could be separated in NP, annulus fibrosus (AF) and cartilaginous endplate (CEP), one donor was investigated without discriminated IVD tissue.

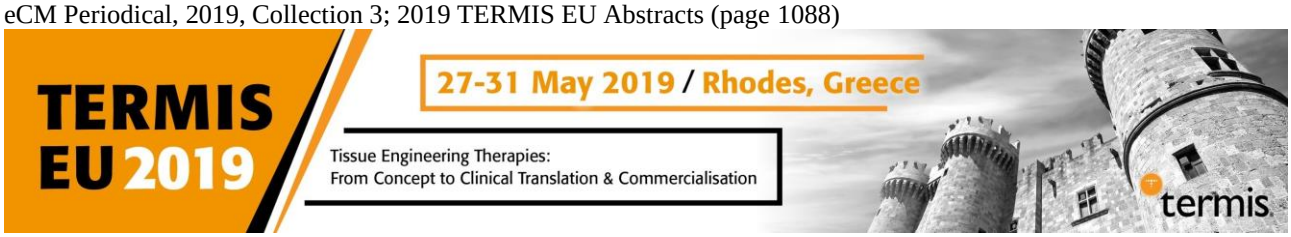
RESULTS & DISCUSSION: In six of the seven comparisons a mean up-regulation of Interleukin 6 (IL-6) was detected (mean ± SEM of all comparisons: 88.8 ± 79.4-fold in DISH-IVD compared to controls). Early Growth Response 2 (EGR2) and Insulin-like Growth Factor 1 (IGF1) were up-regulated in DISH-IVD donors (i.e., 20.5 ± 12.4-fold and 19.0 ± 19.5-fold, respectively). The two Growth and Differentiation Factors 5 and 6 (GDF5 and 6) were down-regulated in two of the three DISH-IVDs (i.e., -21.9 ± 16.2-fold and -8.2 ± 4.2-fold, respectively).

CONCLUSIONS: Most interestingly, the DISH-IVD cells showed a considerable change in IGF1 and IL-6. IGF1 was already determined as a serum marker for rheumatic diseases, such as DISH. These results are unexpected considering the fact that the ossification occurs in the neighboring ligaments and enthesis leaving the inner part of the IVD macroscopically unaffected.

ACKNOWLEDGEMENTS: Financial support was received from direct funds from the Clinical Trial Unit, Inselspital, Bern University Hospital, and by a grant of the Swiss Society of Orthopaedics and Traumatology to C. E. A.

REFERENCES

[1] Resnick D et al. 1978. AM J Roentgenol 131:1049-1053



3D bioprinting of hepatocytes co-culture systems – towards biofabrication of liver models

R. Abdelgaber, A. Lode, M. Gelinsky
Presenting Author: Rania Abdelgaber: rania.abdelgaber@tu-dresden.de
Centre for Translational Bone, Joint & Soft Tissue Res., Technische Universität Dresden, Germany

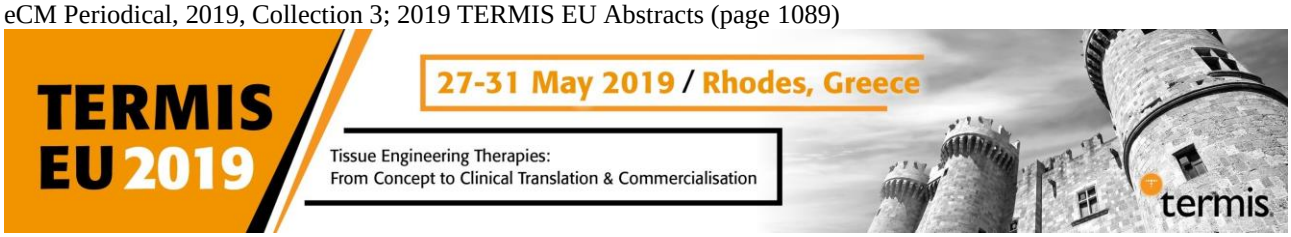
INTRODUCTION: 3D bioprinting has emerged as a tool to develop biomimetic constructs that resemble the native microenvironment of tissues. Thus, the behavior and function of printed cells in a 3D environment can be improved compared to in vitro 2D cultures. Aim of this study is to design a liver model mimicking in vivo microenvironment by fabrication of constructs based on cell laden hydrogels and to further characterize their biological response in terms of viability and liver specific functions. Core/shell 3D bioprinting has been utilized for the fabrication of tissue constructs with spatial compartmental distinction between core and shell phases with encapsulation of different cell types in each phase. In an attempt to further mimic in vivo hepatic microenvironment, hepatocytes were co-cultured with supporting fibroblasts in a core/shell fashion were the two cell types are in close proximity. Such co-cultures are known to maintain hepatocyte viability and enhance their functionality.

METHODS: For the fabrication of core/shell scaffolds, BioScaffolder 3.1 (GeSiM mbH, Radeberg, Germany) was used. Human HepG2 hepatocyte-like cells were encapsulated and processed in 3% HP-LVM alginate/9% methyl cellulose/20% Matrigel (alg-MC-matrigel) hydrogels. After plotting, scaffolds were stabilized via Ca^{2+}-mediated crosslinking and were incubated in (DMEM) supplemented with FCS, penicillin and streptomycin for up to 4 weeks Those HepG2 containing constructs were analyzed for cell viability by live-dead staining (calcein AM/ethidium homodimer-1), for cytoskeletal morphology via confocal laser scanning fluorescence microscopy, and concerning liver specific functionality, albumin secretion and E-cadherin expression were investigated. To characterize the influence of fibroblasts co-culture on the functionality of hepatocytes, NIH-3T3 fibroblasts were encapsulated in the core with HepG2 cells encapsulated in the shell. 3D printed scaffolds were crosslinked and cultured in DMEM for 4 weeks. Viability of both cell types and functionality of the hepatocyte-like cells were assessed.

RESULTS & DISCUSSION: Using the alg-MC-matrigel bioink, volumetric 3D printed scaffolds were successfully fabricated. HepG2 cells showed survival rates of around 60% after 24 h of incubation in medium. In comparison to 2D cultures of HepG2, cells encapsulated in 3D printed constructs showed enhanced functionality concerning albumin secretion. In 2D cultures HepG2 which are epithelial-like in shape tend to grow in clusters. In case of the bioprinted constructs, cells started to grow in aggregates/clusters at day 7 especially around macropores of the scaffolds where oxygen and nutrients are abundant. These clusters where spheroidal in shape and exhibited a 3D structure adapted to the geometry of the scaffold. HepG2 cells printed in core/shell fashion with fibroblasts as supporting cells in the core showed enhanced albumin secretion & cadherin expression.

CONCLUSIONS: 3D bioprinting is a promising approach to fabricate liver models which mimic the functional characteristics better than 2D in vitro cell cultures. Functionality of hepatocyte-like cells was further improved by co-culture of hepatocytes with fibroblasts using core/shell bioprinting technology. Co-culturing hepatocytes with non-parenchymal cells (e.g. fibroblasts) maintained hepatocyte viability and function, whereas hepatocytes cultured alone lost their characteristics.

ACKNOWLEDGEMENTS: European Social Fund (ESF) and the Free State of Saxony.



3-D structures with tunable porosity for in vitro anisotropic neural models

R.F. Canadas[1,2,4], T. Ren[4], A. Tocchio[4], A.P. Marques[1,2,3], J.M. Oliveira[1,2,3], R.L. Reis[1,2,3*], U. Demirci[4,5]
Presenting Author: Raphaël F. Canadas, raphael.canadas@i3bs.uminho.pt
[1]3B's Research Group – Biomaterials, Biodegradable and Biomimetics, Headquarters of the European Institute of Excellence on tissue Engineering and Regenerative Medicine, AvePark, 4805-17 Barco, Guimarães, Portugal [2]ICVS/3B's- PT Government Associate Laboratory, University of Minho, Guimarães-Braga, Portugal [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Guimarães, Portugal [4]Bio-Acoustic MEMS in Medicine Laboratory, Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Palo Alto, California 94304, USA; [5]Electrical Engineering Department by courtesy, Stanford University, Stanford, California 94305, USA

INTRODUCTION: Organized networks are common in nature, where cells can be found isotropically or anisotropically distributed [1]. While progress was achieved organizing cells in two-dimension (2-D), reported fabrication techniques for aligned networks in 3-D are limited [2]. Here, we describe the use of a biomimetic extra-cellular matrix system to engineer anisotropic in vitro neural models.

METHODS: Polymeric blends of methacrylated gelatin and gellan gum were varied. Crosslinking and freezing were used as a mechanism to tune pore orientation, pore size and mechanical properties. 3-D neural constructs were developed culturing primary neurons isolated from embryonic mouse cortex. Heterotypic cultures with endothelial cells were performed in a custom microfluidic device.

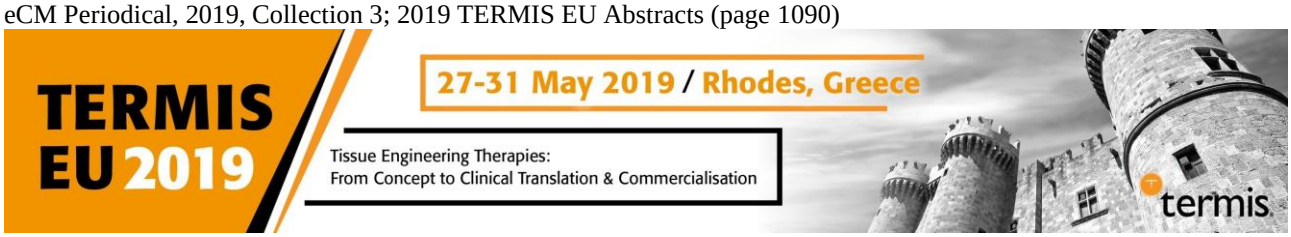
RESULTS & DISCUSSION: The degradation rate and mechanical properties were controlled by adjusting the ratio of the polymeric blend, while the 3-D anisotropic structure and its pore size were tuned by varying the temperature of the freezing step and guiding the crosslinking. The top-down fabricated oriented porous structures in a single step process resulted in pore sizes ranging from 18μm to 376μm overcoming the resolution of similar techniques and ranging the size of human neocortex columns. As a model system, neurons were injected in the 3-D oriented porous systems creating 3-D neural anisotropic constructs at a large millimeter scale, which has not been achieved before. Further, we also aligned primary neurons interfaced with endothelial cells under dynamic conditions controlled by a microfluidic platform designed for co-cultures as an organ-on-chip type model.

CONCLUSIONS: The presented system can potentially be used to model several tissues in vitro, especially anisotropic neural tissues characterized by aligned neurites outgrowing from primary neurons. Degradation and mechanical properties were tuned by material engineering techniques with a meaningful neuronal organization. Degenerative conditions in the brain result in degradation, regional stiffness changes, altered neuron networks morphology and ultimately neuronal function loss as observed during aging, and degenerative disorders.

ACKNOWLEDGEMENTS: Funding from FLAD and the FCT grant SFRH/BD/92565/2013.

REFERENCES

[1] Griffith LG & Swartz MA. Nat Rev Mol Cell Biol. 2006; 7:211-224
[2] Bouyer C et al, Adv Mater. 2016; 28:161-167



Surface characterization of polymeric nanostructurated scaffolds for periodontal regeneration

R. Osorio[1], M. Toledano-Osorio[1], E. Osorio[1], A.L. Medina-Castillo[2], M. Toledano[1]
Presenting Author: Raquel Osorio, rosorio@ugr.es
[1]Department of Stomatology, Dental School, University of Granada, Spain [2]NanoMyP. Spin-Off Enterprise from University of Granada, Spain

INTRODUCTION: Guided tissue regeneration in periodontal disease remains clinically unpredictable. A major limitation of employed resorbable materials is the inability to exert spatiotemporal control over the wound-healing process. Employed resorbable membranes show a fast rate of biodegradation. Degradation products also alter remineralization. Novel polymeric membranes were designed and created by electrospining. Functionalization of the polymeric membranes through the incorporation of bioactive components (calcium, zinc, silicon) was performed to enhance their osteoconductive properties [1]. Doxycycline was also doped on tissues to account for antibacterial properties. The aim of this study was the surface characterization of novel matrices by means of bioactivity, nanomechanical properties and topography.

METHODS: Polymeric matrices were created and loaded with silicon, zinc, calcium and doxycycline. Doping efficacy was probed. To probe for membranes' bioactivity, the method proposed by Kokubo (ISO 23317:2012). Nanomechanical properties mappings were conducted using a Hysitron Ti Premier nanoindenter equipped with nano-DMA III (quasistatic force setpoint F_q=2 µN, sinusoidal force of amplitude F_A=0.10 µN and frequency f=200 Hz). Atomic Force microscopy was employed for topography analysis.

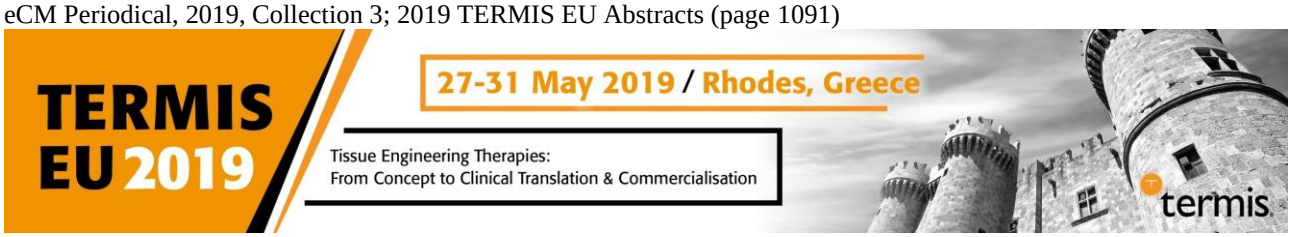
RESULTS & DISCUSSION: Bioactivity of tissues was demonstrated in all doped tissues, but it was drastically increased in the presence of silicon. Calcium and phosphorous nanocrystals were encountered at the EDX spectra on nanofibers surfaces. SiO_2 tissues doped with Zn or Dox attained the highest amount of calcium/phosphate deposits. Mechanical storage moduli of ion-doped tissues were within the range 10 to 15 GPa. Nanofibers were randomly distributed and their diameter was around 300 nm.

CONCLUSIONS: Silicon doping and zinc complexation on tissues facilitated phosphate groups binding and calcium attraction from media. Doxycycline is known to form chelates with metallic ions such as calcium ions. Mechanical storage moduli of tissues are similar to that of calcified trabecular bone (15 GPa) [2], values that highly differ from the storage modulus calculated for cross-linked collagen scaffolds (1GPa). This is important, as substrate stiffness can modify cell behavior, and cells may probe and respond to mechanics in fibrillar matrices [3]. Nanofibers diameter is similar to that of mineralized collagen fibers on trabecular bone. It may be concluded that silicon modified tissues doped with zinc or doxycycline are candidates to accomplish guided tissue regeneration in periodontal patients. In vivo research is needed to confirm the effectiveness.

ACKNOWLEDGEMENTS: Financial support was received from MINECO/AEI/FEDER/UE. Project MAT-2017-85999-P.

REFERENCES

[1] Osorio R et al. Clin Oral Invest. 2017; 21(9):2695-2707
[2] Polly BJ et al. Calcif Tiss Int. 2012; 90:286-293
[3] Baker BM et al. Nat Mater. 2015; 14:1262-1268



Hyaluronan photocrosslinked hydrogels for improved osteochondral repair: a 8 weeks pre-clinical study on a mini-pig model

R. Beninatto[1], L. Gao[2], C. Barbera[1], M. Pavan[1], M. Cucchiarini[2], D. Galesso[1], H. Madry[2]
Presenting Author: Riccardo Beninatto, rbeninatto@fidiapharma.it
[1]Fidia Farmaceutici S.p.A., 35031, Abano Terme, Italy [2]Center of Experimental Orthopaedics, Saarland University Medical Center and Saarland University, D-66421 Homburg/Saar, Germany

INTRODUCTION: Hyaluronic acid hydrogels play an essential role in the fields of tissue engineering and regenerative medicine. In the present study, a solution of a photocrosslinkable coumarin-hyaluronan (HA) derivative [1] was applied to chondral lesions in the knee of mini-pigs, and its efficacy and safety for cartilage repair was assessed at 8 weeks.

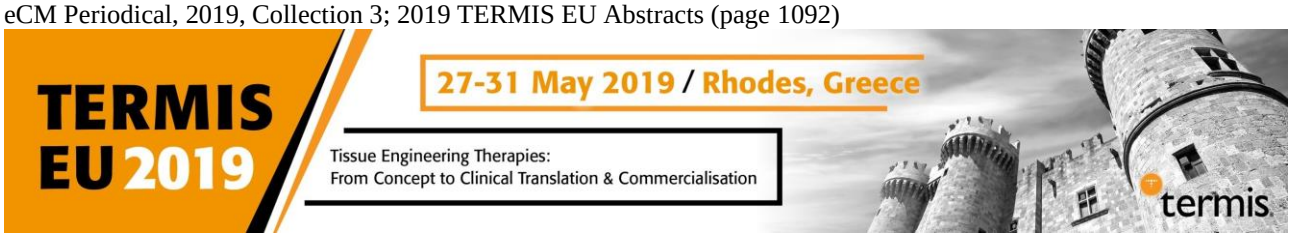
METHODS: Standardized circular full-thickness chondral defects (Ø: 5 mm) were created in the trochlear groove of mini-pigs and treated by (1) debridement, (2) debridement and microfracture (MFX), (3-4) debridement, microfracture, and two different prototypes of photocrosslinkable coumarin-HA solutions (p30 and p40), which were activated for the in situ gelation through 5 min near-UV irradiation (365 nm). The osteochondral repair was assessed at 8 weeks postoperatively using established macroscopic, histological, immunohistochemical, and micro-computed tomography analyses [2]. For the histological evaluation, sections were stained with Safranin-O/Fast Green protocol. A total of 320 stained sections (8 sections per defect) were analyzed applying the complex cartilage repair score described by Sellers et al [3].

RESULTS & DISCUSSION: Semi-quantitative histological analysis of cartilage revealed a significantly better overall structure of the repair tissue upon treatment with coumarin-HA p30, compared to the other three treatment groups. Enhanced defect architecture and surface architecture were observed within the repair tissue of the MFX + coumarin-HA p30 group compared to MFX alone. Furthermore, improved new subchondral bone formation was seen in p30 treated defects, compared to MFX. No significant difference of all µ-CT parameters was detected among the four treatment groups. Compared with the normal osteochondral unit, all treatment methods induced a general attenuation of µ-CT parameters of the subchondral bone plate.

CONCLUSIONS: The collected data illustrates the beneficial effect of a new UV-photocrosslinked hyaluronan hydrogel on early cartilage repair at 8 weeks in vivo in a translational animal model.

REFERENCES

[1] Beninatto R et al. Materials Science & Engineering C. 2018;96:625-34.
[2] Gao L et al. Sci. Rep. 2016;6:32982.
[3] Sellers R S et al. J. Bone Joint Surg. Am. 1997;79:1452-63.



Platelet lysate paradox: Loss of phenotype, but improved redifferentiation of articular chondrocytes

M. Rikkers[1], K. Dijkstra[1], R. Levato[1], D.B.F. Saris[1,2,3], J. Malda[1,4], L.A. Vonk[1]
Presenting Author: Riccardo Levato, r.levato-2@umcutrecht.nl
[1]Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands [2]Developmental Bioengineering, University Twente, Enschede, The Netherlands [3]Department of Orthopaedics, Mayo Clinic, Rochester MN, United States [4]Department of equine sciences, Utrecht University, Utrecht, The Netherlands

INTRODUCTION: Osteoarthritis and focal cartilage defects in the knee occur frequently. With increasing life expectancy, the need for therapies other than knee replacement is growing. Since the intrinsic repair capacity of cartilage is limited, other ways to stimulate cartilage regeneration are being explored. Platelet-rich plasma (PRP) is a blood product containing high growth factor levels and can be used in versatile applications [1]. PRP can be injected intra-articularly for the treatment of knee osteoarthritis. Moreover, platelet lysate (PL) is used for the expansion of cells for cell therapy [2]. This study aims to investigate the potential of using PL to stimulate cartilage matrix production by chondrocytes in vitro. In addition, the potential of PRP as a 3D cell carrier is explored.

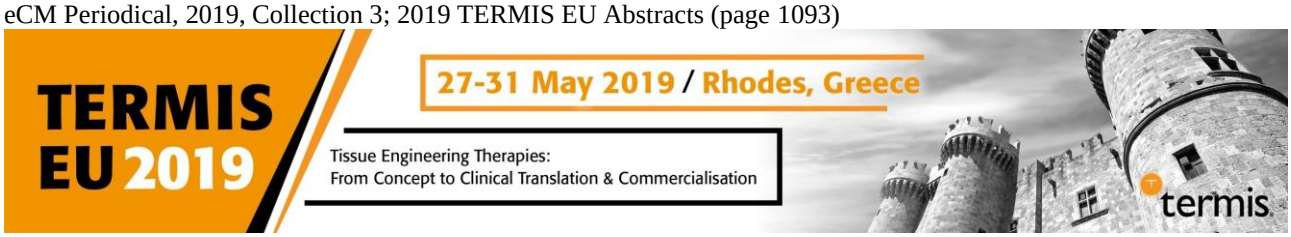
METHODS: PRP and PL were prepared from human blood and platelet enrichment in PRP was determined. Human chondrocyte monolayers were subjected to a range of PL concentrations for 7 days. Cell proliferation and morphology were assessed. Expression of chondrogenic genes was determined by RT-PCR. Next, chondrocytes were brought back into 3D culture and cartilage matrix production was assessed after 28 days. Outcomes were cartilage extracellular matrix (ECM) formation by biochemical assays (glycosaminoglycans (GAG), collagen, and DNA quantification), gene expression analyses and histology. Next, PL was used at 1% and 5% as a supplement for redifferentiation of chondrocytes in pellets with similar outcomes on cartilage ECM production. Finally, PRP was used to make chondrocyte-loaded 3D gels.

RESULTS & DISCUSSION: PL had a dose-dependent effect on chondrocyte proliferation, but expression of chondrogenic markers was decreased. When brought back into 3D pellet culture, GAG production after 28 days was significantly higher for chondrocytes that were expanded with 1% PL compared to controls. When used for redifferentiation of chondrocyte pellets, PL decreased GAG and collagen production. This was confirmed by (immuno)histochemistry. Finally, chondrocyte-containing PRP gels showed similar results in terms of low GAG and collagen production. Again, this was confirmed by histology.

CONCLUSIONS: Platelet lysate stimulates chondrocyte proliferation in 2D monolayer and cartilage ECM production in subsequent 3D pellet culture. However, this does not work for high levels of PL. Furthermore, PL and PRP are not suitable for redifferentiation of chondrocytes.

REFERENCES

[1] Dohan Ehrenfest et al. Trends Biotechnol. 2009; 27(3):158-67
[2] Prins HJ et al. Tissue Eng Part A. 2009; 15(12):3741-51



TNFα pretreatment increased the chondrogenic potential of mesenchymal stem cells under inflamed condition

C.Voskamp[1], W.J.L.M. Koevoet[2], G.J.V.M. van Osch[1,2], R. Narcisi[1]
Presenting Author: Roberto Narcisi, r.narcisi@erasmusmc.nl
[1]Department of Orthopaedics, Erasmus MC, Rotterdam, NL [2]Department of Otorhinolaryngology, Erasmus MC, Rotterdam, The Netherlands

INTRODUCTION: Mesenchymal stem cells (MSCs) exposed to TNFα during chondrogenesis have a reduced differentiation capacity. However, when MSCs are stimulated with TNFα during expansion phase, they have an increased proliferation potential [1] and an increased short-term survival rate in vivo [2], suggesting a possible role of TNFα in priming MSCs to better survive in vivo. However, the effect of TNFα priming on chondrogenesis of MSCs is poorly investigated. The aim of this study was to investigate the effect of TNFα pretreatment on MSCs chondrogenesis, even under the presence of TNFα during differentiation.

METHODS: Human MSCs were obtained from bone marrow of patients undergoing a total hip replacement (N=10). MSCs were expanded with 1 ng/ml FGF2 and 0, 1, 10 or 50 ng/ml TNFα for 24 hrs or 5 days. Next, the MSCs were chondrogenically differentiated for 4 weeks by standard pellet culture containing 10 ng/ml TGFβ, in the presence or absence of 1 ng/ml TNFα. Gene expression analysis, GAG and DNA biochemical analysis and histology were performed at the end of the culture to asses chondrogenesis.

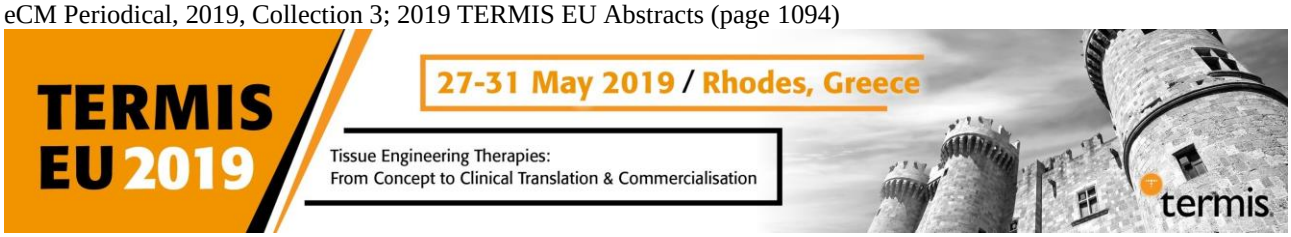
RESULTS & DISCUSSION: GAG/DNA analysis and histology (GAG and COL2) showed that MSCs expanded without TNFα had a reduced matrix production when they were exposed to TNFα during chondrogenesis. Interestingly, after chondrogenic differentiation in the presence of 1 ng/ml of TNFα, MSCs pre-cultured with 10 ng/ml or 50 ng/ml of TNFα for 5 days have an increased GAG and COL2 deposition compared to MSCs pre-cultured without TNFα (Figure 1), and 1.9-fold increase in GAG/DNA. No significant difference was observed after pretreatment for 24 hrs. These results indicate that TNFα pretreatment for 5 days primes the MSCs to deal with the presence of TNFα during chondrogenesis. Furthermore after differentiation without TNFα, we observed that MSCs pretreated with 50 ng/ml TNFα for 5 days also have an increased GAG content (1.8-fold increase) and an increased COL2A1, SOX9, ACAN expression. This suggests that 5 days pretreatment with TNFα increases the chondrogenic capacity of MSCs, regardless the presence of TNFα during chondrogenesis.

CONCLUSIONS: This study showed that TNFα pretreated MSCs have an increased chondrogenic capacity. Given the positive effect on chondrogenesis, TNFα pretreatment can be beneficial for MSC-based therapies to treat cartilage defects.

ACKNOWLEDGEMENTS: This work is funded by the Dutch Arthritis Foundation, Mrace Erasmus MC and NWO.

REFERENCES

[1] Bai X et al. Cell Mol Med. 2017; 21(9):2077-91.
[2] Giannoni P et al. Tissue Eng Part A. 2010; 16(2):489-99



Biomechanics of decellularized blood vessels

R. Simsa[1,2], Andreas Teuschl[3], N. Bergh[2], P. Fogelstrand[2]
Presenting Author: Robin Simsa, robin.simsa@verigraft.com
[1]VERIGRAFT AB, Gothenburg, Sweden [2]Department of Molecular and Clinical Medicine/Wallenberg Laboratory, University of Gothenburg and Sahlgrenska University Hospital, SE-413 45, Gothenburg, Sweden [3]Austrian Cluster for Tissue Regeneration, Vienna, Austria; Department of Biochemical Engineering, UAS Technikum Wien, Vienna, Austria; City of Vienna Competence Team Bioreactors, UAS Technikum Wien, Vienna, Austria

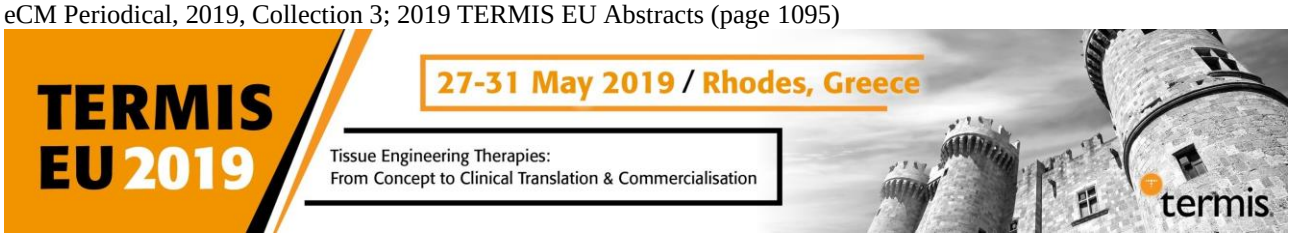
INTRODUCTION: Decellularization (DC) of native tissue is a method to generate 3D scaffolds for medical applications. DC however can affect the mechanical properties of tissue, with implications for cell behavior in vitro or in vivo. In this study, we decellularized blood vessels with different fluid application methods and observed effect on biomechanics and protein retention.

METHODS: Porcine vena cavae were decellularized with a previously optimized method, utilizing the detergents TritonX and TnBP as well as DNase. The veins were decellularized in following perfusion settings: (1) static, (2) agitation or (3) perfusion with different perfusion speeds. Blood vessels were analyzed for cell removal, cytotoxicity, extracellular matrix (ECM) protein retention and biomechanics.

RESULTS & DISCUSSION: DNA quantification and histological stainings showed that all perfusion conditions completely decellularized the veins. Glycosaminoglycans were less present following DC compared to the native tissue in all groups. In contrast, the adhesion proteins fibronectin and vitronectin were retained. Scanning electron microscopy analysis showed no obvious alterations of the extracellular matrix (ECM) of decellularized veins, except for the group perfused with the highest velocity, which showed signs of tearing, indicating damage to the ECM. A cytotoxicity assay showed no negative effects of decellularized tissue on cell viability, indicating sufficient removal of detergents. Biomechanical analysis showed that maximum tensile strength and burst pressure were not different between native and DC groups, but the perfusion-DC groups showed an increased elastic modulus, indicating a higher local stiffness with increased perfusion velocities.

CONCLUSIONS: In conclusion, our data indicates that perfusion-DC negatively affects blood vessel biomechanics while static or dynamic DC retains native biomechanical properties.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 722779 and was conducted within the "Training 4 Cell Regenerative Medicine" (T4CRM) network.



Decellularized brain extracellular matrix as a hydrogel for neuronal stem cell growth and differentiation

R. Simsa[1,2], T. Rothenbücher[3], C. Salado[4], A. Martínez Serrano[3], J. M. Canals Coll[4], P. Fogelstrand[2]
Presenting Author: Robin Simsa, robin.simsa@verigraft.com
[1]VERIGRAFT AB, Gothenburg, Sweden [2]Department of Molecular and Clinical Medicine/Wallenberg Laboratory, University of Gothenburg and Sahlgrenska University Hospital, Sweden [3]Universidad Autonoma de Madrid, Spain [4]Universitat de Barcelona, Spain

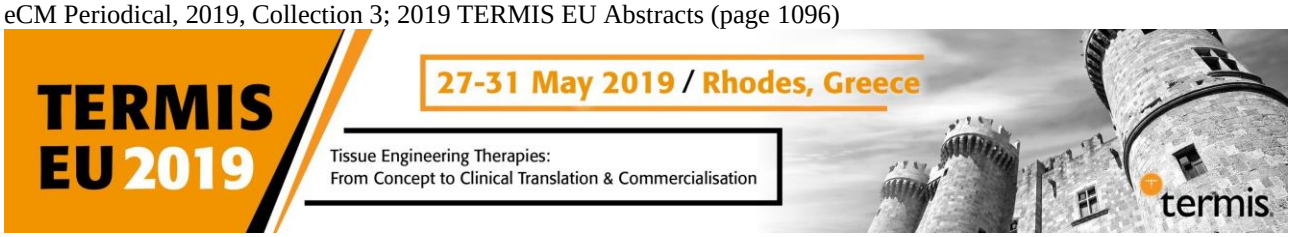
INTRODUCTION: The extracellular matrix (ECM) is the natural environment of cells and has the potential to guide cell proliferation and differentiation by ECM structure and composition. In this study, we investigated the potential of decellularized brain ECM to act as a scaffold for neuronal stem cells. For this purpose, we processed brain ECM into a hydrogel, characterized hydrogel properties and performed cell viability experiments.

METHODS: Porcine brains were decellularized using a sodium deoxycholate/TritonX based method, lyophilized and milled into a fine powder. The ECM powder was furthermore solubilized by pepsin degradation and neutralized with NaOH to form a hydrogel at 37°C. Different concentrations of hydrogel were tested for stiffness, water holding capacity, and clotting kinetics by live-image analysis. Furthermore, we tested the growth and viability of neuronal stem cells embedded within the brain ECM hydrogel.

RESULTS & DISCUSSION: Porcine brains could successfully be decellularized, as indicated by low DNA content (reduction of >90%) and the absence of visible nuclei in DAPI section or whole tissue staining. Different methods for generation of brain hydrogel were tested, of which pepsin-degradation showed to be the most reliable. Lyophilization following pepsin neutralization lead to an ECM powder which can easily be diluted in water in concentrations of >3 mg/mL to readily form a hydrogel at 37°C. Clotting kinetics showed that clotting process can be accelerated by concentration of ECM hydrogel as well as by salt concentration and temperature. Stiffness of ECM hydrogel at different concentrations showed to be linear increased with higher concentrations, as observed with dynamic rheometric analyzer. Furthermore, cultivation of stem cells within the ECM-hydrogel lead to high cell viability and alignment within 3 days.

CONCLUSIONS: In conclusion, our data indicates that the brain ECM can be utilized as a hydrogel for neuronal stem cell growth and proliferation. Furthermore, we showed that the properties of the hydrogel can be closely manipulated according to the experimental requirements by different concentrations of ECM and salts. Further experiments concerning the differentiation potential of ECM-hydrogel compared with MatriGel will be performed.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 722779 (T4CRM).



BARX1 enhanced the dentinogenic and repressed osteogenic differentiation of mesenchymal stem cells from dental root apical papilla (SCAPs) in vitro

R. Dong[1], X. Lin[1, 2], Z.P. Fan[1]
Presenting Author: Rui Dong, dongrui95@126.com
[1]Laboratory of Molecular Signaling and Stem Cells Therapy, Beijing Key Laboratory for Tooth Regeneration and Function Reconstruction of Oral Tissues [2]Department of Implant Dentistry, Capital Medical University School of Stomatology, Beijing, China

INTRODUCTION: BARX1, a member of the homeodomain family of transcription factors, plays crucial roles in the tooth development [1,2]. Our previous study found that BARX1 is highly expressed in dental tissue-derived mesenchymal stem cells (MSCs), suggesting a potential function in regulating the differentiation of dental tissue-derived MSCs for dentin formation or tissue regeneration [2,3]. In this study, we used MSCs from dental root apical papilla (SCAPs) to investigate the functions of BARX1.

METHODS: SCAPs were isolated, cultured, and identified as previously described [2,3]. We over-expressed of BARX1 in SCAPs by retroviruses infection. After selected by 2 ug/ml puromycin, the expressions of Barx1 were observed by western blot. The dentinogenic markers including DSPP and DMP1, and osteogenic marker-BSP were measured by real-time RT-PCR.

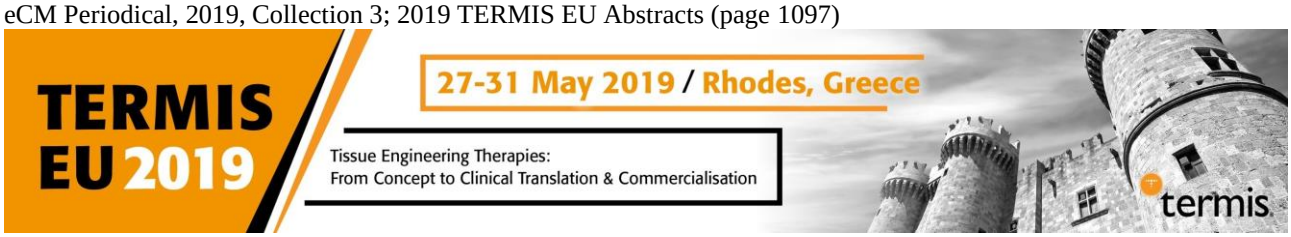
RESULTS & DISCUSSION: Western Blot result showed that BARX1 was ectopically expressed in SCAPs. The real-time RT-PCR results showed that over-expression of BARX1 down-regulated the mRNA expressions of BSP at 3 and 7 after osteogenic induction, and up-regulated DSPP at 0 and 3d and DMP1 at 0, 3, 7, and 21d at the mRNA level in SCAPs.

CONCLUSIONS: BARX1, a transcription factor expressed in mesenchyme of molar primordia, is involved in regulating tooth morphogenesis, plays a role in development of tooth and craniofacial mesenchyme originated from neural crest [1,2]. Our findings suggested that BARX1 was an enhancer of dentinogenic differentiation and inhibitor of osteogenic differentiation in SCAPs. This indicated that BARX1 may be a key regulator to control dentinogenic differentiation in MSCs.

ACKNOWLEDGEMENTS: This work was supported by grants from National Natural Science Foundation of China (81570936 to R Dong), and High-Level Talents of the Beijing Health System (2013-3-034 to R Dong).

REFERENCES

[1] Tsukada Y et al. NATURE 2006; 439: 811-6.
[2] Fan Z et al. Nat Cell Biol.2009;11(8):1002-9.
[3] Lin X et al. Cell Prolif. 2018:e12522. doi: 10.1111/cpr.12522.



Revascularization promoted by platelet pro-angiogenic factors or hyaluronan oligomers – A step towards endodontic regeneration using injectable systems

P.S. Babo[1,2]; C.R. Silva[1,2]; A.L. Silva[1,2]; R.M.A. Domingues[1,2,3]; J. Rouwkema[4]; R.L. Reis[1,2,3]; M.E. Gomes[1,2,3]

Presenting Author: Rui MA Domingues, rui.domingues@i3bs.uminho.pt

[1]B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães 4805-017, Portugal [4]Department of Biomechanical Engineering, Faculty of Engineering Technology, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

INTRODUCTION: Revascularization of pulp canal after endodontic treatment attains to rescue teeth from mid-term extraction fate, and can be promoted by the injection of hydrogels containing adequate cues. Herein are explored the proangiogenic effect of platelet-origin mediators modulated release [1] or hyaluronan oligomers instructive cues [2] within hydrogels aiming endodontic regeneration.

METHODS: Hyaluronic acid (HA) hydrogels incorporating PL were produced by mixing 2% aldehyde-modified HA solution containing aldehyde-functionalized cellulose nanocrystals (CNCs) with 2% hydrazide- functionalized HA dissolved in human platelet lysate (PL). Were assessed the sprouting of human dental pulp cells (hDPCs) or DPCs/HUVECs 1:1 pellets encapsulated in the HA hydrogels, and the neovascularization in a chicken choriallantoic membrane (CAM) assay. Also, low (LMW) or high (HMW) molecular weight (6 kDa and 230 kDa; Lifecore) HA were immobilized over methacrylated gelatin hydrogels (GelMA) and the response of HUVECs in terms of viability and arrangement analyzed.

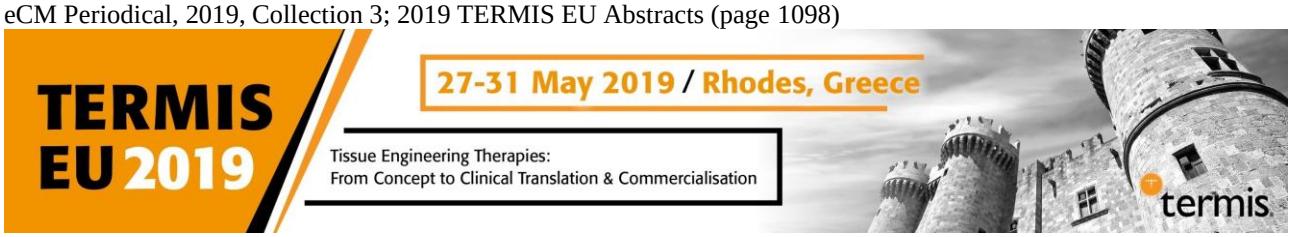
RESULTS & DISCUSSION: PL incorporation enhanced the sprouting of DPCs both in single and co-cultures ($p>0.001$). Moreover, the combined effect of PL and DPCs encapsulation promoted the ingrowth of vascularized tissue into the hydrogels. The immobilization of LMW-HA over GelMA hydrogels enhanced the metabolic activity and promoted the organization of HUVECs into capilar-like structures.

CONCLUSIONS: Our findings from in vitro studies show that both the incorporation of human-origin proangiogenic growth factors and the creation of instructive paths of HA oligomers within injectable hydrogels, might promote the revascularization required for endodontic regeneration.

ACKNOWLEDGEMENTS: Financial support from FCT (UTAP-ICDT/CTM-BIO/0023/2014; RMAD grant SFRH/BPD/112459/2015; PSB grant from project PTDC/CTM-BIO/4706/2014-POCI-01-0145-FEDER 016716); and ERDF NORTE-01-0145-FEDER-000021. SH-CHSJ (Porto, Portugal) for the platelet concentrate batches.

REFERENCES

[1] Fortunato TM et al. Sci Rep. 2016;6(1):25326
[2] Slevin M et al. J Biol Chem. 2002;277(43):41046–59



Three-dimensional invasion assay of glioma stem cells under interstitial flow conditions in a microfluidic device

T. Nunokawa[1], N. Namba[1], R. Sudo[1,2]
Presenting Author: Ryo Sudo, sudo@sd.keio.ac.jp
[1]School of Integrated Design Engineering, Keio University, Yokohama, Japan, [2]Department of System Design Engineering, Keio University, Yokohama, Japan

INTRODUCTION: Glioma, a malignant brain tumor, has poor prognosis, as it diffuses into the surrounding normal brain tissue. Since this invasion mechanism is still unclear, it is important to investigate the control factor of glioma invasion. Recently, we have developed a three-dimensional (3D) culture model of glioma stem cells in a microfluidic device, and demonstrated that endothelial cells promoted 3D invasion of heterogeneous glioma cells populations [1]. There are increasing evidences that biomechanical stimuli, such as interstitial flow, play important roles in cancer cell invasion. Therefore, in the present study, we investigated the effect of interstitial flow on 3D invasion of glioma stem cells.

