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Abstracts List

TS001
The influence of aerobic exercise on the number and differentiation of blood rat endothelium progenitor cells (EPCs)

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Objectives: It is known that exercise has the ability to change the skeletal muscle fibre type. The fibre transformation induced by aerobic exercise is from type II to type I. It has been described that in these conditions there is also an increase in the capillary density. However, the source of the endothelial cells in the formation of these capillaries is unclear. A possible explanation is that exercise increases production and differentiation of EPCs from bone marrow. Therefore, the aim of this work is to study the influence of exercise on the number and differentiation of EPCs and its correlation with capillary density in soleus muscle.
Methods: In order to achieve our goal we used male Wistar adult rats, which were trained for 8 weeks in a treadmill aerobic protocol. The animals were divided in two groups: a group with exercise and a control one. The control group was submitted to a mild exercise (during the same time protocol) just to maintain the motor skills of the trained rats, and avoid central nervous system interferences. Blood was collected from the jugular vein (after anaesthesia) using 6% EDTA as anticoagulant at the end of the exercise protocol (after 8 weeks). At the end of the experiments the animals were sacrificed by anaesthetic overdose, and the soleus muscle was removed for histological and molecular analysis. The histological analysis included a hematoxilin-eosine staining (to verify muscles integrity and/or exercise-induced changes) and an ATPase staining to count and measure the different fibre types. The muscle capillaries were analysed by immunoassay. The EPC's in total blood were analysed by flow cytometry using CD45, CD34, CD133 and CD146.
Results and Discussion: The results showed that exercise increased the number of type I with a reduction in type II skeletal muscle fibres. We also verified, as expected, that the C:F ratio increases in the soleus muscle of the trained group. As for EPC's in total blood, we found that the markers for immature cells (CD34 and CD133) maintained the percentage after training but the cells $CD146^+$ decreased in the exercise group when compared to control group. This decrease was correlated with the C:F ratio.
Conclusions: Thus, our results suggest that exercise increases the EPC's turnover and differentiation into endothelial cells to form new vessels.

TS002
The influence of aerobic exercise on the number and differentiation of blood rat endothelium progenitor cells (EPCs). Effect of simultaneous treatment with ibuprofen

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Objectives: This work aims at studying the influence of exercise on the number and differentiation of EPC's and its correlation with capillary density in soleus muscle. Since many of the gym frequenting uses anti-inflammatory drugs to avoid pain during training, it is also our goal to study the influence of ibuprofen in these mechanisms.
Methods: We used male Wistar adult rats that were trained during 8 weeks in a treadmill aerobic protocol. The animals were divided into three groups: a group with exercise, exercise plus ibuprofene (40 mg/kg) and a control one. The control group was submitted to a mild exercise (during the same time protocol) just to maintain the motor skills of the trained rats and avoid central nervous system interferences. Blood was collected using 6% EDTA as anticoagulant at the end of the exercise protocol. At the end of the experiments the animals were sacrificed by anaesthetics overdose, and the soleus muscle was removed to histological and molecular analysis. The histological analysis included a hematoxilin-eosine staining (to verify muscles integrity and/or exercise-induced changes) and an ATPase staining to count and measure the different fibre types. The muscle capillaries were analysed by immunoassay. The EPCs in total blood were analysed by flow cytometry using anti-CD45, anti-CD34, anti-CD133 and anti-CD146.
Results and Discussion: The results showed that exercise increased the number of type I with a reduction in type II skeletal muscle fibres, but less in the ibuprofen group. As expected, the C:F ratio increased in the soleus muscle of the trained group and in a lower amount in the ibuprofen treated group. As for EPC's in total blood, we found that the markers for immature cells (CD34 and CD133) maintained the percentage after training but the cells $CD146^+$ decreased in the exercise group when compared to control group. In the ibuprofen group the percentage of cells with $CD34^+$, $CD133^+$ and $CD146^+$ were lower than in the control but equal to the exercise group. This decrease of $CD146^+$ cells was correlated with the C:F ratio.
Conclusions: Our results suggest that exercise increases the EPCs turnover and differentiation into endothelial cells to form new vessels. However, the junction of ibuprofen to exercise decreases the exercise effects when used alone. This may suggest that the endothelium differentiation may be related to the COX pathway.

TS003
Fetal stem cells obtained from amniotic fluid and wharton's jelly expanded using platelet lysate for tissue engineering applications

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Extra-embryonic tissues, such as amniotic fluid (AF) and Wharton's Jelly (WJ) of umbilical cord, offer many advantages over both embryonic and adult stem cell sources. These tissues are routinely discarded at parturition and the extracorporeal nature of these cell sources facilitates its isolation. Also the comparatively large volume of tissue facilitates the isolation of larger numbers of stem cells. Autologous approaches to use MSCs, namely from bone marrow, have difficulties regarding the limited numbers of cells from the patient but also its limited expansion potential. Furthermore, fetal stem cells appear to have even more pronounced immunomodulatory properties than adult MSCs. This allogeneic escape mechanism may be of therapeutic value, because transplantation of allogeneic human MSCs in stock would be readily available, as opposed to the required expansion stage (involving both time and logistic effort) of autologous cells. Cell expansion protocols are based on the use of media supplemented with fetal bovine serum (FBS) as a source of nutrientes and growth factors. The animal serum is not completely safe, once there is a possibility of contamination by animal viroses, prions or others source of contaminants. Additionally, it was described that FBS used systematically in MSCs subcultivation induces more humoral immune response. Moreover, anti-FBS antibodies could be detected in patients after receiving MSCs expanded in FBS. Platelet lysate (PL) has enormous possibilities in cell therapy, namely because of the high concentration of growth factors that promotes higher cell expansion. A recent study showed that proliferation of MSCs was much higher on PL gel compared to tissue culture plastic. The immunomodulatory properties of MSCs are maintained when expanded in culture medium supplemented with PL. Based on these premises we isolated fetal stem cells from AF obtained from amniocentesis and WJ from umbilical cords. These cells were plated and expanded in low density numbers in basal culture medium either supplemented with FBS or with PL. In each passage cells were counted for proliferation kinetics and prepared for flow cytometry analysis. Expanded populations were analysed both for population size and phenotype and for the characteristic MSCs surface markers (CD34, CD45, CD73, CD44, CD106, CD105, CD29, CD90, CD31), markers related with immune response (HLA-DR, 80, 83, 86) and embryonic markers SSEA-4 and TRA-1-60.