METHODS: Mouse glioma stem cells [2] were cultured in a microfluidic device, which was made of polydimethylsiloxane and a cover glass. There are two microchannels and the intervening gel scaffold which is a mixture of type I collagen gel and Matrigel. Medium reservoirs were connected to the microchannels and interstitial flow was generated by 5 mmH_2O across the gel scaffold. Media were changed every day. Cell invasion into the gel was observed by phase-contrast microscopy, and morphological features of invading cells were quantitatively analyzed. Immunofluorescence staining was performed to investigate the mechanism of flow-enhanced cell invasion and the stained samples were analyzed using a confocal microscope.

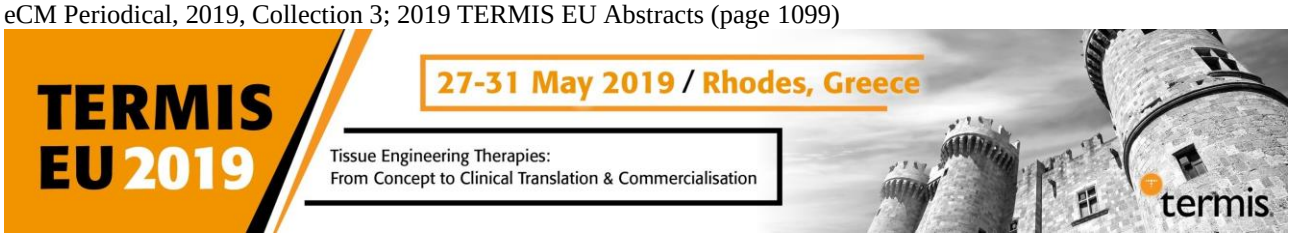
RESULTS & DISCUSSION: Glioma stem cells seeded in a microchannel attached to the surface of the gel scaffold, and invaded into the underlying gel. When the cells were cultured under interstitial flow in the channel-to-gel direction (forward, Figure 1), invasion distance increased compared to static culture. In contract, gel-to-channel (reverse) flow attenuated cell invasion compared to static culture. We also found that invading cells in the forward flow conditions formed characteristic protrusions. Immunofluorescence staining revealed that integrin β1 highly expressed at the protrusions. In addition, expression of FAK and phosphorylated Src was upregulated in the forward flow conditions.

CONCLUSIONS: Our results suggested that invasion activity of glioma stem cells was regulated by the direction of interstitial flow. The flow-enhanced invasion might be involved in integrin-mediated signaling pathways.

ACKNOWLEDGEMENTS: This research was supported by AMED, Japan (JP19gm5810026).

REFERENCES

[1] Chonan Y et al. Integr Biol. 2017; 9(9):762-73
[2] Sampetrean O et al. Neoplasia. 2011; 13 (9): 784-91



Different roles for MEK isotypes in ESWT signal response

S. Nebel[1], T. Machacek[1], H. Redl[2], D. Rünzler[1]
Presenting Author: Sabrina Nebel, nebel@technikum-wien.at
[1]Faculty of Life Sciences, University of Applied Sciences Technikum Wien, Vienna, Austria [2]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology/AUVA Research Center, Vienna, Austria

INTRODUCTION: Extracorporeal shockwave therapy (ESWT) is known to improve wound healing by activation of the ERK-cascade, which is linked to proliferation, differentiation and survival [1]. Recent findings showed that the upstream activators of ERK, MEK1 and MEK2, although highly similar, are not functionally redundant [2]. Herein, we investigated if the two MEK isotypes play different roles in the signal response to ESWT.

METHODS: MEK1 or MEK2 knockout (KO) cell lines were established by transfection (Metafectene Pro) of a pCAG-eCas9-GFP-U6-gRNA vector, expressing guide-sequences specific for either mek1 or mek2, into HEK293T cells, followed by clonal expansion. KO was confirmed by Western blotting and Sanger sequencing. 16 h prior to ESWT, cells were starved in medium containing 0.2% FCS to eliminate growth factor induced background signal. Established KO cell lines were harvested, adjusted to 10^6 cells per 0.5 mL and treated with Dermagold ESWT-device (100 pulses; 0.19 mJ/mm²; 3 Hz). Protein was isolated 5 min afterwards for analysis of ERK and MEK activation via Western Blot.

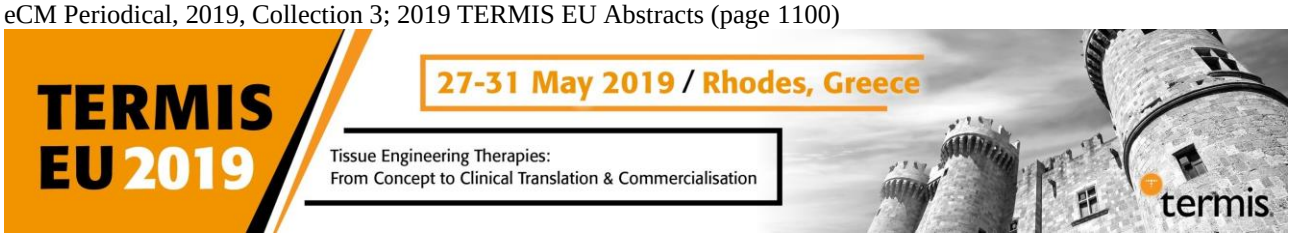
RESULTS & DISCUSSION: Western Blot revealed multiple clonal cell lines with no detectable expression of the targeted MEK isotype (10 for mek1, 19 for mek2). Subsequent Sanger sequencing was used to verify a gene knockout and to analyze genomic sequence alterations. Premature stop codons on both alleles could be detected in two KO cell lines for both mek1 (B8, B10) and mek2 (KE2, JC9). Total protein expression of the remaining MEK isotype stayed unchanged. Treatment with extracorporeal shockwaves revealed significantly lower ($p<0,0002$) activation levels of total MEK (normalized to GAPDH) in MEK1 ablated cells compared to the control. Interestingly, this decrease did not reduce ERK activation levels. In cells lacking MEK2, similar levels of MEK as well as ERK phosphorylation could be detected, compared to wildtype cells.

CONCLUSIONS: Our data clearly indicate different roles of MEK1 and MEK2 in the signaling response to ESWT.

ACKNOWLEDGEMENTS: This study was sup-ported by FFG COIN Disease Tissue (FFG#845443).

REFERENCES

[1] Weihs A et al. J Biol Chem. 2014; 289(39):27090-104
[2] Nowacka JD et al. Oncotarget. 2016; 7(49):80113-80130



Catechol-based free-standing film for tissue regeneration

S. Suárez-García[1], J. Sedó[1], J. Saiz-Poseu[1], S. Nürnberger[2], D. Ruiz-Molina[1]
Presenting Author: Salvio Suárez-García, salvio.suarez@icn2.cat
[1]Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Campus UAB, Bellaterra, 08193 Barcelona, Spain [2]Department of Trauma Surgery, Austrian Cluster for Tissue Regeneration, Medical University of Vienna, Währinger Gürtel 18-20, A-1090, Vienna, Austria

INTRODUCTION: Catechol-based systems provide novel properties such as adhesion, bioactivity and wettability modification [1,2]. Herein, we present the generation of a universally functionalizable film in one-pot reaction which is especially attractive due to its potential versatility and ease of application.

METHODS: Thin free-standing films were synthesized in the air-liquid interphase by dissolving benzene-1,2-diol (10 mmol) and hexamethylenediamine (10 mmol). The films can be handled and deposited on different (bio)substrates. Additionally, the films can be functionalized with (bio)molecules of interest such as polyethylene glycol, folic acid and growth factors.

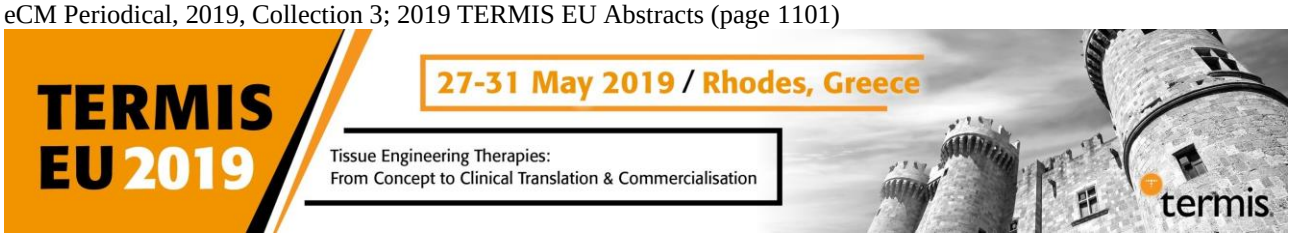
RESULTS & DISCUSSION: The catechol-based films were fully characterized by different techniques (FT-IR, XPS, UV-Vis, SEM). The thickness of the films can be controlled over the time achieving sizes from tens (77 ± 14 after 2 h of reaction) to hundreds (487 ± 16 nm after 48 h of reaction) nm. Once synthesized, the films can be easily transferred to different substrates allowing the modification of the surface properties. The tuneability of the reactivity of the films, lets the in situ and post-functionalization. Furthermore, the films are biocompatible and biodegradable in the cell culture.

CONCLUSIONS: A functionalizable polydopamine-like film was successfully obtained by cross-linking polymerization of pyrocatechol with HMDA under oxidizing conditions. These results demonstrate the potential of the catechol-based films as a universal material that can act as a flexible platform for further functionalization with, for example, biomolecules of interest in biomaterials science, amongst others.

ACKNOWLEDGEMENTS: Supported by grant MAT 2015-70615-R from the Spanish Government funds and by the European Regional Development Fund (ERDF). ICN2 is funded by the CERCA Program/Generalitat de Catalunya. ICN2 is supported by the Severo Ochoa program from Spanish MINECO (Grant No. SEV-2017-0706). S. S.-G. acknowledges the support from MINECO BES-2015-071492 grant. The authors acknowledge the support form the Cost ENBA CA15216.

REFERENCES

[1] Saiz-Poseu J. et al. Angew. Chem. Int. Ed. 2018; 57:2–21
[2] Suárez-García S. et al. Biomimetics 2017; 2: 22



Combinatorial presentation of peptide sequences allows differentiation of mesenchymal stem cells towards distinct chondrogenic phenotypes

S. Camarero-Espinosa[1], J. Cooper-White[1,2]
Presenting Author: Sandra Camarero-Espinosa, s.camarero-espinosa@maastrichtuniversity.nl
[1]Australian Institute for Bioengineering and Nanotechnology (AIBN), Cnr College Rd & Cooper Rd, Building 75, Brisbane QLD 4072, Australia [2]University of Queensland, School of Chemical Engineering, Brisbane, Qld, Australia

INTRODUCTION: Articular cartilage is a multi-zonal material in which chondrocytes assume different phenotypic states depending on the zone they are, i.e., persistent, proliferative and hypertrophic chondrocytes. These distinct chondrocytes deposit an extracellular matrix (ECM) that is specific to each of the zones and is responsible of the non-linear mechanical properties of the tissue. Tissue engineering strategies to regenerate cartilage have so far resulted on the formation of an isotropic neocartilage that lacks adequate differentiation status of the cells and insufficient mechanical properties.

METHODS: We hypothesized that mimicking the biochemical environment of cells in the different zones of cartilage will drive the differentiation of MSCs towards the distinct chondrogenic phenotypes. We performed a systematic study by presenting to human mesenchymal stem cells (hMSCs), varying ratios and concentrations of peptide sequences derived from the ECM (collagen I and IV and, decorin and heparin sulphate cell recognition sites) and cell adhesion molecules (E- and N-cadherin), and characteristic of bone and cartilage. After 2 and 3 weeks of culture, we analysed the ECM deposition and morphology of cultured cells by inmunohistological experiments and the cell phenotype by polymerase chain reaction (PCR).

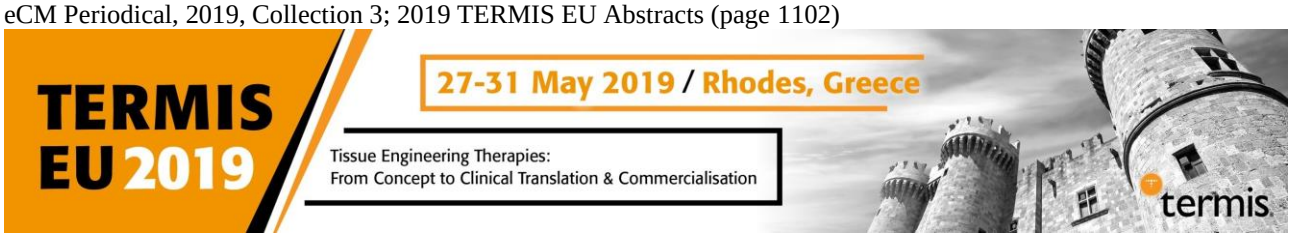
RESULTS & DISCUSSION: We used an adamantyl-modified block copolymer that readily self-assembles onto hexagonally packed nanodomains to conjugate peptide-modified ß-cyclodextrin molecules on a controlled manner. Analysis of the phenotype and ECM deposition by MSCs differentiated on these substrates allowed us to understand the effect of the different peptides on cell morphology and organization (clustered vs spread), the role of the presented peptide density on cell organization and ECM deposition, and the optimal combination of peptides to differentiate hMSCs towards persistent, proliferative and hypertrophic chondrocytes.

CONCLUSIONS: We show that fine tuning chondrogenic differentiation of hMSCs is possible via the controlled presentation of spatial and chemical cues, deciphering the role of individual ECM-derived peptides in this process.

ACKNOWLEDGEMENTS: This work was supported by the Swiss National Science Foundation (P2FRP2_158734) and the Australian Research Council Discovery Grants Scheme (DP140104217).

REFERENCES

[1] Li H, et al. Biomacromolecules. 2014;15:43-52.
[2] Camarero-Espinosa et al. Submitted.



Myrtus extracts and its bioactive molecules orchestrate stem cell pluripotency in stressing conditions

S. Cruciani[1,§], S. Santaniello[1,§], A. Fadda[2], G. Sarais[3], M. Mulas[4], M. Maioli[1,5]
Presenting Author: Sara Cruciani, sara.cruciani@outlook.com
[1]Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy [2]Institute of Science of Alimentar Productions (ISPA), Consiglio Nazionale delle Ricerche (CNR), traversa la Crucca, 3, 07100 Sassari [3]Department of Life and Environmental Sciences, University of Cagliari, Via Ospedale 72, 09124, Cagliari, Italy [4]Department of Agriculture, University of Sassari, Via De Nicola 9, I-07100 Sassari, Italy [5]Center for Developmental Biology and Reprogramming- CEDEBIOR, University of Sassari, Viale San Pietro 43/B, 07100, Sassari, Italy

INTRODUCTION: Bioactive molecules are emerging as novel tools able to counteract different pathological cell conditions [1]. In particular, tannins and flavonoids, largely represented in the Mediterranean plant as Myrtus communis, are well known antioxidants [2], useful in counteracting aging and inflammation.
Within this context, it has been shown that mesenchymal stem cells could be inhibited in their regeneration capabilities by an inflammatory microenvironment [3]. In the present work we evaluate the effects of Myrtus extracts, obtained from the waste of the liquor production, on stem cells behavior under stressing condition.

METHODS: Adipose derived stem cells (ADSCS) were exposed for 12-24 and 48h with 0,5 mg/ml extracts, derived from pulp berry and industrial waste, and then induced to senescence by H_2O_2 treatment. Were then evaluated ROS production, the expression of inflammatory cytokines and sirtuin-dependent epigenetic changes by real time RT-PCR, together with stemness related gene modifications. Finally, stem cell senescence was analyzed by the β-galactosidase assay.

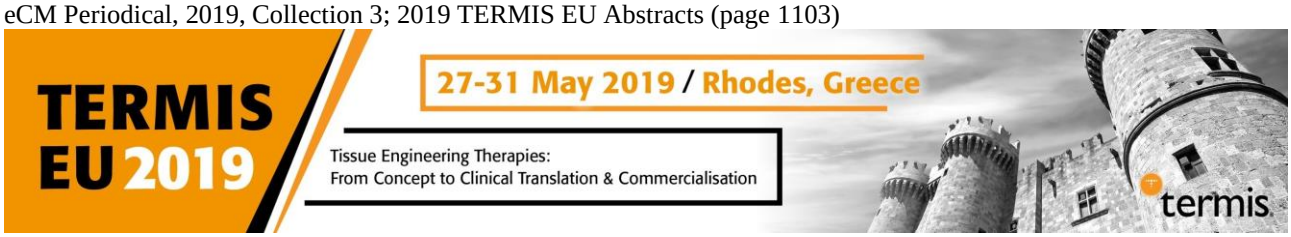
RESULTS & DISCUSSION: Extracts exposure significantly decreased nitric oxide production and IL-6 expression, counteracting inflammation induced by oxidative stress. Real-time PCR analysis showed upregulation of pluripotency related genes (Oct4-Sox2 and Nanog) in cells cultured in presence of extracts compared with untreated cells. Moreover, after 48h of treatment, cells exhibited increased levels of HSP90 and SIRT1 activity, protecting cells from oxidative stress damages. These results were confirmed by β-galactosidase assay, which demonstrated that Myrtus products might counteract the premature senescence elicited by H_2O_2 treatment.

CONCLUSIONS: Industrial myrtle by-Products exert an anti-senescence and protective activity for the high contents of antioxidants molecules, still relevant even after the industrial utilization. Our results suggest a potential use of Myrtus extracts in modulating stem cell pluripotency and inflammatory response, to implement stem cell potential in regenerative medicine.

ACKNOWLEDGEMENTS: The authors are grateful for the financial support from Eldor Lab SRL, Milan, Italy; Fondazione di Sardegna, Project "Myrtus 2.0: from waste to resource (Bioactivity of myrtle by-products)" and P.O.R. FSE 2014/2020 Regione Autonoma della Sardegna.

REFERENCES

[1] An-Qi Liu et al. Int. J. Mol. Sci. 2018, 19(4), 1016
[2] Nassar MI et al. Pharmacognosy Res., 2010, 2(6): 325–329
[3] Günter Lepperdinger. Curr Opin Immunol. 2011, 23(4): 518–524



Reduction of the initial blood volume in the preparation of platelet rich fibrin (PRF): A proof of the low speed centrifugation concept and a new perspective for in vitro research

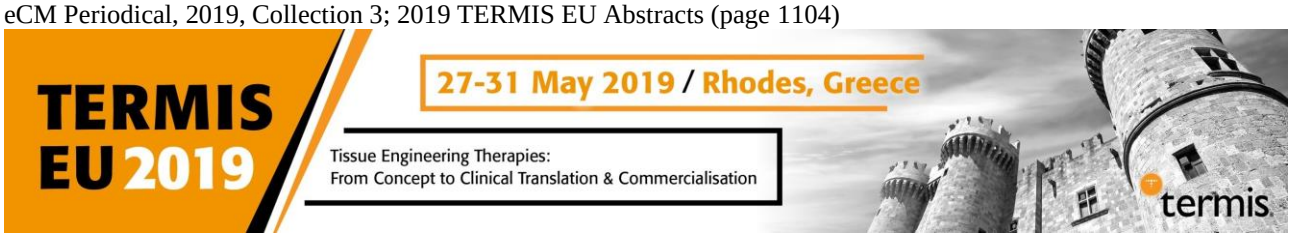
S. Al-Maawi1, R. Sader, CJ. Kirkpatrick, S. Ghanaati
Presenting Author: Sarah Al-Maawi, Sarah.Al-Maawi@kgu.de
Frankfurt Orofacial Regenerative Medicine (FORM) Lab, Department for Oral, Cranio-Maxillofacial and Facial Plastic Surgery, Medical Center of the Goethe University Frankfurt, Frankfurt, Germany

INTRODUCTION: Platelet rich fibrin is a blood concentrate system, gained by the centrifugation of patients' own peripheral blood. The low speed centrifugation concept (LSCC) showed that reducing the applied centrifugal force (RCF) leads to significant improvement of the PRF bioactivity. The initial blood volume required to prepare PRF was 10 ml. The present study aimed to investigate the composition, regenerative capacity and therapeutic effect of PRF produced using 3 ml tubes to reduce the required initial blood volume.

METHODS: Peripheral blood was collected form 6 healthy volunteers after informed consent. 3 tubes of 10 ml served as control and 3 tubes of 3 ml were used in the test group. 3 centrifugation protocols according to LSCC were investigated (High-RCF: 710 xg; Low RCF: 177 xg and Medium RCF: 44 xg; all for 8 minutes). For each protocol 1 tube of 10 ml and 1 of 3 ml were centrifuged at the same time. The PRF bioactivity was evaluated using automated cell count for blood cells and quantification of growth factor (GF) release (PDGF-BB, EGF and TGF-ß1) for 7 days. The therapeutic effect of PRF was evaluated by cultivating primary human osteoblasts (hpOBs) using PRF-conditioned medium (PRF-CM) for 7 days. In this part, hpOBs cultured using DMEM+20%FCS served as appositive control. Cell proliferation and differentiation was measured on day 3 and 7.

RESULTS & DISCUSSION: in both test (3ml tubes) and control groups (10ml tubes) PRF matrices prepared using a high RCF contained significantly lower number of Platelets and leukocytes compared to those of low RCF. No statistically significant difference was observed in the number of platelets when comparing the test and the control group in the respective preparation protocol. However, the control group (10 ml, low RCF) contained statistically significantly higher number of leukocytes compared to the test group (3 ml, low RCF). PRF matrices of high or medium RCF released significantly lower GFs compared to those of low RCF. On day 5 and 7 statistically significantly higher TGF-ß1 concentrations were measured in the control group (10 ml tube, low RCF) compared to the test group (3ml tube, low RCF). However, EGF and PDGF-BB no statistically significant differences were measured at any time point. In both test and control groups, hpOBs that was cultured with PRF-CM of high or medium RCF showed statistically significantly lower proliferation compared to hPOBs cultured with PRF-CM of low RCF or DMEM+20%. No statistically significant differences were found between the test and the control groups. No statistically significant differences were found between PRF-CM (low RCF) and DMEM+20%FCS. Alizarin staining and quantification on day 7 showed that hpOBs cultured using PRF-CM of low RCF underwent statistically significantly higher differentiation compared to hpOBs cultucred with PRF-CM of high, medium RCF or DMEM+20%FCS.

CONCLUSIONS: The present study proved that PRF-matrices produced using 3ml tubs show comparable bioactivity and therapeutic effect to those prepared using 10 ml tubes. PRF-CM showed comparable results to DMEM+20%FCS in the proliferation of hpOBs more positive effect in the differentiation. These data outline new perspectives for the use of PRF-CM in the in vitro cell culture research and minimal invasive PRF indication e.g. in pediatric regenerative medicine.



Characterization of extracellular matrix hydrogels from different tissues with the focus on neural tissue repair

S. Kubinová[1], K. Kekulová[1,2], K. Výborný[1,2], Z. Koci[1]
Presenting Author: Sarka Kubinova, sarka.kubinova@iem.cas.cz
[1]Institute of Experimental Medicine, CAS, Prague, Czech Republic [2]2nd Medical Faculty, Charles University, Prague, Czech Republic

INTRODUCTION: Extracellular matrix (ECM) hydrogels, prepared by tissue decellularization, represent natural injectable materials suitable for neural tissue repair [1]. However, it is not known whether tissue specific CNS-derived ECMs are advantageous for the neural tissue repair when compared to the ECMs derived from the other tissues.

METHODS: We compared ECM hydrogels derived from human umbilical cord (UC), porcine urinary bladder (UB), brain and spinal cord (SC) in terms of the composition, and mechanical and biological properties with the focus on neural tissue repair.

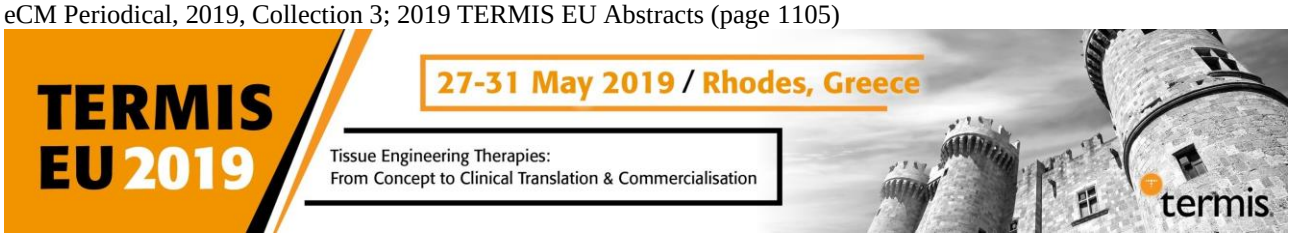
RESULTS & DISCUSSION: The ECM hydrogels did not differ from each other in the concentration of collagen, while the highest content of glycosaminoglycans (GAGs) was found for UC-ECM. In spite of a different GAGs content, all ECM hydrogels similarly promoted the migration of human mesenchymal stem cells (MSCs) and differentiation of neural stem cells, as well as adult dorsal root ganglion (DRG) axon growth in vitro [2]. We also did not find significant differences between the regenerative potential of UB-ECM and SC-ECM in vivo in the treatment of spinal cord injury [3]. However, the degradation of growth-inhibitory chondroitin sulfate proteoglycan by an enzyme chondroitinase ABC significantly improved in vitro DRG axon growth only in SC-ECM, but not in UC-ECM and UB-ECM hydrogels.

CONCLUSIONS: Based on these results, we propose that for the neural tissue repair, tissue specific ECMs derived from CNS, such as SC-ECM, might not be superior over the non-specific ECMs.

ACKNOWLEDGEMENTS: Supported by MEYS CZ.02.1.01/0.0./0.0/15_003/0000419, MEYS of the CR: LO1309.

REFERENCES

[1] Medberry CJ et al. Biomaterials. 2013;34: 1033-1040
[2] Kočí Z et al. Tissue Eng Part C Methods. 2017;23(6):333-345
[3] Tukmachev D et al. Tissue Eng Part A. 2016;22(3-4):306-17



Intervertebral disc regeneration using bioactive microparticles with leaf-stacked structure

M.J. Kim[1], J.S. Kim[2], J.H. Lee[2], H.C. Lee[2], J.H. Lee[3], K.M. Huh[4], S.H. Oh[1,5]
Presenting Author: Se Heang Oh, seheangoh@dankook.ac.kr
[1]Dept. of Nanobiomedical Science, Dankook Univ., Cheonan, Korea [2]College of Veterinary Medicine, Gyeongsang National Univ., Jinju, Korea [3]Dept. of Advanced Materials and Chemical Engineering, Hannam Univ., Daejeon, Korea [4]Dept. of Polymer Science and Engineering, Chungnam Univ., Daejeon, Korea [5]Dept. of Pharmaceutical Engineering, Dankook Univ., Cheonan, Korea

INTRODUCTION: Low back pain which restricts daily life activities and has a high incidence/prevalence is a major health problem. Damage or degeneration of intervertebral disc (IVD) which consists of an external annulus fibrosus (AF) and an internal nucleus pulposus (NP) is commonly considered as the main cause of low back pain [1].

METHODS: We developed a novel PCL microparticles with leaf-stacked structure throughout entire particles (ELSS) using PCL solution (in tetraglycol) by simple heating/cooling method, which can allow sustained release of growth factor for sufficient periods [2]. The release pattern of transforming growth factor-β_3 (TGF-β_3) from the ELSS and chondrogenic differentiation behavior of human mesenchymal stem cells (hBMSCs) by continuously released TGF-β_3 were studied. To investigate whether the hBMSCs/ TGF-β_3/ELSS can provide appropriate environment for IVD regeneration, we used canine with IVD defects as animal models.

RESULTS & DISCUSSION: Prepared microparticles have unique leaf-stacked structure throughout entire particles (100-300 μm). It is observed that the TGF-β_3 loaded in ELSS particles is continuously released for 18 days even without any specific and difficult immobilization. The TGF-β_3-loaded ELSS particles provide suitable environment for chondrogenic differentiation of hBMSCs. hBMSCs/TGF-β_3/ELSS group shows more enhanced IVD regeneration behavior than other groups at in vivo animal study using canine model.

CONCLUSIONS: Our findings suggest that our hBMSCs/TGF-β_3/ELSS system may provide an appropriate environment for regeneration of damaged IVD, and thus a promising therapeutic tool to reduce pain and burden of patients with IVD disease.

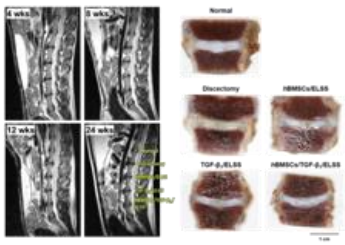
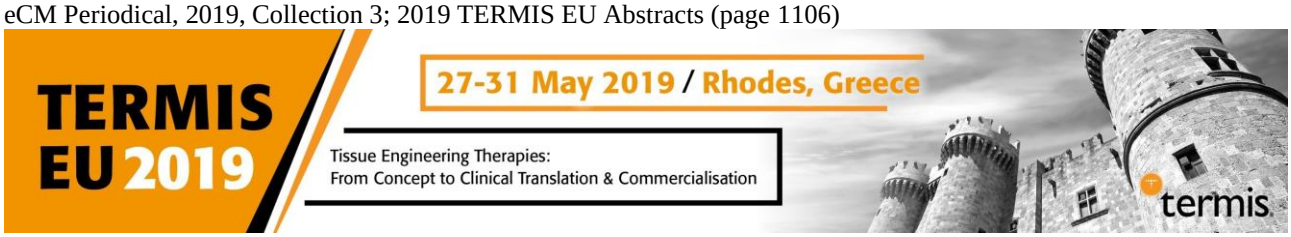


Figure 1: Representative IVD regeneration behaviors of each group (MRI and morphology results).

ACKNOWLEDGEMENT: This research was supported by grants from the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A1A02085564).

REFERENCES

[1] Priyadarshani P et al. Osteoarthr. Cartilage 2016; 24:206-12
[2] Kim HY et al. ACS Appl. Mater. Interfaces 2018;10:21091-102



Oxygen-releasing hollow microparticles for effective bone regeneration

H.Y. Kim[1], S.Y. Kim[1], H.Y. Lee[1], J.H. Lee[2], J.H. Byun[3], K.M. Huh[4], S.H. Oh[1,5]
Presenting Author: Se Heang Oh, seheangoh@dankook.ac.kr
[1]Dept. of Nanobiomedical Science, Dankook Univ., Cheonan, Korea [2]Dept. of Advanced Materials and Chemical Engineering, Hannam Univ., Daejeon, Korea [3]Dept. of Oral and Maxillofacial Surgery, Gyeongsang National Univ. Hospital, Jinju, Korea [4]Dept. of Polymer Science and Engineering, Chungnam Univ., Daejeon, Korea [5]Dept. of Pharmaceutical Engineering, Dankook Univ., Korea

INTRODUCTION: Insufficient oxygen supply to cells in tissue-engineered three-dimensional (3D) scaffolds may be the result of limited oxygen diffusion and/or slow ingrowth of blood vessels; this low oxygen environment (hypoxia) poses the biggest challenge to producing clinically applicable tissues/organs at relevant volumes.

METHODS: We prepared hollow microparticles (HPs) loaded with an emulsion of the oxygen carrier perfluorooctane (PFO; PFO-HPs) for the timely supply of oxygen to surrounding cells. To investigate whether PFO-HPs provide a suitable environment for the cell survival and maintenance of osteogenic differentiation potency of human periosteal-derived cells (hPDCs) under hypoxia, hPDCs seeded on PFO-HPs were incubated under hypoxia. To determine whether PFO-HPs can supply sufficient oxygen for the survival of hPDCs and new bone formation by differentiation of hPDCs into bone cells, we used miniature pigs with mandibular defects as animal models.

RESULTS & DISCUSSION: PCL HPs were 100-500 μm spherical structures with an empty inner space like a balloon, and the average size of the PFO emulsion was ~300 nm. The PFO-HPs prolonged the survival and preserved the osteogenic differentiation potency of hPDCs under hypoxia. hPDCs seeded onto PFO-HPs formed new bone at a faster rate and with a higher bone density than other groups.

CONCLUSIONS: Our findings suggest that PFO-HPs provide a suitable environment for the survival and maintenance of differentiation ability of hPDCs at bony defects without vascular networks until new blood vessel ingrowth occurs, thus enhancing bone regeneration. PFO-HPs are a promising system for effective delivery of various functional cells, including stem cells and progenitor cells, to regenerate damaged tissues/organs.

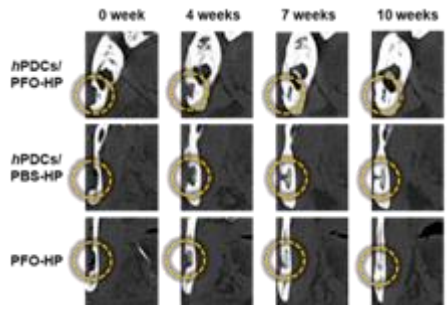
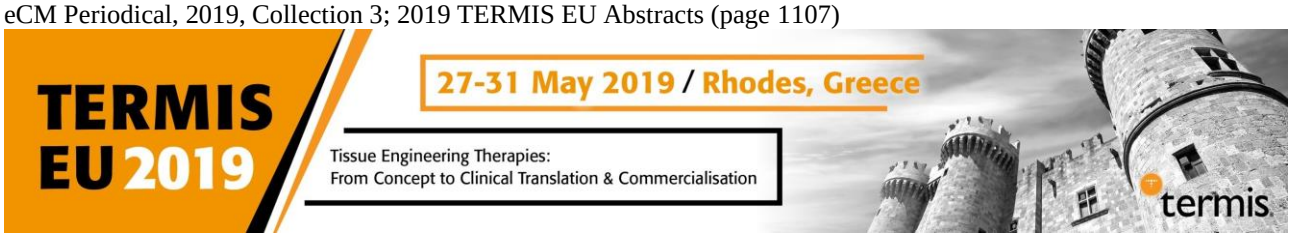


Figure 1: Representative bone regeneration data of each group (dashed circle, defect or reconstructed bone).

ACKNOWLEDGEMENT: This research was supported by grants from the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A1A02085564).



Evaluation of polycaprolactone-associated human nasal septum-derived chondrocytes as a therapeutic agent for cartilage defect repair

D. H. Kim[1], S. H. Hwang[2], S. W. Kim[1]
Presenting Author: Se Hwan Hwang, yellobird@catholic.ac.kr
[1]Department of Otolaryngology-Head and Neck Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea [2]Department of Otolaryngology-Head and Neck Surgery, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

INTRODUCTION: In the field of tissue engineering, biodegradable scaffolds have been used as supporting tools for cell proliferation and/or appropriate matrix formation (i.e., protein synthesis) to fill tissue defects with cells. In this study, we manufactured a complex of human nasal septal cartilage (hNC) with polycaprolactone (PCL) for transplantation into cartilaginous skeletal defects and evaluated their characteristics.

METHODS: Nasal septum tissue was obtained from five patients aged ≥ 20 years who were undergoing septoplasty. hNCs were isolated and subcultured for three passages in vitro. To formulate the cell–PCL complex, we used type I collagen as an adhesive between chondrocyte and PCL. Immunofluorescence staining, cell viability and growth in the hNC–PCL complex, and mycoplasma contamination were assessed.

RESULTS & DISCUSSION: hNCs in PCL showed viability ≥ 70% and remained at these levels for 9 h of incubation at 4°C. Immunostaining of the hNC–PCL complex also showed high expression levels of chondrocyte-specific protein, Type II collagen, Sox9, and aggrecan during 24 h of clinically applicable conditions.

CONCLUSIONS: The hNC–PCL complex may be a valuable therapeutic agent for implantation into injured cartilage tissue, and can be used clinically to repair cartilaginous skeletal defects. From a clinical perspective, it is important to set the duration of the implantation process at ≤ 9 h to achieve effective functional implantation.

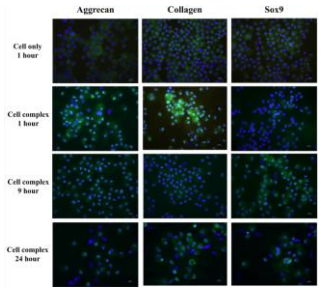
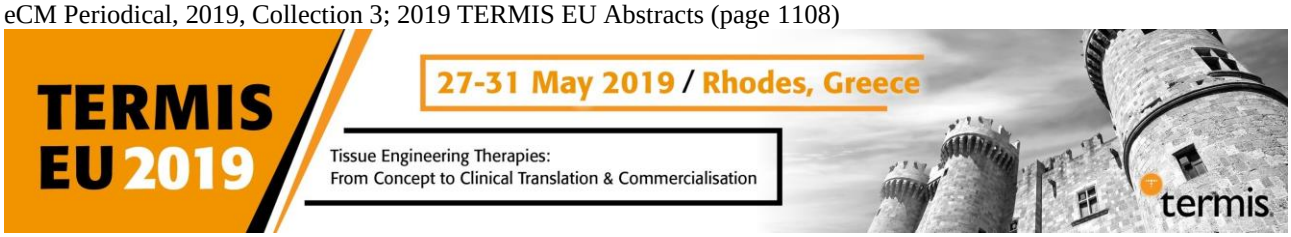


Figure 1: The expression of ECM protein of hNCs- PCL was analyzed by immunostaining in the serum free condition. Immunofluorescence staining for Type II collagen, Sox9 and Aggrecan showed that approximately most of hNCs expressed these proteins during follow up period.

ACKNOWLEDGEMENTS: This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education (2018R1D1A1B07045421).



Lipid functional microgels for the coating of insulin secreting β-cell organoids

Y. Inceoglu, M.B. Akolpoglu, S. Kizilel
Presenting Author: Seda Kizilel, skizilel@ku.edu.tr
Koç University, Chemical and Biological Engineering, Sariyer, Istanbul, 34450, Turkey

INTRODUCTION: Type 1 diabetes (T1D) is an autoimmune disease characterized by destruction of pancreatic β cells. Construction and immunoprotection of beta cell organoids are eligible strategies for islet therapy research in T1D [1,2]. In this study, we have investigated coating of β-TC-6 islet organoids with PEG-lipid microgels through hydrophobic interactions.

METHODS: β-TC-6 islet organoids were formed by hanging drop method. PEG microgels were synthesized through water-in-water (W/W) technique with different ultrasonic powers (30%, 60%) and emulsification durations (10, 30, 45 min) for size optimization. As a lipid functionality acrylate-PEG-DSPE was covalently attached to the microgel. Emulsion was then photopolymerized with 514 nm visible light. Islet organoids were coated by PEG-lipid microgels with different DSPE (2.5 mM, 5mM) and microgel (10 mg/ml, 20 mg/ml) concentrations, addressed here as 2.5DSPE10, 5DSPE10, 2.5DSPE20 and 5DSPE20.

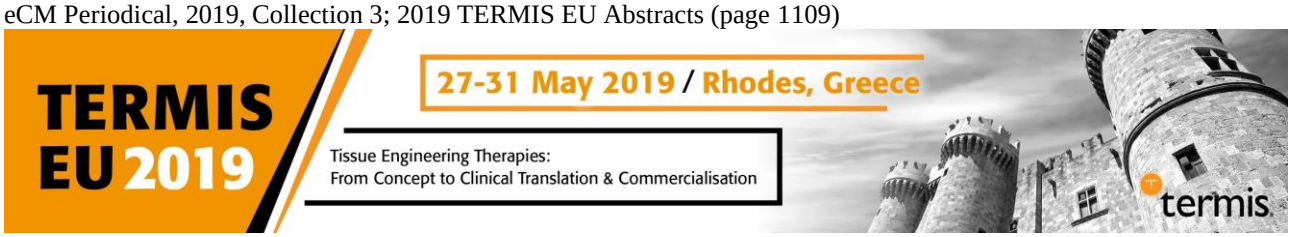
RESULTS & DISCUSSION: Wet emulsion microscopy images of PEG-lipid microgels were analyzed by Image J and size distribution was found as 2-3 μm. The smallest microgel diameter was obtained as 2.13 μm with 60% power and 30 min ultrasonication. PEG-lipid microgels were fluorescently labeled with fluorescein-o-acrylate to confirm the deposition of microgels around organoids by hydrophobic interactions between cell membrane and DSPE group. Viability of the organoids increased when DSPE concentration was doubled from 2.5 mM to 5 mM. We observed highest apoptotic activity for in cells coated with 5DSPE20. Stimulation index for all groups were higher than 1 which indicates organoids are functional and can secrete insulin in response to high glucose before and after PEG-lipid microgel coating.

CONCLUSIONS: For all conditions studied, coated β-TC-6 islet organoids retained their insulin secretion function and viability. This approach is promising for immunoisolation of islet organoids to generate thin protective barriers without disturbing the cell membrane.

ACKNOWLEDGEMENTS: We acknowledge financial support by Scientific and Technological Research Council of Turkey (TUBITAK) under 1001-Scientific and Technological Research Projects Funding Program (SBAG 116S442).

REFERENCES

[1] Bal T et al. Macromol Biosci. 2018; 18(5): 1800001
[2] Kepsutlu B et al. Curr Pharm Biotechnol. 2014; 15(7): 590-608



Bioadhesive PEGylated chitosan nanoparticles for TRAIL induced gene therapy in glioblastoma (GBM)

N. O. Dogan[1], U. Bozuyuk[1], A. Cingoz[2], F. Seker[2], T. Bagci-Onder[2], S. Kizilel[1]
Presenting Author: Seda Kizilel, skizilel@ku.edu.tr
[1]Koç University, Chemical and Biological Engineering, Sariyer, Istanbul, 34450, Turkey [2]Koç University, School of Medicine, Sariyer, Istanbul, 34450, Turkey

INTRODUCTION: Chitosan is a well-known bioadhesive polymer that is a suitable candidate for drug and gene delivery systems due to its tunable properties and cationic nature [1]. Here, we aim to synthesize biofunctional chitosan nanoparticles together with water soluble polymer PEG and plasmid DNA (pDNA) encoding TNF-related apoptosis inducing ligand (TRAIL). TRAIL is well-known for inducing apoptosis in cancer cells rather than healthy cells [2]. Hence this proposed system will have potential to be used as a gene delivery vehicle to promote apoptosis and decrease the viability of glioblastoma multiforme.

METHODS: Transfection studies were performed on HEK293-T and FMC-U87MG cell lines. HEK293-T and FMC-U87MG cells were co-cultured at a ratio of 1:4 and seeded in a 24-well plate as triplicates. After 24 h, transfection medium containing serum-free media and nanoparticle solution were added onto the cells and incubated for overnight. Cells' fluorescence and bioluminescence were measured after 48 h of post-transfection.

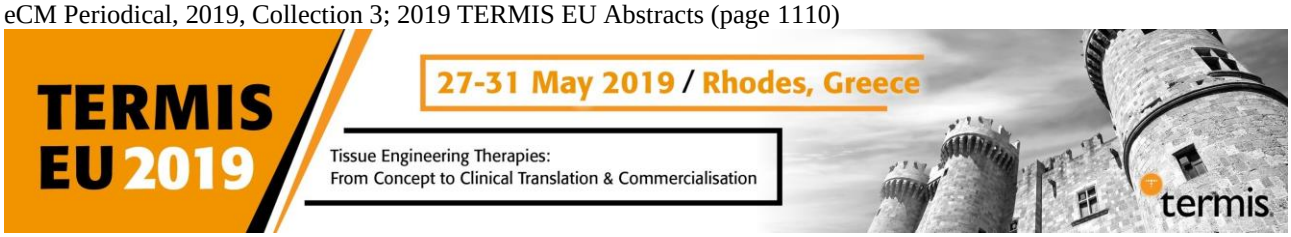
RESULTS & DISCUSSION: We propose transfection of GBM cells using chitosan-PEG-TRAIL nanoparticle system, where apoptosis in GBM cells can be achieved through co-culture of GBM cells with HEK293-T. Based on this approach, transfected HEK293-T cells were used to achieve secretion of TRAIL protein. For co-culture experiments, we used engineered U87MG cells that express the fusion of mCherry and firefly luciferase along with HEK293-T cells. We observed increased amount of GBM cell death with the secretion of TRAIL. We also found that after 24 h of post-transfection, only 25% of GBM cells remained in the medium.

CONCLUSIONS: We concluded that chitosan-PEG-TRAIL nanoparticle system significantly inhibited U87MG proliferation when these cells were co-cultured with HEK293-T.

ACKNOWLEDGEMENTS: We acknowledge financial support by Scientific and Technological Research Council of Turkey (TUBITAK, project number: 116M995), COST Action-European Cooperation in Science and Technology–ENBA CA15216.

REFERENCES

[1] Bozuyuk U et al. ACS Applied Materials & Interfaces 2018; 10(40):33945-55
[2] Erkoc P et al. Macromolecular Bioscience 2018; 17, 1600267.



Stromal vascular fraction delivery in hyaluronic acid-gelatin loaded biphasic calcium phosphate scaffolds for enhanced bone regeneration

S.-S. Park[1], B.T. Lee[1,2]
Presenting Author: Seong-soo, Park, sungsu2546@naver.com
[1]Department of regenerative Medicine, College of Medicine Soonchunhyang University, Cheonan, Chungnam, Republic of Korea, [2]Institue of Tissue Regeneration, College of Medicine, Soonchunhyang University, Chungnam, Republic of Korea

INTRODUCTION: Stromal Vascular Fraction (SVF) is well known as adipose tissue –derived heterogeneous cells due to its high potential clinical applications. Based on our previous studies using Hyaluronic Acid-Gelatin loaded Biphasic Calcium Phosphate scaffold (HyA-Gel/BCP), we confirmed that ECM like HyA-Gel hydrogel loading was significantly improved the new bone formation by the fast attachment and migration of osteoblast cells. In this study, we focused on the effect of autologous SVFs loading on osteogenesis in HyA-Gel/BCP scaffolds by in-vitro and in-vivo approaches.

METHODS: SVF were isolated from the inguinal fat pad of rats. Isolated SVFs were seeded on the pre-conditioned HyA-Gel/BCP scaffolds and cultured for 3 days in incubator (37℃, CO_2 5%). Isolated SVFs were analyzed by Flow cytometer (CD31, CD73, CD45). Cell attachment was estimated by Scanning Electron Microscopy. In-vitro biocompatibility was analyzed by MTT assay for 1 days, 5 days and 7 days. In-vivo studies were performed by craniotomy for 4 and 8weeks using sprague-dawley rats.

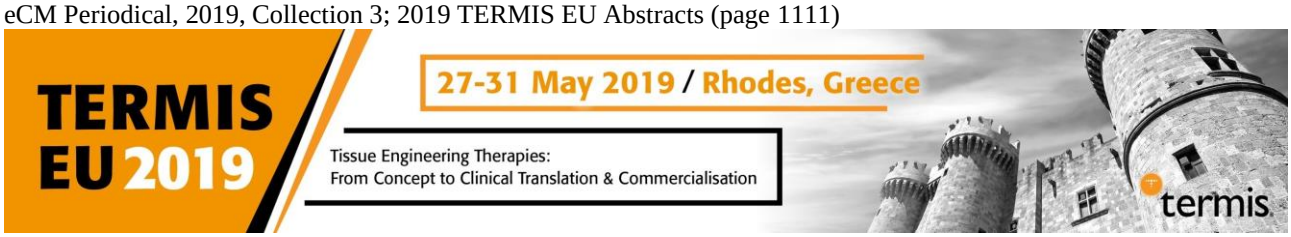
RESULTS & DISCUSSION: Stromal vascular fractions were successively isolated from the inguinal-fat-pad of rats. From the in-vitro studies, the isolated and cultured SVFs expressed the typical MSC markers such as $CD73^+$, $CD45^-$ and $CD31^-$. SVFs were cultured on the HyA-Gel/BCP scaffolds for 3 days, 5 days and 7 days. From the SEM observation, SVFs were attached and expanded actively on the scaffolds after 5 and 7 days. The results of MTT assay indicated that SVFs were well proliferated without toxicity on HyA-Gel/BCP scaffolds. In-vivo experiments were performed by using 3 groups: control, HyA-Gel/BCP and SVF loaded HyA-Gel/BCP groups. Most of bone grafted zones using HyA-Gel/BCP and SVF loaded HyA-Gel/BCP groups were covered with new bones without any inflammation and foreign body reactions. Detailed quantitative analysis by u-CT analysis indicated that the value of BV/TV ratio in SVF loaded HyA-Gel/BCP scaffolds showed higher than that of HyA-Gel/BCP group.

CONCLUSIONS: Due to the excellent biocompatibility, the isolated autologous SVFs were attached and proliferated actively on the HyA-Gel/BCP scaffolds. In-vivo experiment using craniotomy on rat skulls showed that SVFs loaded HyA-Gel/BCP scaffold improved remarkably the new bone formation. Thus we can say that autologous SVFs loading on scaffolds will have a potential for clinical application.

ACKNOWLEDGEMENTS: Financial support was received from National Research Foundation of Korea (NRF) (No. 2015R1A6A1A03032522)

REFERENCES

[1] Nguyen Linh et al. Tissue Eng Part A 2014; 13, 14 (20):1993-2004



Macroporous polymer-based hydrogels for applications in peripheral nerve regeneration

S. Oliveira Formoso[1], L.A. Fielding[1]
Presenting author: Sergio Oliveira Formoso, sergio.oliveiraformoso@postgrad.manchester.ac.uk
[1]School of Materials, The University of Manchester. Manchester, M13 9PL, UK

INTRODUCTION: Lesions to the peripheral nervous system are considered a key area for the development of regenerative treatments. Although a myriad of factors can cause neuropathies (traumatic injuries, iatrogenic effects, and diabetes are responsible for most of the cases), current treatments do not allow for total regeneration. Disability, sensory deficiencies, development of neuropathic ulcers, pain, as well as higher levels of stress and depression arises as major side effect. Herein, we show a methodological characterization of poly(2-hydroxyethyl methacrylate) (PHEMA) based hydrogels envisioned as luminal fillers with potential for peripheral nerve regeneration [1–3].

METHODS: Macroporous hydrogels based on PHEMA were synthetized via redox-initiated free radical polymerization. Rheological analysis and swelling behaviour studies in different liquids (deionised water, minimum essential media, phosphate buffer saline, and ethanol) have been performed. Scanning electron microscopy images were acquired in order to visualize any changes in porosity. Incorporation of glycerol methacrylate (GMA) as a comonomer was studied to corroborate any improvements on mechanical properties and swelling behaviour. Polyethylene glycol dimethacrylate was used as a crosslinker.

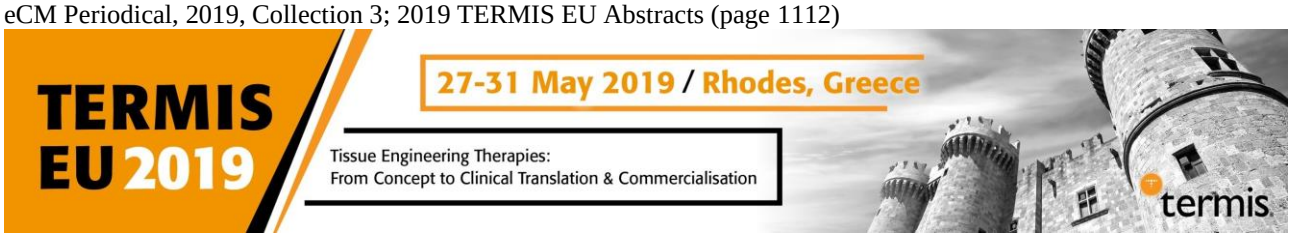
RESULTS & DISCUSSION: HEMA and GMA were copolymerized via redox free radical polymerization to form soft and mechanically compliant hydrogels. These hydrogels, with elastic moduli in the range 5-10 kPa, can withstand the mechanical forces imposed during suturing and have reduced swelling, compared with PHEMA macroporous hydrogels, to prevent disruption of other structures. In addition, macroporous PHEMA hydrogels with elastic moduli in the range 0.9-1.6 kPa were synthetized using the same method. Although they are able to incorporate large quantities of liquids - such as deionized water, minimum essential media and phosphate buffer saline, their shape does not change and therefore, the possible constriction forces that could be imposed to the cellular environment are greatly reduced. SEM images were acquired to corroborate the presence of pores.

CONCLUSIONS: These hydrogels show potential for further functionalization to support axonal regrowth and Schwann cell infiltration via incorporation of molecules present in the extracellular matrix of the nerve such as laminin, or short polypeptides such as RGD or IKVAV. In addition, the project envisions the creation of micro- and nanotopographical cues by changing the crosslinker molecule to a degradable moiety.

ACKNOWLEDGEMENTS: Financial support was received from EPRSC and MRC Centre For Doctoral Training in Regenerative Medicine (studentship grant number: EP:L014904/1).

REFERENCES

[1] Faroni A et al. Adv Drug Deliv. Rev. 82(2015):160-167
[2] Carriel V et al. Expert Rev. Neurother. 14(2014):301-318
[3] Plant G W et al. Brain Res. 671(1995):119-130



Development of a bifunctional PCL-based barrier membrane for guided tissue engineering

S. Dikici[1*], B. A. Dikici[1*], G. C. Reilly[1], S. MacNeil[1], F. Claeyssens[1]
Presenting Author: Serkan Dikici, sdikici1@sheffield.ac.uk
[1]Department of Materials Science and Engineering, Kroto Research Institute, University of Sheffield, Sheffield, United Kingdom *Both authors contributed equally to this work

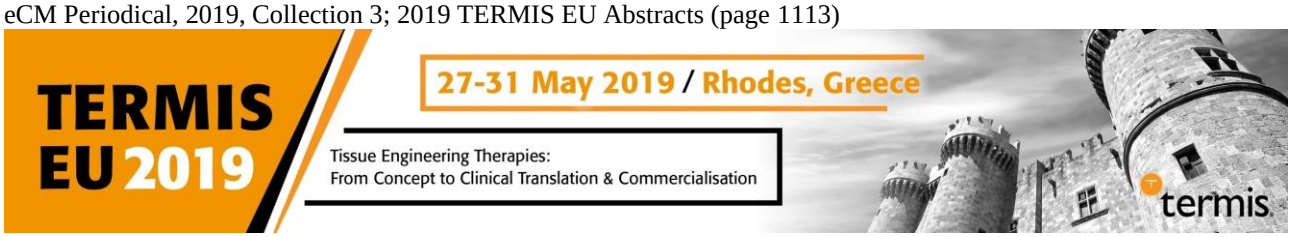
INTRODUCTION: Bone grafts are often inserted into defect sites in the jaw to encourage bone formation prior to inserting periodontal implants. As bone is relatively slower growing than the surrounding gum tissue then a barrier membrane (BM) is often used to prevent soft tissue overgrowing the site on grafts where bone formation is required.
Our aim was to develop a bilayer synthetic biodegradable BM that would act as a physical barrier for preventing epithelial invasion for up to 4 weeks without limiting the diffusion of waste and nutrients, and which would guide bone formation by ingrowth into a highly porous and interconnected bone-like structure.

METHODS: Each layers of the bilayer scaffold were firstly characterized separately. For layer 1 (L1), a pre-polymer of PCL was synthesized and methacrylated to obtain a photocurable polymer (PCLM). L1 was produced by casting of PCLM PolyHIPE under UV. MLOs (Murine long-bone osteocytes) were cultured on L1 for 21 days to assess cell viability and cell infiltration. Collagen and mineral deposition to scaffolds were measured. Resazurin reduction assay was performed for assessment of cell viability on each side. PolyHIPE scaffolds were finally assessed using an ex vivo chick femur defect model to show fully interconnected structure of the scaffolds. To produce a barrier layer (L2), we first electrospun PCL dissolved in 4 separate solvent compositions to find the best solute to manufacture random. We compared fiber dimeters and bead formation using SEM images. Human dermal fibroblasts (HDFs) were cultured on L2 to assess the barrier properties over 28days.

RESULTS & DISCUSSION: The results of the fluorescent and histological images proved that HDFs were not able to penetrate into the L2, while L1 encouraged the penetration and ingrowth of both MLOs and chick cells.

CONCLUSIONS: The bifunctional BM was successfully produced via emulsion templating and electrospinning. This novel bioresorbable membrane has potential to be used in GTR applications by acting as a barrier for epithelial cells at least for up to 4 weeks while allowing bone cells to grow in the implant zone.

ACKNOWLEDGEMENTS: Financial support was received from Turkish Ministry of National Education.



Maturation and subtype specification of cardiomyocytes differentiated from human pluripotent stem cells

S.C. Choi[1], J.H. Choi[1], J.H. Kim[1], C.Y. Park[1], H.J. Joo[1], S.J. Hong[1], D.S. Lim[1]
Presenting Author: SeungCheol Choi, choisc86@gmail.com
[1]Department of Cardiology, Cardiovascular Center, College of Medicine Korea University, Seoul, Korea

INTRODUCTION: Human pluripotent stem cells (hPSCs)-derived cardiomyocytes (CMs) resemble immature embryonic or fetal CMs rather than mature adult CMs [1]. Therefore, generation of mature and subtype CMs from hPSCs is important for in vitro modeling of adult-onset cardiac disease and drug discovery [2,3]. Here, we identified maturation factors that could induce maturation and subtype specification of hPSC-CMs.

METHODS: We optimized differentiation protocols of hPSCs to CMs by evaluating cell seeding densities, concentrations of a Wnt activator, CHIR99021 and a Wnt inhibitor, IWP2, incubation times, and different media under feeder-free culture conditions. hPSCs were treated with various cytokines, growth factors, anti-oxidants, hormones, small molecules and signal pathway inhibitors. Maturity and atrial-like, ventricular-like, and nodal-like characteristics of hPSC-CMs were analyzed by qRT-PCR, immunostaining, flow cytometry, Western blot, and microscope imaging software.

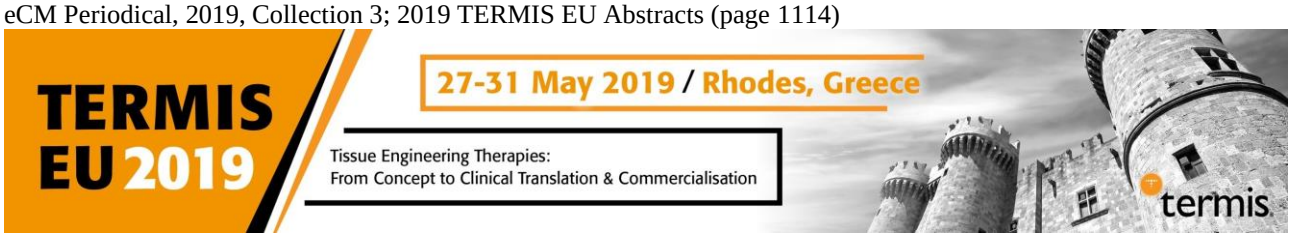
RESULTS & DISCUSSION: Factors that enhance maturation and subtype specification of hPSC-CMs were selected by mRNA expression levels of a mature CM marker (cTnI), a ventricular CM marker (MLC2v), atrial CM markers (MLC2a, ANP, IRX4), a nodal CM marker (TBX18), smooth muscle cell markers (SMA, SM22), and an endothelial cell marker (CD31) (Figure 1). CM maturation and subtype specification factors were further confirmed by immunostaining, flow cytometry and Western blot with a ventricular marker (MLC2v), a sarcomere marker (sarcomeric alpha-actinin), and a T-tubule marker (caveolin-3). Beating CMs forming tubular-like structures were observed in maturation factor-treated groups compared to control group.

CONCLUSIONS: We identified several key molecules that enhance maturation and subtype specification of hPSC-CMs. These factors that could generate mature and subtype CMs are useful for cell-based in vitro disease modeling, drug screening and toxicity testing.

ACKNOWLEDGEMENTS: This work was supported by the Korean Government (NRF-2018R1D1A1B07050742).

REFERENCES

[1] Yang X et al. Circ Res. 2014;114(3):511-23
[2] Hartman ME et al. Adv Drug Deliv Rev. 2016;96:3-17
[3] Jiang Y et al. Mol Cells. 2018;41(7):613-21



Establishment of optimal cryopreservation condition of murine ovarian follicles

S.Y. Ku[1], Y. Y. Kim[1], Y. J. Ryu[1], Y. J. Kim[2], H. Kim[1], C. S. Suh[1]
Presenting Author: Seung-Yup Ku, jyhsyk@snu.ac.kr
[1]Department of Obstetrics and Gynecology, Seoul National University Hospital [2]Department of Obstetrics and Gynecology, Korea University Guro Hospital, Seoul, Korea

INTRODUCTION: Cryopreservation of germ cells is one of the leading technologies for assisted reproductive technology (ART), however, they are focused on embryos and oocytes. Ovarian follicle cryopreservation s one of the emerging issues to overcome current limitations and this is prerequisite for establishment of next generation ART [1]. In this study, we established optimal cryopreservation condition for pee-antral murine follicles.

METHODS: Murine ovaries and ovarian follicles of pre-puberty stage were isolated using mechanical dissociation. Cryopreservation was performed as status of whole ovary, half ovary and isolated sing follicle, respectively. The cryo-solution was consisted with addition of 10% of ethylene glycol (EG), DMSO and 1M sucrose. The thawing was performed after 1 week of cryopreservation and ovaries and half ovaries were dissociated into follicles. The follicle culture media was consisted according to our previous studies [2,3,4] and factors X and Y was added. After maturation of each follicle, hCG (human Chorionic gonadotropin) and epidermal growth factor (EGF) were treated for ovulation.

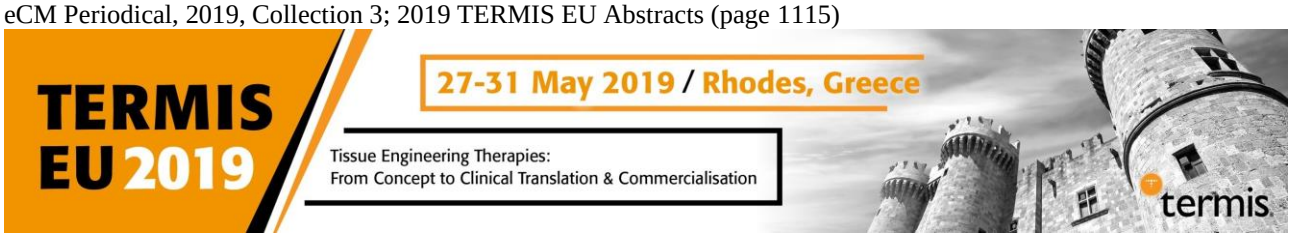
RESULTS & DISCUSSION: Cryopreservation of whole ovaries and half-ovaries were significantly shrunken the ovaries and hard to isolate follicles and the cells seemed to be damaged as shown very dark color. Thawed follicles from cryopreserved as follicles demonstrated the survival and expansion in vitro. The addition of factors, X and Y, was significantly enhanced the survival. The thawed follicles successfully ovulated matured oocytes. And the matured oocytes was fertilized successfully in vitro.

CONCLUSIONS: Collectively, these data clearly illustrate the beneficial effect of factor X and Y for thawing of ovarian follicle after cryopreservation and the optimal status of murine ovary for long-term preservation.

ACKNOWLEDGEMENTS: This research was supported National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2016R1E1A1A01943455).

REFERENCES

[1] Kim YY et al. Tissue Eng Part B Rev. 2017;23(2):142-58.
[2] Kim YJ et al. Hum Reprod. 2013;28(11):3050-61.
[3] Kim YJ et al. J Tissue Eng Regen Med. 2017;11(11):3100-10.
[4] Park KE et al. Reprod Sci. 2013;20(8):909-16.



Bone morphogenic proteins and osteogenic differentiation of stem cells

S.S. Ebadi[1,2†], A. Ahmadi[1,2†], H. Niknejad[1]
Presenting Author: Seyed-Shayan Ebadi, ss.ebadi@sbmu.ac.ir
[11]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [†]Co-first author

INTRODUCTION: Bone morphogenic proteins (BMPs) are a group of cytokines belonging to the transforming growth factor-β (TGF-β) superfamily. As the BMPs play a major role in Skeletal development and bone formation, disruption of their signaling induce different types of malformations [1]. Furthermore, BMPs have been unveiled as one the most potent growth factors in bone tissue engineering. Recently, 14 types of BMPs have been identified which regulate multilineage specific of stem cells including adipogenesis, osteogenesis, chondrogenesis and neurogenesis [2, 3]. The objective of this study was to compare osteogenic feature of different types of BMPs and evaluate their specific pathways.

METHODS: We investigated databases including "Pubmed", "Scopus", "Google Scholar" and "Web of Sciences" for related articles. Key words which were used for search were include "Bone morphogenic protein", "BMP", "Osteogenic differentiation", "Bone tissue engineering", "Bone regeneration", "Osteogenesis", "Signaling transduction" and "Signaling pathway". Totally, 260 paper were found and finally 130 of them were chosen based and their relevancy, date of publication and number of citations.

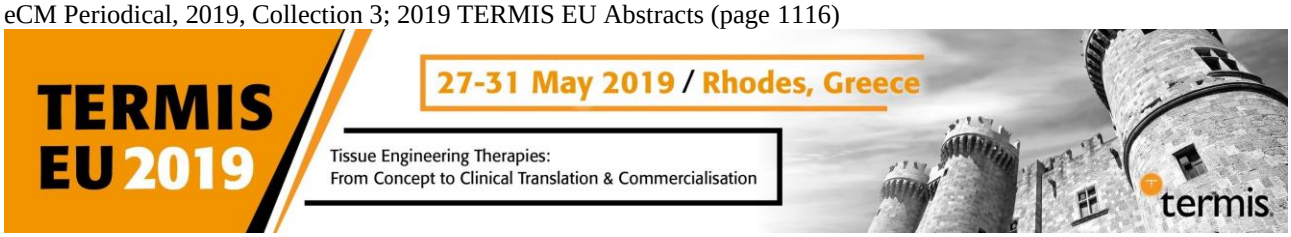
RESULTS & DISCUSSION: We found that among 14 types of BMPs, BMP-2, BMP-6, BMP-7 and BMP-9 are the most potent osteogenic factors. In addition, utilization of BMP-9 has induced higher rate of mineralization and calcium content of stem cells compare to other types of BMPs. Resistance of BMP-9 to BMP-3 and noggin, as two well-known BMP antagonists, revealed that the differentiation pathway of BMP-9 is different from other osteogenic BMPs.

CONCLUSIONS: This study showed that BMP-9 is the most potent ostogenic BMP. Furthermore, it regulates bone formation in different intracellular pathway compare to other osteogenic BMPs.

ACKNOWLEDGMENTS: The authors would like to thank the personnel of Erfan hospital for assistance in this research. We also thank Ms Tayebi for her assistance during professional laboratory works. Research reported in this publication was supported by Research Grant Committee from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFRENCES

[1] Lamplot JD et al. Am J Stem Cells. 2013; 2(1):1.
[2] Scarfì S et al. World J Stem Cells. 2016; 8(1):1.
[3] Bond AM et al. Dev Neurobiol. 2012; 72(7):1068-8



Cell Spray and Wound Healing: current approaches and technology

A. Esfandpour[1,2†], S. Motamedi[1,2†], S.S. Ebadi[1,2†], H. Niknejad[1]
Presenting Author: Seyed-Shayan Ebadi, ss.ebadi@sbmu.ac.ir
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [†]Co-first author

INTRODUCTION: Utilization of cell therapy in order to heal the refractory wounds has been emerged during recent decades. Among copious types of cell delivery methods, cell spraying has showed promising way compare to other [1]. However, new methods and technologies are growing fast which specify the need for further evaluations. Therefore, the objective of this study was to evaluate various methods and technologies in cell delivery with focus on cell spraying.

METHODS: A review of literature was conducted using Web of Science, Pub Med, and Google scholar from 2005 to the end of 2018. The keywords were “cell spray”, “stem cells”, “cell aerosols”, “cell delivery”, “keratinocyte cell spray”, “fibroblast cell spray”, and “wound healing”.

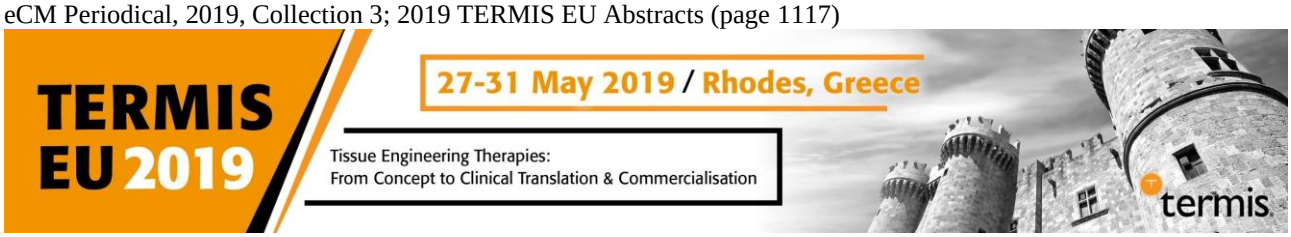
RESULTS & DISCUSSION: An overview of cell therapy by cell spraying method for chronic ulcers and different grades of burns is presented in this review. In addition, the differences between the details of cell spraying methods including various cell sources and spray-on cellular compounds are also summarized. Finally, the results of animal experiments and clinical trials are expressed and compared with each other.

CONCLUSIONS: Techniques used for management of wounds have differed from autologous full-thickness grafts and mesh expansion of split-thickness skin grafts [2] to new conservative methods. However, reducing removed skin to cover the exposed areas [1] and wound re-epithelialization time [2] were required to optimize transplantation outcomes. Spray-applied cell therapy has recently drawn great attention as a less invasive method approaching these ideals. According to studies, although cell spray does not alter the basic surgical indications and principles of epidermal replacement [1], promotion of carrier and application technique would bring it to the near future cell transplantation techniques.

ACKNOWLEDGEMENTS: The authors thank Dr. Tayebi for their assistance in this research. Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Gravante G, et al., Burns. 2007 1;33(8):966-72.
[2] Esteban-Vives R, et al., Burns. 2018 1;44(3):549-59.



Biocompatible conducting nanochitosan/polypyrrole–alginate scaffold for nerve tissue engineering

A. Manzari-Tavakoli [1†], S. Shafiee[2†], A. Moghimi[1], B. Farhadihosseinabadi[4], H. Niknejad[3]
Presenting Author: Sepehr Shafiee, Sepehr.Shafiee@gmail.com
[1]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran [3]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [4]Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [†]Co-first author

INTRODUCTION: Tissue engineering is a novel scientific approach for repair or replace damaged organs and lost tissues. Scaffolds with suitable electrical properties are critical for the success of nerve tissue engineering. The objective of this study was to fabricate a conducting scaffold based on polypyrrole. In order to enhance cell interactions, polypyrrole was blended with alginate and nanochitosan. [1-2]

METHODS: 0.1 M pyrrole in 1 N HCl solution was added to 3% Alg solution and stirred. Then, $FeCl_3$ was added drop wise and solution was stirred for 5 h. This polymer blend was isolated from the reaction mixture by dialyzed to remove oxidant and unreacted substances. 3 wt% nanochitosan solution was prepared by dissolving nanochitosan in acetic acid solution and finely powdered 1 wt% PPy–Alg was added. The samples were further lyophilized in a freeze dryer system. The freeze-dried samples were then cross-linked with glutaraldehyde.

RESULTS & DISCUSSION: The electrical conductivity of Nanochitosan/polypyrrole–alginate composite has been measured by four-probe set up. The electrical conductivity of Nanochitosan /polypyrrole–alginate composite was found $1.3x10^{-4}$ ±0.00003 (0.556 mA), $3.1x10^{-4}$ ±0.00002 (1.133 mA) and $6.2x10^{-4}$ ±0.00002 (2.266 mA) which was comparable to natural conducting scaffolds.

CONCLUSIONS: According to the results of this study, it can be concluded that the developed nanochitosan/PPy–Alg composite scaffold has electrical conductivity which is suitable to serve as a substrate of stem cell for nerve tissue regeneration.

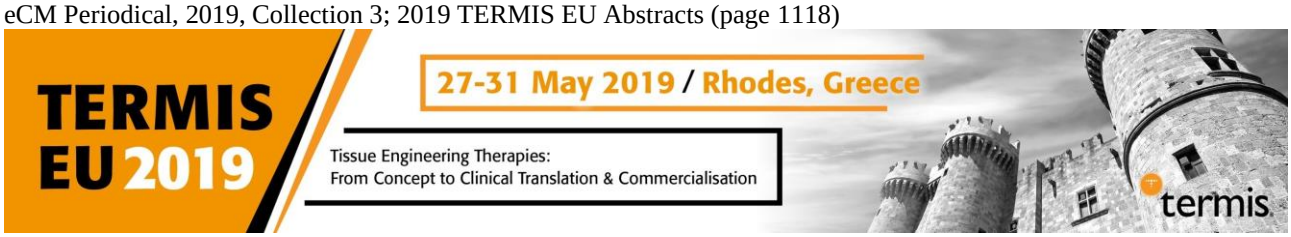
		Conductivity (σ.S/cm)
0.556 mA	1.133 mA	2.266 mA
$1.3x10^{-4}$ ±0.00003	$3.1x10^{-4}$ ±0.00002	$6.2x10^{-4}$ ±0.00002

Table 1: Conductivity Measurements of Alginate –Pyrrole-Nanochitosan Composite

ACKNOWLEDGEMENTS: The authors would like to thank Dr. R. Sedghi in the Department of Chemistry, Dr. M. Manzari-Tavakoli in the Medicinal Plants and Drugs Research Institute and Dr. B.Noushin and Dr. R. Tarasi in the Department of Pharmacology. Research reported in this publication was supported by Elite Research Grant Committee from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Azizian S et al. Carbohydrate polymers. 2018; 202, 315-322.
[2] Modaresifar K et al. Artificial cells, nanomedicine, and biotechnology. 2017; 46 (8), 1799-1808.



Recent advances on tissue and whole-organ decellularization protocols

M.R. Malekpour[1,2,†], S. Shafiee[1,†], M. Abbasi-Kangevari[1,2], B. Jambar -Nooshin[1,3], R. Mazloomnejad-Meybodi[1], H. Niknejad[1]
Presenting Author: Sepehr Shafiee, Sepehr.Shafiee@gmail.com
[1]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran [2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran [3]Department of Tissue Engineering, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical sciences, Tehran, Iran, [†]Co-first author

INTRODUCTION: The idea of tissue decellularization is a promising approach to obtain the biologic scaffolds while preserving the extracellular matrix (ECM) integrity and bioactivity [1-2]. However, the currently applied decellularizing methods alter tissue structure as well as physical and biochemical properties of the remaining ECM, which in turn affect the host response to the material. Minimization of these undesirable effects is the objective of decellularization approaches [3]. The focus of this study was to present the recent advances on decellularization protocols of tissues and whole-organs.

METHODS: A literature search was conducted on PubMed, Scopus, and Google Scholar. Keywords were: 'Optimized Decellularization', 'Whole-Organ Decellularization', 'Tissue Decellularization', 'Acellularization', and 'Biological Scaffold'. The search was limited to journal articles published in English from 2010 to the end of 2018.

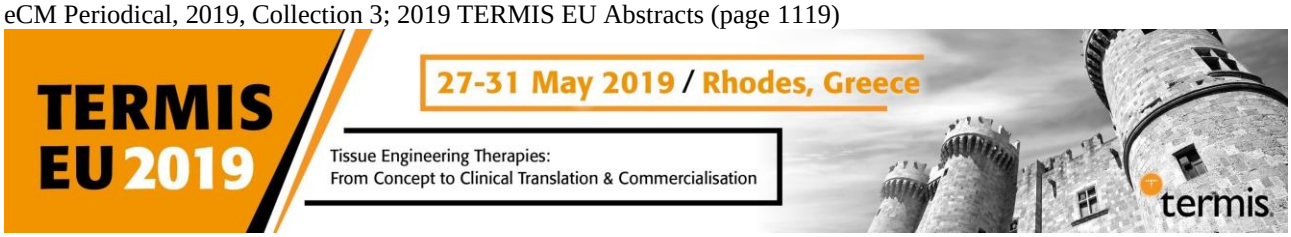
RESULTS & DISCUSSION: Two main factors which contribute to protocols efficacy are decellularization agents and techniques of applying the agents. Commonly used agents include chemical, biological molecules, and physical approaches. Frequently employed techniques were whole-organ perfusion, pressure gradient induction, agitation, and treatment with supercritical fluids. The most effective agents and techniques depend mainly on tissue characteristics including cellularity, density, lipid content, and thickness.

CONCLUSIONS: The optimal decellularization of tissues requires a combination of different agents and techniques, including chemical, biological, and physical approaches. However, further works will be required to optimize the combinations of approaches for complete removal of cell components.

ACKNOWLEDGEMENTS: Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran

REFRENCES:

[1] Mazza G, et al. Sci Rep. 2015; 5:13079
[2] Becker M, et al. Int J Mol Sci. 2018;19(4):1032
[3] Zambon JP, et al .Acta Biomater. 2018; 75:226-234



Proof of the low speed centrifugation concept in rodents: New perspectives for in vivo research

S. Ghanaati, S. Al-Maawi, C. Herrera-Vizcaino, R Sader, C. J. Kirkpatrick
Presenting Author: Shahram Ghanaati, shahram.ghanaati@kgu.de
Frankfurt Orofacial Regenerative Medicine (FORM) Lab, Department for Oral, Cranio-Maxillofacial and Facial Plastic Surgery, Medical Center of the Goethe University Frankfurt, Frankfurt, Germany

INTRODUCTION: Blood concentrates are obtained by centrifugation of the patient's own peripheral blood. These concentrates revealed increasing importance in the regenerative medicine and tissue engineering. Many limitations are faced in preclinical research concerning the volume of blood needed to produce platelet-rich fibrin (PRF) matrices (10 mL). This study evaluated for the first time three different centrifugation protocols to obtain injectable PRF (i-PRF) matrices using a small blood volume of 3 mL.

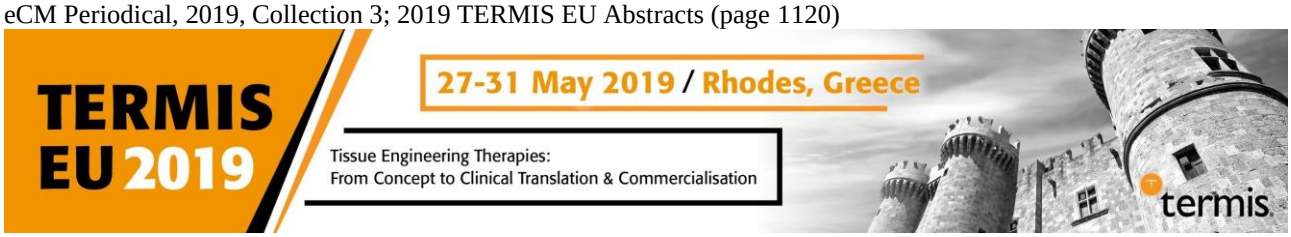
METHODS: Small tubes were specially produced for this study. Blood was collected from 24 Wistar rats (ca. 200 g) for this study using the cardiac puncture technique. From each animal, two tubes were collected: the first was prepared according to the standard PRF protocol without anticoagulation and used for in vitro culturing and histology; the second was anticoagulated and used for automated cell count. Three different relative centrifugation forces (RCFs; high [710 g], medium [177 g], and low [44 g]) were evaluated in this study (n = 8 per RCF). The obtained PRF matrices were cultured over 10 days, and the supernatants were collected for quantification of growth factors (GFs), vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF-b1), and platelet-derived growth factor-BB (PDGF-BB).

RESULTS & DISCUSSION: The results showed that it is possible to produce i-PRF using a small blood volume of 3 mL. Reducing the RCF led to a significant increase in the number of platelets and leukocytes, specifically lymphocytes, within the i-PRF matrices. GF release (VEGF, TGF-b1, and PDGF-BB) was continuously measurable in all evaluated i-PRF matrices over 10 days. The i-PRF matrices prepared using low RCF released significantly more GF compared to those prepared using a medium or high RCF over 10 days ($p < 0.05$). These findings are in accordance with the previously described low-speed centrifugation concept (LSCC), which was first established using human blood, and may serve as a proof of concept of the LSCC in another species, that is, rats.

CONCLUSIONS: Reducing the initial blood volume provides many possibilities to implement PRF matrices in preclinical research as an autologous system. However, further studies are needed to evaluate the therapeutic effects of PRF matrices obtained using 10 mL blood compared to those obtained using 3 mL blood.

REFERENCES

[1] Ghanaati et al. 2011 Biomaterials 32(32):8150-8160
[2] Choukroun and Ghanaati (2017). Eur J trauma Emerg Surg
[3] El Bagdadi et al. (2017). Eur J Trauma Emerg Surg Submitt. 2016



The recent advances on the role of placenta stem cell and comparison with other sources in cell therapy of wound healing

M. Kazemzadeh Hemmesi[1,2†], S. Shariatzadeh[1,2†], H. Niknejad[1]
Presenting Author: Siavash Shariatzadeh, siavash.shariatz@gmail.com
[1]Department of Pharmacology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Iran, [†]Co-first author

INTRODUCTION: Appropriate treatment and management of a wound and its consequent scar increases the patient's quality of life and also reduces the costs in health care system. Since injured skin is regenerated by self-renewal and differentiation of skin progenitor stem cells, applying stem cells into wounds is a current therapeutic alternative. In this study, we focused on the advances about role of stem cell types on wound healing process.

METHODS: Articles retrieved from EMBASE, PubMed and Google scholar; published in English between years 2008 and 2018; Using following keywords: "stem cell", "Placenta", "cell therapy", "non-healing wound", "wound healing" and/or "burn". Through the large number of studies, 115 original articles reviewed.

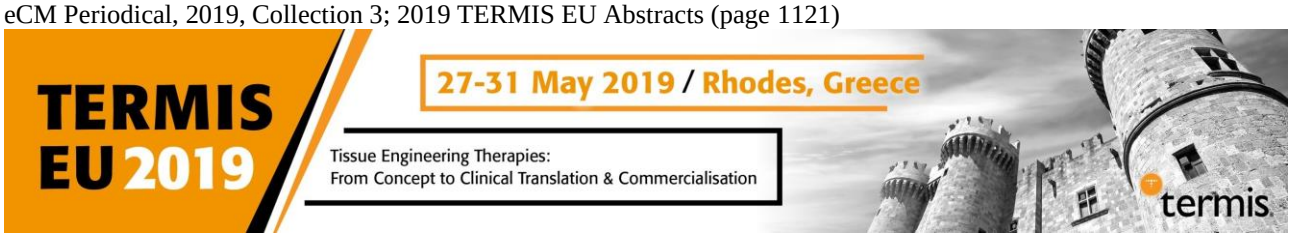
RESULTS & DISCUSSION: Stem cells can be derived from a variety of origins including bone marrow, adipose tissue, cord blood and extra-fetal tissue, embryonic and induced pluripotent stem cells (iPSCs), menstrual blood, and skin. Stem cells empower wound healing process by the following mechanisms: 1) Inducing angiogenesis by both releasing growth factors and direct differentiation to vasculature cells, 2) Differentiation into fibroblasts and keratinocytes [1] and 3) Inducing inflammation by cytokine release [2]. Bone marrow mesenchymal stem cells (BM-MSCs) and adipose-derived mesenchymal stem cells (AD-MSCs) have been more frequently studied in wound healing. Extraction of BM-MSCs from iliac crest is difficult whereas AD-MSCs can be simply achieved from peripheral fat tissue. Despite the pluripotent nature of embryonic stem cells and their differentiation into keratinocytes are favorable, their usage is still controversial due to the probable potential of immune rejection, teratoma formation and also ethical concerns. Yet using iPSCs reported to provoke less immunoreactivity in comparison to the other stem cell types. Stem cells from the placenta also considerably affect skin regeneration, while can circumvent the challenging problems of the other sources.

CONCLUSIONS: Wound healing process does have its particular needs based on the properties of each wound. While any of stem cell types imposes common or distinct healing mechanisms, usage of each stem cell type on a particular wound, will result in a broad range of clinical efficacy. Although there have been much advances in understanding of placenta stem cells therapeutic features in wound healing, it seems there are still a lot to find out by future investigations.

ACKNOWLEDGEMENTS: The authors would like to thank T. Tayebi for her assistance in this research. Research reported in this publication was supported by Researcher Grant Committee under award number [963951] from the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

REFERENCES

[1] Nie C et al. Cell transplantation. 2011;20(2):205-16
[2] Chen L et al. PloS one. 2008;3(4):e1886



The effect of human amnion-derived exosomes on angiogenesis

D. Hayati[1†], M. Valizadeh[1†], S. Shariatzadeh[2†], A. Jafari[2], H. Rajabi-Maham[1], H. Niknejad[2]
Presenting Author: Siavash Shariatzadeh, Siavash.shariatz@gmail.com
[1]Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran [2]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, †Co-first author

INTRODUCTION: Human Amniotic membrane (HAM) has various potentials for therapeutic purposes including angiogenesis and anti-angiogenesis which is surface dependent. Recently exosomes have gained a lot of attention to be as a carrier for a variety of macromolecules which control intracellular communication. The aim of this study was to evaluate the influence of Amnion-derived exosomes on angiogenesis [1-3].