TS004
Cell sheet engineering for reproducing the bone marrow hematopoietic stem cell niche

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Hematopoietic stem and progenitor cells (HSPC) are multipotent cells responsible for the maintenance and renewal of the hematopoietic lineage in the adult body. The fate of these stem cells is closely regulated by their surrounding microenvironment, or niche. The importance of the microenvironment for HSPC function has been long recognized by researchers that more than 30 years ago attempted to emulate it in 2D using a layer of bone marrow stromal cells to culture hematopoietic cells for long time periods (Dexter-type cultures). However, all the models based on feeder layers are less than perfect in recreating the hematopoietic microenvironment. The use of growth factor cocktails provided some promising results concerning the maintenance and proliferation of some cell populations but still struggle to deliver the correct microenvironment for the maintenance of suitable HSPC populations. Part of the problem of the current systems lies on the lack of the third dimension. At the same time, the proposed three-dimensional methodologies using scaffolds to engineer the bone marrow (BM) microenvironment present very limited results probably due to the scaffolding matrices' intrinsic limitations. Therefore, an engineered BM microenvironment capable of acting as a functional HSPC niche would provide a tremendous tool for the study of hematopoiesis as well as for obtaining and maintaining HSPC. Using osteogenic cell sheets, we have previously demonstrated that it was possible to induce the ectopic formation of mature bone tissue with a clear bone marrow, avoiding the use of scaffolds. In the present work, we studied the potential of using osteogenic cell sheets to build *in vitro*, a 3D microenvironment capable of providing HSPC a suitable niche for their survival and proliferation. For this, we used bone marrow stromal cells and adipose-derived stem cells to produce the osteogenic cell sheets and human umbilical cord blood as a source of hematopoietic stem cells.

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TS005
Improved regeneration of mice full-thickness excisional wounds by human adipose stem cells cell-sheets

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Stem Cells have emerged as a powerful element for generating skin tissue and for promoting enhanced wound healing through the differentiation into relevant skin cell lineages and/or by paracrine interactions. Adipose stem cells (hASCs) in particular, appear as an attractive cell source for skin regeneration, due to their natural abundance, relatively easy methodology of isolation and secretion of factors important for the restoration of healthy skin. Common approaches for stem cell delivery, comprehending the use of direct injection of single stem cell suspensions or the use of biomaterials-based strategies, comprise in some cases, poor engraftment of those cells or associated inflammatory processes that lead to a reduced effect over skin restoration. An innovative alternative comprehends the use of Cell Sheet (CS) engineering that, by taking advantage of temperature-responsive culture surfaces, allows the non-invasive harvest of cultured cells, as intact sheets, along with their deposited extracellular matrix, facilitating the direct transplantation to host tissues. This study exploited the potential of CS Engineering for fabricating 3-layered hASCs CS to fully regenerate mice full-thickness excisional wounds. The outcome of the transplanted cell machinery and the success of this technology were evaluated at relevant timepoints considering wound healing parameters such as re-epithelialization, angiogenesis, neotissue quality formation and hASCs contribution for the skin tissue regeneration. Overall, the created constructs showed good stability *in vitro*, were easily attached to the wound bed and showed to play a significant and specific role over epidermal regeneration through paracrine effects. *Acknowledgements* The authors would like to acknowledge for the financial support by Skingineering (PTDC/SAU-OSM/099422/2008), Portuguese Foundation for Science and Technology (FCT) funded project.

TS006
Fish scales patterning guiding hASC growth

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Marine organisms and materials exhibit interesting properties for their use in biomedical applications, following biomimetic approaches [1,2]. For instance, the white seabass (*Lates Calcarifer*) scales exhibit a very interesting pattern, which inspire their use as a guiding platform for cellular growth, in a tissue regeneration approach. Moreover, fish scales are composed of hydroxyapatite and type I collagen fibrils, equivalent to the materials that one can find in human bone, as thus their use in bone tissue engineering is envisaged. The main goal of the present work was the assessment of fish scales, combining intrinsic features such as patterns, hydroxyapatite and collagen in different means, as cell culture supports aiming at guiding cell growth and extracellular matrix deposition and mineralization. In this sense, human adipose derived stem cells (hASCs) were cultured onto seabass scales, under osteogenic and non-osteogenic conditions. Fish scales supported cell adhesion and cytoskeleton organization defined by the surface patterning. Moreover, hASCs were able to proliferate along the time of culture and to differentiate towards the osteogenic lineage depositing and mineralizing the characteristic extracellular matrix. This work constitutes the first step to demonstrate the value of the intrinsic properties of seabass scales for exploitation in the biomedical field and in particular for bone tissue engineering.

References

1. TH Silva *et al.*, Materials of marine origin: a review on polymers and ceramics of biomedical interest, International Materials Reviews 2012, *in press*. DOI: 10.1179/1743280412Y.0000000002
2. TH Silva *et al.*, Biomaterials from Marine-Origin Biopolymers", in J.F. Mano (Ed.): Biomimetic Approaches for Biomaterials Development, Wiley-VCH, 2012, in press.