METHODS: HAM was isolated from the placenta and cultured into a serum-free 12 well plate. The condition media was collected after 24 hours and their exosomes were isolated using Exocib kit. The exosomes were characterized by transmission electron microscopy and dynamic light scattering. The angiogenesis activity of Human amnion-derived exosomes(hA-Exo) was assessed by In vivo rat dorsal skinfold chamber.

RESULTS & DISCUSSION: Transmission electron microscopy of hA-exo showed a typical cup-shaped morphology (figure1). The number of vessel sprouts and their length were affected significantly by the compositions of hA-exo.

CONCLUSIONS: This study showed that hA-exo affect angiogenesis which can be as a candidate for cardiovascular disease therapy.

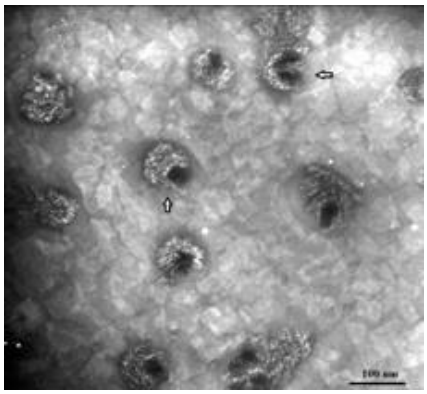
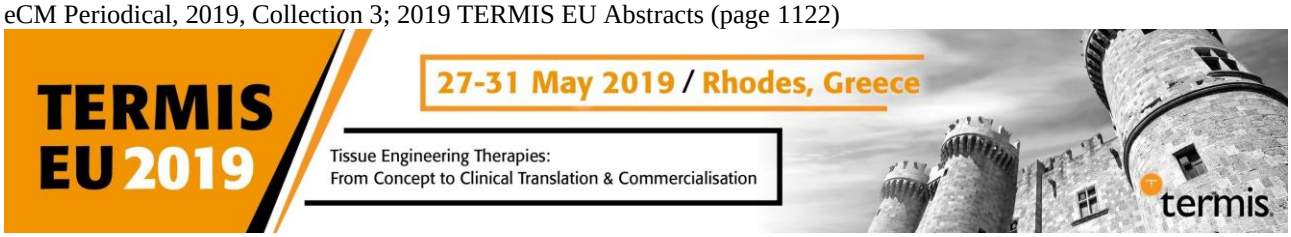


Figure 1: TEM showing cup-shaped morphology of exosomes, scale bar = 100nm

ACKNOWLEDGEMENTS: We would like to thank Ms. Tayebi and the operation room personnel of Erfan hospital for their assistance in this research.

REFERENCES

[1] Niknejad H et al. Placenta. 2013;34(4):340-5
[2] Mamede AC et al. Cell Tissue Res. 2012; 349(2):447-58
[3] Madeo M et al. Nature Communications. 2018;9: 4284



Secretome of 3-D and 2-D cultured MSCs in xeno-free conditions

S. Shanbhag[1], S. Suliman[1], K. Mustafa[1]
Presenting Author: Siddharth Shanbhag, siddharth.shanbhag@uib.no
[1]Tissue Engineering Research group, University of Bergen, Norway

INTRODUCTION: Three-dimensional (3-D) culture of mesenchymal stromal cells (MSCs) represents a novel strategy to enhance their regenerative properties for tissue engineering applications, by more closely simulating the in vivo microenvironment vs. 2-D monolayer culture [1]. MSCs exert their regenerative effects mainly via paracrine mechanisms [2]. The objective of this study was to investigate differences in the gene expression and cytokine profile (secretome) of 3-D and 2-D cultured MSCs in vitro.

METHODS: Human bone marrow MSCs (BMSCs) and gingiva-derived progenitor cells (GPCs) were isolated and characterized for immunophenotype and multipotency, in xeno-free medium supplemented with pooled human platelet lysate (PL). Passage 2 cells were subsequently cultured either in 2-D monolayers or as 3-D spheroids for different time periods to assess cell viability (live/dead stain) and gene expression. Conditioned medium (CM) from 3-D and 2-D cultured cells was collected after 48h PL-free culture; contents were measured via a Multiplex assay and normalized to the amount of DNA to account for differences in cell numbers in 3-D and 2-D conditions.

RESULTS & DISCUSSION: BMSCs and GPCs cultured in PL demonstrated characteristic stromal phenotype and tri-lineage differentiation potential. 3-D spheroids were formed and maintained for up to 7 days with high viability (Figure 1). Expression of stemness- (Sox2, Oct4, Nanog) and osteogenesis-related genes (Runx2, BMP2) was significantly upregulated in 3-D vs. 2-D cultures ($p<0.05$). Multiplex analyses of CM revealed differential secretion of several cytokines, chemokines and growth factors, which were generally higher in 3-D vs. 2-D cultured cells; notably, secretion of FGF-2, SCGF- β, PDGF-BB, HGF and VEGF was significantly higher in 3-D vs. 2-D cultures of both GPCs and BMSCs ($p<0.05$).

CONCLUSIONS: 3-D culture enhances the in vitro secretory profile and stemness-related gene expression of MSCs compared to 2-D monolayers. Whether this is translated to enhanced regenerative effects of MSCs in vivo, should be investigated in future studies.

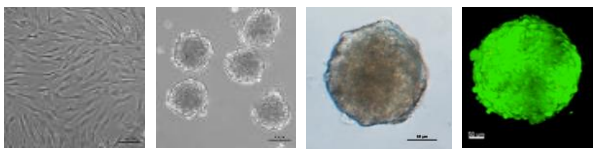
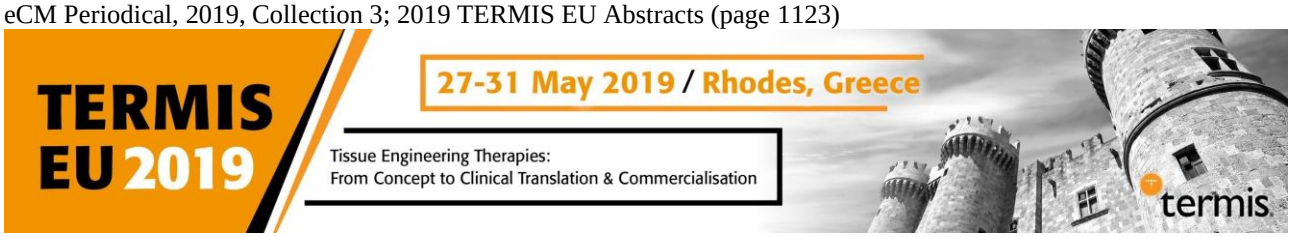


Figure 1: Morphology of 2-D (a) and 3-D (b, c) cultured GPCs. Cell viability after 7 days (d) suspension culture showing mostly live (green) and few dead (red) cells.

ACKNOWLEDGEMENTS: Financial support was received from Helse Bergen and the ITI Foundation (1117/2015).

REFERENCES

[1] Cesarz Z. et al. Stem Cells Int. 2016; 2016: 9176357.
[2] Haumer A. et al. Adv Drug Deliv Rev. 2018; 129:285-294.



Agarose as a potential scaffold for adipose derived mesenchymal stem cells.

S. Castro-Piedra[1], J. Morales-Sanchez[1].
Presenting Author: Silvia E. Castro-Piedra, scastro@tec.ac.cr
[1]Center for Biotecnology Research, Costa Rica Institute of Technology, Costa Rica

INTRODUCTION: The use of adipose derived stem cells (ASC) has been studied as a potential cell therapy with excellent outcomes [1]. These cells can be transplanted directly in the wound site or in combination with a biomaterial that acts as scaffold for them. Agarose have been reported as a biocompatible material that could be used in Regenerative Medicine [2]. Herein, we analyzed two different types of agarose in order to determine the best one to use as a scaffold for ASC transplantation.

METHODS: Scaffolds made with agarose gel (1.5%) of two different melting points (High and Low) were prepared in 24 well plates and were allowed to gel during 24 h at 37 ºC. Then, murine ASC were seeded at 40,000 cells/well, and were allowed to attach for 24 h. After the seeding, the scaffolds were evaluated during four weeks, considering attachment and proliferation.

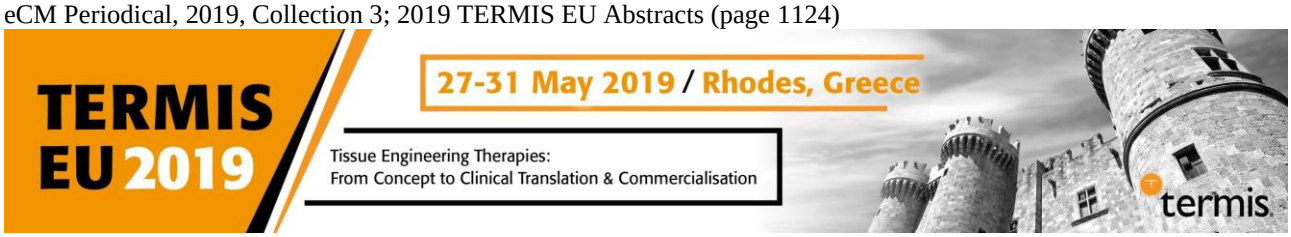
RESULTS & DISCUSSION: Even if the gel was stable during the four weeks, ASC seeded on high melting point agarose gel showed cell surface projections, the culture proliferated as lumps and no migration was observed. On the other hand, ASC seeded on the low melting point agarose gel adopted fibroblast-like morphology, they didn't proliferate in lumps, also both migration and spreading were observed. In low melting point agarose gel, scaffolds showed lower stability, and it lost structure during the third week.

CONCLUSIONS: Murine ASC were able to attach in both agarose scaffolds but they adopted different morphology and growth dynamics, due to the mechanical differences of the agaroses indicating that the strength of the scaffold can affect the behavior of ASC. Thus, is crucial to study how interaction of ASC with biomaterials could change their regenerative properties. This work represents the first steps of research using ASC in combination with biomaterials to improve wound healing for Costa Rican population.

ACKNOWLEDGEMENTS: Finantial support: Vicerrectoría de Investigación, Instituto Tecnológico de Costa Rica. Project No. 1510077.

REFERENCES

[1] N. Bertozzi et al. Annals of Medicine and Surgery 2017: 20 41-48.
[2] S. Sakai et al. J of Bioscience and Bioengineering. 2017:103 22-26.



Design of injectable thermosensitive gels for the localized and controlled delivery of biomolecules in chronic wounds treatment

A. Grivet Brancot[1], O. Lima[2], M. Boffito[1,2], S. Sartori[1,2], G. Ciardelli[1,2], S. Bronco[1]
Presenting Author: Simona Bronco, simona.bronco@pi.ipcf.cnr.it
[1]Istituto per i Processi Chimico-Fisici, Consiglio Nazionale delle Ricerche, Pisa (Italy) [2]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino (Italy)

INTRODUCTION: Chronic skin wounds (CSWs) are a global health problem, affecting patients' quality of life and healthcare system finance. In this context, there is an urgent need of innovative treatments to trigger healing and tissue regeneration. In this contribution, new thermosensitive injectable sol-gel systems were designed as potential carriers of biomolecules (i.e. platelet lysate) in CSWs treatment.

METHODS: Two different PUs were synthesized [1] starting from two commercially available poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers (Poloxamer P407 and F68), to modulate thermosensitivity in the resulting gels. An aliphatic diisocyanate and an amino acid derived diamine (L-lysine ethyl ester) were used as diisocyanate and chain extender, respectively. The obtained PUs (PU_P407 and PU_F68) were chemically characterized by Size Exclusion Chromatography (SEC) and Fourier Trasformed Infrared (FTIR) spectroscopy. PU aqueous solutions were characterized by tube inverting, gelation time test at 37°C, injectability and rheology to assess their temperature-driven sol-to-gel transition. Swelling and stability to dissolution were also evaluated. Model proteins (bovine serum albumin, horseradish peroxidase, -HRP-) were encapsulated and their release was studied by bicinchoninic acid assay (BCA assay)

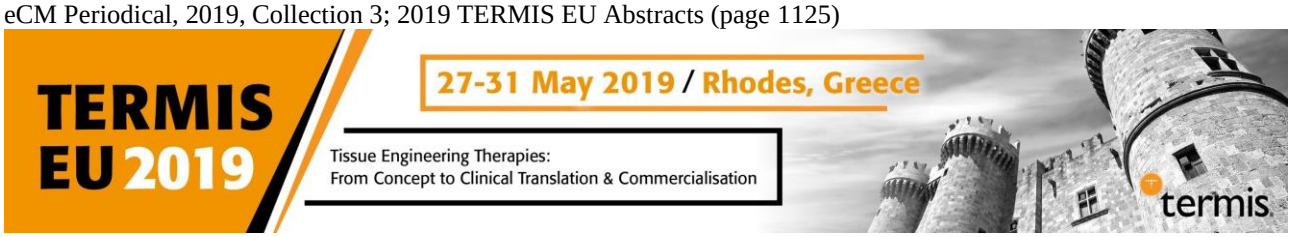
RESULTS & DISCUSSION: PUs were successfully synthesized as assessed by SEC and FTIR spectroscopy. Sol-gel systems with gelation temperature in the range 20-35°C, sol-to-gel transition at 37°C within 10min and injectability through commercial needles (G22, G18, G14) at 5, 25 and 37°C were designed. Rheological characterization showed a full development at 37°C for PU_P407 gels, while PU_F68 systems were still in the sol phase, despite their gel-like behavior due to their rubber consistence. Consequently, gel residence time in aqueous environment for PU_F68 systems was limited to 1-3 days, reaching 7-14 days for PU_P407 gels. Hence, encapsulation and release studies with model proteins were performed only on PU_P407 gels, demonstrating their capability to load and release biomolecules in a controlled and sustained way.

CONCLUSIONS: Injectable thermosensitive sol-gel systems were successfully designed as promising platforms in the treatment of CSWs.

ACKNOWLEDGEMENTS: This work was financially supported by PREVISION "Prognostic evaluation of the use of three-dimensional bioactive scaffolds and injectable gels for the treatment of chronic lesions of the skin by means of new biomedical imaging technologies" project, financed by Fondazione Pisa.

REFERENCES

[1] Boffito M et al. Poly Inter. 2016; 65(7):756-769.



3D-printing of platelet rich plasma based ink for cartilage tissue regeneration

S. Carluccio[1], M.E. Palamà[1], C. Gentili[1]
Presenting Author: Simonetta Carluccio, simonetta.carluccio@edu.unige.it
[1]Department of Experimental Medicine DIMES, University of Genoa, Italy

INTRODUCTION: Therapies to regenerate human articular cartilage defects are still challenging due to poor tissue reparative potential and no self-renewing chondrocyte expansion in vitro. Among the innovative trends used for cartilage repair, in the last decade, Platelet Rich Plasma (PRP) has emerged as a promising therapeutic agent to promote tissue regeneration, since the growth factors contained in platelet granules trigger proliferative stimulus on human chondrocytes while maintain their somatic differentiation potential [1]. Recently, tissue engineering and regenerative medicine fields are pushing on 3D bioprinting as novel method for the 3D fabrication of living tissue-like structures based on the capability to create cell-laden scaffolds with predesigned architecture and distribution of biological factors [2]. Here we produce a new type of bioactive-ink for 3D printing with the aim of encapsulating human articular chondrocytes in enhanced scaffolds able to promote cellular functions and finally chondrogenesis by supplying a sustained quantity of growth factors.

METHODS: Scaffolds were realized by a 3D-extrusion bioprinter, starting from a nanofibrillated cellulose and alginate ink mixed with PRP (2×10^6 platelets/ml) in combination with human primary articular chondrocytes. The realized constructs were then cross-linked in a $CaCl_2$ solution (50mM) subsequently to the printing, thus obtaining a freestanding structures. PRP growth factor release through the constructs was characterized prior to encapsulate cells. Chondrocyte-laden scaffolds were cultivated in chondrogenic medium (containing TGFβ) and assessed for cell viability and proliferation in vitro. The ability to sustain the in vivo chondrogenesis was checked by subcutaneous implantation of the printed constructs in nude mice for 1 month followed by histological analysis of those recovered.

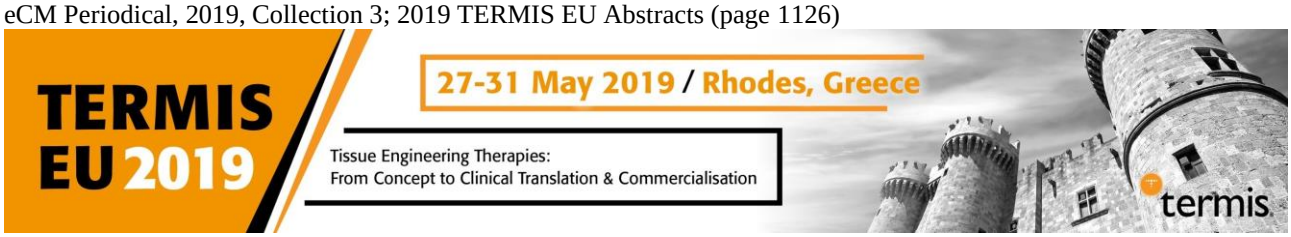
RESULTS & DISCUSSION: Embedding of PRP in the printed constructs supports the chondrocyte viability and growth in the scaffold in comparison to control constructs realized without adding of biological factors. The gradual and controlled release of growth factors from platelets is achieved during culture time, thus obtaining a beneficial micro-environment for cell functions and their interactions. The in vivo test demonstrates cellular production of a glycosaminoglycan-positive matrix and organization in a hyaline-like cartilage tissue more consistent than control group without platelet derivatives.

CONCLUSIONS: 3D bioprinting allows to realize custom-made and autologous cartilage-like constructs available in the injury treatment or even surgery. The introduction of PRP improves the biological function of a standard printable ink and it offers the opportunity for the controlled and localized delivery of growth factors with the aim to stimulate cartilage regeneration and survival.

ACKNOWLEDGEMENTS: This research is supported by Starstem H2020 project.

REFERENCES

[1] Pereira RC et al. Tissue Eng Part A. 2013; 19(11-12): 1476-88
[2] Murphy SV, Atala A Nat. Biotechnol. 2014; 32(8): 773



Improvement in interfacial performance of titania doped hydroxyapatite onto Ti6Al4V alloy substrate with carbide adhesion layers

S. Kulesza[1], L.R. Constantin[2], A. Vladescu[2,3], V. Pruna[4], I. Titorencu[4], M. Bramowicz[1], C.M. Cotrut[5,3]

Presenting author: Sławomir Kulesza, kulesza@matman.uwm.edu.pl

[1]University of Warmia and Mazury in Olsztyn, Olsztyn, Poland [2]National Institute for Optoelectronics, Magurele, Romania [3]National Research Tomsk Polytechnic University, Tomsk, Russia [4]Institute of Cellular Biology and Pathology "Nicolae Simionescu" of the Romanian Academy, Bucharest, Romania [5]University Politehnica of Bucharest, Bucharest, Romania

INTRODUCTION: Orthopedic implants made of Ti alloys often suffer from poor adhesion to the tissues that can be overcome by using additional biocompatible coatings from calcium phosphates. However, such coatings do not stick sufficiently to the metallic surfaces, hence very thin interfacial layers of transition metal carbides can be added to increase adhesion, corrosion resistance and cell proliferation of the implants.

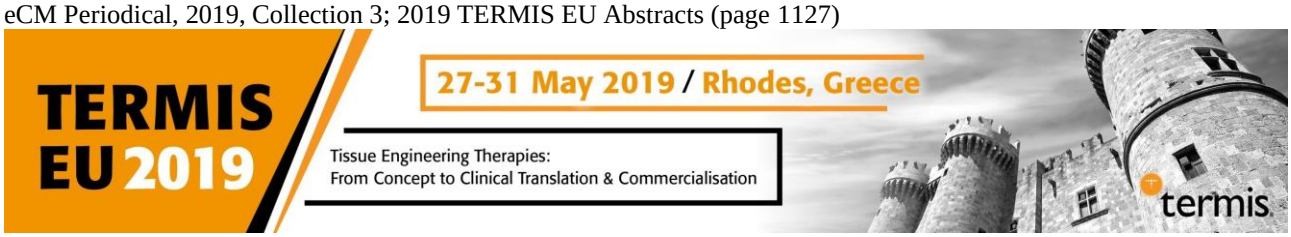
METHODS: The coatings were deposited by RF magnetron sputtering on Ti-6Al-4V discs using HAP, TiO_2 and NbC, ZrC or TiC high-purity targets. The adhesion was evaluated by an examination of the load at which the film flaking starts using the UMT-TriboLab platform (Bruker) The corrosion behavior was tested in SBF solution using a PARSTAT 4000 Potentiostat. Biological tests were carried out on human osteosarcoma cells line MG63 (American Type Culture Collection) and to estimate the proliferation level, the DNA amount was measured.

RESULTS & DISCUSSION: The presence of the interfacial carbide layer does not prevent the formation of the HAP phase. In addition, complex coatings exhibit increased resistance to cracking and spallation with respect to the reference, indicating beneficial effect of that layer to the adhesion between d-HAP and Ti6Al4V alloy. All coatings with carbide interfaces exhibited also higher breakdown potential values than the reference coating and uncoated substrate, which can prove that the addition of a metallic carbide interlayer improves anti-corrosion performance. Similar observation concerns higher proliferation level after both 3 and 7 days of culture, compared to the reference.

CONCLUSIONS: Presented data clearly illustrate that carbide-based adhesion layers might be promising solutions to improve bonding performance between various HAP coatings and Ti6Al4V orthopaedic implants.

REFERENCES

[1] Ratner B D et al. Ann Rev Biomed Eng. 2004;6:41-75.



Elevated WNT5A and WNT11 during MSC chondrogenesis: WNT inhibition lowers BMP and Hedgehog activity and reduces hypertrophy

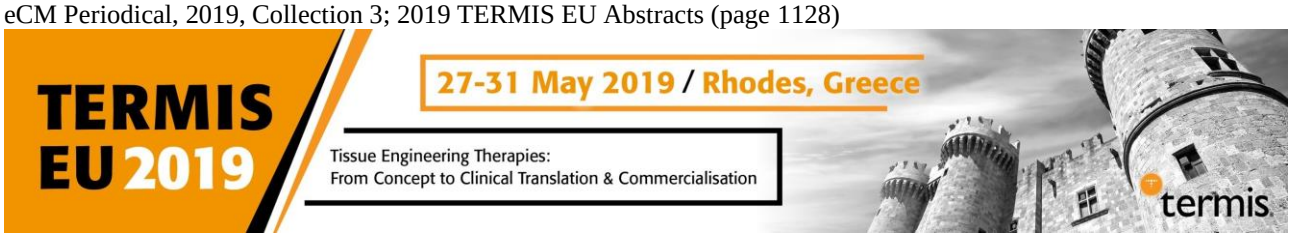
S. Diederichs[1], V. Tonnier[1], W. Richter[1]
Presenting Author: Solvig Diederichs, solvig.diederichs@med.uni-heidelberg.de
[1]Research Center for Experimental Orthopaedics, Center for Orthopaedics, Trauma Surgery and Paraplegiology, Heidelberg University Hospital, Heidelberg, Germany

INTRODUCTION: During in vitro chondrogenesis, bone marrow-derived mesenchymal stromal cells (MSCs) proceed along the endochondral pathway and develop an inherent mineralization activity leading to bone formation at ectopic sites, which is undesired for cartilage regeneration. In contrast, articular chondrocytes (ACs) re-differentiate into a stable articular phenotype and form no bone under the same conditions. The signals driving MSCs into hypertrophy are still incompletely understood. WNT signaling controls endochondral ossification during limb development. We here aimed to illuminate the relevance of WNT signaling for endochondral MSC differentiation, specific candidate drivers in the complex WNT network and the potential crosstalk with other presumably pro-hypertrophic pathways, i.e., BMP and hedgehog (HH) signaling. Better knowledge will allow improved articular cartilage neogenesis in vitro and provide novel therapeutic strategies for regeneration of cartilage tissue in damaged joints.

METHODS: Expression of 71 WNT network genes was assessed via microarray at day 28 of human MSC chondrogenesis and AC re-differentiation and genes of interest verified via qPCR in independent time course experiments. β-catenin, WNT5A and WNT11 levels were detected by Western blotting. WNT signaling during MSC chondrogenesis was blocked with IWP-2 (2µM from day 14 on) and effects were compared with our most potent anti-hypertrophic treatment, pulsed PTHrP(1-34), by monitoring endochondral markers, BMPs, IHH and their targets via qPCR. Ectopic bone formation was assessed after subcutaneous implantation into immune deficient mice via micro CT and histology.

RESULTS & DISCUSSION: 11 WNT genes showed a more than 2-fold different mean expression between re-differentiated ACs and MSC-derived chondrocytes. 9 out of these were higher expressed in MSC-derived chondrocytes. Assessment of WNT ligands showed that high levels of non-canonical WNT5A early and WNT11 late discriminated MSC chondrogenesis from AC re-differentiation. Also, β-catenin seemed incompletely silenced in the MSC group. This suggested higher WNT activity in MSC-derived chondrocytes. WNT inhibition with IWP-2 supported chondrogenesis according to proteoglycan deposition and COL2A1 expression and reduced the pro-hypertrophic transcription factor MEF2C and multiple downstream targets including IBSP and ALPL mRNA, and ALP activity. In addition, IWP-2 treatment lowered BMP4, BMP7 and the BMP target gene ID1. In addition, IHH along with its target GLI were suppressed. This demonstrated that WNT activity drove MSC hypertrophy in crosstalk with BMP and HH signaling. WNT inhibition almost matched the strong anti-hypertrophic capacity of PTHrP(1-34) pulses, yet, hypertrophic marker expression remained above AC level and in vivo mineralization and ectopic bone formation were reduced but not eliminated.

CONCLUSIONS: WNT inhibition was anti-hypertrophic but not powerful enough to re-direct MSC chondrogenesis to articular cartilage neogenesis. Thus, in view of the here discovered crosstalk, a combinatorial inhibitor approach may be promising to fully silence the pro-hypertrophic network during MSC chondrogenesis.



The effect of substance P/Heparin conjugated PLCL polymer coating of bioinert ePTFE vascular grafts on the recruitment of both ECs and SMCs for accelerated regeneration

D.H. Kim[1,2], S.H Kim[1,2,3,*]
Presenting Author: Soo Hyun Kim, soohkim@kist.re.kr
[1]KU-KIST Graduate School of Converging Science and Technology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul, 02841, Republic of Korea [2]Center for Biomaterials, Korea Institute of Science and Technology, Seoul 02792, Republic of Korea [3]Department of Biomedical Engineering, Korea University of Science and Technology (UST), Daejeon 305-350, Republic of Korea

INTRODUCTION: Currently ePTFE vascular grafts are mostly used for treatment of vascular diseases. However, the issues of poor long-term patency and restenosis can arise after bypass surgery. Through a polymer coating, we intend to remodel bioinert ePTFE vacular grafts by creating an environment in which SMCs and ECs could grow together with bioactive molecules capable of instructing the surrounding tissues to migrate into the graft, inducing angiogenesis, and promoting regeneration.

METHODS: ePTFE vascular grafts put into Heparin conjugated PLCL polymer solution and/or Substance P conjugated PLCL polymer solution. Subsequently, Hep-PLCL coated ePTFE, SP-PLCL coated ePTFE, Hep/SP-PLCL coated ePTFE were implanted subcutaneously in SD-rats for 2weeks and 4weeks.

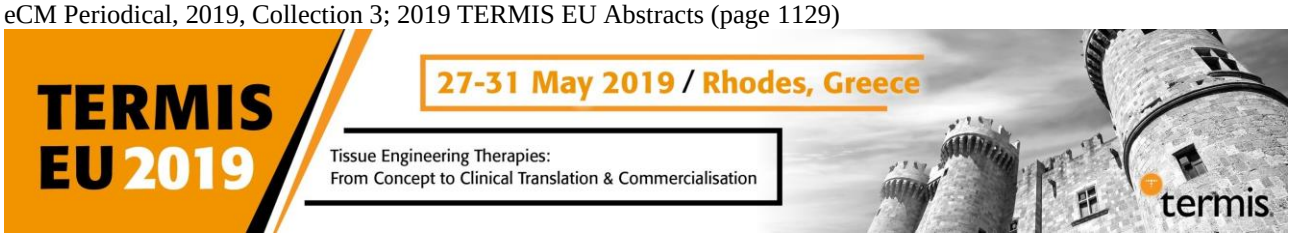
RESULTS & DISCUSSION: SEM and toluidine blue and anti-SP staining demonstrated that the surface and pore of ePTFE were uniformly coated. H&E staining illustrated significant increase of recruited cells into the grafts in SP-PLCL coated group and Hep/SP-PLCL coated group. The double staining of vWF and α-SMA revealed a significant increase of recruited ECs and SMCs in Hep/SP-PLCL coated ePTFE. CD68 and CD206 double staining reveled that Hep/SP-PLCL coating induced differentiation into anti-inflammatory M2 macrophage and inhibited pro-inflammatory macrophage infiltration. The MSC specific markers CD90 and CD105 were significantly more expressed in SP-PLCL and Hep/SP-PLCL coated ePTFE than the other groups.

CONCLUSIONS: Hep/SP Coatings allow grafts to be remodeled by creating a microenvironment where cells can grow by infiltrating into the grafts while also greatly enhancing angiogenesis. In particular, Hep/SP-PLCL showed markedly improved vascular remodeling through the recruitment of mesenchymal stem cells (MSCs), vascular cells (ECs, SMCs) and M2 macrophages.

ACKNOWLEDGEMENTS: This work was supported by the KU-KIST Graduate school of Converging Science and Technology Program and KIST Institutional Program (Project No. 2E27930)

REFERENCES

[1] Yu H et al. J. Vasc. Surg. 2003;38(3):557-563
[2] Kim et al. Biomaterials. 2013;34(6):1657-68
[3] Kohara H et al. Biomaterials. 2010;31(33):8617-25



Autophagy is needed during the differentiation of adipose derived stem cells to functional smooth muscle cells for use in bladder engineering

S. Salemi, D. Mohr- Haralampieva, C. Millan, B. Kranzbühler, T. Sulser, D. Eberli
Presenting Author: Souzan Salemi, souzan.salemi@usz.ch
Laboratory for Urologic Tissue Engineering and Stem Cell Therapy, Department of Urology, University Hospital Zurich, Switzerland

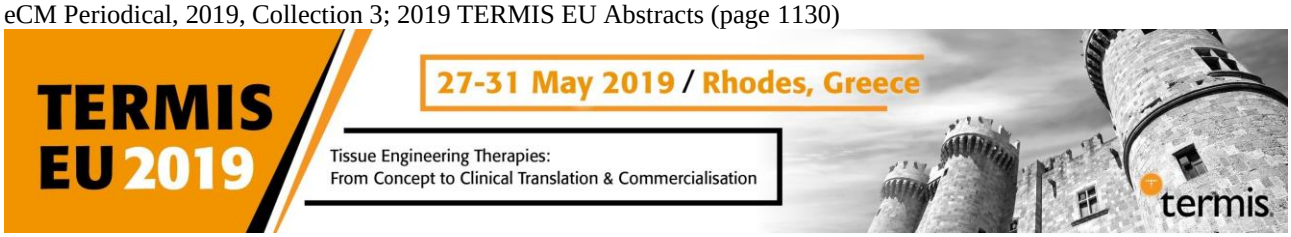
INTRODUCTION: Tissue engineering using smooth muscle cells may provide a treatment option for diseases with smooth muscle pathology such as bladder dysfunction, urinary incontinence, and erectile dysfunction. As autologous smooth muscle cells (SMC) cannot be harvested from organs with end-stage diseases there is a need for other cell sources. Adipose derived stem cells (ADSC) can be easily harvested and differentiated into smooth muscle tissue. We have shown that autophagy, a conserved lysosomal degradation pathway, is required for cell survival and differentiation of adult stem cells. ADSC undergoing differentiation to SMC efficiently remodel their cytoskeleton and shape in an energy-consuming process. We investigated functional role of autophagy during differentiation and remodeling of ADSCs to SMC in vitro.

METHODS: Human and rat ADSCs were characterized and induced towards SMC using induction medium for 1 to 6 weeks. The changes in gene and protein expression level for SMC markers: calponin, smoothelin, α-SMA, MyH11; and autophagy related markers: LC3, Atg5, Beclin1 and p62 were investigated by real time PCR, ICC and WB.

RESULTS & DISCUSSION: Upon induction, up-regulation of Atg5-Atg12 and free Atg5 was observed during 4-6 weeks. This was supported by an increase in conversion of cytosolic LC3I to membrane-bound LC3II protein. At the same time the contractile proteins calponin, MyH11 and smoothelin were up regulated during 1-3 weeks and decreased after 5-6 weeks of differentiation detected by WB and ICC. Pharmacological blocking of autophagy by 3-methyladenine during the differentiation abolished the differentiation capacity of stem cells.

CONCLUSIONS: Our study demonstrates that autophagy plays an important role in ADSC differentiation to SMC. Influencing autophagy by pharmacological agents might be used to further optimize the induction to SMCs.

ACKNOWLEDGEMENTS: Financial support was received from Helmut Horten and Niedermaier foundations.



Distinct effect of neurotrophic factors on neuronal protection and axonal outgrowth

K. Prautsch[1,3], L. Degrugillier[1,3], D. Schaefer[1,2], R. Guzman[3], D. Kalbermatten[1], S. Madduri[1,2,3]
Presenting Author: Srinivas Madduri, srinivas.madduri@usb.ch
[1]Department of Plastic, Reconstructive Surgery, University Hospital Basel, Spitalstrasse 21, 4021, Basel, Switzerland [2]Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, 4021, Basel, Switzerland [3]Department of Biomedical Engineering, University of Basel, Gewerbestrasse 14, 4123 Allschwil, Switzerland

INTRODUCTION: The promotion of axonal survival and outgrowth is crucial in order to ensure peripheral nerve regeneration and has therefore been subject of intensive research during the past 20 years. One of the factors that drive neurite elongation are neurotrophic factors (NTF) which are retrogradely transported from the injured axonal tip to the neuronal cell soma. In this in vitro study, these survival and growth stimulatory factors were to be investigated for their neurotrophic potential on chicken embryonic dorsal root ganglia (DRG).

METHODS: DRG explants from 10 days old chicken embryos were isolated and cultured in culture medium enriched with either nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4) at a concentration of 10ng/ml. As control, DRG explants were grown in NTF-free culture medium. After 48h of incubation time, axonal outgrowth was visualized by tubulin beta-III immune staining and the growth response was quantitatively analyzed by measuring the length and area of neurite outgrowth[1,2,3].

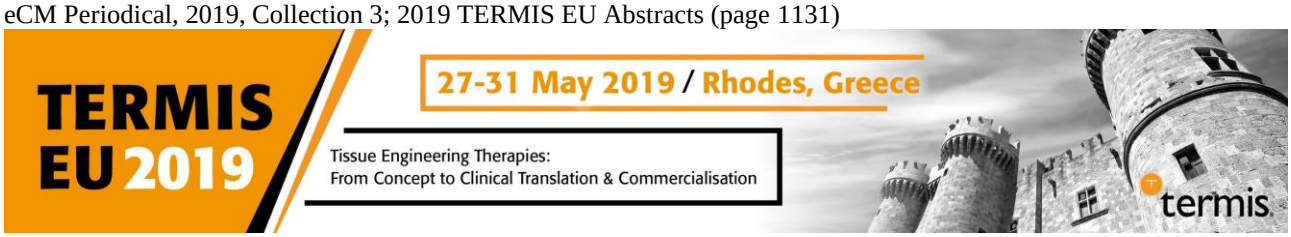
RESULTS & DISCUSSION: Striking differences can be observed in the axonal outgrowth of DRG explants for distinct experimental conditions in terms of axonal count, elongation, density and branching. Interestingly, NT-3 and BDNF exerted the most potent neurotrophic effect as evidenced by axonal length and growth-area when compared to all the other conditions.

CONCLUSIONS: This in vitro study revealed the regenerative potential held by NT-3 and BDNF that even exceeded the stimulatory effect of potent and widely acknowledged growth factors such as NGF and GDNF. Further experiments in order to study the growth kinetics and the cell survival rate of NT-3 and BDNF treated neuronal cells are in progress.

ACKNOWLEDGEMENTS: SNSF nº 31003A_133076/1; CTI nº 16168.1 PFLS-LS.

REFERENCES

[1] Madduri. et al., Biomaterials. 2010, 31:2323-34
[2] Madduri. et al., Biomaterials. 2010, 31:8402-09
[3] Madduri. et al., Journal of controlled release. 2010, 143:168-174



Bioactive fibrin conduits for long gap peripheral nerve regeneration

K. Prautsch[1,3], L. Degrugillier[1,3], D. Schaefer[1,2], R. Guzman[3], D. Kalbermatten[1], S. Madduri[1,2,3]
Presenting Author: Srinivas Madduri, srinivas.madduri@usb.ch
[1]Department of Plastic, Reconstructive Surgery, University Hospital Basel, Spitalstrasse 21, 4021, Basel, Switzerland [2]Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, 4021, Basel, Switzerland [3]Department of Biomedical Engineering, University of Basel, Gewerbestrasse 14, 4123 Allschwil, Switzerland

INTRODUCTION: Adipose derived stem cells (AdSC) are known to enhance peripheral nerve regeneration when delivered in nerve conduits through distinct mechanisms. This study aimed at evaluating the synergistic effect of AdSC and nerve growth factor (NGF) in vitro and in vivo[1,2,3] on axonal outgrowth.

METHODS: AdSCs were pre-treated with NGF ($AdSC^{NGF}$). Dorsal root ganglia (DRG) were cultured in conditioned medium ($CM\text{-}AdSC^{NGF}$) obtained from $AdSC^{NGF}$. Axonal outgrowth from DRG was visualized by immunostaining and evaluated by measuring the length and area of neurite outgrowth. For in vivo study, four rats per group were operated creating a 15mm gap on left sciatic nerve. $AdSC^{NGF}$ were either integrated into the wall of FC or injected into the conduit's lumen within a soft fibrin glue solution. Nerve ends were attached by a single suture to the conduit. Functional gait analyses were performed weekly. After four weeks, gastrocnemius muscles and sciatic nerves were explanted. The wet-weight of the muscles was measured and nerve tissue was process for immunostaining and microscopic analysis[1].

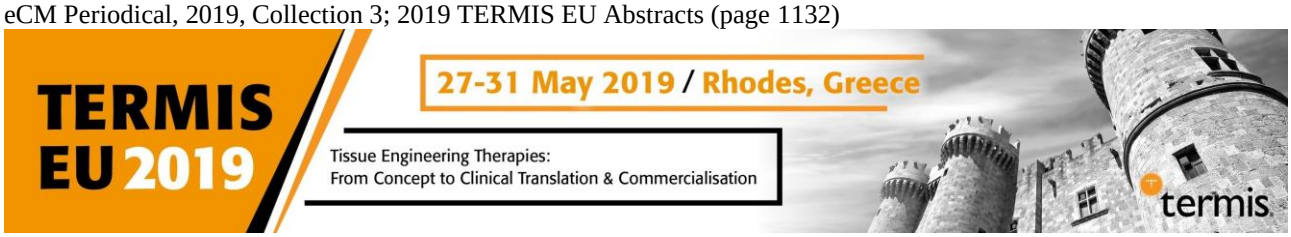
RESULTS & DISCUSSION: The secretome ($CM\text{-}AdSC^{NGF}$) derived from $AdSC^{NGF}$ exhibited significant axonal outgrowth from DRG explants when compared to controls. In vivo, all fibrin conduits, grafted for bridging the long-gap injuries, showed contraction. Muscle weigh loss was comparable across all groups. The distal nerve cross sections in experimental groups indicate incomplete nerve regeneration, whereas proximal cross sections show an axonal count close to autografts.

CONCLUSIONS: The study revealed the synergistic effect of AdSC's derived secretome in the presence of NGF on axonal outgrowth in vitro. The suitability of fibrin conduits for treating long nerve gaps is to be evaluated. Although, the 15 mm long gap could not be crossed by regenerating axons within the 4 weeks, the axonal count in proximal part of the nerve and the reduced muscle weight loss indicate the potential of bioactive fibrin conduits for supporting axonal outgrowth over long-gaps.

ACKNOWLEDGEMENTS: SNSF nº 31003A_133076/1; CTI nº 16168.1 PFLS-LS.

REFERENCES

[1] Madduri. et al., Biomaterials. 2010, 31:2323-34
[2] Madduri. et al., Biomaterials. 2010, 31:8402-09
[3] Madduri. et al., Journal of controlled release. 2010, 143:168-174



Assessing the properties of collagen II scaffolds as a function of species, tissue and gender

Z. Wu[1,2], S. Korntner[1,2], A.M. Mullen[3], I. Skoufos[4], A. Tzora[4], D.I. Zeugolis[1,2]
Presenting Author: Stefanie Korntner, stefanie.korntner@nuigalway.ie
[1]Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway [2]Ireland, Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland [3]Teagasc Research Centre, Ashtown, Ireland [4] Technological Education Institute (TEI) of Epirus, Arta, Greece

INTRODUCTION: Porcine and fish by-products in particular are rich sources for collagen, which is the main component of the extracellular matrix (ECM). Although there are studies investigating different collagen derived from various tissue sources for the purpose of creating biomaterials, the comparison of biophysical, biochemical and biological properties of type II collagen isolated from cartilaginous tissues has yet to be assessed. In addition, it has been shown from previous studies that sex steroid hormones affect the collagen content in male and female animals, herein, type II collagens from male and female porcine cartilage were assessed in order to investigate gender effects on the property of collagen scaffolds. Therefore, the aim is to assess the properties of type II collagen scaffolds as a function of species, tissue and gender for cartilage regeneration.

METHODS: Type II collagen was extracted from male and female porcine trachea, auricular, articular cartilage and cartilaginous fish through acid-pepsin digestion at 4°C. Collagen sponges were created via freeze-drying. Sponges were seeded with human adipose derived stem cells to assess chondro-inductive potential of collagen sponges.

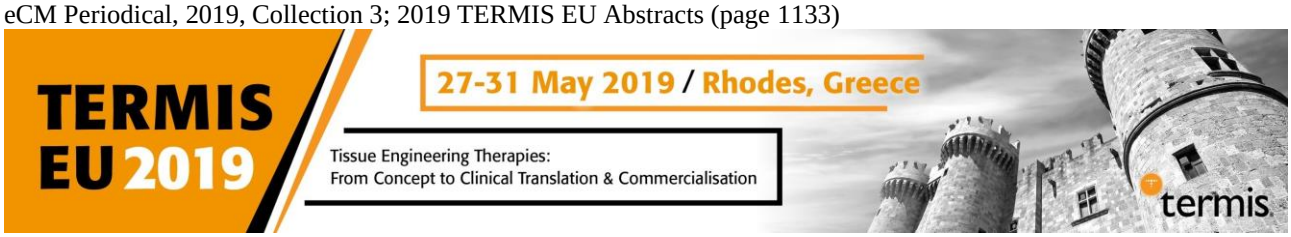
RESULTS & DISCUSSION: SDS-PAGE was performed to assess the purity of extracted collagen solution. In comparison with commercially available collagen types, SDS-PAGE confirmed the purity and molecular weight of collagen polypeptide chains of freshly isolated collagen from food waste. Previous studies demonstrated that higher denaturation temperature of collagen indicates higher thermal stability, which has been evaluated as a critical characteristic of collagen based medical devices. Cross-linker significantly increased thermal stability of collagen sponges in all groups, which means the crosslinking system that we used in this study helped to increase the denaturation temperature of collagen biomaterials. Collagen isolated from male and female articular cartilage exhibited higher resistance towards enzymatic degradation. All groups of collagen sponges support the proliferation and chondrogenic differentiation of human adipose derived stem cells.

CONCLUSIONS: In conclusion, collagen sponges support the proliferation and differentiation of human adipose derived stem cells to different extents.

ACKNOWLEDGEMENTS: Zhuning Wu is in receipt of a Teagasc Walsh Fellowship. This work forms part of the ReValueProtein Research Project (Grant Award No. 11/F/043) supported by the Department of Agriculture, Food and the Marine (DAFM) under the National Development Plan2007–2013 funded by the Irish Government. This work is also funded by Science Foundation Ireland (Grant Number: 13/RC/2073).

REFERENCES

[1] Mukaida T et al. Journal of orthopaedic science. 2005; 10(5), pp.521-528.



Optimal Isolated condition of adipose derived stromal vascular fraction for the development of automated systems

S. J. Lee[1], K. J. Kim[2], S. H. Moon[2], Y. H. Ryu[1], Y. N. Han[1], E. J. Kim[1], W. H. Jang[1], J. W. Rhie[2]
Presenting Author: Su Jin Lee, dltnwlsgod1004@hanmail.net
[1]Department of Biomedicine and Health Sciences, College of Medicine, The Catholic University of Korea [2]Department of Plastic and Reconstructive Surgery, College of Medicine, The Catholic University of Korea

INTRODUCTION: Adipose tissue has been reported to contain adult stem cells and several other cell types. Recently, adipose derived stromal vascular fraction (SVF) have been isolated from adipose tissue and applied in various research fields [1-3]**.** Among the different methods used to obtain stem cells, enzyme concentration and the duration of enzymatic treatment vary depending on the researcher; SVFs isolated conditions have not yet been optimally established. Therefore, we aimed to develop an automated system for the isolation of SVF.

METHODS: The yield and viability of SVF were evaluated by treating adipose tissue with 0.1% and 0.2% collagenase type I enzyme for 20, 40, and 60 min. Flow cytometry and colony forming units (CFUs) were analyzed for Adipose stem cells (ASCs) obtained by culturing the separated cells until passage 3. A cytokine array was performed to investigate the correlation between colony-forming ability and stemness.

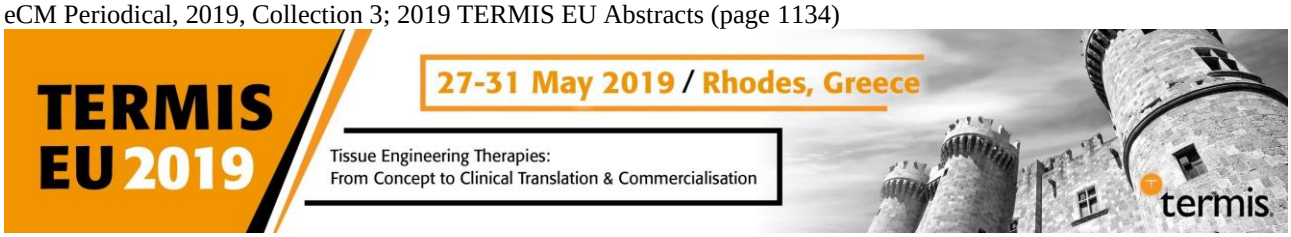
RESULTS & DISCUSSION: Enzyme reaction with a collagenase concentration of 0.1% was increased number of SVF compared with 0.2% collagenase. However, an increase in enzyme reaction time to 60 min significantly reduced cell survival compared with the 40 min treatment. The CFU values were also higher when the processing time was 40 min compared with the 60 min in cells cultured up to passage 3. In addition, the higher CFU groups exhibited increased expression of growth factors, such as interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1, than lower CFU groups.

CONCLUSIONS: These results will provide researchers with an efficient method for the ASC isolation and can be useful reference for development of automatic system.

ACKNOWLEDGEMENTS: This work was financially supported by National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017M3A9E2060428 and 2017R1A6A3A11030383) and by a Ministry of Trade Industry and Energy of Korea (10062127)

REFERENCES

[1] Wang JM et al. Exp Ther Med. 2017;13(3):1039-1043.
[2] Kilroy GE et al. J Cell Physiol. 2007;212(3):702-709.
[3] Yang HJ et al. Cells Tissues Organs. 2014;199(5-6):373-383



BMP-2 release from PLGA scaffolds fabricated by supercritical carbon dioxide and surface-selective laser sintering

S.A. Minaeva[1], E.N. Antonov[1], A.V. Vasilyev[2], T.B. Bukharova[2], V.K. Popov[1]
Presenting Author: Svetlana Minaeva, minaeva.svetlana@gmail.com
[1]Institute of Photonic Technologies FRC "Crystallography and Photonics" RAS, Moscow, Russia
[2]Research Centre for Medical Genetics, Moscow, Russia

INTRODUCTION: Bone morphogenetic protein 2 (BMP-2) is one of the most effective osteogenic inductors in bone tissue engineering. Encapsulation of BMP-2 into biocompatible and bioresorbable materials protect it from rapid degradation under the influence of the environment and provide its uniform controlled release into surrounding tissue in a given local area. The purpose of our study was the development of porous bioactive scaffolds based on polylactoglycolic acid and BMP-2 using the methods of supercritical fluid (SCF) polymer plasticization followed by its foaming and surface-selective laser sintering. Comparative studies of BMP-2 release kinetics was performed in physiological saline.

METHODS: Polylactoglycolic acid porous (ca. 60 vol. %) scaffolds (PDLG 7507 PURAC Biochem BV) containing 10 µg/ml BMP-2 (Acron Biotech, USA, cat. AK8356) were manufactured by the supercritical carbon dioxide plasticization followed by foaming method at 33±0,1°C. Part of the scaffolds was subjected to additional processing by surface-selective laser sintering (SSLS) [1]. Fabricated scaffolds have a disk-like shape with a diameter of 5 mm and a thickness of 2 mm. The release kinetics of BMP-2 was determined using enzyme-linked immunosorbent assay (ELISA) using a standard ELISA kit for determining the concentration of BMP-2 (R&D, USA) in physiological saline.

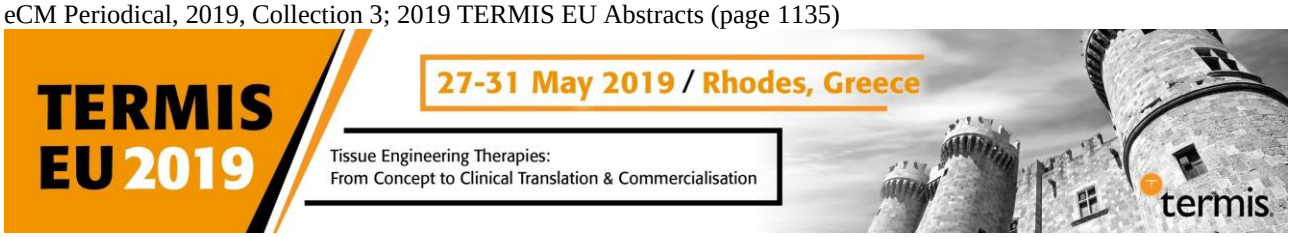
RESULTS & DISCUSSION: Scaffolds fabricated by SCF only provided a smooth release of BMP-2 over 15 days. During this time, almost 100% of the encapsulated protein was released from the polymer. Laser sintering promotes longer retention of BMP-2 in the scaffolds. The peak release was observed from 13 to 15 days.

CONCLUSIONS: SSLS allows to maintain the biological activity of the protein within the volume of sintered particles [2]. Scaffolds obtained with SSLS have a dense surface layer that provides delayed release of protein. The combination of SCF and SSLS scaffold manufacturing methods allows the formation of structures with a controlled release rate of biologically active substances.

ACKNOWLEDGEMENTS: This work was supported by the Ministry of Science and Higher Education within the State assignment FSRC «Crystallography and Photonics» RAS in part of supercritical fluid BMP-2 encapsulations and within the State assignment FSBI «Research Centre for Medical Genetics» in part of BMP-2 release research and by Russian Foundation for Basic Research (Grant No. 18-32-20184) in part of laser assisted scaffolds design.

REFERENCES

[1] Antonov E. N. et al. Laser Physics. 2006. 16(5):774-787
[2] Antonov E. N. et al. Adv mater. 2005; 17(3):327-330



Metabolic imaging of IPS cells during dermal differentiation

S. Rodimova[1], D. Reunov[1], E. Kalabusheva[2], E. Dashinimaev[2], A. Meleshina[1]
Presenting Author: Svetlana Rodimova, srodimova123@gmail.com
[1]Institute of biomedical technologies, Privolzhsky research medical university, Nizhny Novgorod, Russia [2]Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

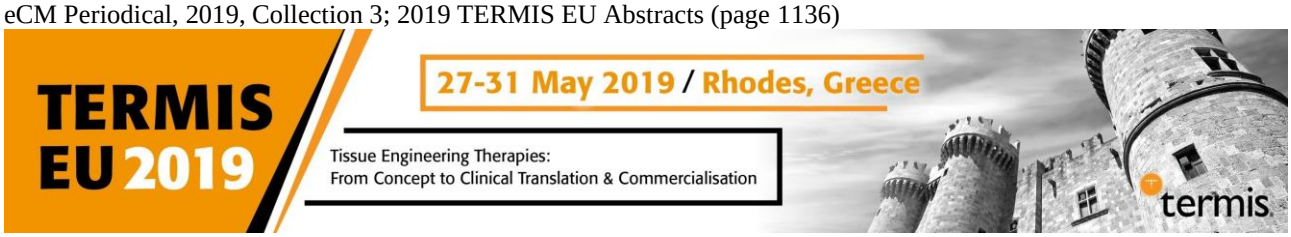
INTRODUCTION: The effective control of the stem cell metabolic status plays an important role in the determination of the certain stage of the differentiation, what is the actual problem of the cell biology and the regenerative medicine. In the process of the cell differentiation, the metabolic pathways are switched from the glycolysis to the oxidative phosphorylation. Presently, methods of the multiphoton microscopy with the fluorescent lifetime imaging actively introduced into biomedical researches. This method allows to evaluate the activity of cellular metabolic pathways based on the fluorescence lifetime and the contributions of free and bound forms of NAD(P)H during cell differentiation.

METHODS: In this study we evaluated the cellular metabolism of induced pluripotent stem (iPS) cells during dermal differentiation based on fluorescence lifetime of NAD(P)H using FLIM. IPS-KYOU line was purchased from ATCC cell Bank (USA). IPS-KYOU line were characterized using immunocytochemical analysis and quantitative real time RT-PCR in according to the expression of specific markers. This cell line was used as a base cell culture for obtaining directly differentiated dermal MSCs and keratinocyte precursors. iPS cells fluorescence imaging was performed using a confocal laser scanning microscopy LSM 880 (CarlZeiss, Germany) with FLIM mode. The excitation wavelength was set to 750 nm for cellular autofluorescence signals, with an emission signal range of 455 to 500 nm. The data were analyzed with the commercially available SPCImage software. The fluorescence lifetimes and their contributions (free and protein-bound forms of NAD(P)H: a_{free} NADH, a_{bound} NAD(P)H) for the areas of interest were calculated by finding the global minimum of the $\chi 2$ value for bi-exponential fittings.

RESULTS & DISCUSSION: During keratinocyte differentiation was shown a statistically significant increase in the contribution of bound NAD(P)H, indicating the rise of the oxidative phosphorylation contribution to the total cellular metabolism- a_{bound} (iPS) = 15.09±2.65 %; a_{bound} (keratinocyte precursors) = 21.02±1.34 %. However, in dermal MSCs derived from iPS no significant changes were observed in the contribution of the bound NAD(P)H - a_{bound} (dermal MSCs) = 15.71± 1.39 %, indicating the same glycolytic metabolism as in iPS.

CONCLUSIONS: The results extend the general understanding of functional changes in the process of iPS differentiation and open up new ways for the development of treatment strategies in regenerative medicine and stem cell therapy.

ACKNOWLEDGEMENTS: This work has been financially supported by Russian Science Foundation (grants No. 17-75-20178).



Multiphoton microscopy and FLIM analysis of liver structure and function during regeneration

S. Rodimova[1], D. Kuznetsova[1], N. Bobrov[2], D. Reunov[1], V. Zagainov[2], E. Zagaynova[1]
Presenting Author: Svetlana Rodimova, srodimova123@gmail.com
[1]Institute of biomedical technologies, Privolzhsky research medical university, Nizhny Novgorod, Russia [2]The Volga District Medical Centre of Federal Medical and Biological Agency, Nizhny Novgorod, Russia

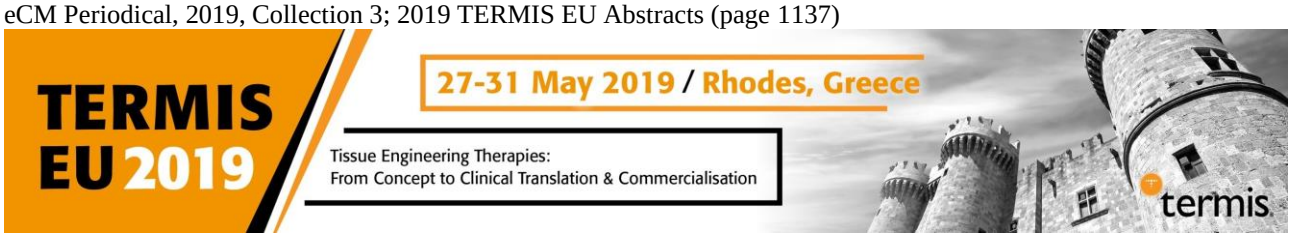
INTRODUCTION: It is a well-known fact that the liver has a high regenerative potential, and now an increasingly topical task is to study the mechanism of regeneration of liver tissue and search for methods of stimulation of this process. Traditional methods are not sufficient for accurate description of internal structure of heterogeneous cell populations and the dynamics of biological regenerative processes of the liver. Presently, methods of multiphoton microscopy with fluorescent lifetime imaging actively introduced into biomedical researches.

METHODS: It is highly informative, non-destructive and minimally invasive techniques, that allows to study a large number of processes occurring at the level of tissues and cells Also, these methods afford to analyze the molecular and cellular composition, as well as to assess the state of connective tissue fibers due to their ability to generate a second optical harmonic. In our work, we investigated metabolic changes and characteristics of collagen fibers in rat model of 30% and 70% hepatectomy at different stages of regeneration based on the fluorescence intensity level and the fluorescence lifetimes of metabolic cofactors (NAD(P)H, FAD) and second harmonic signal by the method of multiphoton microscopy with FLIM and SHG modes.

RESULTS & DISCUSSION: In this study, metabolic changes and formation of collagen fibers in rat model of liver resection were investigated based on fluorescence intensity level of metabolic cofactors (NAD(P)H, FAD) and second harmonic signal by multiphoton microscopy with FLIM and SHG modes. A separate analysis of NADH and NADPH were presented to assess the contribution of energy metabolism, as well as to evaluate the activity of lipogenesis in hepatocytes.

CONCLUSIONS: The obtained data can be used to develop new criteria for evaluation of metabolic and structural changes of hepatocytes aimed at personalized medicine in the future.

ACKNOWLEDGEMENTS: This work was supported by the Russian Science Foundation grant 19-15-00263.



The characterization of collagen, silk fibroin and chitosan based materials for biomedical applications

S. Grabska[1], A. Sionkowska[1,]
Presenting Author: Sylwia Grabska, sylwiagrabska91@gmail.com
[1]Department of Biopolymer and Cosmetic Chemistry, Faculty of Chemistry, Nicolaus Copernicus University in Toruń, Poland

INTRODUCTION: Natural compounds can be used to obtain biomaterials as scaffolds, hydrogels or films. Due to low physico-chemical properties of single biopolymers there is a need to modify these materials by another biopolymers addition [1]. The materials can be also modified by cross-linking agent addition. The blending of collagen with other polymers can lead to preparation of new materials suitable for biomedical applications [2]. Materials based on collagen (Coll), silk fibroin (SF) and chitosan (CTS) were tested as the potential forms for tissue engineering purposes.

METHODS: The aim of the study was to characterize scaffolds obtained by freeze-drying method from collagen, silk fibroin and chitosan mixture after the cross-linking by EDC/NHS addition. Materials were characterized by the FTIR-ATR spectroscopy, porosity and density measurements, swelling behavior and mechanical parameters measurements. Collagen was prepared in our laboratory from tail tendons of young rats. Silk fibroin was prepared from Bombyx mori cocoons in our laboratory following the method described by Kim et. Al. with slight modifications [3]. Chitosan was supplied by the company Sigma-Aldrich (Poznań, Poland). The biopolymers were mixed together in appropriate weight ratio. The biocompatible scaffolds were obtained by freeze-drying method.

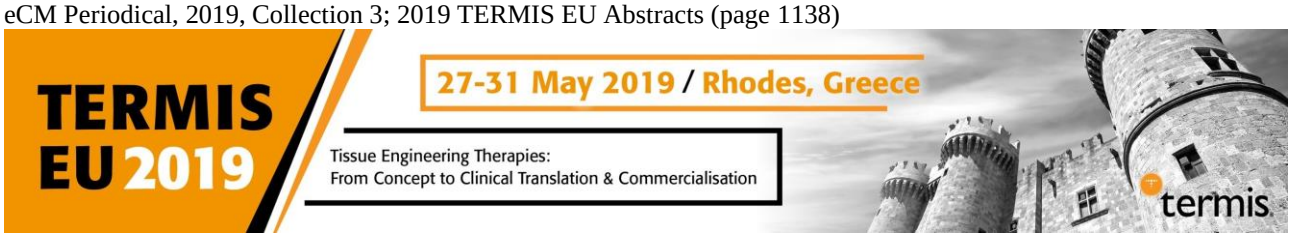
RESULTS & DISCUSSION: FTIR-ATR spectroscopy showed that between components of the blend there are interactions. Swelling tests were prepared. It was found that 3D composites made from collagen, silk fibroin and chitosan are hydrophilic with a high swelling ability. Cross-linking of such biopolymeric materials alters the swelling degree and porosity of materials. The cross-linking process has significant effect on the density of the materials. 3D materials based on Coll/SF/CTS are rigid and inflexible.

CONCLUSIONS: Strong interactions between two and three components in polymer blend can lead to new materials. The modification of physico-chemical properties can be a consequences of the strong interaction between the polymeric compounds. The properties of prepared materials depend on the mixture composition and content of the cross-linking agent.

ACKNOWLEDGEMENTS: Authors would like to thank for financial support from the PROM project from Polish National Agency for Academic Exchange.

REFERENCES

[1] Sionkowska A. Prog Polym. Sci. 2011; 36: 1254-1276.
[2] Kaczmarek B et al. Int J Biol Macromol. 2018; 107: 470-477.
[3] Kim UJ et al. Biomater. 2005; 26: 2775-2785.



Low-intensity LED irradiation as a therapeutic agent on the in vitro model of Parkinson's disease

P.Y. Bikmulina[1], N.V. Kosheleva[2,3], A.I. Shpichka[1], I.M. Zurina[1,3], T.M. Zharikova[1], P.S. Timashev[1,4,5], Y.A. Rochev[1,6]

Presenting Author: Tatiana Zharikova, zharikova.tm@gmail.com

[1]Institute for Regenerative Medicine, Sechenov First Moscow State Medical University, Moscow, Russia [2]Faculty of Biology, MV Lomonosov Moscow State University, Moscow, Russia [3]FSBSI Institute of General Pathology and Pathophysiology, Moscow, Russia [4]Federal Research Centre 'Crystallography and Photonics', Russian Academy of Sciences, Institute of Photonic Technologies, Moscow, Troitsk, Russia [5]Department of Polymers and Composites, N. N. Semenov Institute of Chemical Physics, Moscow, Russia [6]NUI Galway, Galway, Ireland

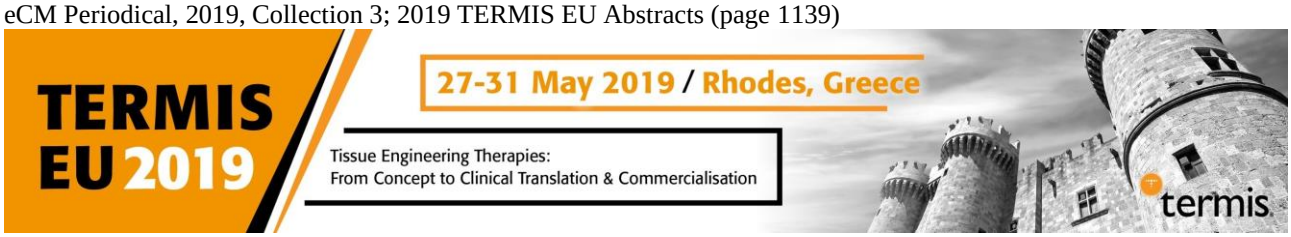
INTRODUCTION: Parkinson`s disease (PD) is a long-term neurodegenerative disorder that affects about 1% of people over the age of 60. Degeneration of dopaminergic neurons, associated with mitochondrial dysfunction, oxidative stress, and apoptotic cell death, has a leading role in PD pathophysiology. Etiology of PD is still not completely understood. For example, the use of pesticides, particularly rotenone (a cytotoxic agent that inhibits complex I of the electron transport chain of mitochondria) is a cause of sporadic PD in people living in countryside. Low-intensity LED irradiation (LILI) is widely used for therapeutic purposes to stimulate cell metabolism, proliferative activity, and viability. The main target of LILI in cells is mitochondria. Therefore, the mechanisms of LILI can be studied on in vitro models using cellular respiration inhibitors, such as rotenone. This work aimed to study the effects of LILI on rotenone-induced PD model in vitro.

METHODS: The monolayer culture of human neuroblastoma cell line Sk-N-BE(2) was used in the work. Growth culture medium consisted of DMEM/F12 and 10% FBS. Continuous LILI was performed with two modes of LED light 1) 633nm, 1200s, 22kJ/m2 and 2) 840nm, 600s, 21kJ/m2 2 hours before the culture was treated with the rotenone. Mitochondrial activity was suppressed by overnight culture exposure to rotenone (0,01-150μM). MTT and Alamar Blue assays were used to study cell viability. Apoptosis was detected by the Muse Annexin V and Dead Cell Assay Kit (Sigma, USA). Cells stained with Hoechst 33258 (Sigma, USA) and MitoTracker Green FM (ThermoFisher, USA) were monitored via live light time-lapse microscopy in CellInsight CX7 device (ThermoFisher, USA). Oxygen consumption rate (OCR) was measured by Seahorse assay.

RESULTS & DISCUSSION: Both LILI modes had a stimulating effect on the neuroblastoma cell line. The preliminary irradiation with 633 nm LEDs before rotenone treatment increased cell viability by 15% compared to non-irradiated culture. Irradiation at a wavelength of 840 nm affected a higher concentrations of toxin (half-lethal dose - 50 μM), increasing the cell viability by 20% on average. OCR in neuroblastoma cell line increased by 15% and 20% after irradiation with 633nm and 840nm LEDs, respectively. The irradiation of cells resulted in elevation of mitochondrial activity by 15%.

CONCLUSIONS: On the in vitro rotenone-induced PD model it was shown that LILI of impaired cells improved their viability and metabolic activity without cytotoxic effects on intact cells. LILI can find application as a therapeutic agent for PD.

ACKNOWLEDGMENTS: The study was supported by the Russian Science Foundation (15-15-00132, cell viability assays) and the Russian Foundation for Basic Research (17-02-01248, LED irradiation).



Bone marrow- versus adipose tissue-derived mesenchymal stem cells for the treatment of corneal failure in a rabbit model of limbal stem cell deficiency

T. Nieto-Miguel,[1,2] S. Galindo,[2,1] M. López-Paniagua,[2,1] JM. Herreras,[2,1,3] C. García-Vázquez,[2] E. Rey,[1,2] M. Calonge[2,1]
Presenting Author: Teresa Nieto-Miguel, tnietom@ioba.med.uva.es
[1]Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine, Carlos III National Institute of Health, Spain [2]IOBA (Institute of Applied Ophthalmobiology), University of Valladolid, Spain [3]Department of Ophthalmology, Clinic University Hospital, Spain

INTRODUCTION: Limbal stem cell deficiency (LSCD) occurs as a result of limbal epithelial stem cell destruction or dysfunction, leading to vision loss and chronic inflammation of the ocular surface. Our research group has recently demonstrated that transplantation of bone marrow-derived mesenchymal stem cells (BM-MSC) onto the ocular surface of patients suffering from LSCD can safely and effectively help repair the corneal epithelium [1]. However, adipose tissue-derived MSC (AT-MSC) are more accessible, more cost-effective and a safer source of MSC than bone marrow. The aim of this work was to compare the efficacy of BM-MSC versus AT-MSC for the treatment of corneal failure due to LSCD in rabbits.

METHODS: Eighteen New Zealand white rabbits were transplanted with 250,000 MSC on amniotic membrane (9 with BM-MSC; 9 with AT-MSC) 3 weeks after inducing them a LSCD [2]. Nine non-transplanted animals formed the control group. Conjunctival invasion, corneal neovascularization, opacification, and epithelial defects were weekly examined on a slit lamp. Histopathology analyses at the end of follow-up (11 weeks) evaluated the level of tissue damage and the presence of lymphocytes (as a sign of inflammation) and goblet cells (as a sign of conjunctivalization) in limbal and corneal areas.

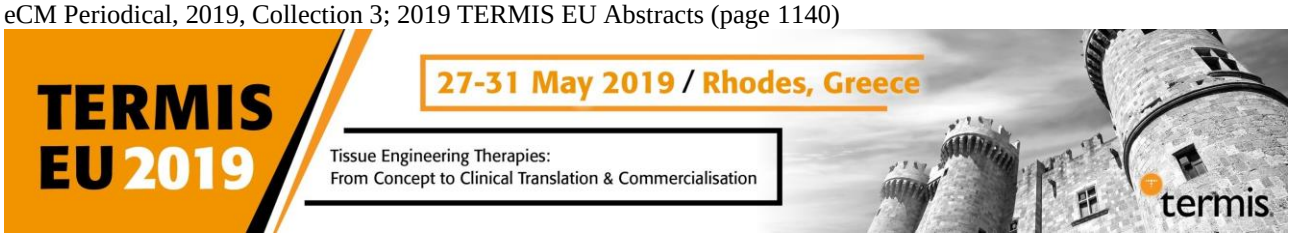
RESULTS & DISCUSSION: The BM-MSC-transplanted group showed less neovascularization and less corneal opacity at weeks 6-8, and 6-9, respectively than the untreated group. The AT-MSC-treated group had less conjunctival invasion and corneal opacity at weeks 6-8 and 6-10, respectively than the control group. There were no differences in the epithelial defects among the 3 groups. BM-MSC- and AT-MSC-transplanted groups showed higher number of corneal and limbal epithelial layers, less disorganization of the stroma, fewer inflammatory cells in the corneal stroma, and fewer goblet cells in the limbal or corneal epithelium than the untreated group.

CONCLUSIONS: Transplantation of BM-MSC and AT-MSC in a LSCD rabbit model reduced the development of corneal opacity, and partially restored the damaged limbal and corneal tissue structure. BM-MSC were superior in reducing the development of corneal neovascularization whereas AT-MSC better prevented conjunctival invasion. Both types of MSC seem valid alternatives for the treatment of LSCD.

ACKNOWLEDGEMENTS: Ministries of Economy and Competitiveness (SAF2015-63594-R MINECO/FEDER, EU) and of Science and Innovation, Spain (SAF2010-14900); Carlos III National Institute of Health, Spain (CIBER-BBN, CB06/01/003 MINECO/FEDER). Regional Center for Regenerative Medicine and Cell Therapy, Castilla y León, Spain.

REFERENCES

[1] Calonge M et al. Transl Res. 2019; doi: 10.1016/j.trsl.2018.11.003. (In Press)



Therapeutic ion loaded mesoporous nanoparticles for treating infections and biofilms in chronic skin wounds

T. E. Paterson1,2, K. Zheng3, P. Balasubramanian3, A. Bari4, S. Fiorilli4, A. J. Bullock2, A. R. Boccaccini3, C. Vitale-Brovarone4, S. MacNeil2, J. Shepherd1
Presenting Author: Thomas Paterson, t.paterson@sheffield.ac.uk
[1]School of Clinical Dentistry, University of Sheffield, Sheffield, UK [2]Kroto Research Institute, Department of Materials Science and Engineering, University Sheffield, Sheffield, UK [3]Institute of Biomaterials, University of Erlangen-Nuremberg, Erlangen, Germany [4]Department of Applied Science and Technology, Politecnico di Torino, Italy

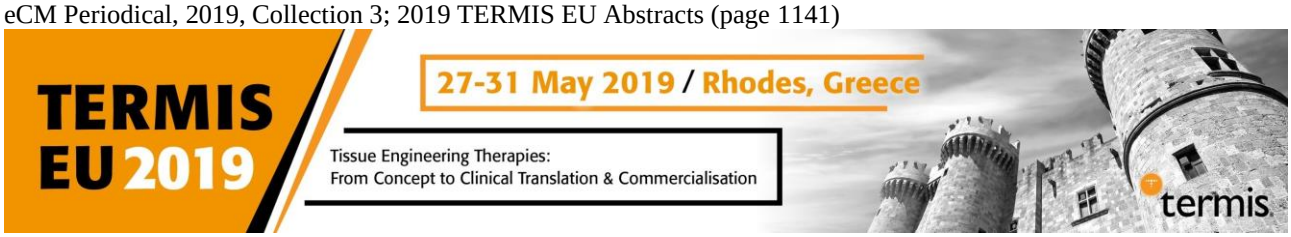
INTRODUCTION: Biofilms present within chronic skin wounds are difficult to treat and prevent wound healing. Silver and copper ions have been shown to be reliable antimicrobials. However, these antimicrobial agents are ineffective unless delivered to the site of infection. Mesoporous nanoparticles are one such delivery system that could act as a platform for delivering therapeutic ions. This project evaluates silver- and copper-containing mesoporous nanoparticles for their antimicrobial effectiveness, including testing them in a 3D tissue engineered skin infection model.

METHODS: Mesoporous glasses nanoparticles were manufactured using sol-gel methodology and then loaded with Ag+ and Cu2+. ICP was used to confirm ion loading. Monolayer fibroblast toxicity studies were conducted. Samples were tested using standard antimicrobial assays and tissue engineered infected skin models. The clinically relevant bacteria Staphylococcus aureus and Pseudomonas aeruginosa were used in the testing. SEM, viable colony count, PrestoBlue™, and crystal violet stain were used to assess biofilm viability. Biofilm formation and biofilm disruption were both examined.

RESULTS & DISCUSSION: Mesoporous nanoparticles successfully released ions and were non-toxic to mammalian cells at a concentration toxic to bacteria. Mammalian cells in tissue engineered skin were more resilient to silver releasing mesoporous nanoparticles than fibroblast monolayers. Silver nanoparticles were effective at preventing biofilm formation yet unable to disrupt established biofilms. Copper was equally effective against established and forming biofilms but was less effective than silver at preventing biofilm formation. In contrast to standard 2D assays, tissue engineered infection models showed the difficulty in treating established P.aeruginosa infections.

CONCLUSIONS: The mesoporous nanoparticles were non-toxic to mammalian cells at a concentration that exhibited antimicrobial properties. Tissue engineered skin was more resistant to treatment than simple 2D toxicity or antimicrobial tests. Depending on the bacteria microbiome, treating chronic infected wounds with these therapeutic containing mesoporous nanoparticles could reduce healing time by treating bacterial infections.

ACKNOWLEDGEMENTS: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 685872-MOZART (www.mozartproject.eu).



Biophysical characterization of poly (l-lactic) acid microcarriers with and without modification of chitosan and nanohydroxyapatite

L. Li[1], T. Liu[1], X. Li[1], K. Song[1*]
Presenting Author: Tianqing Liu, liutq@dlut.edu.cn
[1]State Key Laboratory of Fine Chemicals, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian 116024, China

INTRODUCTION: Nowadays, microcarriers are widely utilized in drug delivery, defect filling, and cell culture [1-2]. Also, many researchers focus on the combination of synthetic and natural polymers and bioactive ceramics to prepare composite biomaterials for tissue engineering and regeneration [3].

METHODS: Poly (l-lactic) acid (PLLA) was fully dissolved in dichloromethane at a ratio of 1:10 (W/V) prior to being added into gelatin aqueous solution. A certain amount of nanohydroxyapatite (nHA) was added to the polymer solution, followed by mechanical agitation for two hours with a lid on to reduce the volatilization of dichloromethane. Then the gelatin-poly (l-lactic) acid suspension was stirred for 5h. The liquid beads gradually solidified and precipitated at the bottom of the beaker with the volatilization of organic solvent. Finally, the wet microcarriers were collected and dried in a vacuum drying oven.

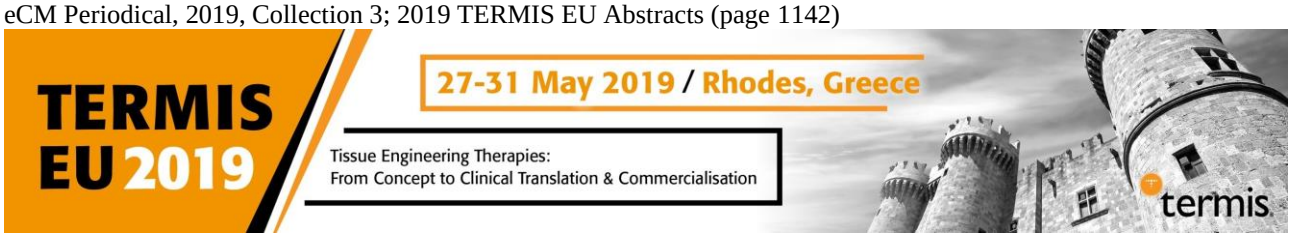
RESULTS & DISCUSSION: In this study, PLLA, PLLA/nHA and PLLA/nHA/Chitosan (PLLA/nHA/Ch) were prepared based on physical doping and surface modification. Their physicochemical properties and functional performances in MC3T3-E1 cell culture were compared. Statistical results showed that the average diameter of PLLA microcarriers was 291.9 ± 30.7 μm, and that of PLLA/nHA and PLLA/nHA/Ch microcarriers decreased to 275.7 ± 30.6 μm and 269.4 ± 26.3 μm, respectively. The surface roughness and protein adsorption of microcarriers were enhanced with the doping of nHA and coating of chitosan. The cells-carrier cultivation stated that the PLLA/nHA microcarriers had the greatest proliferation-promoting effect, while the PLLA/nHA/Ch microcarriers performed the strongest attachment with MC3T3-E1 cells. Besides, the cells on the PLLA/nHA/Ch microcarriers exhibited optimal osteogenic expression.

CONCLUSIONS: Generally, chitosan was found to improve microcarriers with superior characteristics in cell adhesion and differentiation, and nanohydroxyapatite was beneficial for microcarriers regarding sphericity and cells proliferation.

ACKNOWLEDGEMENTS: Financial support was received from the National Natural Science Foundation of China (31670978/21676041), and the Natural Science Foundation of Liaoning (20180510028).

REFERENCES

[1] Sart S et al. Biotechnol Progr. 2013; 29: 1354–1366
[2] Zhang Y et al. Acta Pharm Sin B. 2018; 8:34–50
[3] Shahin-Shamsabadi A et al. Mat Sci Eng C. 2018; 90: 280–288



Development of an alginate hydrogel for 3D printing

T. Robinson[1], J. M. Huyghe[2]
Presenting Author: Tiffany-Rae Robinson, 18035884@studentmail.ul.ie
[1]The Bernal Institute, University of Limerick, Limerick, Ireland

INTRODUCTION: Alginate is a naturally derived polysaccharide material with a relatively low cost, excellent biocompatibility, and optimal chelating abilities. Furthermore, it has the potential to be used as a bioink in 3D printing, and is currently a choice material in tissue engineering and regenerative medicine applications [1-3].

METHODS: Alginic acid sodium salt from brown algae with medium viscosity (Sigma Aldrich) was crosslinked with Ca^{2+} to form an interpenetrating network (IPN) hydrogel. Multiple formulation methods of the hydrogel were examined including variations of the Ca^{2+} crosslinking agent and polymerization methods. The first hydrogel was formed via use of $CaSO_4$ and was polymerized by UV light. The second hydrogel was formed via use of $CaCl_2$ and was polymerized by an incubation period of 6 hours at 37°C. Both $CaSO_4$ and $CaCl_2$ were able to form a hydrogel, though differences in the clarity and mechanical properties were observed.

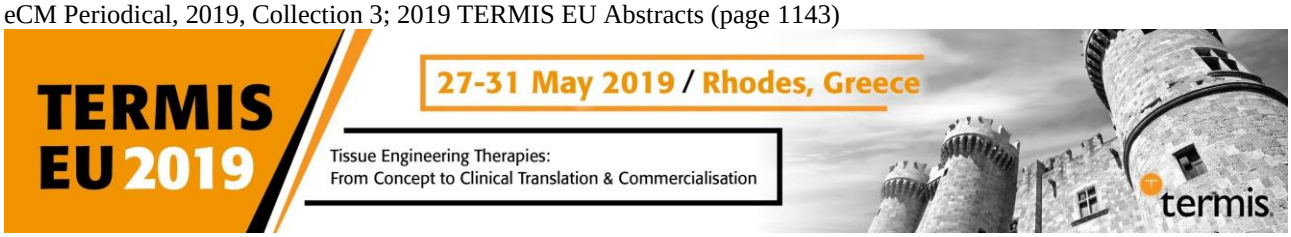
RESULTS & DISCUSSION: Qualitatively, the hydrogel formed via use of $CaCl_2$ was more uniform in structure and possessed better clarity than the hydrogel formed by $CaSO_4$. It is believed that this is due to the solubility of each compound in deionized water. Quantitatively, the swelling capabilities and compression strengths of both hydrogels were analyzed. The swelling capabilities were examined via weight analysis over a time range at: 5 minutes, 10 minutes, 20 minutes, 30 minutes, 1.5 hr, 1 day, 2 days, and 4 days. Additionally, after 4 days of swelling the gel underwent compression analysis. The $CaSO_4$ hydrogel had swelling capabilities of 144.6% and a compression strength of 12.4kN/m^2 when swollen. The $CaCl_2$ hydrogel is still currently undergoing testing.

CONCLUSIONS: Collectively, this data indicates that formulation of an IPN hydrogel synthesized from sodium alginate is possible via use of Ca^{2+} crosslinking. The mechanical properties, including viscosity, of the gel must be further examined and defined to transition this material towards becoming a bioink suitable for 3D printing by extrusion techniques.

ACKNOWLEDGEMENTS: The author would like to acknowledge Sally Regan and Laura Enright for their previous research contributions. The author would like to acknowledge Eanna Fennel and Emmet O'Reilly for use and guidance of lab equipment.

REFERENCES

[1] H. Li, Y. J. Tan, K. F. Leong, and L. Li, "3D Bioprinting of Highly Thixotropic Alginate / Methylcellulose Hydrogel with Strong Interface Bonding," 2017.
[2] K. Y. Lee and D. J. Mooney, "Alginate: Properties and Biomedical Applications," Prog. Polym. Sci., vol. 37, pp. 106–126, 2012.
[3] J. Sun and H. Tan, "Alginate-based biomaterials for regenerative medicine applications," Materials (Basel)., vol. 6, no. 4, pp. 1285–1309, 2013.



Leukocyte- and platelet-rich fibrin is an autologous biomaterial that improves survival and neurite outgrowth of dorsal root ganglion neurons

T. Vangansewinkel[1], P. Gervois[1], M. Lo Monaco[1], Y. Dillen[1], R.B. Driesen[1], G. Merckx[1], H. Kemps[1], A. Bronckaers[1], E. Wolfs[1], I. Lambrichts[1]
Presenting author: Tim Vangansewinkel, tim.vangansewinkel@uhasselt.be
[1] Morphology Research Group, Biomedical Research Institute, UHasselt, Diepenbeek, Belgium

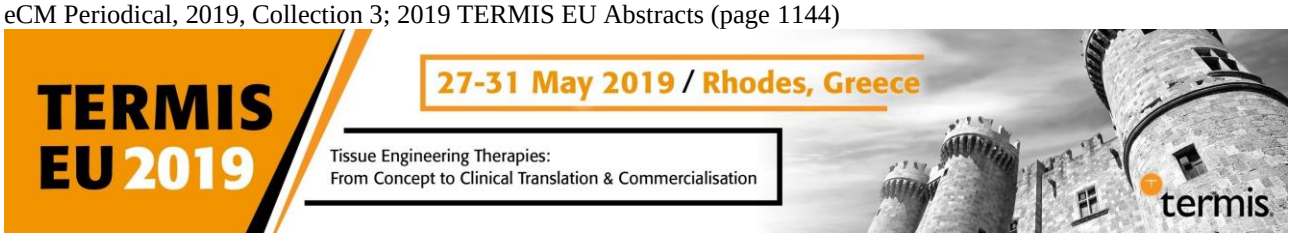
INTRODUCTION: Different strategies such as autografts and nerve conduits are employed in the clinic to rejoin nerve endings after injury, but these bridging therapies are suboptimal and have several limitations. New therapeutic strategies to establish repair of injured nerves are therefore needed. For this purpose, our research group aims to combine Schwann cell differentiated human dental pulp stem cells with biomaterials such as Leukocyte- and Platelet-Rich Fibrin (L-PRF). L-PRF is a second-generation platelet concentrate that is characterized by a dense fibrin network rich in platelets, leukocytes and many soluble mediators. Platelet concentrates are innovative tools in regenerative medicine and their success in tissue repair and regeneration mainly depends on the release of growth factors. Recently, we showed that L-PRF has potent angiogenic properties and the goal of this project was to investigate the neurotrophic potential of this biomaterial.