TS007
Mechanical liposuction vs. laser liposuction vs. surgeries – the best method of adipose tissue harvesting for ADSC isolation

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Objectives: One of the major goals of tissue engineering and regenerative medicine is search and characterize the new sources of mesenchymal stem cells, which will be better than bone marrow. Adipose tissue is easily accessible and abundant source of stem cells hence their isolation creates new opportunities for regeneration of injured tissues. The aim of this study was the comparison of the Adipose Derived Stem Cells (ADSCs) quality harvested during mechanical liposuction (ADSCsML), laser liposuction (ADSCsLL) and surgeries (ADSCsS) and to select facilely available mesenchymal stem cell source with the highest proliferative potential and ability to differentiate.
Methods: The human adipose tissue was obtained during planned surgical procedures in patients with pathological obesity ($n = 30$), mechanical liposuction ($n = 8$) and laser liposuction ($n = 10$). ADSCs after isolation were cultured in DMEM/Ham's F-12 medium supplemented with 20% (up to the first passage) or 10% (after first passage) fetal bovine serum (FBS), 1% antibiotic and basic fibroblast growth factor (bFGF). To characterize their properties the proliferation rate, differentiation potential and phenotype analysis (CD34, CD44, CD45 and CD90 expression) were performed. Results and Discussion: ADSCs harvested by three methods underwent rapid adhesion to the surface of culture dishes and began to proliferate. The proliferation rate was stable during 14 days of culture. The cells proliferated at least to the sixth passage. The success of culture establishment was 86.7%, 100% and 100% for ADSCsS, ADSCsLM and ADSCsLL, respectively. The average of cell number per 1 g/mL of tissue, at the first passage, was 2.3×10^5, 1.1×10^5 and 9.7×10^3 for ADSCsS, ADSCsLL and ADSCsLM, respectively. ADSCs cultured in medium supplemented with differentiation factors toward osteogenic, adipogenic and chondrogenic lineages showed ability to multilineage differentiation. All ADSCs types were CD44+, CD90+, CD34+ and CD45- (at third passage).
Conclusions: The results obtained in that study support the findings that adipose tissue harvested by three considered methods contains rapidly growing cells with high differentiation potential. However, concerning average of cells number at the first passage, adipose tissue collected during surgeries seems to be the most efficient. Nevertheless, liposucation is less invasive procedure so that ADSCs isolated from lipoaspirates obtained from a larger number of patients require more detailed investigation.

TS008
Microparticles loaded Gellan gum hydrogel matrices: engineering tissues for nucleus pulposus regeneration

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The intervertebral disc central core is made by a gel-like tissue structure composed of more than 80% of water, Nucleus Pulposus (NP). Proteoglycans such as versican and especially aggrecan are the main constituents of the NP matrix as well as collagen type II. The purpose of this work is creating novel Gellan gum-based (GG) hydrogel formulations. GG microparticles (MPs) dispersed in a GG matrix are the novelty for finding application as NP substitute. The ongoing experiment comprises de GG functionalization through methacrylated groups addition. In order to optimize some properties of GG, the functionalization will allow us to improve the water solubility and photopolymerization *in situ* of the biomaterial. High acyl (HA) and Low acyl (LA) Gellan Gum (GG) at different ratio [75%:25% (v/v); 50%:50% (v/v), 25%:75% (v/v)], HAGG 0.75% and LAGG 2%, were mixed in order to prepare solutions to be used as formulations of GG MPs/hydrogels matrix. The GG MP/hydrogel matrix formulations were characterized by dynamic mechanical analysis (DMA), swelling behaviour and degradation rate. The toxic effect of GG MPs/hydrogel discs leachables onto the cells was investigated *in vitro* using a mouse lung fibroblast-like cell (L929 cells) line. Live/Dead cell viability assay was performed to assess the encapsulation efficacy; meanwhile DAPI/Phalloidin staining was performed to evaluate cell morphology. The Methacrylated Gellan Gum (GG-MA) was prepared following the protocol [1]. Mechanism reactions occurred in presence of glycidyl methacrylate by addition to a solution of LAGG at 2% (w/v). The reaction was running over 24 h at room temperature controlling the pH at 8.5 with sodium hydroxide 1 M. GG MPs size was measured using a stereo microscope by staining the MPs with Toluidine Blue-O. This method also allowed evaluating the MPs dispersion and matrix cohesion. From DMA analysis it was observed that the range of 50–500 mg/mL of incorporated MPs is the optimal concentration to reinforce GG matrices. It was demonstrated the non–cytotoxic effect of MPs/hydrogels over L929 cells. In fact, L929 cells were successfully encapsulated in all GG formulations GG MPs and remaining viable over 72 h of culturing. The resulting product from methacrylation reaction was evaluated by nuclear magnetic resonance to assess the reaction efficiency and the degree of substitution. Methacrylated Gellan gum and GG MP/hydrogel matrix are promising hydrogels to be used in tissue engineering strategies for treatment of the degenerated NP.

Reference

1. Silva-Correia J, 2011 Journal of Tissue Engineering and Regenerative Medicine, 5(6): e97–e107.

TS009
Dendrimer-based nanoparticles in tissue engineering and regenerative medicine approaches

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Dendrimeric macromolecules are synthetic and spherical systems that have been attracting great deal of attention as nanocarriers for intracellular drug delivery (DDS) [1]. These possess several advantages as nanocarriers, as it possibly incorporating smart functionalities such as targetability and stimuli-responsive drug release. Our group has been proposing the use of dexamethasone (Dex)-loaded carboxymethylchitosan/polyamidoamine dendrimer nanoparticles (CMCht/PAMAM NPs) [2,3] for controlling the differentiation of stem cells towards the osteogenic lineage. In this work, the biocompatibility of the Dex-loaded CMCht/PAMAM NPs were investigated, *in vitro* and *in vivo*. CMCht/PAMAM NPs were produced as described by Oliveira *et al.* [4]. Dexamethasone (Dex) was loaded into the NPs by means of using a precipitation route. Dex-loaded NPs were chemically bound to fluorescein isothiocyanate (FITC) for styding their intracellular fate. The cellular uptake of NPs was investigated using different cell types namely rat bone marrow stromal cells (RBMSCs) and Human Osteosarcoma cell line (SaOS-2), for times up to 14 days of culturing. The internalization of the FITC-labelled and Dex-loaded CMCht/PAMAM dendrimer NPs waere investigated using fluorescence microscopy and flow cytometry (FACS) analyses. FITC-labelled NPs (1 μg/g and 10 μg/g) were injected intraveneously on Wistar rats and its biodistribution in different organs was evaluated up to 72 h. Results have revealed that Dex-loaded CMCht/PAMAM dendrimer NPs are non-cytotoxic, *in vitro* and *in vivo*. The *in vivo* studies also revealed that CMCht/PAMAM dendrimer NPs are stable in circulation. The histological study has shown that NP's are uptaken by cells from different tissues/organs namely, brain, liver, kidney and lung. This study has revealed that the dexamethasone-loaded CMCht/PAMAM dendrimer nanoparticles are non-toxic and demonstrated a great ability for being uptaken by different cell types. Moreover, we have demonstrated that the dexamethasone-loaded CMCht/PAMAM dendrimer nanoparticles are able to control stem cells osteogenic differentiation. As a final conclusion, the developed nanocarriers are advantageous as intracellular nanocarrier tools for preprogramming the stem cells fate *ex vivo*. The developed dexamethasone-loaded CMCht/PAMAM dendrimer nanoparticles showed great promise for application in bone tissue engineering strategies as it allowed us to produce de novo bone tissue *in vivo*.