METHODS: L-PRF was prepared by collecting blood samples of healthy human donors in glass-coated tubes, which were immediately centrifuged at 2700 rpm for 12 min. The resulting L-PRF clots were either compressed to collect exudate or cultured for 24h, 48h, 96h or 144h to obtain conditioned medium (CM). The morphology of L-PRF was characterized via electron microscopy and by performing immunostainings for CD41, fibrin and fibronectin. Next, an antibody array and ELISA was performed on exudate and CM to analyze the release of growth factors by L-PRF. Finally, we examined the effect of L-PRF on cell survival and neurite outgrowth by culturing rat DRG neurons under serum deprived conditions, and stimulating them with various concentrations of exudate and CM.

RESULTS & DISCUSSION: L-PRF consists out of two definable domains: a fibrin-rich zone that contains clusters of activated platelets and a leukocyte-rich area. It is a porous scaffold formed by fibrin fibers that also colocalize with fibronectin. Secretome analysis revealed that L-PRF contains many growth factors such as nerve growth factor, vascular endothelial growth factor, platelet-derived growth factor, and brain-derived neurotrophic factor, which are slowly released from the clot ($n = 4$-6). Moreover, neurite outgrowth and survival of DRG neurons was significantly increased by L-PRF CM in a dose-dependent manner ($n = 6$, p value < 0.01).

CONCLUSIONS: L-PRF slowly releases growth factors that support neuronal cell survival and stimulate neurite outgrowth. L-PRF is an autologous biomaterial that has several advantages in terms of biocompatibility and safety. It can be easily obtained from the patient's own blood which makes it ideal in clinical applications. Our findings indicate that L-PRF may be suitable as a scaffold for nerve repair but this will be evaluated in future experiments.

ACKNOWLEDGEMENTS: Financial support was received from FWO Flanders 12U7718N, G029112N, 1134717N, 1508015N, G026112N & G0C1916N; BOF grants BOF16DOC06 & BOF16 OCNA02; and FSR-confin Uhasselt-UNamur.



Visible light crosslinking of RGD modified hyaluronan

T. Bobula[1], P. Boháčová[1], L. Kovářová[1], H. Vágnerová[1], V. Hrobař[1], S. Karel[1], M. Pravda[1], V. Velebný[1]
Presenting Author: Tomas Bobula, bobula@contipro.com
[1]Contipro a.s., Dolni Dobrouc, 401, Dolni Dobrouc, Czechia

INTRODUCTION: Photochemical crosslinking is advantageous due to temporal and spatial control over the gelation [1]. These advantages allow to prepare size and shape structured scaffolds using light processing techniques [2]. Hyaluronan (HA) is an important structural component of ECM. Therefore it is a suitable scaffolding material. But native HA does not form a gel nor support cell adhesion. Therefore it requires chemical modification according to the special demands of a particular application [3]. In this work we prepared cell-adhesive and covalently crosslinked hydrogels of HA suitable for advanced tissue engineering applications.

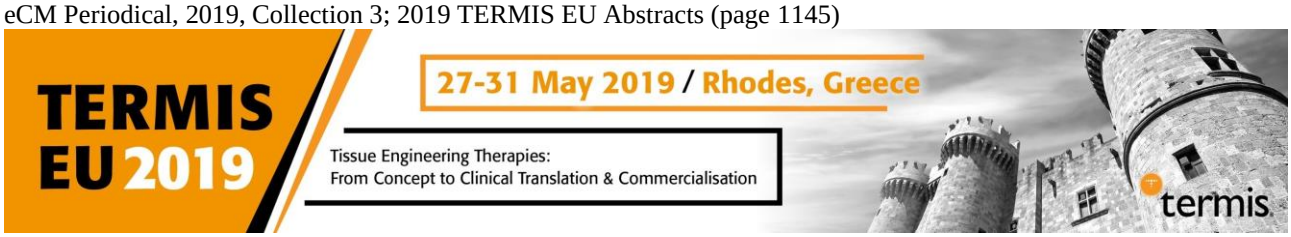
METHODS: Hydroxyphenyl RGD oligopeptides were synthesized by solid phase synthesis and conjugated with oxidized HA via reductive amination. The structural characterization of the derivatives was elucidated by NMR and Mw by Secmalls. The hydrogels were prepared from the precursor PBS solutions (1-3% w/v) with riboflavin as a photosensitizer using LED source (1.3-26.3 W/cm^2, 445 nm). Viscoelastic properties of the hydrogels were analyzed by AR-G2 rheometer using strain sweep mode. The swelling ratio of the samples was analyzed in PBS at r.t. Cell adhesion (3T3 fibroblasts, 20000 cells/well) was monitored microscopically and evaluated by ATP test.

RESULTS & DISCUSSION: Two adhesive derivatives of HA (RGD, RGD-Ahx) with DS = 4.5% and Mw = 396 kDa, 370 kDa were synthesized. They differed by the steric accessibility of a crosslinkable hydroxyphenyl group, which was increased in RGD-Ahx by the introduction of 6-aminohexanoic acid spacer. Both derivatives were subjected to photocrosslinking and the effect of material and process parameters on the gel properties was examined. The sterically less hindered RGD-Ahx provided 60% stiffer (G' = 160 Pa) and 20% less swelled hydrogels (Q_e = 80%). The optimal crosslinking conditions in relation to viscoelastic and sorption properties were determined: riboflavin conc. (8.7 µg/ml), polymer conc. (10 mg/ml), light intensity (13.2 W/cm^2) and irradiation time (60 s). 2D cell assays clearly showed the difference in adhesion between both RGD derivatives and a negative control without RGD sequence.

CONCLUSIONS: This work showed efficient and light controllable preparation of the functional HA scaffolds.

REFERENCES

[1] Bobula T et al. Carbohydr Polym. 2017; 161:277-285
[2] Loebel C et al. Biomacromolecules. 2015;16(9):2624-2631
[3] Zaviskova et al. J Biomed Mater Res A. 2018;106A:1129-1141



Regulation of spheroid cavitation in the in vitro cultivation of salivary gland progenitor cells

H.-W. Lee [1], Y.-C. Hsiao [2], T.-H. Young [1], T.-L. Yang [3]*
Presenting Author: Tsung-Lin Yang, yangtl@ntu.edu.tw
[1]Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei, Taiwan [2]Department of Ophthalmology, Zhongxing Branch, Taipei City Hospital, Taipei, Taiwan [3]Department of Otolaryngology, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan

INTRODUCTION: Dysfunctional salivary glands (SGs) caused by diseases or therapeutics may lead to serious clinical illness related to xerostomia.[1] Cell therapy based on autologous progenitor cells are thus regarded as efficient and feasible solution. When SG progenitor cells are prepared to form salispheres, the spheroid-like structures, cavitation occurs during in vitro cultivation, which hampers their stemness. The study aims to explore the ways of regulation spheroid cavitation formation.

METHODS: The murine submandibular glands (SMGs) were retrieved from the adult mice, and prepared for salishpere formation.[2] Fabrication of chitin biomaterials in distinct forms was performed as the methods previously reported.[3] The change of salisphere phenotypes and the properties of cellular organization, stemness, and differentiation potentials were analyzed.

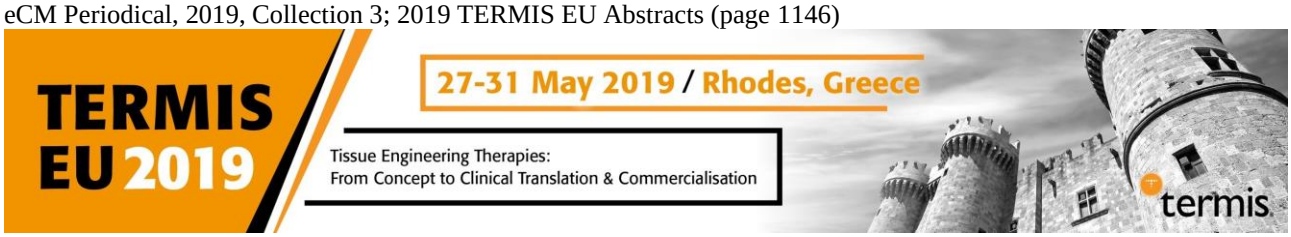
RESULTS & DISCUSSION: When the salispheres were cultured in the environment fabricated with chitin biomaterials, their numbers and the sizes increased. However, the incidence of cavitation was not affected. The effect was facilitated by high concentrations of chitin biomaterials, and fabricated as hydrogels. The salispheres were regulated in either intercellular interaction, or polarization to maintain the spheroid structure. The stemness properties of culture salispheres were preserved as well by showing characteristic features. The results demonstrated that the phenomena of spheroid cavitation of the salivary gland progenitor cells could be regulated by fabricated biomaterial environments, which helped to maintain the original properties of salispheres during in vitro preparation.

CONCLUSIONS: The data presented in the current study confirmed the advantages of applying biomaterial-fabricated culture environment to regulate the spheroid cavitation of salispheres during in vitro preparation.

ACKNOWLEDGEMENTS: The authors thank the MOST and the NTUH for their financial support.

REFERENCES

[1] Vissink A et al. Crit Rev Oral Biol Med. 2003;14(3):199-212.
[2] Lombaert IM et al. PloS one. 2008;3(4):e2063.
[3] Yang TL et al. Biomed Eng-App Bas C. 2008;20(287-96.



Hydrothermal microwave synthesis and characterization of hydroxyapatite nanoparticles for biomedical applications

A. Chodara[1,2], U. Szałaj[1,2], S. Kuśnieruk[1], J. Wojnarowicz[1], T. Chudoba[1], W. Łojkowski[1]
Presenting Author: Urszula Szałaj, u.szalaj@labnano.pl
[1]Laboratory of Nanostructures, Institute of High Pressure Physics, Polish Academy of Sciences, Warsaw, Poland, [2]Faculty of Materials Engineering, Warsaw University of Technology, Warsaw, Poland

INTRODUCTION: Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) is an inorganic component of bones and teeth. Hydroxyapatite possesses exceptional biocompatibility and bioactivity properties with respect to bone cells and tissues, probably due to its similarity with the hard tissues of the body. Nowadays hydroxyapatite is one of the most often applied bio-nanomaterials, e.g. in bone implants, scaffold layers, drug delivery agent, dental materials. Various processes for the preparation of HAP powders have been developed in the past [1-2].

METHODS: Hydroxyapatite nanopowder were synthesized by hydrothermal synthesis using microwave reactor MSS2 (Microwave Solvothermal Synthesis). The starting materials include pharmaceutical grade substrates: calcium hydroxide $Ca(OH)_2$ and orthophosphoric acid H_3PO_4 as substrates to obtain ceramic nanoparticles.

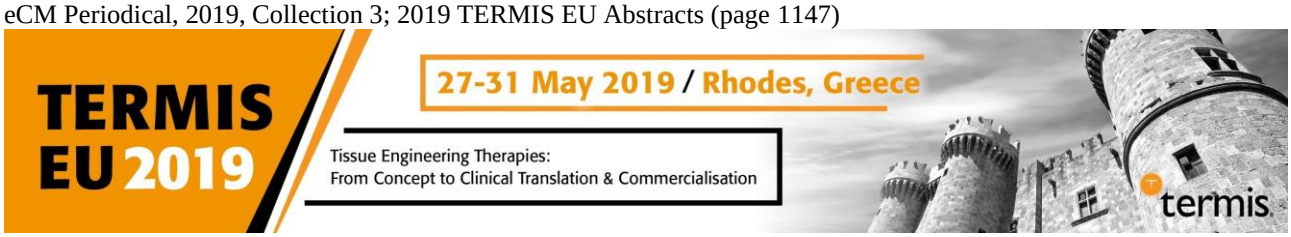
RESULTS & DISCUSSION: Obtained nanoparticles have average grain size in range of 8 - 45 nm. Thanks to a wide variety of grain size crystallinity it can be used in different application depending on desired resorption time of hydroxyapatite. The water is the single byproduct. Nanopowder has been characterized by several methods: X-ray diffraction (Phase Purity), SEM (morphology), BET (Specific Surface Area) and helium pycnometry (Skeleton Density).

CONCLUSIONS: Microwave synthesis allows easily and precisely control the grain size of nanoparticles. The mean grain size was controlled by synthesis conditions, such as time, pressure and temperature. Obtained hydroxyapatite is similar to the bioapatite.

ACKNOWLEDGEMENTS: This work was carried out in the Nanoligabond - Artificial tendons and ligaments fixation to bone tissue using nanotechnology approach - project financed by the European Regional Development Fund.

REFERENCES

[1] Kuśnieruk S et al. Beilstein J. Nanotechnol. 2016; 26(1):1586-1601
[2] Szczes A. et al. Advances in Colloid and Interface Science 2017;249:321-330



Novel approach for quantification of pore-size for scaffolds (>16 cm2) using ImageJ software for quality control

V. Sharma[1], D. Moulding[2], E. García-Gareta[1], L. Hook[3]
Presenting Author: Vaibhav Sharma, sharmav@raft.ac.uk
[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood, HA6 2RN, United Kingdom [2]UCL Great Ormond Street Institute of Child Health (ICH), 30 Guilford Street, London WC1N 1EH [3]Smart Matrix Ltd, Leopold Muller Building, Mount Vernon Hospital, Northwood, HA6 2RN, United Kingdom

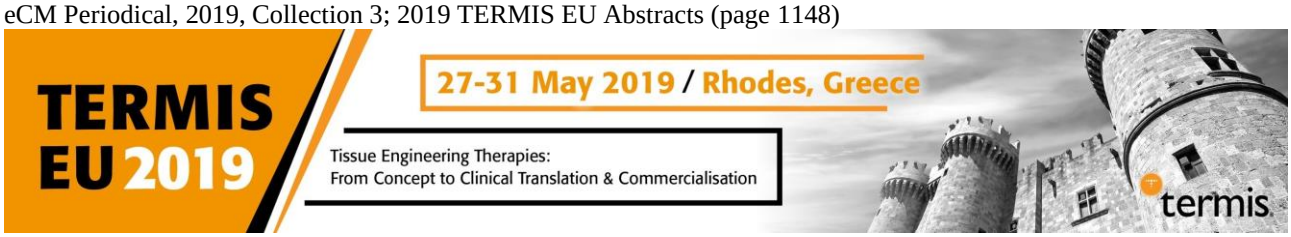
INTRODUCTION: With the advancement of digitalization and computer programming, image processing has become the latest tool to understand and quantify pore size, pore size distribution, porosity and microstructure of bio-aggregates in tissue-engineered scaffolds. Techniques available to analyse the microstructure of scaffolds include optical light microscopy, scanning electron microscopy, X-ray spectroscopy and field emission scanning electron microscopy. To quantify these micro-structures post microscopic analysis very challenging. Different image processing softwares like MATLAB, ICY, Avizo, Image Pro are available however, these programes are widely used to quantify pores over small sample areas with homogenous pore structure. The analysis becomes difficult when the size of the scaffolds increases (>16 cm2) and if the pore distribution is gradient.

METHODS: In this study, we present a method using ImageJ software to quantify pore size distribution of porous scaffolds with gradient porosity distributed over an area of 1 – 100 cm^2 by utilising laser scanning confocal microscopy images.

RESULTS & DISCUSSION: A macro was created in ImageJ to test this method. A plugin called tubeness is used to perform the analysis. After tubeness processing to identify pore boundaries, images are thresholded then skeletonized. A watershed is run to separate overlapping pores, before measurement of pore area and shape descriptors. Using this method, pore distribution of a fibrin-alginate scaffold (49 cm2 > 1x108 pixels) was quantified within three minutes on a standard PC and measured over 227,000 pores.

CONCLUSIONS: Such tools offer the potential of repeatability, accuracy and effectiveness in data quantification which will be valuable for quality control during scaffold manufacturing processes.

ACKNOWLEDGEMENTS: This work was supported by the Restoration of Appearance and Function Trust (RAFT, UK, registered charity number 299811) and Smart Matrix Limited.



Microscopic challenges to understand structural characteristics of protein based scaffolds for research and translational applications

V. Sharma[1], D. Moulding[2], N. Kohli[1], L. Hook[3], E. García-Gareta[1]
Presenting Author: Vaibhav Sharma, sharmav@raft.ac.uk
[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood, HA6 2RN, United Kingdom [2]UCL Great Ormond Street Institute of Child Health (ICH), 30 Guilford Street, London WC1N 1EH [3]Smart Matrix Ltd, Leopold Muller Building, Mount Vernon Hospital, Northwood, HA6 2RN, United Kingdom

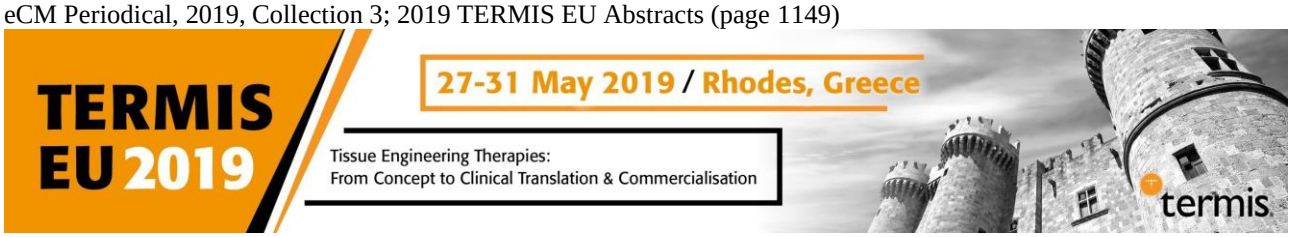
INTRODUCTION: To successfully commercialize tissue engineered scaffolds it is essential to have a complete understanding about the specifications of the product. Scaffolds are extensively used as structural support to boost the function of damaged tissue in the field of tissue engineering and regenerative medicine (TERM). The complex structures and material properties result in distinct challenges with regard to analyzing and assessing the behavior of these materials. Imaging technologies have been widely used as a central tool in the field of TERM. Conventional methods such as histological and immunohistochemical techniques have been used to accumulate data on the engineered scaffolds but have failed to provide accurate information since they are invasive and can be used only on small samples rather than the entire scaffold. Imaging techniques should maintain the natural state of the material, should be quantitative, and allow three-dimensional (3D) analysis.

METHODS: To compare the structural features of a porous 3D 10*10 cm fibrin-alginate dermal scaffold different techniques ranging from scanning electron microscopy, atomic force microscopy, laser scanning confocal microscopy (LSCM), microCT, two photon microscopy, wide field microscopy and light microscopy were used.

RESULTS & DISCUSSION: Our results suggest that apart from LSCM, all the other techniques either altered the structure of the scaffold or could only visualize a small area and were time consuming. LSCM visualized the entire area of the scaffold (10*10 cm) in its natural state within 90 minutes at a high resolution, collecting multiple images in digital form from serial sections and finally representing the 3D structure of the scaffold.

CONCLUSIONS: This study discusses the difficulties involved in imaging 3D porous scaffolds and highlights the advantages and validity of LSCM.

ACKNOWLEDGEMENTS: This work was supported by the Restoration of Appearance and Function Trust (RAFT, UK, registered charity number 299811) and Smart Matrix Limited.



Silk fibroin and argentine clays for bone reconstruction:a novel hybrid material for bones

E. Bertoli[1,2], V.E. Bosio[2]
Presenting Author: Valeria E. Bosio, vbosio@gmail.com
[1]Theroretical and Applied Physico-Chemical Research Institute (INIFTA), National University of La Plata & CCT-CONICET La Plata, Bs. As., Argentina [2]Biomaterials in Tissue Engineering Lab (BIOMIT), CINDEFI, University of La Plata & CCT-CONICET La Plata, Bs. As., Argentina

INTRODUCTION: Reconstructed Silk Fibroin (SF) from Bombyx mori is a biopolymer with biodegradability and biocompatibility for long-term medical implants. The versatility of this material for different structures and the possibility to be degraded with the time shows an advantage to rebuild damaged bones by Tisse Engineering (TE). However, a biomimicry of natural 3D bone structure made with silk needs to be mechanically reinforced. Herein, we ventured to developing a hybrid material with Montmorilonite (MMT), a natural clay from argentine environments, and SF, assessing the influence of these clays in the already known silk porous structures.

METHODS: Silk porous sponges were prepared by salt-leaching method following previously reported protocol [1] with some modifications. NaCl salt was sieved with metal mesh to obtain particle size distributions between 500 and 590 µm and added into SF aqueous solution (with or without Polyvinyl Alcohol (PVA), Alendronate (Alen) or MMT at a 2:1 (w/v) ratio), in tube-shaped containers. Deposition and inclusion methods were used to add MMT to the SF scaffolds. After that, scaffolds were drying at room temperature for 2 hs without stirring before their stored. Characterization of the new materials was carried out by SEM, FTIR, TGA, DSC, Compression resistance analysis & Encapsulation of deformable liposomes loaded with fluorescein.

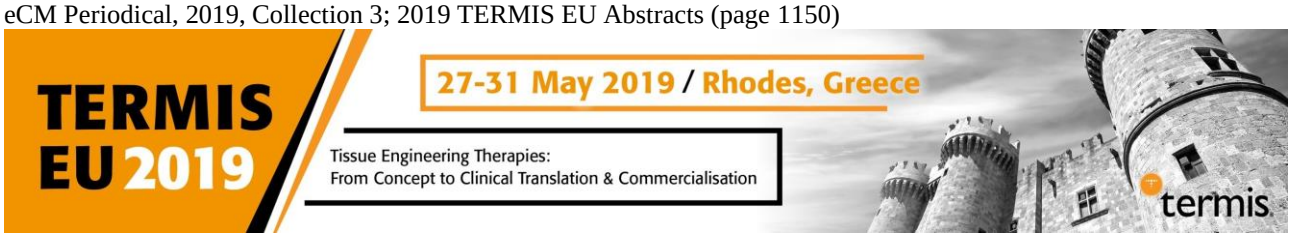
RESULTS & DISCUSSION: All the SF-based scaffolds containing PVA, Alen or MMT presented significant increase ($p < 0.001$) of compression resistance with better results when MMT was present. FT-IR, TGA/DSC analysis suggest interactions between PVA and MMT with the SF correlated with a significant increase in the thermal stability of the hybrid materials compared with SF scaffolds. To evaluate the capacity of the hybrid scaffolds of loading/release cargo molecules during bone tissue regeneration process, deformable liposomes with fluorescein as model drug were included and studied in the systems, showing the capability of these materials to load and release drugs without loosing the structure and mechanical properties.

CONCLUSIONS: The results illustrate the beneficial effect of argentine natural MMT to enhance the properties of SF porous materials to be used as bone scaffolds for TE.

ACKNOWLEDGEMENTS: Financial support was received from CONICET and ANCyT.

REFERENCES

[1] Bosio V.E. et al. J Mater Chem B. 2017; 5:1227-35.



Ovine amniotic epithelial cells transcriptome portrait

V. Di Lollo[12], M. Orsini[2], M. Ancora[2], C. Cammà[2], N. Bernabó[1], A. Canciello[1], V. Russo[1], M. Mattioli[1], B. Barboni[1]
Presenting Author: Valeria Di Lollo, vdilollo@unite.it
[1]Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy

INTRODUCTION: Amniotic Epithelial Cells (AEC) represent an emerging source in stem cells. However, AEC regenerative potential can be largely affected by the in vitro amplification. In order to standardize the AECs-based regenerative protocols, an advanced NGS approach of ovine cell characterization has been used to compare the transcriptome of 3 passages amplified oAEC obtained under standardized conditions (CTR) or by adding progesterone (P_4) in order to preserve the epithelial native phenotype during expansion.

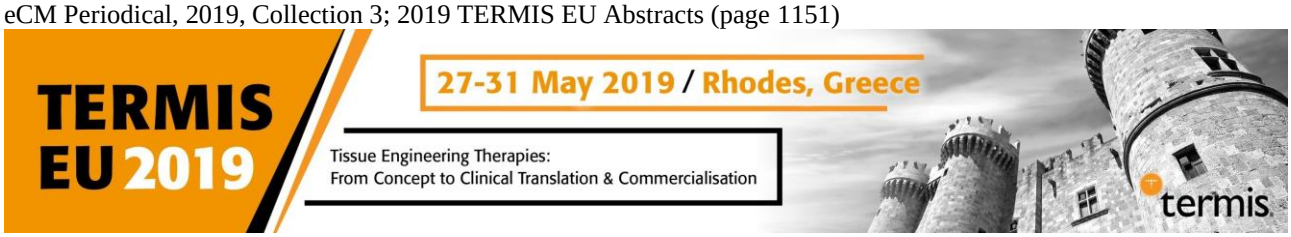
METHODS: The transcriptome profile has been performed by RNA sequencing (NextSeq500 Illumina platform). Differentially Expressed Genes (DEGs), identified using Tophat2-Cufflink2 pipeline [2], were by in house developed scripts to obtain enriched Gene Ontology (GO) terms and pathways.

RESULTS & DISCUSSION: The transcriptional landscape profiles showed 495 and 753 DEGs in CTR and P4, respectively, belonging to 3 main enriched GO-modules. In order to identify the mechanisms controlling biochemical pathways, we firstly derived a gene-gene network from DEGs. The networks were analyzed to detect both local and general hubs, which represent most connected nodes. They were compared to the bottleneck nodes that play a key role in controlling information throughout the networks. Integrating expression levels into the so obtained controlling structure (hubs, bottlenecks, DEGs), we identified a pattern of genes (listed in Figure1) characterizing the two oAECs transcriptomes.

CONCLUSIONS: For the first time, we described the whole transcriptome portrait of oAECs by using RNA-seq. The results clearly defined most biological processes and molecular pathways having a key-role in the two morphologically different oAECs. We, also, provided useful information to understand essential mechanisms involved in tissue regeneration. This approach can suggest a research framework in sheep, considered a consolidated model organism in regenerative medicine [3] filling lacking of information about the ovine genome and gene sequences.

REFERENCES

[1] Canciello A. et al. Sci.Rep.2017; 7: 3761
[2] Trapnell C. et al. Nat.Prot.2012; 7: 562-578
[3] Barboni B. et al. Placenta 2018.



In vitro expansion of mesenchymal stem/stromal cells using media supplemented with unfractionated heparin-free platelet lysate

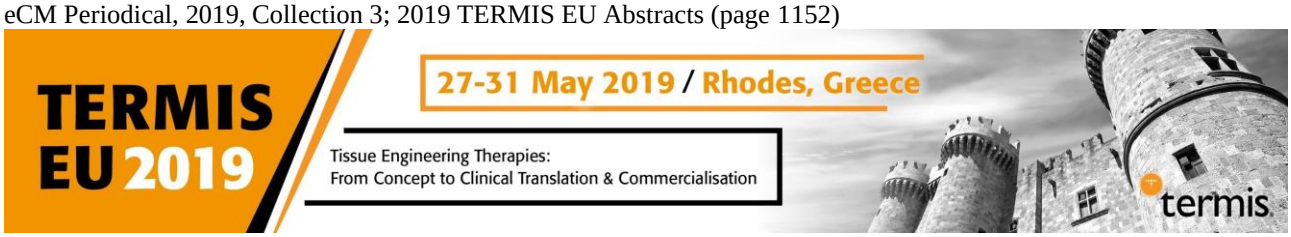
V. Alonso-Camino[1], B. Clarke[1], J. Nielsen[1], L. Smith[1], J. Vanderwielen[1], W. Mirsch[1]
Presenting Author: Vanesa Alonso-Camino, alonso-camino.vanesa@millcreekls.com
[1]Mill Creek Life Sciences, LLC, Rochester, Minnesota, USA

INTRODUCTION: Human platelet lysate (hPL) arose as a xenogeneic-free alternative to FBS to be used as a supplement for expansion of human mesenchymal Stem Cells (MSCs) used in clinical applications. The use of the first generation Hpl involves the addition of heparin to the cell culture media to prevent clotting. Heparin is purified and refined from swine according to cGMP methods and is one of the most widely used drugs in humans. hPL with heparin (PLTMax®) has been usedall over the world for more than 30 clinical trials. There has been no overt requirement for removal of heparin. However; some quality systems have requested its removal or replacement from cell culture processes in an effort to remove all xenogeneic components.

METHODS: To eliminate the need for heparin addition, Mill Creek Life Sciences developed PLTGold®, a step forward in the evolution of hPL. PLTGold® contains all the growth factors and proteins necessary for cell growth, but with reduced turbidity and no clot formation. Adipose derived and bone marrow derived mesenchymal stem cells (MSCs) were grown in media supplemented with either PLTMax® or PLTGold®. Growth kinetics, phenotype and capacity to undergo differentiation were compared for cells grown in each medium.

RESULTS & DISCUSSION: The chemical (pH and Osmolality) and biochemical analysis (Total protein content) performed for PLTGold® showed values comparable to those obtained for PLTMax®. MSCs grown in media supplemented with PLTGold® maintained the characteristic healthy MSC phenotype, as observed for cells grown in media supplemented with PLTMax®. The analysis of growth factors in PLTGold® revealed values that fell within the reference range established for PLTMax®. Real time imaging of cell growth using PLTGold® as a media supplement to grow adipose-derived and bone marrow derived MSCs showed increased cell growth kinetics (reduced cell doubling time) compared to cells grown in media supplemented with FBS or Human AB Serum. The performance of PLTGold® was comparable to the original hPL PLTMax®. After long term expansion of adipose derived and bone marrow derived MSCs in media supplemented with PLTGold®, both types of cells still maintained multipotency, with capacity to undergo adipogenesis, chondrogenesis and osteogenesis.

CONCLUSIONS: We report here PLTGold®, a new Human Platelet Lysate with the following characteristics: Xenogeneic free; Heparin-free; Unfractionated; Clot-free; Contains all the growth factors present in PLTMax®; Performance standards are similar or better than the original hPL PLTMax®. These characteristics make PLTGold® an ideal candidate to grow MSCs under cGMP conditions for use in the clinic.



In vitro expansion of human primary endothelial cells for clinical use using PLTGold® human platelet lysate

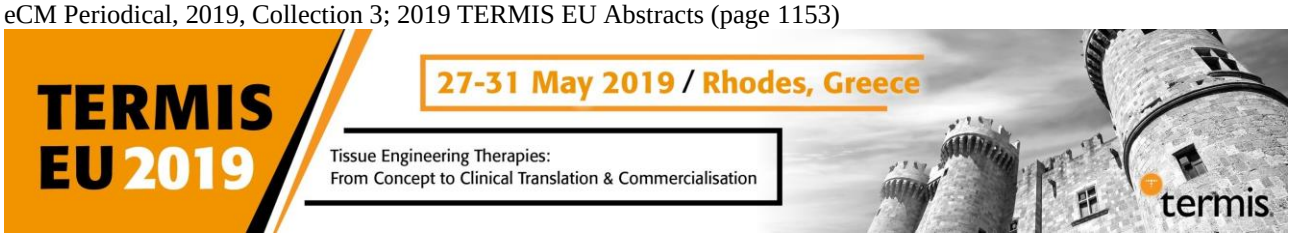
V. Alonso-Camino[1,2], W. Mirsch[1]
Presenting Author: Vanesa Alonso-Camino, alonso-camino.vanesa@millcreekls.com
[1]Mill Creek Life Sciences, LLC, Rochester, Minnesota, USA

INTRODUCTION: Stem/progenitor cell based therapy is a leading strategy in regenerative medicine as reflected by the large and ever growing number of clinical trials indexed. The prospective use of exogenous endothelial cells to promote tissue repair is gaining attention in particularly in applications of regenerative medicine in which vasculogenesis plays a critical role. Some examples include large area wound healing, repair of the ischemic heart, vascular transplant and organ recellularization. In addition, as cells therapy progresses, combination cell therapies will likely play a bigger and more important role as tissue and organ engineering demonstrate more promise. To date, media available in the market to grow endothelial progenitor cells or endothelial primary cells are not GMP compliant or contain xenogeneic elements such as fetal bovine serum (FBS) or even bovine brain extract (BBE). However, the use of animal serum containing products to grow cells that will be used for clinical applications involves a series of risk factors ranging from immunogenic responses against exogenous antigens to the transmission of diseases. For this reason, there is a lack of available resources to support the use of endothelial cells in translational medicine.

METHODS: To determine if we could obtain a cGMP complete medium to grow endothelial cells, we supplemented EndoGo™ XF Medium (Biological Industries) with 5% PLTMax® or 5% PLTGold®. This combination was used to grow human umbilical vein endothelial cells (HUVEC) in comparison with the main product available in the market to grow endothelial cells. This competitor medium contains 2% Fetal Bovine Serum (FBS) and Bovine Brain Extract (BBE).

RESULTS & DISCUSSION: HUVEC grew rapidly in the EndoGo™ XF + PLTMax® combination (with an average doubling time of 26h) and demonstrated improved growth rate compared to the competitor medium. HUVEC maintained their typical endothelial cell morphology thorough the experiment. Besides the differences in cell growth between both products, cells grown in the competitor medium went into senescence and started detaching from the plate before reaching confluence, whereas cells grown in EndoGo™ XF + PLTMax® kept dividing even after reaching confluence. To eliminate the need for heparin addition, Mill Creek Life Sciences developed PLTGold®, a step forward in the evolution of hPL. PLTGold® is xenogeneic-free, Heparin-free, unfractionated and clot-free. Growth of HUVEC in EndoGo™ XF + 5% PLTMax® was compared with growth in EndoGo™ XF + 5% PLTGold®. The average cell doubling times obtained for medium supplemented with PLTGold® was 28h compared to the average of 26h obtained for PLTMax®. However, even when growth was slightly slower in EndoGo™XF supplemented with PLTGold® compared to the same medium supplemented with PLTMax®, cells continued exponentially growing until reaching 100% confluence without any indication of senescence or cell death as opposed to what happened when using the competitor medium containing FBS and BBE.

CONCLUSIONS: EndoGo™ XF + PLTGold® is the only completely xenogeneic-free and cGMP compliant medium available in the market to grow human endothelial cells. It also demonstrated improved growth rate compared to other media commercially available.



Mucoadhesive oromucosal slowly disintegrating bilayered patch with octenidine for oral ulcers treatment

V. Štěpánková[1], J. Chmelař[1], V. Velebný[1]
Presenting Author: Veronika Štěpánková, veronika.stepankova@contipro.com
[1]Contipro a. s., Dolní Dobrouč, Czech Republic

INTRODUCTION: Oral ulcers are common, with an estimated prevalence of 4% in the world. Active substances are applied as topical pastes, gels or mouthwashes [1]. Disadvantages of these mentioned forms are their limited time of contact with the ulcers and the need to avoid drinking and eating for several tens of minutes. The aim of our work was to develop a bilayered mucoadhesive slowly disintegrating patch containing an antimicrobial agent octenidine that would cover the ulcer for at least one hour and allow fluid intake.

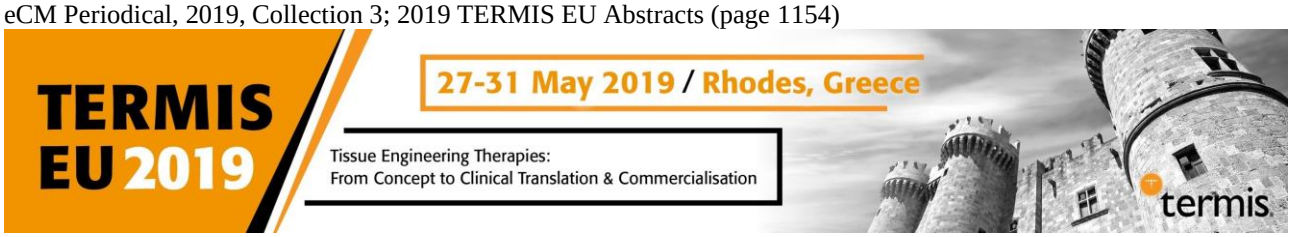
METHODS: Mucoadhesive films containing hydroxypropyl methylcellulose (HPMC) were prepared by solution casting. Parameters to select the final composition were visual evaluation of the solutions and fabricated films, their thickness and homogeneity, and evaluation of mucoadhesive properties. Active layer was prepared in the form of a lyophilizate from native hyaluronic acid (1 MDa) and octenidine (OCT). HPLC analysis was used to evaluate the amount and homogeneity of OCT. Antimicrobial activity of the lyophilizate was tested using diffusion plate method. Bilayered patch was created by moistening one side of the lyophilizate and the film using 90% (v/v) isopropanol and putting them in contact. Pressure was applied for a few seconds and the patch was left to dry at elevated temperature. Its surface pH was measured. MTT assay was used to assess cytotoxicity effect. Each form and the final patch were subjected to GC analysis to determine residual organic solvent, TGA analysis to determine dry mass, determination of weight per square meter and dissolution in human saliva. Prepared bilayered patches were tested by volunteers. They were positioned onto different places in oral cavity.

RESULTS & DISCUSSION: Clear solutions and visually homogenous films without any precipitation were prepared from HPMC, Eudragit, Eurocert Ponceau 4R and glycerol (200:75:1:0.06 w/w). Specifically, HPMC K15M and E4M (1:3 w/w) were combined with Eudragit RL100 and RS100 (1:9 w/w). Prepared films were 85±10 µm thick and their weight per square meter was 110±10 g/m^2. The dry matter was 94±1 %. In vitro dissolution test performed in Petri dishes in the presence of human saliva showed no fragmentation, satisfactory mucoadhesive force and over an hour dissolution time of the film. Weight per square meter of hyaluronan and OCT in the lyophilizate were 50-60 g/m^2 and 0.03-0.04 g/m^2, respectively. Antimicrobial activity against selected bacterial strains was demonstrated. pH of moistened bilayered patch was around 5, which is in the limit ($4 \leq pH \leq 8$) for safe use in the oral cavity. The content of residual solvents was below the limit of 0.5% (w/w) and cytotoxicity was excluded. In vitro dissolution tests of complete patches showed no difference compared to films tested alone, but the in vivo test revealed faster dissolution, detachment of the film from the active layer and subsequent adhesion of the film to other parts of the oral cavity.

CONCLUSIONS: Bilayered patch was successfully prepared and in vitro testing proved its safety. In vivo test by volunteers revealed the need to improve the cohesion of the layers and to increase hydrophobicity of external side of the film to prevent unspecific adhesion.

REFERENCES

[1] Ahmed M. U et al. Bangladesh Journal of Plastic Surgery 2010; 1(2): 23-29



In vivo assessment of cell-based bioartificial nerve substitutes for peripheral nerve repair

V. Carriel[1,2], J. Chato-Astrain[1,2], O.D. García-García[1,2], D. Durand-Herrera[1,2], E. Miralles[3], F. Campos[1,2], M.A. Martín-Piedra[1,2], I. Garzón[1,2], M. Alaminos[1,2], A. Campos[1,2]
Presenting Author: Víctor Carriel, vcarriel@ugr.es
[1]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [2]Instituto de Investigación Biosanitaria ibs.GRANADA, Spain, [3]Division of Clinical Neurophysiology, University Hospital San Cecilio, Granada, Spain

INTRODUCTION: Peripheral nerves are delicate organs which are frequently affected by traumatic injuries. Currently, nerve autograft is considered as the gold standard technique to repair critical nerve gaps with variable success rate [1]. Over the recent years several tissue engineering approaches demonstrated promising results and point out the potential usefulness of tissue engineering in peripheral nerve repair. The aim of this study was to create and evaluate a fibrin/agarose and adipose stem cell-based bioartificial substitute in peripheral nerve repair. Furthermore, the peripheral nerve regeneration profile was determined by using functional and histological quality controls.

METHODS: For this in vivo study, 20 adult male Wistar rats were used. Under general anesthesia a segment of 10-mm was removed from the left sciatic nerve of 15 animals. Defects were repaired by using nanostructured fibrin/agarose hydrogels containing cells (Nano) [1], NeuraGen® conduits filled with Nano (Coll-Nano) and autografts (n=5 each). Furthermore, 5 animals were used as native controls. After 12 weeks, animals were subjected to pinch test, toe-spread test and electromyography. After 14 weeks, animals were euthanized for histological and morphometry analyses of implanted grafts and distal muscles [2].

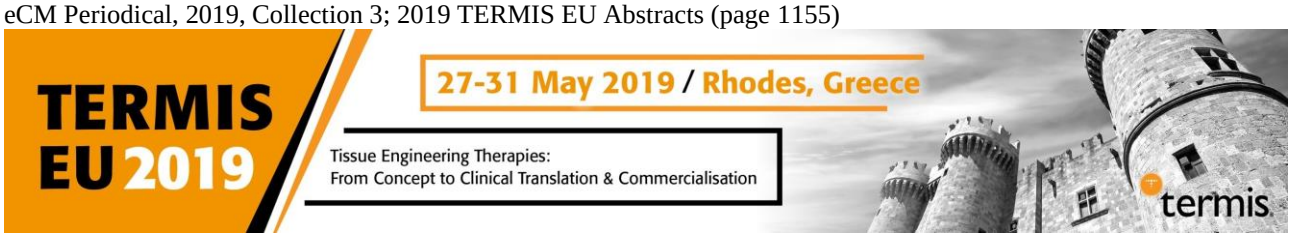
RESULTS & DISCUSSION: Functional analyses showed slight differences between engineered strategies as compared to autografts, especially for the use of Nano substitutes. Electromyography revealed clear signs of reinnervation of distal muscles in Auto and Nano groups which were significantly superior to Coll-Nano group. Histology demonstrated an active nerve tissue regeneration process at the middle portion of implanted grafts, being these results comparable between Auto and Nano groups and both superior than Coll-Nano group. Muscle morphometry revealed less percentage of weight and volume loss in Auto group followed by Nano and finally Coll-Nano group respectively, and differences were confirmed by muscle histology

CONCLUSIONS: In this in vivo study we demonstrated that nanostructured fibrin/agarose hydrogels containing adipose stem cells can promote functional recovery, muscle reinnervation and tissue regeneration in a comparable manner to autograft technique. However, future studies are needed to demonstrate the potential usefulness of these substitutes in the repair of critical nerve defects.