References:
1. J.M. Oliveira, et al., 2010, Progress in Polymer Science, 35:1163–1194.
2. J.M. Oliveira, et al., 2010, Bone, 46:1424–1435.
3. J.M. Oliveira, et al., 2011, Nanomedicine: Nanotechnology, Biology, and Medicine, 7:914–924.
4. J.M. Oliveira, et al., 2008, Advanced Functional Materials, 18:1840–1853.

TS010
Controlling mesenchymal stem cell proliferation and differentiation through glycosaminoglycan engineered surfaces

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Proliferation and differentiation of mesenchymal stem cell (MSCs) are regulated by its "niches" - the specific microenvironment that accounts for tissue maintenance and repair. Glycosaminoglycans (GAGs), as components of niches, bind non-covalently growth factors and trigger multiple downstream signaling pathways controlling cellular activities. Unraveling the chemical structures of GAGs like hyaluronic acid (HA), chondroitin sulfate (CS) and heparin (Hep) has shown that they all have similar disaccharide units, but differ in the quantity and location of sulfate groups, which greatly influences their interactions with proteins and cells. Here we developed a 2D model to study GAG effects on mesenchymal stem cells' (MSCs) proliferation and differentiation, which may pave the way for applications in tissue engineering. GAGs were oxidized using periodate to generate dialdehydes, shown by FT-IR and H1-NMR spectroscopy, which were covalently immobilized on amino-silane modified substrate by reductive amination. Surface wettability increase after immobilization of hydrophilic ox-GAG molecules. Swelling surfaces exhibited relative flatten to aggregated structures, as investigated by AFM, with the increase of sulfation of immobilized molecules. Meanwhile the roughness increased from 0.35 nm to 1.42 nm. Zeta-potentials of ox-GAGs immobilized surfaces decreased with ox-HA<ox-CS<ox-Hep, indicating that the amount of sulfate groups increased. Proliferation of multipotent C3H/10T1/2 mesenchymal mouse cells was enhanced on ox-Hep while inhibited on ox-HA. Confocal laser scanning microscopy images showed that cells on ox-Hep had the largest spreading area, by 24% beyond the others, whereas the smallest form factor of 0.59. Alizarin red-S staining showed that red dye bonded to nodules on each surface, indicating osteogenic differentiation in the presence of rhBMP-2, with ox-HA<ox-CS<<ox-Hep. It can be assumed that the larger amount of sulfates on ox-Hep contributed to the binding of BMP-2, which protected active protein-glycan complex from being suppressed by antagonists, thus significantly enhance osteogenesis of MSCs. In summary, GAG immobilized surfaces with growth factors can mimic natural ECM-protein interactions and enhance growth factor activity, which seems to be a useful method to engineer MSCs' proliferation and differentiation for tissue repair and regeneration.

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TS011
Novel cellulose and chitosan sulfates promoting the activity of growth Factors FGF-2 and BMP-2

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Growth factors regulate proliferation and differentiation of cells and play important role as tools in tissue engineering. Particular emphasis has been put on the application of heparin as component of surface coatings and tissue engineering scaffolds to produce systems for controlled release of growth factors with heparin binding domains like FGF, VEGF and BMP. However, heparin is not an ideal material due to the variability of its sulfate content, molecular weight and bioactivity. Here we present novel derivatives of cellulose and chitosan that possess bioactivity comparable or greater than that of heparin with first insights into the mechanism of their bioactivity. Cellulose and chitosan were chemically modified to obtain derivatives that differ in the degree and substitutions site of sulfates and partly also carboxylates. The chemical structure and derivatisation degree was studied with Raman and NMR spectroscopy. Studies with the growth factors FGF-2 that possesses not only mitogenic but also moderate angiogenic effect and 3T3 fibroblasts revealed that the binding and mitogenic activity of derivatives was enhanced with increasing sulfate content. The introduction of carboxylic groups to the O-6-position of glucose in analogy to heparin was not effective. Further studies with BMP-2 demonstrated an osteogenic activity of derivatives with a maximum at intermediate sulfation degree, which was evident by the alkaline phosphatase activity of C2C12 mouse myoblast cell line and expression of osteogenic genes. Studies on the mechanism of action of derivatives vs. FGF-2 revealed that particularly a protection from proteolytic digestion seem to prolong activity of growth factors. Since all assays described above were solution based further studies were carried testing whether cellulose sulfates can be used as bioactive surface coatings. Cellulose sulfates with high bioactivity were selected and used to form multilayers with chitosan using the layer-by-layer technique. It was found that intermediate sulfated cellulose sulfates were particularly effective because a long-term release was detected. Overall, the novel derivatives seem to be suitable for construction of bioactive surface coatings or tissue engineering scaffolds promoting angiogenesis and bone formation.

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TS012
Development of a bilayered system for periodontal regeneration using tissue engineering approaches

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Periodontitis is a prevalent gram negative infection disease that causes the destruction of the tooth supportive tissues. Effective treatment of periodontal disease is important, since periodontal disease is correlated with several systemic diseases. However, adult periodontal tissues have a low potential of self-renewing and regeneration. Concerted efforts have been made to accelerate periodontal tissue regeneration, using a plethora of techniques including grafting materials, signalling molecules and cell-based tissue engineering. Nevertheless, a strategy for predictable reconstruction of normal structure and functionality of periodontal damaged tissue is still missing. In this work, we propose the development of a bilayered system for the regeneration of alveolar bone and periodontal ligament. This system consists of a bilayered composite made of calcium phosphate (CaP) cement incorporating hyaluronic acid microspheres loaded with Platelet Lysates (PL) and a hydrogel layer based on PL, harbouring mesenchymal stem cells (MSCs). The advantage of this strategy lies in the ability to develop a system that can be easily injected and which provides adequate mechanical support, both initially and during new tissue ingrowth. After the degradation of the HA microspheres incorporated in the CaP cement, a fully interconnected network can be created, which leads to rapid penetration of bone-forming cells into the CaP cement. Additionally, the distinct degradation rates of the components of the bilayered system allow a controlled release of the entrapped growth factors and further accelerate the periodontal tissues remodelling process, mimicking the physiologic wound healing process. The data collected suggests that it is possible to fabricate the cement composite layer incorporating PL from which a number of growth factors are released in a controlled manner. Moreover, the cement composites incorporating HA microspheres loaded with PL show low cytotoxic values and induce the expression of early markers of osteogenic differentiation in human adipose-derived stem cells (hACS).

TS013
Development of bioactive constructs through cell crosslinking for tissue regeneration

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Injectable systems are particularly attractive for a minimally invasive approach in tissue engineering applications. Many different methods for *in situ* crosslinking have been investigated however the ability of cells to promote hydrogel formation has not been fully explored. This study addressed the hypothesis that cells can promote crosslinking of chitosan microparticles forming a tridimensional network. Through covalent immobilization we were able to functionalize particles with specific antibodies, used to promote the attachment of cells and growth factors of interest. CD90 anti-human antibodies that are highly expressed by human adipose stem cells (hASCs) were successfully conjugated with chitosan microparticles. A tridimensional hydrogel was obtained by the assembly of the modified chitosan microparticles with hASCs and was stabilized by the crosslinks established by the entrapped cells. The degree of crosslinking of the structure could be regulated by the cell concentration in each construct. Moreover we believe that a combination of microparticles tailored with specific growth factors will increase the stability of the construct and promote cell differentiation. It is well established that platelets are an important source of autologous growth factors that can modulate cell growth and differentiation. In this study we propose antibody-conjugated particles as a method to select specific growth factors from the mixture obtained from platelet lysates. The obtained construct simultaneously provides support for stem cell growth as well localized and sustained presentation of factors to modulate cell differentiation. We intend to design a novel multifunctional injectable system that may be customized by combining particles with different growth factors for a specific application.

TS014
Liquified multilayered capsules incorporating microparticles for cell adhesion sites

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Polymeric multilayered capsules (PMCs) have found great applicability in various applications due to their versatile wall functions, capability to load active substances and unique permeability. PMCs are based on the sequential adsorption of polyelectrolytes by the layer-by-layer (LbL) technique, followed by the elimination or liquefaction of the template core. The principle of the strategy is to physically isolate a wide range of materials, including cells, proteins and/or therapeutic molecules, from the outside environment. This selective permeability is mediated by the LbL membrane, which allows the diffusion of nutrients, oxygen, waste products and metabolites, while avoiding the entrance of high molecular weight immune system components. However, when living cells are encapsulated, the existing methodologies still have to address a main issue related to the fact that most cells are anchorage-dependent and, thus, cannot grow in suspension. Therefore, although the liquified environment ensures the diffusion of essential molecules for cell survival, on the other hand liquified environments are deprived from cell adhesion sites. To overcome this main drawback, we hypothesized that liquified and flexible capsules combined with encapsulated microparticles to provide cell adhesion sites are a promising attempt. To test this hypothesis, hierarchical structures featuring (i) an external shell combining three polyelectrolytes, namely poly(L-lysine) (PLL), alginate (ALG) and chitosan (CHT) prepared by LbL, and (ii) incorporating surface functionalized poly(L-lactic) acid (PLLA) microparticles were developed. The construction of the multilayered structure by quartz-crystal microbalance with dissipation monitoring was monitored. Additionally, the mechanical performance of capsules was evaluated. Results show that the combined assembly of PLL, ALG and CHT resulted in a more resistant and thicker film with an exponential build-up growth regime compared to the assembly without PLL. The ability of the optimized capsules to support cell survival was assessed. L929 cells were encapsulated and cell viability and proliferation assays were performed. Results show that capsules containing PLLA microparticles revealed an enhanced metabolic activity, biocompatibility and proliferation. We believe that the developed approach will offer new possibilities to the existing bioencapsulation strategies. Different microparticles loaded with growth factors and other biomolecules of interest can be encapsulated in order to customize and control different cellular functions, such as differentiation of stem cells into the desired lineage, according to the target application field.

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TS015
Gellan gum – hydroxyapatite composite hydrogels for bone tissue engineering