ACKNOWLEDGEMENTS: This study was supported by the Spanish "Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica," from the National Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), Grant Nos FIS PI17-0393, co-financed by "Fondo Europeo de Desarrollo Regional (FEDER)," European Union.

REFERENCES

[1] Carriel V et al. J Tissue Eng Regen Med. 2017;11(5):1412-1426.
[2] Chato-Astrain J et al. Front Cell Neurosci., 18 December 2018.



Differential behavior of human hyaline and elastic cartilage-derived chondrocytes during aggregates formation using agarose microchips

D. Durand-Herrera[1,2], D. Sánchez-Porras[1], A.B. Páez[1], J. Chato-Astrain[1,2], O.D. García-García[1,2], F. Campos[1,2], M.A. Martín-Piedra[1,2], I. Garzón[1,2], M. Alaminos[1,2], V. Carriel[1,2].
Presenting Author: Víctor Carriel, vcarriel@ugr.es
[1]Department of Histology, Tissue Engineering Group, Faculty of Medicine, University of Granada, Granada, Spain [2]Instituto de Investigación Biosanitaria ibs.GRANADA, Spain [3]Division of Clinical Neurophysiology, University Hospital San Cecilio, Granada, Spain

INTRODUCTION: 3D cellular aggregate generation on agarose chips is a useful cell culture alternative in tissue engineering approaches. The aim of this study was to generate, characterize and compare the structural and biological properties of human hyaline and elastic cartilage-derived chondrocytes spheroid aggregates as potential alternative for cartilage tissue engineering.

METHODS: Human hyaline and elastic cartilage-derived chondrocytes were isolated from cartilage biopsies. Tissue samples were in collagenase and cells were obtained by centrifugation. Spheroid aggregates were generated by seeding 2.5 x 10^5 cells in agarose microchips containing approximately 1250 micro-wells with an average diameter of 400 μm [1]. The spheroid aggregates were controlled daily by phase contrast microscopy analysis. Cell viability, proliferation and cell damage were determined by Live/dead, WST-1 and DNA release methods; structure and histological properties were analyzed at 4, 7, 14, 21, 28 days of ex vivo culture.

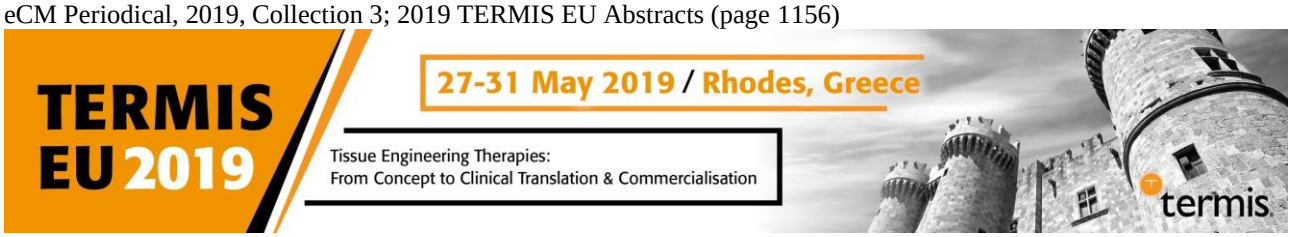
RESULTS & DISCUSSION: Chondrocytes from elastic and hyaline cartilage were able to self-assemble and form stable aggregates from day 3 onward. Viability assessment revealed high cell viability and proliferation rate in both types of aggregates over the time. Histology revealed a uniform cell distribution pattern in each, but hyaline-derived aggregates resulted more compacted than elastic-derived structured, which showed a looser pattern. Histochemistry confirmed the progressive synthesis over the time of acid proteoglycans in both types of aggregates.

CONCLUSIONS: This study demonstrated that human hyaline and elastic cartilage-derived chondrocytes are a suitable cell source for the generation of stable and functional aggregates. These chondrocyte-based aggregates could be promising alternatives to generate functional and biomimetic cartilage bioartificial models for tissue engineering applications.

ACKNOWLEDGEMENTS: This study was supported by Grant CS PI-0257-2017 from Consejería de Salud, Junta de Andalucía, Spain and by IDI-20180052 (Agarmatriz), leaded by Hispanagar, SA, Burgos, Spain, through CDTI, Ministry of Economy and Competitiveness, Spain, Programa Operativo Pluriregional de Crecimiento Inteligente (CRIN). D. Durand is supported by "Consejo Nacional de Ciencia y Tecnología" (CONACYT), scholarship program from Mexican government 580680/694261.

REFERENCES

[1] Durand-Herrera D et al. Histochem Cell Biol. 2018;150(4):379-393.



IFN-γ primed mesenchymal stem cells from paediatric patients (pMSCs) exhibit immunomodulatory abilities towards allogeneic B and T cells

V. Palomares Cabeza[1,2,3], M.J. Hoogduijn[2], R. Kraaijeveld[2], M. Franquesa[4], E. Wolvius[1], E. Farrell[1], P. Brama[3]
Presenting author: Virginia Palomares Cabeza, v.palomarescabeza@erasmusmc.nl
[1]Department of Oral and Maxillofacial Surgery, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands [2]Department of Internal Medicine, Erasmus MC, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands [3]School of Veterinary Medicine, University College Dublin, Dublin, Ireland [4]REMAR Group and Nephrology Service, Germans Trias i Pujol Health Science Institute & University Hospital, Badalona, Spain

INTRODUCTION: Mesenchymal stem cells from paediatric patients (pMSCs) are an attractive cell source in regenerative medicine, due to their higher proliferation rates and better differentiation abilities compared to adult MSCs (aMSCs) [1]. We have previously characterized the immunomodulatory abilities of undifferentiated pMSCs on T cells under co-culture [1]. Luk et al. reported that undifferentiated aMSCs can inhibit B cell proliferation and maturation under inflammatory conditions. In this study, we aim to clarify the immunomodulatory effect of pMSCs towards B and T cells in an inflammatory microenvironment.

METHODS: Bone marrow derived pMSCs or aMSCs were primed for 3 days with 50 ng/mL of IFN-γ. To analyse the interaction between pMSCs and T cells under inflammatory conditions, CD3/CD28 stimulated peripheral blood mononuclear cells (PBMCs) were co-cultured with primed or unprimed pMSCs. To investigate B cell responses, quiescent B cells obtained from spleens by CD43 negative selection were stimulated with anti-IgM, anti-CD40, IL-2 and co-cultured with either IFN-γ primed or unprimed pMSCs or aMSCs. The immunophenotype of pMSCs upon IFN- γ stimulation, and T and B cell proliferation after 5 and 7 days of co-culture, respectively, were analysed by FACS. IgG production by B cells was determined by ELISA.

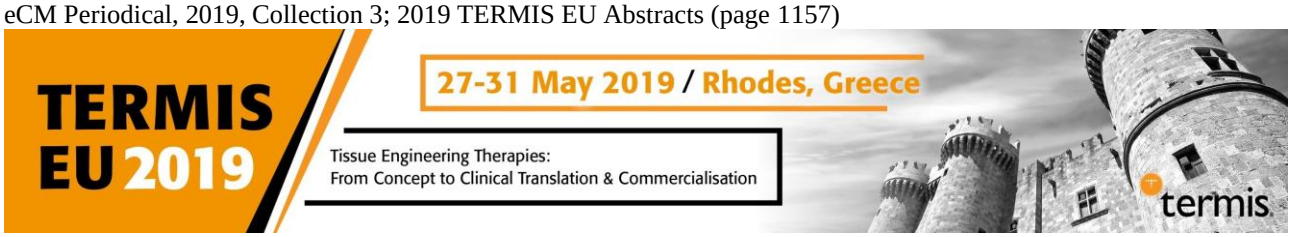
RESULTS & DISCUSSION: pMSCs upregulated the expression of HLA-ABC, HLA-DR and the co-stimulatory molecules CD80 and CD86 upon IFN-γ priming. IFN-γ addition on pMSCs did not seem to further decrease the proliferation rates of $CD4^+$ nor $CD8^+$ stimulated T cells compared to unprimed pMSCs. IFN-γ primed pMSCs but not unprimed pMSCs strongly inhibited naïve ($CD19^+CD27^-$), memory ($CD19^+$ $CD27^+$), and total B cell proliferation. Antibody-producing plasmablast ($CD19^+CD27^{hi}$, $CD38^{hi}$) formation and IgG production was also significantly inhibited by IFN-γ primed pMSCs compared to unprimed pMSCs.

CONCLUSIONS: Collectively, these results show that pMSCs have immunomodulatory effects upon the adaptive immune response which can be influenced by the inflammatory microenvironment. This knowledge can be useful in regenerative medicine in tailoring the pretreatment of pMSCs to best modulate the immune response for a successful implant engraftment and avoidance of a strong allogeneic immune response.

ACKNOWLEDGEMENTS: Financial support was received from the AO foundation (AOCMF-15-27F)

REFERENCES

[1] Knuth et al. Tissue Eng Part C Methods. 2018 24(6):313-321



Genetically modified human mesenchymal stem cells for articular cartilage repair

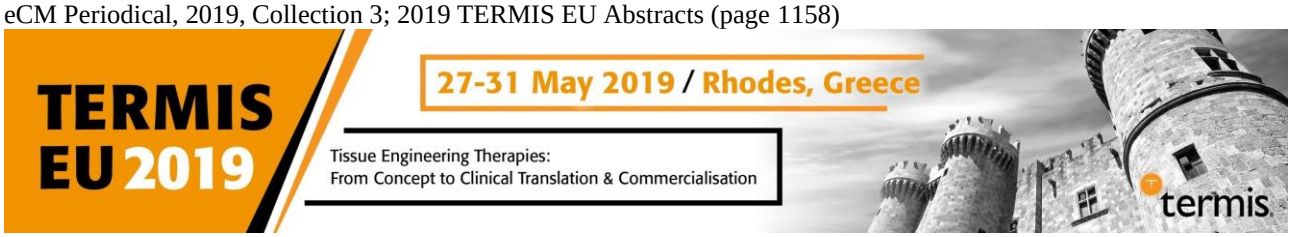
V. Kordium[1,2]. O. Toporova[1,2], O. Deriabina[2], V. Fishchenko[3], I. Pokholenko[1,2], N. Shuvalova[2], O. Botsul[3], P. Morgunov[1,2], A. Totchilovski[4]
Presenting Author: Vitalii Kordium, v.kordium@gmail.com
[1]Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine [2]State Institute of Genetic and Regenerative Medicine NAMSU, Kyiv, Ukraine [3]National Pirogov Memorial Medical University, Vinnytsya, Ukraine [4]BioTexCom Center for human reproduction, Kyiv, Ukraine

INTRODUCTION: Mechanical articular cartilage trauma are one of the most common in the knee joint injuries and makes up 35 - 55%. Condylar fractures of the knee joint in most cases provoke the development of deforming arthrosis. Current techniques to repair injured cartilage cannot completely regenerate the natural cartilage functions. In recent years mesenchymal stem cells (MSCs) have been proposed as a potential regenerative cell therapy for patients with cartilage damage. Genetic modification of MSCs to express certain anti-inflammatory cytokines can significantly enhance their regenerative potential. The purpose of this study was to evaluate the effectiveness of human MSCs overexpressing interleukin-10 on the model of acute lesion of the knee cartilage in rats.

METHODS: Human MSCs were isolated from umbilical cords by explant method. At the second passage cells were characterized by morphology, MSCs surface markers and trilineage differentiation. hIL-10/GFP-encoding plasmid was created and umbilical cord MSCs were transfected using TurboFect. MTT assay was applied to measure cytotoxicity of poliplexes at 24h post transfection. Transfection effectiveness of polyplexes have been evaluated by flow cytometry of 48 h post transfection culture, and quantitative green fluorescent protein expression was calculated against recombinant eGFP by Bio-Tek Synergy HT. IL-10 concentration was determined using enzyme-linked immunosorbent assay“Interleukin-10 -IFA-BEST ”Kit in conditioned serum-deprived culture supernatants of transfected MSCs after 48 hours of culture. Intraarticular injections of $1x10^5$MSCs-IL-10 were performed in rats with mechanical damage of articular cartilage. Histological and biochemical methods are used to assess the therapeutic effects of genetic modified MSCs.

RESULTS & DISCUSSION: Transfected MSCs have shown high viability (94-96%). More than 95% of transfected MSCs were positive for stem cell markers
(CD73, CD90, CD105) and negative for CD34, CD45. The results of ELISA revealed that considerable amounts of human IL-10 (640 pg per$1x10^5$cells for 48 h) was secreted from hMSCs/IL-10, while for naive hMSCs its own IL-10 synthesis is much lower - 7 - 9 pg per 10^5cells for 48 h. Histological analysis of damaged tissues after transplantation showed that the formation of mature chondrocytes, pronounced endosteal and periosteal chondrogenesis, the formation of mature endosteal and periosteal cartilage tissue in rats that were transplanted with MSC-IL10 occured in a much shorter time than in control animals. As well as in contrast to the untransplanted control group there was no excessive production of the ossetizing matrix and there were no dystrophic changes of chondrocytes and chondroblasts in experimental rats.

CONCLUSIONS: Intra-articular injection of human MSCs with enhanced synthesis of anti-inflammatory cytokine Il-10 leads to accelerated recovery of damaged cartilage tissue.



A novel anti-adhesion material using LYDEX

W. Hyon[1,2], K. Matsumura[1], S.-H. Hyon[2]
Presenting Author: Woogi Hyon; ygen@bmg-inc.com
[1]School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Ishikawa, Japan [2]BMG Incorporated, Kyoto, Japan

INTRODUCTION: Adhesion of living tissue after surgery not only causes adhesion bowel obstruction, but makes surgery difficult due to poor visual field and bleeding during reoperative surgery. It is also said to cause a number of sequelae such as chronic abdominal pain and infertility [1-3]. Herein, in order to prevent adhesion living tissue, we ventured to evaluate the possibility of application as an adhesion prevention material using a novel self-degradable medical adhesive "LYDEX®" consisting of aldehyde dextran and succinic anhydride treated poly-L-lysine.

METHODS: We evaluated the prevention efficiency from adhesion of the powder type LYDEX in an adhesion model (the intestines). Sprague-Dawley Rat with with body weights (300-310g) were used for the experiment. Width 2.5cm four quarters, about 1mm depth are exfoliated, and the model is produced by filling in addition, the lesion with the burn by the electric knife, after median incision, left side peritoneum and tunica muscularis of rat. The affected part was washed at Ringer's solution after defect creation, and it allowed to stand it for 2 minutes until it applied and hardened LYDEX®. After that, Ringer's solution washed the affected part, it made the closed abdomen. Two weeks later, the abdomen was incised, and the state of adhesion was evaluated with three steps of score.

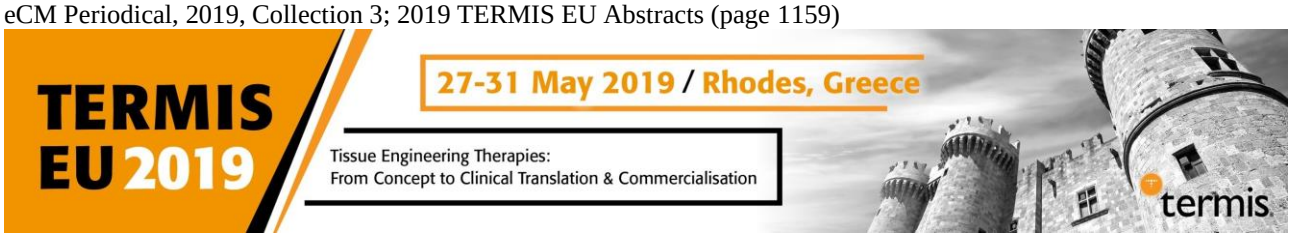
RESULTS & DISCUSSION: The result of the prevention from adhesion carried out by this research is shown in Fig.1. In this figure, we compared various LYDEX®, a SepraFilm, Fibrin glue, and an untreated score. The adhesion score of Fibrin glue was high to the same grade as untreated. In addition, the error bar of SepraFilm was large and it was high-scoring. On the other hand, the score of LYDEX® is low and the Powder type had the lowest score. It was shown that LYDEX® has a SepraFilm of an existing product and the adhesion prevention efficacy more than equivalent also in which type.

CONCLUSIONS: As a result of verifying adhesion prevention performance by applying LYDEX® and ready-made product to an animal model, the possibility of application as an anti-adhesion material was confirmed.

ACKNOWLEDGEMENTS: We did this research under fund support of the Japanese government Ministry of Economy, Trade and Industry. We express gratitude here.

REFERENCES

[1] Shikata J, et al . Jpn J Surg 20 : 660-664, 1990
[2] Beck DE, et al. Dis Colon Rectum 42 : 241-248, 1999
[3] Kamel, R.M. Eur J Obstet Gynecol Reprod Biol, 2010. 150 (2) : 111-8



Indium tin oxide-coated glass modified with carbon nanotubes as disposable working electrodes for dopamine sensing in brain tissue

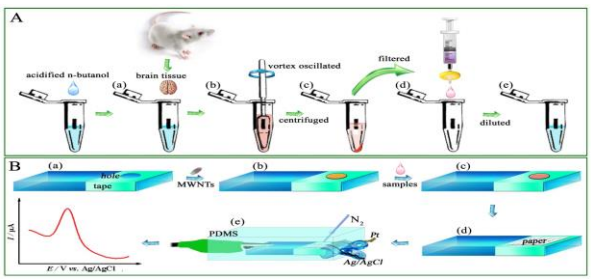
Q. Wang[1,2], Y. Zhou[1,2], C. Sun[1], Y. Yang[1], X. Tang[1]
Presenting Author: Xin Tang, tangxin@ntu.edu.cn
[1]Key Laboratory of Neuroregeneration of Jiangsu and Ministry of Education, Co-Innovation Center of Neuroregeneration, Nantong University, Nantong 226001, P.R. China [2]The Hand and Surgery Research Center, Department of Hand Surgery, Affiliated Hospital of Nantong University, Nantong 226001, P.R. China

INTRODUCTION: The development of inexpensive and simple analytical tools is of practical significance for biological sample analysis. There is still no simple and inexpensive method to measure dopamine in tissues.

METHODS: This work reports the development of a simple and cost-effective electrochemical sensor modified with multi-walled carbon nanotubes (MWNTs) and covered with filter paper for selective and sensitive detection of dopamine (DA) extracted from animal tissues (Fig. 1).

RESULTS & DISCUSSION: The response to DA occurs at a relatively low potential (0.43 V vs. Ag/AgCl) and is not interfered by Noradrenaline (NE) and 5-hydroxytryptamine (5-HT). The influence of electrode area and the amount of assembled MWNTs on the analytical signals were studied. Under optimized experimental conditions, the amperometric response to DA is linear in the 2.5×10^{-8} M to 1×10^{-6} M concentration range, and the detection limit is 5 nM (at a signal-to-noise ratio of 3). The method was further applied to quantify DA in the brain tissue and tendon tissue of rats (Fig. 2). In comparison to conventional methods, the proposed method is simple, rapid and inexpensive. More importantly, the developed sensor is supposed to be a single-use disposable device and electrodes were prepared "as new" for each experiment.

CONCLUSIONS: In our perception, this approach provides an effective way to quantify DA and therefore has a large potential in the field of DA-related diagnostics.



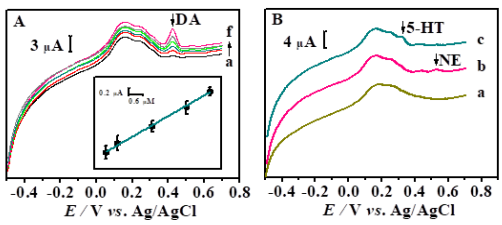
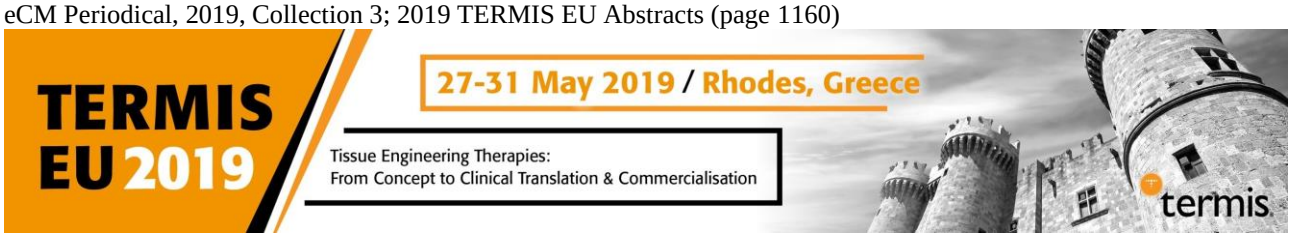


Figure 1: (left) A disposable indium-tin-oxide electrode modified with carbon nanotubes was applied for the sensitive detection of DA extracted from animal tissues. **Figure 2:** (right) (A) DPV curves of different volumes of DA(0 M, 5×10^{-7} M, 10^{-6} M, 2.5×10^{-6} M, 4×10^{-6} M, 5×10^{-6} M) was added to the diluted brain tissue sample(PH=6.0), Inset: Plot of the peak current against the concentration of DA. (B) DPV curves of the diluted tendon tissue sample (PH=6.0) (a). DPV curves of different volumes of 5 μM NE (b) and 5 μM 5-HT (c) was added to the diluted brain tissue sample3 (PH=6.0) (n=3).

ACKNOWLEDGEMENTS: We appreciate the financial support from the National Natural Science Foundation of China (Nos. 81771999 and 81401796)



PAI1 and IHH reduce the pro-angiogenic potential of transient cartilage secretome

Y. Nossin[1], J.L.M Koevoet[1], R. Somoza[2,3], A. Caplan[2,3], B Brachvogel[4], E. Farrell[1], G.J.V.M. van Osch[1]
Presenting Author: Yannick Nossin, y.nossin@erasmusmc.nl
[1]Erasmus MC, University Medical Center Rotterdam, Rotterdam, NL [2]Dept. of Biology, Skeletal Research Center, CWRU, Cleveland, Ohio [3]CWRU Center for Multimodal Evaluation of Engineered Cartilage, Cleveland, Ohio. [4]Universität Köln, Dept. Biochemistry II, Cologne, DE

INTRODUCTION: There are different types of cartilage in the body. Some are stable, such as in articular joints and others are unstable, as in the growth plate and transform into bone. These types of cartilage differ in the invasion by blood vessels that mark the onset of endochondral ossification (EO). Another hallmark of transient cartilage (TC) is chondrocyte hypertrophy, which can be found in a specific zone of the growth plate, the only one invaded by blood vessels. This is induced by secreted factors. Here we look at the impact of selected factors on the angiogenic potential of the secretome of TC.

METHODS: Candidate secreted angiogenic factors from cartilage were identified by comparison of microarray data of the different zones of murine growth plate [1] and gene enrichment analysis to screen for secreted angiogenesis regulating factors. We compared the identified factors with a human microarray that compared TC formed by bone marrow derived mesenchymal stem cells (MSC) with neonatal articular cartilage [2]. The secretome of 3D pellets of MSCs of 3 donors that were chondrogenically differentiated for 21 days [3] was collected and tested for angiogenic capacity with and without blocking the candidates We performed different in vitro assays, the endothelial migration and proliferation assay as well as in vivo assays, the chick chorioallantoic membrane (CAM) assay.

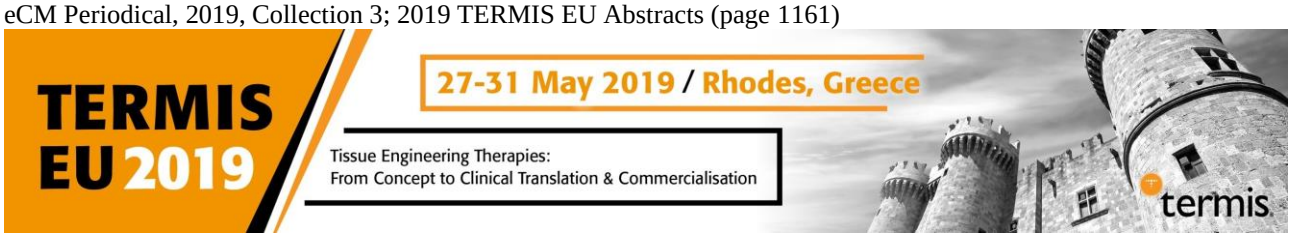
RESULTS & DISCUSSION: We identified 8 factors as candidate angiogenic factors secreted by TC Gene expression in chondrogenic MSC pellets compared to articular chondrocyte pellets by qPCR led to the selection of IHH and PAI1 as most interesting candidates due to their upregulation in TC. MSC derived CM showed a pro-angiogenic potential in the in vitro and in vivo assays. Blocking IHH or PAI1 in the CM further increased its proliferation and migration potential in vitro.

CONCLUSIONS: In the secretome of TC, both IHH and PAI1 had an anti-angiogenic effect, inhibiting mainly endothelial cell proliferation and migration. In conclusion, we provide evidence that IHH and PAI1 play a role in regulating blood vessel formation during EO.

ACKNOWLEDGEMENTS: This project is funded by EU's H2020 research and innovation programme under MSCA No 721432 and partly by a grant from the NIH/NIBIB: 1P41EB021911 "CWRU"

REFERENCES

[1] Belluoccio, D., et al. J Bone Miner Res, 2010. **25**(6): p. 1267-81.
[2] Somoza RA, et al. Tissue Eng Part A, 2018. **24**(3-4): p. 335-350.
[3] Hellingman, C. A.,et al. Cell Transplant, 2011. **20**(6), 925–940.



Development of the bioactive scaffold with cell-derived extracellular matrix (CECM) for wound healing

Y. Choi[1], D. Lee[1], E. Lih[1], M. Kim[1], S. Lee[1], J.H. Lee[2], K. Park[2], H.-H. Jung[1,*]
Presenting Author: Yeji Choi, yjchoi@hansbiomed.com
[1]Advanced Medical Device R&D Center, HansBiomed Co., ltd, Seoul 05836, Republic of Korea
[2]Center for Biomaterials, Korea Institute of Science and Technology, Seoul 02792, Republic of Korea.

INTRODUCTION: The extracellular matrix (ECM) is a complex network of a variety of proteins, proteoglycans, and other macromolecules, where it can provide structural and biochemical support such as cell adhesion, migration, proliferation, and differentiation. There has been especially growing interest in the cell-derived ECM (CECM) due to the potential of paracrine components secreted from specific cells, compared with its of tissue composed of various cells [1,2]. In this study, we have developed a new platform based on the bioactive CECM derived from human lung fibroblasts (WI-38) as a scaffold for wound healing.

METHODS: The CECM scaffolds obtained by WI-38 culture, decellularization and lyophilization. The structure and pore size of the scaffolds were characterized using scanning electron microscopy (SEM). The bioactive molecules such as proteins and growth factors in the CECM scaffolds were identified using immunofluorescence staining (IF), Western blotting (WB), and enzyme linked immunoassay (ELISA). Their potential contribution involved in wound repair was evaluated using in vitro cell study and a full-thickness cutaneous wounds in mice.

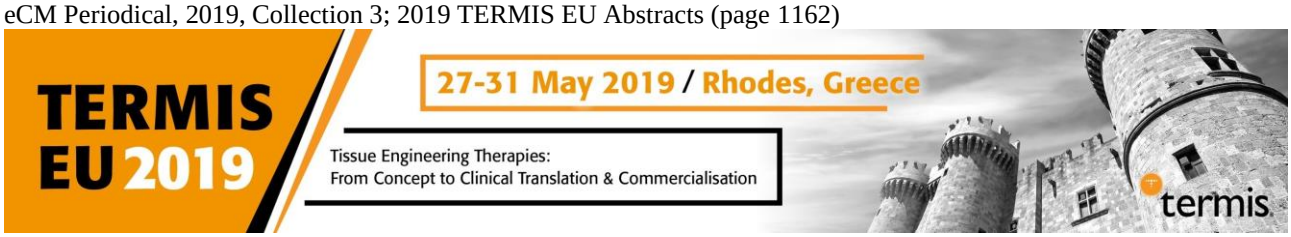
RESULTS & DISCUSSION: To confirm the decellularization process, DNA quantitation and DAPI staining of the CECM were examined. Decellularization resulted in almost complete removal of nuclei and DNA. Six major macromolecules, fibronectin, collagen (I, III, IV), laminin and elastin were identified using IF, WB, and ELISA. The ECM components were abundantly preserved. The CECM scaffolds have the open-porous microstructures with pore sizes ranging 50 μm to 300 μm. The results of cell study showed that the scaffolds were cytocompatible and stimulated cell proliferation. The quantity of CECM in the scaffolds study was considered to be effective biologically in further experiments using an animal models such as diabetic ulcers and burns.

CONCLUSIONS: The bioactive scaffolds based on CECM was successfully developed by decellularization method. The obtain results demonstrated that the CECM scaffolds could be extensively utilized in tissue engineering for clinical applications.

ACKNOWLEDGEMENTS: This research was supported by a grant of the Korea Health Technology R&D Project, funded by the Ministry of Health & Welfare, Republic of Korea (HI17C0882).

REFERENCES

[1] Bae SE et al. Biomac. 2012; 13(9):2811-20
[2] Kim TH et al. Tissue Eng Part A. 2018;24(9-10):830-848



Isolation, culture, and characterization of human adipose derived microvascular endothelial cells

Y. H. Ryu[1], K.-J. Kim[2], S. J. Lee[1], Y.-N. Han[1], E. J. Kim[2], S.-H. Moon[2], J.-W. Rhie[1,2]*
Presenting Author: Yeon Hee Ryu, carmine223@naver.com
[1] Department of Biomedicine & Health Sciences, The Catholic University of Korea, Seoul, South Korea [2] Department of Plastic Surgery, College of Medicine, The Catholic University of Korea, Seoul, South Korea

INTRODUCTION: Adipose-derived stem cells (ASCs) have been gaining fame mainly due to their vast clinical potential, simple isolation methods and minimal donor site morbidity. In particular endothelial cells present in SVF, which are isolated from adipose to obtain the local stem cells, are microvascular endothelial cells that are involved in angiogenesis, symbiotic healing, and inflammation, and have essential angiogenesis capabilities when repairing and rebuilding damaged tissue [1,2]. The aim of this study was a method for reliable isolation of human adipose-derived microvascular endothelial cells (HMVEC-A) has been developed and subsequently characterized in the expression of surface markers associated with endothelial cells.

METHODS: Human adipose-derived microvascular endothelial cells (HMVEC-A) were isolated from Adipose-derived stem cells in fresh human adipose-derived stromal vascular fraction (SVF). After enzymatic digestion to form a single cell suspension, SVF was cultured and ASCs were attached, and the supernatant was harvested to isolate HMVEC-A. And the separated HMVEC-A using anti-CD31 coated Dynabeads high purity culture, and confirmed typical endothelial cells showed cobblestone morphology, CD31, vWF, CD105, CD144, CD146 and eNOS, and normal function of the endothelium was confirmed by the Dil-Ac-LDL uptake measurement and measuring the capillary-like tubes in Matrigel.

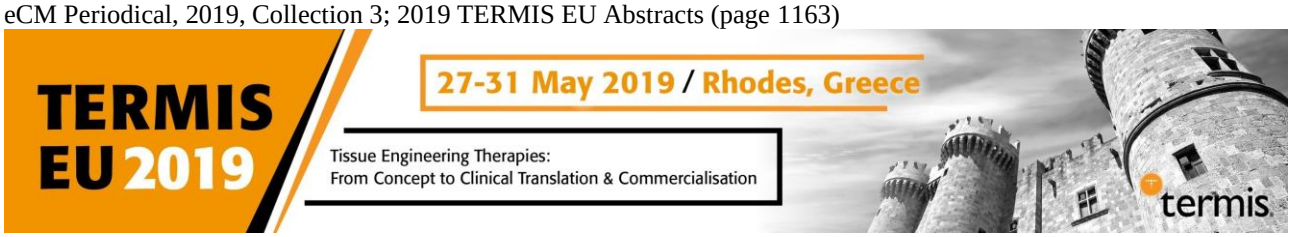
RESULTS & DISCUSSION: This method has enabled the rapid isolation of viable cells to demonstrate a typical endothelial cobblestone morphology during incubation. Cells were confirmed positive for CD31, vWF, CD105, CD144, CD146, eNOS. The cells were able to identify Dil-ac-LDL uptake and form a capillary-like tube structure when cultured in Matrigel.

CONCLUSIONS: In conclusion, microvascular endothelial cells isolated and cultured by the novel method showed high purity and characteristic of vascular endothelial cells. This method has developed a protocol that simplifies and economic isolation and cultures microvascular endothelial cells from adipose tissue while eliminating complex processes.

ACKNOWLEDGEMENTS: This work was financially supported by National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017M3A9E2060428) and by a Ministry of Trade Industry and Energy of Korea (20000325)

REFERENCES

[1] Pill, K., et al., Cell Regeneration, 2015. 4(1): p. 8.
[2] Hodde, J., Tissue Eng, 2002. 8(2): p. 295-308.



Sprayable adhesive nanoparticles for efficient locoregional cancer therapy

Y. Jeong[1], Y. K. Jo[1], B. J. Kim[2], B. Yang[1], K. I. Joo[1], H. J. Cha[1]*
Presenting Author: Yeonsu Jeong, skyyyhu@gmail.com
[1]Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, Korea [2]LSK BioPharma, Salt Lake City, Utah 84111, USA

INTRODUCTION: Adjuvant chemotherapy has been widely used to prevent relapse of cancer after surgical resection of tumor [1]. However, the systemic drug delivery platforms have limitations for clinical translations such as rapid clearance, and systemic toxicity [2]. Therefore, the local delivery of nanoparticles (NPs) can enhance accumulation in tumor, increasing therapeutic effects. Especially, the spraying system allows the uniform placement of NPs onto tumor resection sites in a simple way. For efficient retention on target sites, the adhesion and dispersion properties of NPs are essential in spraying system. Here, we propose sprayable adhesive NPs using mussel adhesive protein (MAP) for effective site-directed cancer therapy.

METHODS: The MAP NPs were fabricated by electrospraying process. The surface adhesion of the MAP NPs was quantified using a Quartz Crystal Microbalane (QCM). For a pH-dependent release profile, the DOX-loaded MAP NPs (MAP@DOX NPs) in dialysis membrane were incubated in buffer of several pH, and then the amounts of released DOX were measured using a fluorescence spectrometer. In addition, in vivo anticancer effects of MAP@DOX NPs were investigated using a tumor xenograft mouse model administered by surgical spraying process.

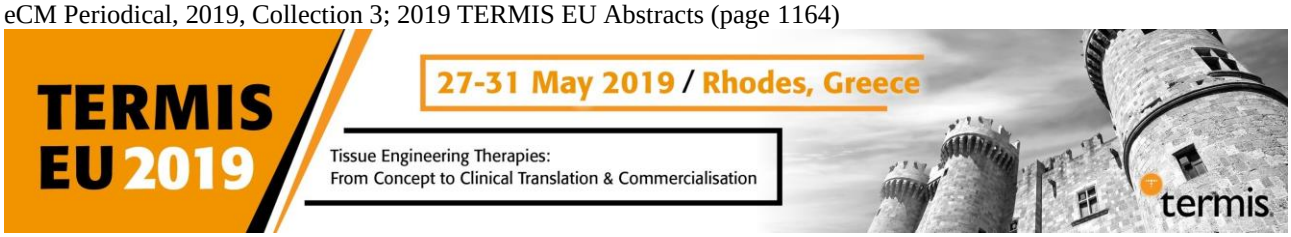
RESULTS & DISCUSSION: The MAP NPs were successfully fabricated and achieved higher adhesiveness compared to BSA NPs. The MAP NPs were sprayed by using an air-pressured atomizer and exhibited high-throughput deposition without breakage of NPs. The MAP@DOX NPs efficiently released DOX in response to the acidic pH condition, and showed effective cytotoxicity on breast cancer cells. For the focal administration of NPs, the MAP NPs were surgically sprayed on tumor tissues and showed efficient retention on tumor sites via their adhesive property. Moreover, the MAP@DOX NPs were effectively inhibited tumor growth compared to free DOX and PBS groups, demonstrating that MAP NP-based spray system could achieve localized drug delivery, improving anticancer effects with a reduced systemic toxicity.

CONCLUSIONS: The sprayable adhesive NPs are expected to be a promising therapeutic strategy for localized adjuvant cancer therapy.

ACKNOWLEDGEMENTS: Financial support was provided by the Marine Biomaterials Research Center grand from Marine Biotechnology Program of the Korea Institute of Marine Science & Technology Promotion funded by the Ministry of Oceans and Fisheries, Korea.

REFERENCES

[1] Fisher B et al. N. Engl. J. Med. 2002; 347(16):1233-1241
[2] Min Y et al. Chem. Rev. 2015; 115(19):11147-11190
[3] Yeonsu J et al. ACS Nano 2018; 12(9):8909-8919



TGF-β1-induced Connexin43 promotes scar formation via the Erk/MMP-1/collagen III pathway

Y. Liu, X. Li
Presenting Author: Yi Liu, lililiuyi@163.com
Laboratory of Tissue Regeneration and Immunology and Department of Periodontics, School of Stomatology, Capital Medical University (CCMU), Beijing

INTRODUCTION: Wound healing can be divided into different phases. Timely initiation and cessation of these stages are the key to successful wound healing; otherwise, scar tissue will be present in the wounded area. Connexins (Cxs) were confirmed to influence and regulate scar formation. The aim of the present study was to investigate the role of Cx43 in the scar formation process and the possible underlying cell signaling pathways.

METHODS: RT-PCR, western blot analysis, immunohistochemistry staining and immunohistofluorescence staining were performed to examine the expression of extracellular matrix components and key proteins of cell signaling pathways.

RESULTS & DISCUSSION: After injury, wounds in the buccal mucosa healed with no scar, whereas skin healed with an evident scar. TGF-β1 expression was gradually increased at the 5th day after injury, along with Cx43 expression, which was progressively increased in the skin and peaked at the scar formation stage. TGF-β1 and Cx43 expression in the oral mucosa was maintained at a low level. The high level of TGF-β1 increased p-Smad2/3 and then induced Cx43, whereas Cx43 overexpression antagonized the phosphorylation of Erk1/2, a downstream protein of Cx43, which affected MMP-1 synthesis. The MMP-1 deficiency led to Collagen III accumulation and facilitated scar formation.

CONCLUSIONS: We demonstrated that TGF-β1-induced Cx43 promoted scar formation via the Erk/MMP-1/Collagen III pathway.

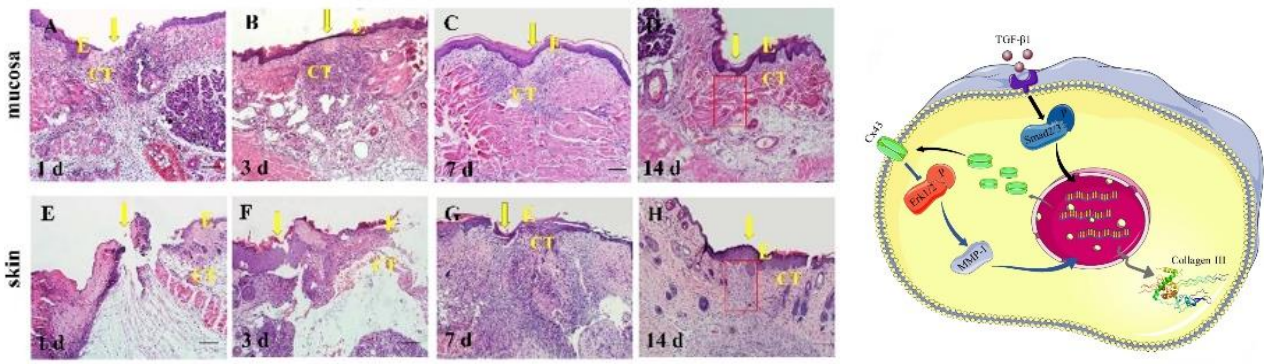
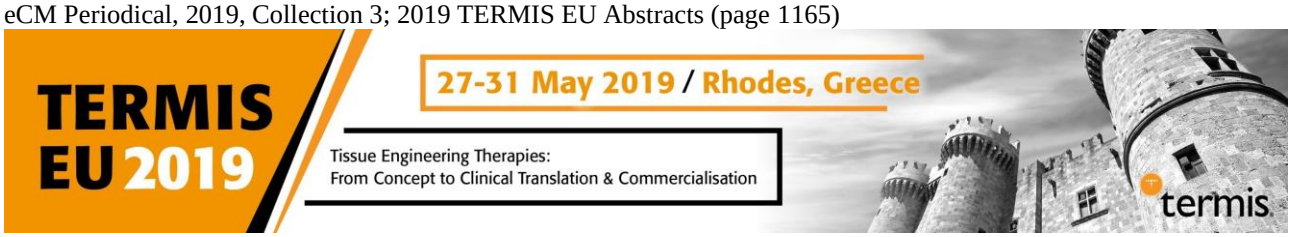


Figure 1: Scar formation was more evident in the skin than the oral mucosa, TGF-β1-Induced Connexin43 Promotes Scar Formation via the Erk/MMP-1/Collagen III Pathway

ACKNOWLEDGEMENTS: Beijing Natural Science Foundation (7172087 to Y.L)



Application of alginate-based bioinks in 3D bioprinting of the human blood-brain barrier model

Y. Li, E. Baumann, J. Huang, A. Jezierski
Presenting Author: Ying (Betty) Li, Betty.Li@nrc-cnrc.gc.ca
Human Health Therapeutic Research Center, National Research Council, of Canada, Ottawa, ON, K1A 0R6, Canada

INTRODUCTION: Advances in 3D hydrogel biomaterials, with bioprintable properties, provides new tools towards modeling the human blood brain barrier and neurovascular function. Due to the complexity of the brain structure, current monolayer transwell BBB models do not recapitulate the three dimensional (3D) micro-environment of the neurovascular unit, such as the cellular heterogeneity, anatomical size and shear stress conditions.To address these limitations, three-dimensional (3D) bioprinting using human induced pluripotent stem cell (iPSC)-derived brain endothelial cells (iBECs) holds immense promise for providing a rapid and robust approach for recapitulating the physiological cellular heterogeneity and structural complexity of the BBB in vivo. In this study, alginate and gelatin, two of the most common biomaterials with biocompatibility, biomimicry and mechanical integrities are employed as printable hydrogel materials to support iBEC attachment and viability.