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The modification of polymeric matrices by adding calcium-phosphate derivatives has been proven an effective strategy for tailoring the properties of scaffolds employed in bone tissue engineering. In this regard and, considering the biomechanics of bone as well as the durotactic response of osteoblasts, this study builds on the hypothesis that the preparation of novel Gellan Gum (GG)-Hydroxyapatite (HA) hydrogel composites could benefit the mechanical profile of matrices as well as the cell-substrate interaction in favor of cell recruitment and growth. To this purpose, HA microparticles at different concentrations (10 and 20%) were successfully incorporated into GG based hydrogels. The composites were characterized by Scanning Electron Microscopy (SEM) coupled with Energy Dispersive Spectroscopy (EDS), Fourier Transformed Infrared Spectroscopy (FTIR), X-Ray Diffraction Analysis (XRD), Micro Computed Tomography (μ-CT) Analysis and Thermogravimetric Analysis (TGA). *In vitro* degradation and swelling studies were conducted in PBS solution, while the GG/HA composites were subjected to Dynamic Mechanical Analysis (DMA). The efficacy of the GG/HA matrices to precipitate apatite in Simulated Body Fluid (SBF) was evaluated, while the cell-matrix interactions were studied by seeding the composites with human osteoblast-like cells. The cell-viability was assayed by staining the cell-loaded composites with calcein-AM, Texas Red-Phalloidin and DAPI-blue and observing by confocal microscope. The image (SEM, μ-CT)-assisted microstructural characterization of the GG/HA composites reveals hydrogels with high porosity (>80%) and average pore size of 260 μm. GG/HA composites demonstrate high water retention ability during swelling studies, while the weight loss did not exceed 8% during the degradation studies. FTIR and XRD detected peaks typical of hydroxyapatite. The thermal curves of the composites disclose an initial weight loss due to moisture removal and two subsequent degradation phases due to the polymeric component decomposition. The matrices exhibit increasing storage modulus (E') and decreasing loss factor (tan δ) as a function of frequency during DMA analysis, while composites containing 20% HA possess always higher E' and lower tan δ values. GG/HA composites develop bioactivity in the form of multiple agglomerates of apatite crystals since the 3rd post-immersion day in SBF. Viability assays indicated that the human osteoblast-like cells seeded on the GG/HA composites were metabolically active. Concluding, the modification of GG-based hydrogels by HA in different concentration appears to result in composites that meet several scaffold-design criteria and to allow the tailoring of their mechanical performance. However, further optimization is required in order to improve cell adhesion and to promote cell growth.

TS016
Is bladder wall regeneration nowadays feasible? Comparison of different biomaterials in bladder wall augmentation

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Introduction: The gold standard for bladder wall creation after radical cystectomy gastrointestinal segments augmentation. However, bowel replacement is associated with many complications**.** The ideal biomaterial for bladder reconstruction has not been found so far. The purpose of this study was to compare the three scaffolds: artificial L–lactide–co–caprolactone (PLC 7015), natural SIS, and composite of amniotic membrane and L–lactide–co–caprolactone (CAM-PLC 7015) in rat bladder wall regeneration.
Methods: Adipose tissue was harvested from 8-week 10 male Wistar rats. Adipose derived stem cells (ADSCs) were seeded in density 3×10^6 cells/cm^2 onto PLC, SIS and CAM-PLC scaffolds and cultured for 5 days in stem cell culture medium. Before implantation the cells were labeled with PKH-26 dye. 28 male Wistar rats were divided into seven equal groups. Augmentation cystoplasty was performed in the earlier created dome defect. Group I: SIS+ 3×10^6ADSCs; II: PLC+ 3×10^6ADSCs; III: CAM-PLC+ 3×10^6ADSCs; IV: SIS; V: PLC; VI: CAM-PLC; VII: control. The reconstructed bladder walls were evaluated in H&E staining and macroscopic estimation. The presence of implanted cells was assessed by fluorescence microscopy. Cystography was performed.
Results: All bladder wall layers has been regenerated in bladders augmented with cell seeded scaffolds. Cystography and histological analysis indicated that the best results were obtained when the bladders were reconstructed with SIS or amniotic membrane. In the bladders substitute with acellular matrices fibrosis and graft contraction occurred. Fluorescence analysis confirmed the presence of implanted cells 3 months after reconstruction and stated the cell migration to spleen, bone marrow and liver.
Conclusions: Adipose tissue is attractive source of stem cells for reconstructive urology. PLC matrix seems to possess poorer regeneration potential comparing to bladder wall restored with SIS or amniotic membrane.

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TS017
Novel bilayered Gellan gum/Gellan gum-hydroxyapatite scaffolds for osteochondral tissue engineering applications

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Osteoarthritis is a major cause of disability during aging. By the age of 60, close to 100% of the population will have histologic changes of degeneration in their knee cartilage (Loeser, 2000). Because of its avascular nature, cartilage has little capacity to self-regenerate. Despite the progress already achieved in osteochondral regeneration, some limitations have to be overcome. The formation of fibrocartilage has to be avoided and the innervation has to be improved. Further, one main feature to be promoted is the induction of vascularization in the bony part but not in the cartilage part and to avoid de-differentiation processes. A promising strategy could pass through the development and optimization of novel culture systems. The ideal approach could integrate scaffolds presenting regions with different physical characteristics, combined with different growth factors to support different stem cells fates, regarding the complex tissue physiology to be regenerate. This work aims to develop novel bilayered gellan gum (GG)/gellan gum-hydroxyapatite (HAp) hydrogels based structures for osteochondral tissue engineering applications. Bilayered GG/GG-HAp hydrogels were produced by joining both solutions of GG 2% (w/v) with and without HAp (20% wt.) for bony and cartilage parts, respectively. The solutions were introduced into a silicone mould with 20:10 mm (height and diameter, respectively). Gelation of GG was promoted by immersion in PBS solution for 24 h. The architecture of the bilayered scaffolds was investigated by micro-computed tomography. Results have shown that the freeze-dried bilayered scaffolds composed by low acyl GG(2%(w/v)/low acyl GG(2%(w/v)-HAp20%(w/w) possess a porosity of 83.4 ± 0.8%, pore size of 279.3 ± 38.6 μm and interconnectivity of 62.2 ± 5.4%. Degradability assays are being performed with the intent to use this system to culture human adipose derived stem cells inducing cell co-differentiation into chondrocytes and osteoblasts. Ultimately, the developed bilayered scaffolds will provide new therapeutic possibilities for the regeneration of osteochondral defects.

TS018
Free-standing multilayer films made of chitosan and alginate for biomedical applications

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Objective: The method for preparing multilayer films by the consecutive deposition of oppositely charged polyelectrolytes has gained tremendous recognition due the user friendly preparation, capability of incorporating high loads of different types of biomolecules in the films, fine control over the materials' structure, and robustness of the products under ambient and physiological conditions. However the preparation of such films needs the assembly on a substrate and, sometimes, cannot be detached from it, which has limited the application of such films in areas as tissue engineering and regenerative medicine. Herein, we report the production of chitosan/alginate (CTS/ALG) free-standing films that can be detached from an underlying inert substrate without any postprocessing step allowing the determination of physical properties of fundamental significance such as ion permeation and mechanical properties.
Methods: In this work, the buildup of free-standing multilayer films made of CTS and ALG was investigated. Several conditions were tested to follow the film growth in order to get thick films. The CTS/ALG free-standing films were characterized by Fourier transform infrared spectroscopy (FTIR) and by scanning electron microscopy (SEM). Permeability tests were performed using FITC-dextran, with several molecular weights, as a drug model molecule. Cell adhesion was assessed using C2C12 myoblast cells over a period of 48 h. Results and Discussion. The produced membranes can be detached from an underlying inert substrate without any postprocessing step. Permeability experiments on these membranes revealed that the permeation of FITC-dextran depended greatly on its molecular weight. The results showed that these films are a good substrate for cell adhesion as spread cells were observed all over the surface, by actin and heochst staining.
Conclusions: The production of free-standing films permits the direct experimental determination of many physical properties of fundamental significance such as ion permeation and mechanical properties that can be tuned for real-world applications. These free-standing films are easy detachable, easy to handle, stable in the presence of physiological solutions and biocompatible, demonstrating potential for applications in tissue engineering and regenerative medicine.

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TS019
Designing silk fibroin-based matrices with ionic liquids for tissue engineering strategies using human adipose stem cells

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Silk (SF) is an attractive biomaterial to be used in tissue engineering applications because of its excellent mechanical properties and biocompatibility [1, 2]. In this work, the cellular response of silk hydrogels produced through dissolution of this protein in ionic liquids (ILs) was investigated. For that, degummed fibers obtained from the cocoons of mulberry silkworm (Bombyx mori) were dissolved in an IL and the solution was gelified in ethanol, followed by IL removal from gels using soxhlet extraction. The fabricated hydrogels were characterized biochemically and biophysically by detecting amino acid composition, FTIR, SEM and mechanical testing (DMA). For *in vitro* assessment, human adipose stem cells (hASCs) were seeded in the hydrogels and cultured for different time periods. The resulting hydrogels have a rubbery consistency, homogeneous surface and viscoelastic behavior. Additionally, no differences on amino acid composition were found, indicating that the silk composition was kept. Confocal images confirmed cell attachment and alignment of actin filaments within the hydrogel matrix with well-develop nuclei. The MTS assay demonstrated the metabolic activity of hASCs in contact with hydrogels up to 28 days. Furthermore, the results of DNA quantification showed that hASCs are able to proliferate during studied period. These results indicated that (i) the efficiency of IL removal resulted in hydrogels with minimal cytotoxicity; and (ii) positive cellular response of the materials surface for the adhesion and proliferation of hASCs. SEM observations corroborated with the results obtained from MTS and DNA suggested that cells are able to migrate at different levels within the structure. These findings indicated that silk hydrogels produced using ILs may be potential candidates for tissue engineering strategies, namely cartilage regeneration.

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References:

1. Silva, S.S., A. Motta, M.R.T. Rodrigues, A.F.M. Pinheiro, M.E. Gomes, J.F. Mano, R.L. Reis, and C. Migliaresi. Biomacromolecules, 2008. **9**(10): p. 2764-2774.
2. Altman, G.H., F. Diaz, C. Jakuba, T. Calabro, R.L. Horan, J.S. Chen, H. Lu, J. Richmond, and D.L. Kaplan. Biomaterials, 2003. **24**: p. 401–416.

TS020
Multilayered polymeric particle production using superhydrophobic surfaces methodology for drug delivery and tissue engineering applications

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Encapsulating technologies that render spherical particles containing cells or relevant moleculeshave been developed to be used in fields such as tissue engineering, pharmaceutics, cosmetics, agriculture, as also in other bio-related applications, namely biosensors and bioreactors. The multiple bioactive agents release, with an important role in tissue regeneration, constitutes an important strategy in tissue engineering. The control of bioactive agents release may be achieved increasing the complexity of the encapsulating particles by adjusting the chemistry and the architecture. In this context, multi-compartmentalized systems able to simultaneously deliver various bioactive agents at different kinetics have emerged and are envisioned to be the next area of development. Multilayered particles exhibiting predefined diameters and layers thickness may offer additional advantages including higher bioactive agents loads, improved molecules stability, and tailored release schedules such as delayed or pulsatile avoiding initial bursts. The most external layers could even act as rate-limiting barriers to further reduce burst release. Since multilayered particles are compartmented, each particle can load multiple bioactive agents isolated from each other. Similarly, more than one type of cells may be immobilized into different compartments. The layers thickness and composition determine the performance of the system. Compared to monocompartment delivery systems, the development of multi-compartmented structures is still immature and intensive efforts are being done to efficiently produce this type of systems. The production of multi-compartmented particles is quite challenging and the existing methodologies involve wet and aggressive conditions that compromise the encapsulation efficiency of bioactive agents and the viability of cells. Herein we report a simple bottom-up approach suitable for preparing multilayered polymeric particles in a very fast way, which involves the use of biomimetic superhydrophobic surfaces. In the present work, concentric multilayered polymeric particles were prepared by adding layers one-by-one, and then their applications as carriers for sequential multiple drug release and as scaffolds for cells immobilization intended in cell therapies or tissue engineering were explored. The results showed that the engineered particles can be loaded with different molecules confined in different compartments for later sequential and time-programmed release. They can also immobilize cells maintaining them viable for long time, being potentially useful for cell-based therapies.