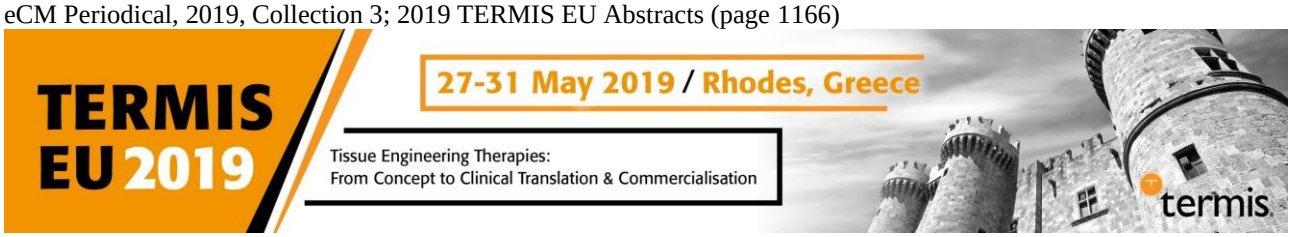
METHODS: Sodium alginate and gelatin was dissolved in human endothelial serum free growth medium with 1% fetal bovine serum (EM) and combined to form a final concentration of 3 w/v% alginate 7 w/v% gelatin solution (A3G7). Pure alginate hydrogel (3 w/v%) was used as control. Human iBECs were derived as previously described [1] and.mixed with the alginate and A3G7 solution to form bioinks with a cell density of 15.5×10^6 cells/mL. Bioink in the form of beads and film were formed by dispensing 200 ~ 500 µL of cell solution into a 24 well plate followed by adding a 60mM $CaCl_2$ solution to crosslink the bioink for 5 mins. Fresh media was added to each well and the plate was placed in an incubator at 37ºC and 5% CO_2. The cell culture media was replaced every day and after 4 days of culture, samples were fixed in formalin and stained with WGA-FITC and Hoechst to visualize cell membrane and nuclei respectively.

RESULTS & DISCUSSION: Fluorescence analysis of WGA-FITC and Hoechst illustrated a significant increase in cell density and attachment of iBECs using alginate-gelatin A3G7 bioink compared to pure alginate. There were more visible areas of cell aggregation in the A3G7 which facilitated cell attachment and cellular viability.

CONCLUSIONS: These results demonstrate the beneficial effect of gelatin in functionalizing gelatin based bioinks for improved cell attachment of iPSC-derived human brain endothelial cells. Optimization of hydrogels, with bioprintable properties, will enable more complex and physiological BBB modeling through deposition of layered 3D cell structures.

REFERENCES

[1] Ribecco-Lutkiewicz M et al Sci Rep. 2018; 8 (30):1837



Mimic clinical used of platelet-rich plasma with different leukocytes concentration and combined with bone marrow-mesenchymal stem cell treat on damaged chondrocytes

Y.-S. Shen[1,2]; Y.-C. Chen[1,3]; C.-H. Chang[1,3]; F.-H. Lin[2]
Presenting Author: YI-SHAN SHEN, katrina60943@gmail.com
1Department of Orthopedics, Far Eastern Memorial Hospital, New Taipei City, Taiwan 2Department of Biomedical Engineering, National Taiwan University, Taipei, Taiwan 3Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Taoyuan, Taiwan

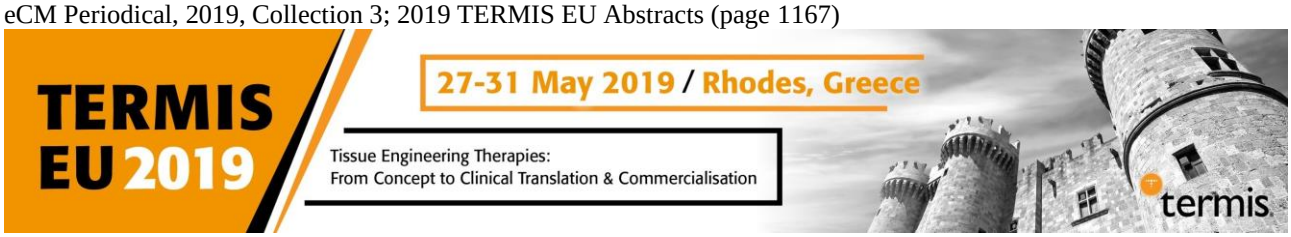
INTRODUCTION: Platelet-rich plasma (PRP) therapy is a new type of growth factor treatment for OA. It is a simple, safe, low-cost and minimally invasive method that provides a natural concentrate of autologous blood growth factors (GFs). In addition, mesenchymal stem cell (MSC) injection in conjunction with PRP might produce a better therapeutic effect due to the growth factor release effect of PRP and the tissue regeneration ability of MSC. In the study we would like to compare the PRP component difference among pure-PRP (P-PRP), leukocyte-poor PRP (LP-PRP) and leukocyte-rich PRP (LR-PRP) isolation system, and evaluate the efficacies of these PRP or PRP/MSC for the treatment of OA in in vitro system.

METHODS: Five kinds of culture conditions would be evaluated in the study including (I) damaged chondrocytes/ PRP/ bone marrow MSCs (BMMSCs) co-culture system, (II) damaged chondrocytes/ PRP culture system, (III) damaged chondrocytes/ BMMSCs co-culture system, (IV) damaged chondrocytes system and (V) normal chondrocyte control group. In system I and III , chondrocytes and BMMSCs were cultured on the bottom layer in the transwell, individually.

RESULTS & DISCUSSION: In this research, the results of complete blood count showed that all – the PRP groups showed less cell numbers in red blood cells and abundant cell numbers in platelets. In the leukocyte-rich PRP group, it showed that the isolated PRP containing more lymphocytes, monocytes, eosinophil and basophil than others. As for leukocyte-poor PRP group, it has only small amounts of lymphocytes. There was no significant difference in the growth factor content of PDGF-AB, TGF-□ and VEGF among these PRP isolation system.
In culture system, we found PRP/MSC co-culture system show synergistic pro-inflammatory performance on Day1and Day3, especially in LR-PRP and LP-PRP groups compare to the damaged chondrocytes group. Only P-PRP groups showed significantly down-regulated inflammatory related genes such as IL-6, IL-1□□and TNF-□□on Day3, and significant different compare to the damaged chondrocytes group at Day 1□In BMMSCs group, results showed that they performed a great anti-inflammatory ability compare to each PRP groups. Besides, the results of three kinds of PRP treated group (system II) were a little different to the system I. The LP-PRP treated groups showed a decreased in the inflammatory responses on Day 3 when comparing to the damaged chondrocytes groups.

CONCLUSIONS: To sum up, PRPs contained some leukocytes may not good for damaged chondrocyte recovery in OA treatment. Besides, when these PRP combined with BMMSCs also induce higher levels in inflammatory responses. Therefore, it seems that pure-PRP or BMMSC might be a better choice for OA patient use in the future.

ACKNOWLEDGEMENTS: Financial support was received from Far Eastern Memorial Hospital (FEMH-2017-C-007).



Human nasal septal chondrocyte (NSC) expansion via NSC-derived matrix and their chondrogenic potential

Y. K. Noh[1,2], I. H. Kim[2], S. W. Kim[3], K. Park[1,4]
Presenting Author: Yong Kwan Noh, yknoh@kist.re.kr
[1]Center for Biomaterials, Korea Institute of Science and Technology, Korea [2]Dept of Biotechnology, Korea University, Korea [3]Dept of Otolaryngology-Head and Neck Surgery, The Catholic University of Korea, Korea [4]Division of Bio-Medical Science and Technology, Korea University of Science and Technology (UST), Korea

INTRODUCTION: Successful cartilage regeneration has long been challenging. Cell sources of human chondrocytes are highly limited, and chondrocytes tend to dedifferentiate when they are heavily expanded in vitro. Upon the significant role of extracellular matrix (ECM) in cartilage regeneration, we have investigated chondrocyte-derived extracellular matrix (CHDM) substrates to proliferate human nasal septal chondrocytes (NSCs) and to keep them with chondrogenic potential.

METHODS: NSCs are cultured until confluence and then treated with 0.15% Triton X-100 and 10 mM NH_4OH (Sigma-Aldrich), then 50 U/mL DNase I and 2.5 µL/mL RNase A (Invitrogen) to obtain decellularized chondrocyte derived ECM (N-CHDM). To make soluble CHDM (S-CHDM), the N-CHDM was digested using 1 mg/ml pepsin (Sigma-Aldrich) in 0.01 N HCl, then treated with 0.1N NaOH for neutralization. NSCs (P5) proliferation on each substrate (TCP, N-CHDM, S-CHDM) was evaluated using cell counting kit-8 (Dojindo). The protein and gene expression of chondrogenic markers were also examined via immunofluorescence and q-PCR, respectively.

RESULTS & DISCUSSION: NSC shows distinct morphology, more focal adhesion (data not shown) and much better cell proliferation on N-CHDM. Cell differentiation was also notably different: more positive signals of collagen type 2 (Col 2) via immunofluorescence staining and significantly upregulated chondrogenic markers (Col 2, Sox9 and Aggrecan) expression on N-CHDM via q-PCR.

CONCLUSIONS: Current results suggest that on the N-CHDM, both proliferation and chondrogenic differentiation of NSC are beneficial, due mainly to the biophysical microenvironments of N-CHDM that are more natural for NSCs.

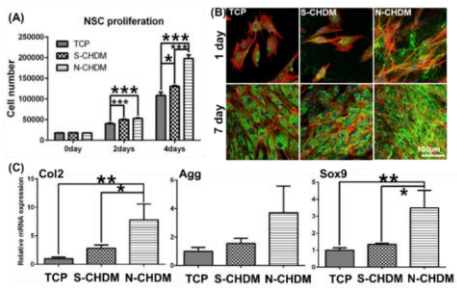
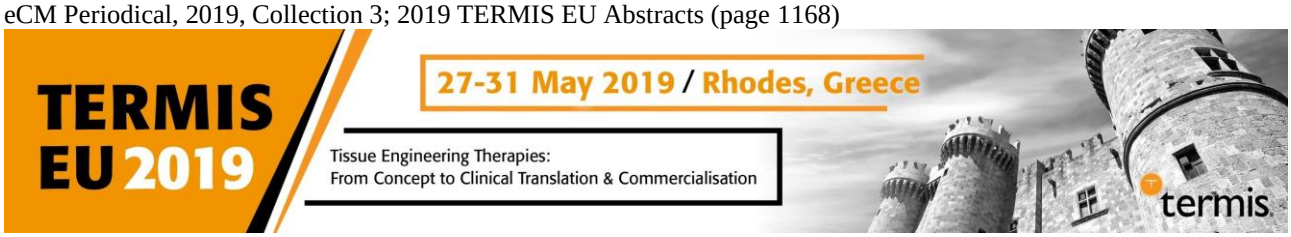


Figure 1: (A) Proliferation of NSCs on each substrate at 0, 2, and 4 day; (B) Immunofluorescence of Col 2 (green), F-actin (red) at 1 and 7 day; (C) Gene expression of chondrogenic markers (Col 2, Aggrecan and Sox9) via q-PCR. Statistical significance (*p<0.05, **p<0.01, ***p<0.001).

ACKNOWLEDGEMENTS: KHIDI, Ministry of Health and Welfare (HI16C0133).



Advanced cardiac tissues from human embryonic stem cells by employing Layer-By-Layer and growth factor treatment

Y. Jang[1], J. Kim[1], Y. Park[1]
Presenting Author: Yongjun Jang, jyj727@korea.ac.kr
[1]Department of Biomedical Engineering, College of Medicine, Korea university, Seoul, South Korea

INTRODUCTION: Engineering cardiac tissues generated from human embryonic stem cells are promising for regenerative medicine, drug testing, and disease modeling. Generation of structurally and functionally similar cardiac tissue to the actual heart could allow efficient improvement of heart studies [1]. Here, we demonstrate a simple method to develop cardiac tissues by employing layer-by-layer (LBL) deposition and accordion-like honeycomb (ALH) patterning with the use of growth factors.

METHODS: $1x10^6$ cells of H9 (human embryonic stem cell) derived cardiomyocytes and cardiac fibroblasts with 400 ul of collagen type I gel are placed in a flat or honeycomb patterned mold, respectively, and centrifuged for 1 min. After 40 min of gelation, RPMI media (Gibco) with or without 20 ng/ml of FGF-4 (Peprotech), 200 ug/ml of ascorbic acid (Sigma Aldrich) were added, respectively, and changed every 2 days. Immunocytochemistry, real-time PCR, and confocal microscopy analysis were conducted by removing the cardiac patches from each mold at given time points.

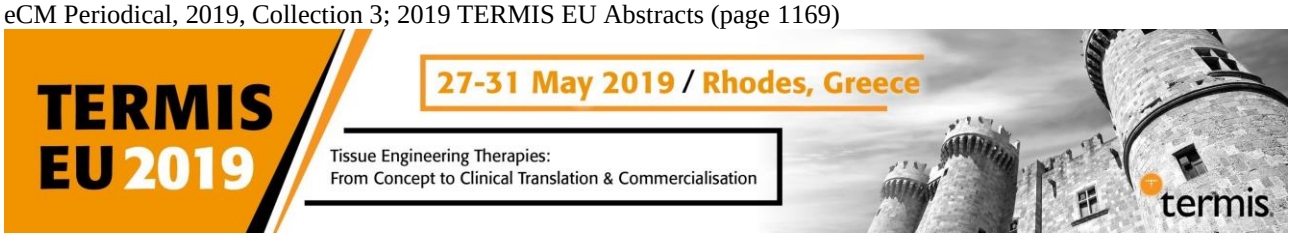
RESULTS & DISCUSSION: The cardiac patches prepared by LBL and ALH show that the expression level of MLC2v (cardiomyocyte maturation marker) is 20 times higher than that of a control group without LBL and ALH. The analysis of their beating video provides the fact that LBL improves consistency of the beating rate by decreasing the variance of contraction and relaxation times among cell patches. ALH further enhances the beating properties by narrowing the variance when combined with LBL, but ALH by itself is not effective.

CONCLUSIONS: The result suggests that a simple method using LBL and ALH is highly beneficial to develop cardiac patches with consistent beating properties. The expression value of MLC2v restates that the method exponentiate the maturation of H9 cells into cardiomyocytes. Thus, our finding has an important implication for engineering functional cardiac patches in vitro and their use in the fields of heart related studies.

ACKNOWLEDGEMENTS: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. NRF-2016M3A9B6947892)

REFERENCES

[1] Engelmayr Jr, George C., et al. Nature materials. 7.12 (2008): 1003.
[2] Weining Bian et al. Biomaterials. 35.12 (2014): 3819-3828.
[3] Liu, Yaowen, et al. Materials Science and Engineering: C 81 (2017): 500-510.



Preparation of polypyrole/silk fibroin conductive composite scaffolds for peripheral nerve regeneration

Y. Zhao, X. Tang, C. Sun, Y. Yang
Presenting Author: Yumin Yang, yangym@ntu.edu.cn
Key Laboratory of Neuroregeneration of Jiangsu and Ministry of Education, Co-Innovation Center of Neuroregeneration, Nantong University, Nantong 226001, P.R. China

INTRODUCTION: The repair of peripheral nerve defects largely depends on engineered nerve grafts. There is still no satisfied materials combining hierarchical structure and electrophysiological property suitable for the peripheral nerve regeneration.

METHODS: In this work, silk was used as raw material, PPy/SF conductive composite scaffold was fabricated by employing 3D bio-printing and electrospinning together (Fig. 1). A 10-mm-long sciatic nerve gap in rats was bridged by PPy/SF to investigate whether PPy/SF conductive composite scaffold was suitable for neural tissue application.

RESULTS & DISCUSSION: Schwann cells can be attached to the surface of the scaffold to maintain their normal configuration-full, spindly and proliferate well on the PPy/SF scaffolds. The conductive composite tissue engineering scaffold facilitates the adhesion and growth of DRG. Immunochemistry (Fig. 2) demonstrated that these PPy/SF promoted peripheral nerve regeneration because electrospun aligned nanoporous SF fibers were responsible for their good hierarchical structure and deposition of PPy enhanced their electrophysiological properties.

CONCLUSIONS: The research provides a new method and idea for the construction of artificial nerve grafts, provides a preliminary research basis for the application of new conductive materials in the field of tissue engineering.

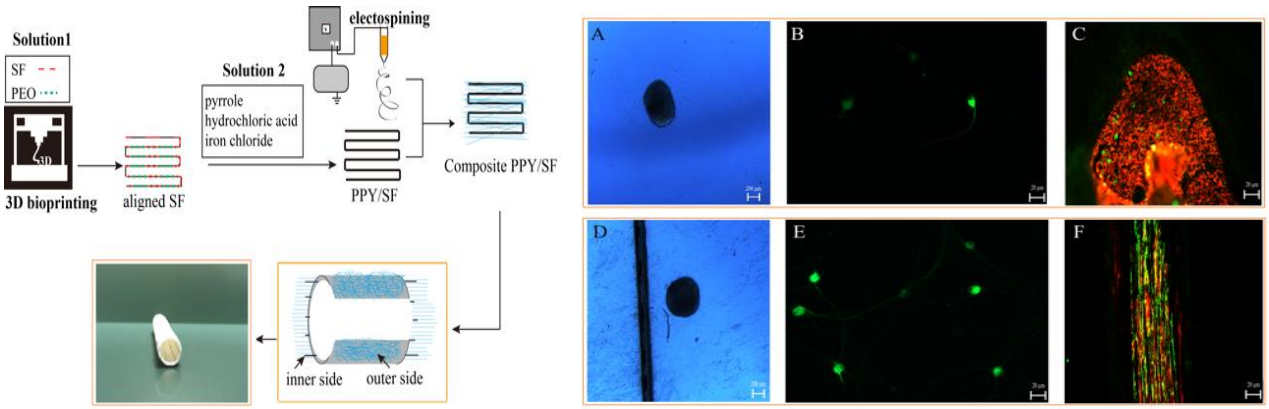
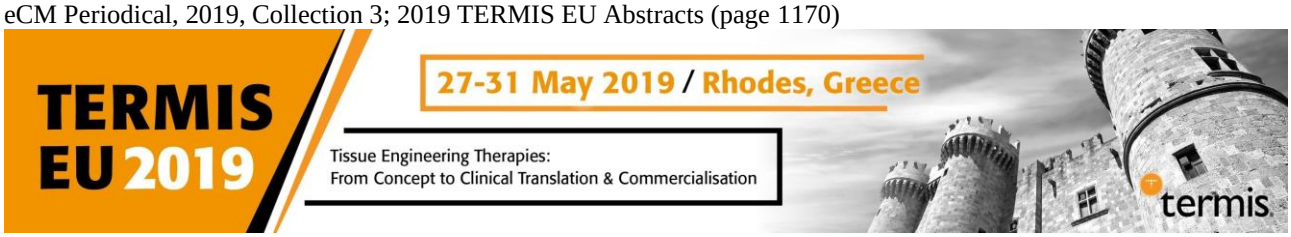


Figure 1: (left) Schematic showing the process of making the conductive polypyrrole/silk fibroin nerve guidance conduits. Figure 2: (right) Growth of DRG co-cultured with conductive composite tissue engineering scaffolds (D) and blank coverslips (A) after 3 days. NF staining of DRG neurons co-cultured with conductive composite tissue engineering scaffolds (E) and blank coverslips (B) after 3 days. Immunofluorescence staining for NF (green) and S100 (red) of longitudinal sections of rat sciatic nerve following PPy/SF scaffolds (F) and silicone tube (C) implantation for 7 days.

ACKNOWLEDGEMENTS: We appreciate the financial support from the National Natural Science Foundation of China (Nos. 31830028, 81671823, 81701835)



Three-dimensional cell culture enhances the differentiation efficiency of insulin producing cells from human induced pluripotent stem cells

Y. Lee[1], S.C. Kim[1,2], I.K. Shim[1]
Presenting Author: Yuna Lee, ynlee426@naver.com
[1]Asan Institute for Life Sciences and [2]Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

INTRODUCTION: Although pancreatic islet transplantation therapy is ideal for diabetes patients, several hurdles prevent it from becoming a standard treatment, including donor shortages and low engraftment efficacy. Insulin producing cells (IPCs) differentiated from stem cells is actively developing as a new islet source but the physiological function of IPC is still not enough to control diabetics in vivo. In this study, to improve the differentiation function of IPCs, we developed three-dimensional (3D) culture technique of IPCs using concave microwell.

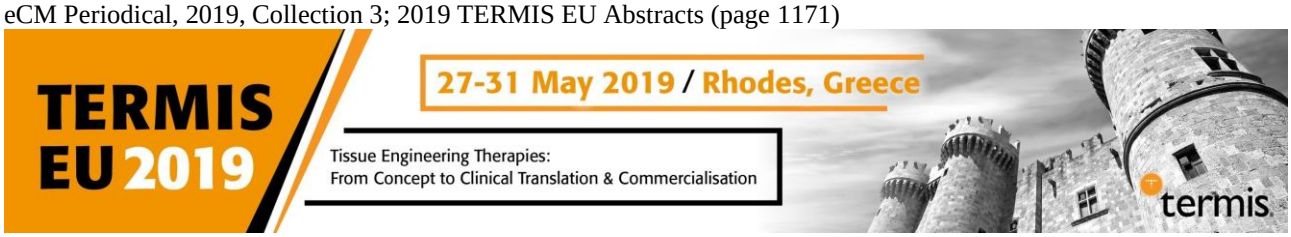
METHODS: Human induced pluripotent stem cells (iPSCs) were differentiated into IPC using small molecules. The cells were maintained on vitronectin coated culture dish in TeSR-E8 media. Human iPSCs differentiated into IPCs through stages in the following order. For pancreatic differentiation, the cells were cultured in RPMI medium containing 1% B27 with 100 ng/ml human activin A and 3 μM CHIR99021 for 24 h (Step1), and then in RPMI medium containing 1% B27 with 100 ng/ml activin A for 48 h (Step2). Subsequently, the medium was replaced with Improved MEM Zinc Option medium containing 1% B27 with 1 μM dorsomorphin, 2 μM retinoic acid and 10 μM SB431542 (Step3). Cells were cultured for 7 days and then these media were replaced with Improved MEM Zinc Option medium supplemented with 1% B27 with 10 μM forskolin, 10 μM dexamethasone and 10 mM nicotinamide (Step4). To enhance differentiation function of IPCs, spheroids were prepared using PDMS concave microwell. For long-term culture, the spheroids were culture in the shaking incubator with a circular motion. The differentiation efficiency of spheroid was compared to 2D monolayer culture by gene expression, insulin secretion, glucose stimulated insulin secretion (GSIS), and histology.

RESULTS & DISCUSSION: In this study, iPSCs successfully differentiated into IPCs. We fabricated 3D IPC spheroids using concave microwell, which was possible to mass-produce spheroids of the desired size. The size of the spheroid was increased in proportion to the number of cells with narrow size distribution. The cells in IPC spheroid showed higher expression of beta cell related transcription factors and insulin genes than the cells from 2D culture. Also, IPC spheroid showed insulin secretion rate in a glucose dependent manner (GSIS index: 1.96±0.68) but IPCs from 2D plate showed no response in glucose concentration (GSIS index: 1.18±0.37). In immunocytochemistry staining, the 3D differentiated IPCs can assemble into an islet like spheroid structure. Insulin positive cells make a cluster in a core, which indicating the post maturation of pancreatic endocrine cells can be regulated by 3D culture condition.

CONCLUSIONS: Using concave microwell chip, IPC spheroids of desired size were fabricated with mass production. Because 3D spheroid has biomimetic structures of natural islets and maintain cell-cell interactions, 3D spheroid culture leads a significant improvement of the maturity of IPCs.

REFERENCES

[1] Y. Kunisada et al. Stem Cell Research. 2012; 8:274-284 2016;6:30746



Bone regeneration using injectable rhFGF-2 gelatin hydrogel for osteonecrosis of the femoral head

Y. Kuroda[1], T. Kawai[1], K. Goto[1], Y. Tabata[2], S. Matsuda[1]
Presenting Author: Yutaka Kuroda, ykuromd@kuhp.kyoto-u.ac.jp
[1]Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[2]Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

INTRODUCTION: Osteonecrosis of the femoral head (ONFH) is a multifactorial disease that can cause femoral head collapse, pain, gait disorders. ONFH is common among young people in their 30s and 40s. In the clinic, although patients are diagnosed, 70%–80% of untreated patients experience femoral head collapse and have to undergo total hip arthroplasty. In the past decades, minimally invasive regenerative therapy has been desired for the early stages of ONFH. The purpose of this study was to evaluate the safety and clinical outcomes of rhFGF-2 gelatin hydrogel.

METHODS: Ten ONFH patients up to precollapse stage 2 underwent a single local administration of 800-μg rhFGF-2 gelatin hydrogel and were followed up for one year. Primary outcomes included adverse events and complications. Secondary outcomes included changes in Harris Hip Scores, VAS pain scores, UCLA scores, radiological changes as determined via X-ray, CT, and MRI images.

RESULTS & DISCUSSION: There were 14 adverse events (five patients). Patients completely recovered from all adverse events without problem. The surgery was performed with a minimally invasive technique (1 cm of skin incision), and walking was allowed from the day after surgery. Mean clinical scores improved significantly after four years compared with before surgery. There was only one case of femoral head collapse, and it had the greatest necrosis volume fraction and was considered to be in the early collapse stage at the time of operation. The other nine cases did not involve ONFH stage progression, and collapse was prevented. CT and MRI images confirmed bone regeneration in the ONFH.

CONCLUSIONS: Clinical application of rhFGF-2 gelatin hydrogel for precollapse stage of ONFH was feasible and safe. Our research is ongoing, further phase II multiple center study has been started in January 2016.

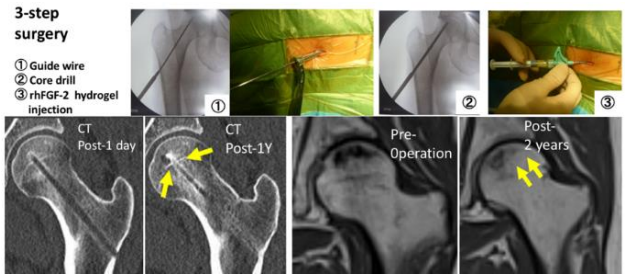
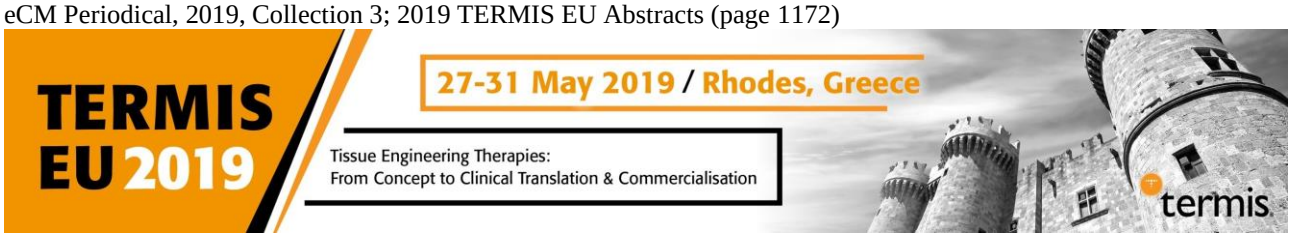


Figure 1: Intraoperative photographs (upper row). Surgical procedure is 3-step. Representative CT and MRI (lower row). Radiographical bone regeneration is observed in the implanted region postoperatively.

ACKNOWLEDGEMENTS: Financial support was received from Japan Agency for Medical Research and Development (AMED) and Translational Research Network Program by Ministry of Education, Culture, Sports, Science and Technology of Japan



Anti-inflammatory drug triamcinolone acetonide inhibits cartilage defect repair

Y.M. Bastiaansen-Jenniskens[1], S. Çapar[1], W. Wei[1], J. A.N. Verhaar[1], L. B. Creemers[2], G.J.V.M. van Osch[1,3]

Presenting Author: Yvonne Bastiaansen-Jenniskens, y.bastiaansen@erasmusmc.nl

[1]Department of Orthopedics, Erasmus MC University Medical Center Rotterdam, The Netherlands [2]Department of Orthopedics, UMCU, Utrecht, The Netherlands [3]Department of Otolaryngology, Erasmus MC University Medical Center Rotterdam, The Netherlands

INTRODUCTION: Inflammation is a response to tissue damage and plays a key role in the initiation of tissue repair. When not resolved however, inflammation also impairs tissue repair. As a result of a traumatic cartilage injury, synovial inflammation occurs. The aim of our study was to investigate whether application of the anti-inflammatory drug triamcinolone acetonide (TAA) improves repair in a mouse cartilage defect model and whether timing of TAA administration is of influence.

METHODS: A full thickness cartilage defect was made in 10 weeks old male DBA/1 mice, a strain known to have cartilage repair capacity. One day or seven days after making the cartilage defect, 25 µg of TAA or saline was administered intra-articularly (4 groups, of 11 mice per group). Eight weeks after creation of the cartilage defect, the experiment was terminated. Knee joints were processed for histological analysis of the synovial thickness and filling of the cartilage defect.

RESULTS & DISCUSSION: Twelve out of 22 of the TAA-treated mice had patella dislocations, versus 3/22 ($p<0.05$) of the saline-treated mice. These mice were excluded from cartilage and synovium analysis. TAA significantly inhibited cartilage defect repair. Control mice that received saline injection demonstrated good defect filling of 83% ± 23% or 90% ± 7% in the animals treated at day 1 and week 1 respectively. In the mice that received TAA 1 day after making the defect, 76% ± 8% of the defect area was filled. In mice that received TAA 1 week after making the defect, 67% ± 15% of the defect area was filled (Figure 1, left). Synovial thickness had the tendency to be decreased in response to intraarticular TAA injection, albeit not statistically significant (Figure 1, right).

CONCLUSIONS: TAA is known to inhibit connective tissue growth, especially after damage, which most likely contributed to the increase in dislocations and the inhibition of cartilage repair, despite its anti-inflammatory properties and application as intra-articular analgesic. Dislocations and inhibition of cartilage repair were independent of the timing of administration. This warrants further investigation into targeted delivery of the TAA, to prevent the negative effects on cartilage regeneration.

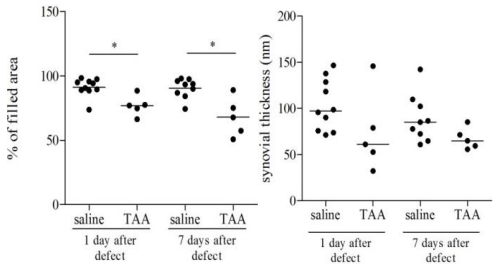
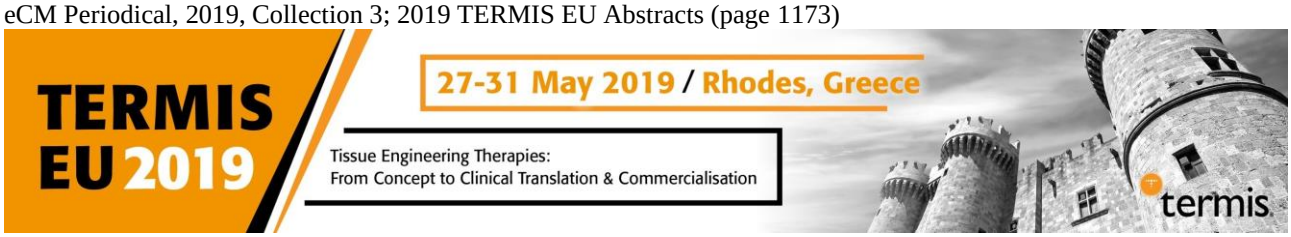


Figure 1: left: Percentage area of the defect filled 8 weeks after making the cartilage defect in response to TAA administration. Right: Synovial thickness at week 8 in response to TAA treatment.



Elastin-like-recombimer hydrogel as a platform for lung regeneration

Z. Soderlund[1], L. E Rendin[1], S. Hajizadeh[2], E. Tykesson[1], A. Ibáñez-Fonseca[3], J.C. Rodríguez-Cabello[3], L. Ye[2], G. Westergren-Thorsson[1]
Presenting Author: Zackarias Söderlund, zackarias.soderlund@med.lu.se
[1]Lung biology, Department of Experimental Medical Science, Lund University, Lund, Sweden [2]Division Pure and Applied Biochemistry, Lund University, Lund, Sweden [3]BIOFORGE Lab, Universidad de Valladolid, Valladolid, Spain

INTRODUCTION: Chronic obstructive pulmonary disease (COPD) is a world-wide health problem, where the tissue is gradually degraded leading to emphysema and loss of tissue integrity [1]. Our goal is to improve regeneration of the damaged tissue by inserting a cell-instructive biomaterial with the ability to activate regeneration in the border zone between destroyed areas and remains of healthy tissue in COPD. Elastin-like Recombinamer (ELR) hydrogels is a new type of biomaterial that have proved to have excellent biocompatibility properties [2]. In addition, the hydrogel has the possibility to add functionality by incorporating factors to guide the production and migration of cells.

METHODS: ELR is a two-part solution, which is a liquid at 4°C and solidifies at 37°C. The ELRs are modified so that one part has an alkyne modification while the second part has an azide modification. When these two parts are mixed at 4°C the alkyne reacts with the azide and creates a covalent bond and a hydrogel is formed. To be able to have a uniform dispersion of human fetal lung fibroblast (HFL1), cells in the hydrogel are mixed with the alkyne part before being mixed with the azide. Cells were then grown for 3 days and evaluated according to morphology and viability. As an alternative to the hydrogel conformation, an ELR-based cryogel was created by forming the hydrogel in subzero temperatures, which creates macro pores inside a conventional hydrogel.

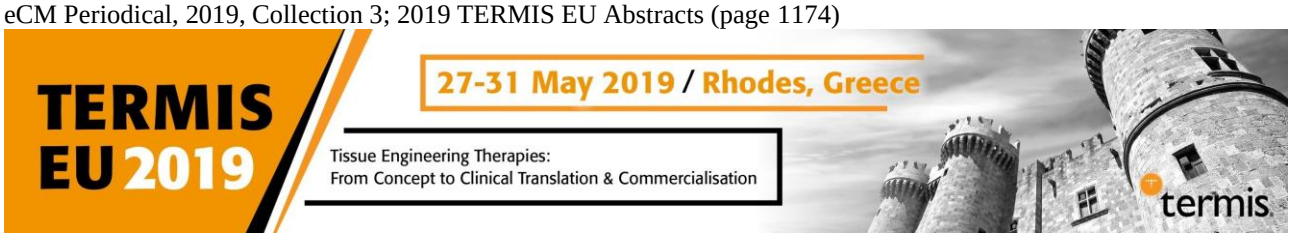
RESULTS & DISCUSSION: HFL1 cells which were grown in the ELR hydrogel showed more in vivo like morphology as well as being uniformly dispersed throughout the hydrogel as shown in Figure 1 compared to cells cultured on top of the hydrogel. A cryogel with large interconnected pores, a structure closely resembling the distal lung tissue, was formed.

CONCLUSIONS: The data showed that an ELR-based hydrogel is a promising synthetic scaffold for lung tissue engineering, mimicking the 3D environment of the extracellular matrix. Work is now being undertaken to add functionality to the scaffold, to incorporate cytokines as well as growth factors into the hydrogel to be able to steer and differentiate cells into the desired state.

ACKNOWLEDGMENTS: Financial support was received from Swedish foundation for Strategic Research, Swedish Heart-Lung foundation, Swedish Research Council, Crafoord foundation, Royal Physiographical Society of Lund, Österlund Foundation.

REFERENCES

[1] O. Hallgren et al. Respiratory Research 2010 11:55
[2] A. Ibanez-Fonseca et al. J Tissue Eng Regen Med. 2018;12:e1450–e1460.



Regenerative potential of iPSCs for musculoskeletal diseases therapy

M. Csöbönyeiová[1], Š. Polák[1], R. Zamborský[2], Z. Varchulová Nováková[3], Ľ. Danišovič[3,4]
Presenting Author: Lubos Danisovic, lubos.danisovic@fmed.uniba.sk
1Institute of Histology and Embryology, Comenius University, Slovakia; 2Department of Orthopaedics, Comenius University, Slovakia; 3Institute of Medical Biology, Genetics and Clinical Genetics, Comenius University, Slovakia; 4Regenmed Ltd., Slovakia

INTRODUCTION: The musculoskeletal diseases (MSDs) are the most common cause of severe long-term pain and physical disability affecting bones, muscles, cartilage, tendons, ligaments and joints. The recent advent of human induced pluripotent stem cells (iPSCs) provides an extensive opportunity to create human-specific models of MSDs. Moreover, human iPSC-based autologous transplantation is considered to be future breakthrough in the field of regenerative medicine.

METHODS: Since successful reprogramming of somatic cells to a pluripotent state by transient expression of four transcription factors (Oct4, Sox2, Klf4, and c-myc) was achieved for the first by Dr. Yamanaka and colleagues [1], the field has rapidly moved forward in order to reduce insertional mutation risks associated with viral delivery methods. The various non-viral approaches represent use of plasmid/episomal/minicircle vectors, direct protein/microRNA delivery and small molecules.

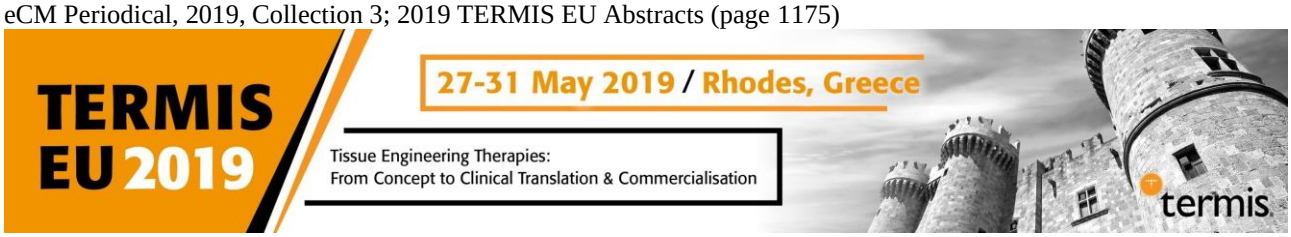
RESULTS & DISCUSSION: One of the major advantages of iPSCs is the ability to create specific MSD models to study their pathophysiology, such as fibrodysplasia ossificans progressiva, Duchene muscular dystrophy, osteosarcoma, Marfan syndrome, autosomal recessive osteopetrosis, etc. Moreover, there have been published various studies demonstrating successful bone, cartilage and muscle repair by iPSC-derived osteto/chondro–blasts and muscle cells, in vitro and in vivo [2][3]. However, the differentiation process of iPSCs into desired cell type remains time-consuming and quite difficult regarding proper cell maturation and purification.

CONCLUSIONS: The accessibility and therapeutic potential of patient's own genetically corrected (CRISPR/Cas9) iPSCs provides a powerful tool for future cell-based regenerative medicine including bone, cartilage and muscle reconstruction. However, there is need for further research focusing on high quality control testing of iPSCs at different levels of their generation to eliminate the risk of tumor formation.

ACKNOWLEDGEMENTS: Supported by APVV-14-0032.

REFERENCES

[1] Takahashi K, Yamanaka. Cell. 2006;126: 663-76
[2] Levi et al. Proc Natl Acad Sci U S A 2012; 109(50):20379-84
[3] Quattrocelli M et al. J Clin Invest. 2015; 125(12):4463-82



Optimized synthesis of polylactide (PLA) from food-waste

C. Gryparis[1], A. Maragkaki[2], V. Raptis[3], C. Bouki[4], C. Tsobanidis[4], V. Harmandaris[3], T. Manios[2], K. Velonia[1]
Presenting Author: Kelly Velonia, velonia@materials.uoc.gr
[1]Department of Materials Science and Technology, University of Crete, Heraklion, 71003 Greece, [2]Department of Agriculture Technological Educational Institute of Crete Heraklion, 71410, Crete, Greece, [3]Institute of Applied and Computational Mathematics, Foundation for Research and Technology-Hellas, Heraklion-Crete, Greece, [4]ENVIROPLAN SA, Athens, Gerakas, 153 44, Greece

INTRODUCTION: Herein we present studies on polylactide (PLA) production from food-waste material at the bench-scale level. Our studies focus on optimizing bioplastic production with respect to energy consumption and added value of the biodegradable polymeric end-product [1].

METHODS: We optimized a food-waste recycling system proposed by Sakai et al [1] in terms of raw material quality, monomer structure and energy consumption. More specifically we studied all parameters involved in the production of PLLA or PLA via fermentation of raw food-waste followed by tin (II) 2-ethylhexanoate catalyzed ring opening polymerization (ROP) of the produced lactate.

RESULTS & DISCUSSION: The temperature required to perform ROP is often the limiting factor for its application in larger scales therefore, our initial studies focused on identifying the lowermost temperature for high molecular weight PLA formation via ROP. Aiming to simplify the overall process, we optimized ROP in terms of monomer structure (i.e. using a racemic mixture rather than a single enantiomer) and evaluated the effect of humidity and/or initiators (i.e. methanol or polyethylene glycol). All products were characterized using NMR spectroscopy and chromatography. The polymerization step was also modelled along the lines of Mehta el al [2],[3] through a model consisting of rate equations for the ROP initiation, propagation and termination steps. Rate constants were determined by fitting to actual data (molecular weight with time, polydispersity) from the experimental process. The equations were solved numerically and predictions were compared to experimental data. Based on the theoretical models, further optimization experiments were performed. By means of the optimal synthetic conditions, enrichment of food-waste was comparatively studied as an attractive alternative for high-added value biopolymers.

CONCLUSIONS: Collectively, our data clearly illustrate the effect of temperature and monomer structure on the production of PLA from food waste.

ACKNOWLEDGEMENTS: This research was co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE – INNOVATE (project code: T1EDK-02746)

REFERENCES

[1] Sakai K et al. J. Ind. Ecol. 2003;7(3-4): 63-74
[2] Mehta R et al. Polym. Plast. Technol. Eng. 2007;46: 257-264
[3] Mehta R. et al. Polym. Plast. Technol. Eng. 2007;46: 933-937