TS021
Mechanical performance and biocompatibility study of methacrylated Gellan gum hydrogels with potential for nucleus pulposus regeneration

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Methacrylated gellan gum hydrogels, obtained either by ionic- (iGG-MA) and photo-crosslinking (phGG-MA), have been investigated as potential biomaterials for supporting *nucleus pulposus* (NP) regeneration and/or repair [1,2]. In previous work, some advantages were attributed to GG-MA hydrogels, such as: (i) the possibility to control endothelial cells infiltration and blood vessel ingrowth's, (ii) tunable and improved mechanical properties, and (iii) *in situ* gelation, within seconds to few minutes. In this study, the mechanical and biological performance of these hydrogels was firstly evaluated *in vitro*. Human intervertebral disc (hIVD) cells obtained from herniated patients were cultured within both hydrogels, for 1 up to 21 days. Dynamic mechanical analysis and biological characterization (calcein-AM staining, ATP and DNA quantification and PCR) were performed after specific times of culturing. A biocompatibility study was also performed *in vivo*, by subcutaneous implantation of acellular iGG-MA and phGG-MA hydrogels in Lewis rats for the period of 10 and 18 days. Tissue response to the hydrogels implantation was determined by histological analysis (haematoxylin-eosin staining). The *in vitro* study showed that both cell loading and culturing time do not have an effect on the mechanical properties of the hydrogels. Regarding their biological performance, the iGG-MA and phGG-MA hydrogels showed to be effective on supporting hIVD cells encapsulation and viability up to 21 days of culturing. Human IVD cells were homogeneously distributed within the hydrogels and maintained its round-shape morphology during culturing time. The *in vivo* biocompatibility study showed that iGG-MA and phGG-MA hydrogels do not elicit any deleterious effect, as denoted by the absence of necrosis and calcification, or acute inflammatory reaction. A thin fibrous capsule was observed around the implanted hydrogels. The results presented in this study indicate that the iGG-MA and phGG-MA hydrogels are stable *in vitro* and *in vivo*, support hIVD cells encapsulation and viability, and were found to be well-tolerated and non-cytotoxic *in vivo*, thus being potential candidates for NP regeneration.

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References:

1. J. Silva-Correia, et al., J Tissue Eng Regen Med, 2011, 5(6):e97-e107.
2. J. Silva-Correia, et al., Tissue Eng Part A, 2012, 18(11–12):1203–1212.

TS022
Advanced mimetic materials for meniscus tissue engineering: targeting segmental vascularization

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Meniscus lesions are among the most common orthopaedic injuries which can ultimately lead to degeneration of the knee articular cartilage. The human meniscus has a limited healing potential, partly due to a poor vasculature, and thus meniscus regeneration using tissue engineering strategies has recently been investigated as a promising alternative to total/partial meniscectomy [1]. Advanced scaffolds for tissue engineering of meniscus should be able to mimic and preserve the asymmetric vascular network of this complex tissue, *i.e.* enable controlling the segmental vascularization during the regeneration process. Novel scaffolds were produced combining a silk polymeric matrix (12 wt%) [2] and the methacrylated gellan gum hydrogel (iGG-MA), which has been shown to be able to prevent the ingrowth of endothelial cells and blood vessels into the hydrogels [3,4]. The angiogenic/anti-angiogenic potential of acellular and cell-laden silk-12 scaffolds combined with iGG-MA hydrogel was investigated *in vivo*, using the chick embryo chorioallantoic membrane (CAM) assay. For producing the cell-laden scaffolds, human meniscus cells (HMC's) were isolated from morphologically intact human menisci using an enzymatic-based digestion and expanded using standard culture conditions. The HMC's-laden hydrogel/silk scaffolds were produced by encapsulating the HMC's into a 2 wt% GG-MA hydrogel, followed by impregnation onto the 12 wt% silk scaffold and ionic-crosslinking in a saline solution. A CAM assay was used to investigate the control of segmental vascularization of the acellular and HMC's-laden hydrogel/silk scaffolds by the effect of GG-MA hydrogel, until day 14 of embryonic development. The *in vivo* study allowed investigating the number of macroscopic blood vessels converging to the implants. The evaluation of possible inflammation and endothelial cells ingrowths was performed by histological (haematoxylin and eosin - H&E - staining) and immunohistochemical methods (SNA-lectin staining). When the silk-12 scaffold was combined with the hydrogel, an inhibitory effect was observed as demonstrated by the low number of convergent blood vessels. Results have shown that iGG-MA hydrogel prevented the endothelial cells adhesion and blood vessels infiltration into the HMC's hydrogel/silk scaffolds, after 4 days of implantation. This study showed that the hydrogel/silk scaffolds enabled controlling the segmental vascularization, thus it can possibly mimic the native vasculature architecture during meniscus regeneration.

References:

1. H. Pereira, et al., Arthroscopy, 2011, 27(12):1706–1719.
2. L.-P. Yan, et al., Acta Biomater, 2012, 8(1):289–301.
3. J. Silva-Correia, et al., J Tissue Eng Regen Med, 2011, 5(6):e97-e107.
4. J. Silva-Correia, et al., Tissue Eng Part A, 2012, 18(11–12):1203–1212.

TS023
Thermosensitive poly-caprolactone scaffolds for 3D differentiation of C2C12 cells and human adipose-derived stem cells

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For tissue engineering (TE) applications, the natural process of regeneration is imitated by using bioresorbable scaffolds that support cellular attachment, migration and proliferation, along with cells capable of differentiation upon exposure to inductive factors. Based on the idea of combining a fully degradable polymer (Poly(ϵ-caprolactone; PCL) with a thermoresponsive polymer (polyethylene glycol methacrylate, PEGMA) a scaffold was developed, which liquefies at 4 °C and solidifies at 37 °C. Adult stem cells, which can be obtained out of human fat tissue, have the ability to be cultured *in vitro* and the potential to differentiate into various lineages, including the osteogenic and adipogenic lineage. There are several advantages using adipose-derived stem cells (ASCs), for instance the possibility to isolate large amounts in an easy way or the possibility to use them in an autologous setting. The mouse C2C12 cell line also features stem cell characteristics and has the potential to differentiate towards the myocyte as well as osteocyte lineage. In this study, the novel thermoresponsive material PCL-PEGMA was combined with C2C12 cells or human ASCs to generate an expandable 3D construct for soft or bone TE, depending on the *in vitro* differentiation conditions. As a first step, biomaterial seeding for C2C12 cells as well as ASCs was optimized and their attachment, survival, distribution and persistence within the 3D material was characterized using viability assays, fluorescence microscopy and scanning electron microscopy. C2C12 cells as well as ASCs attached to the polymers were viable and evenly distributed in all scaffolds. For C2C12 differentiation, the cells were seeded 3D within the scaffold and stimulated with either osteogenic or myogenic differentiation medium for 7 days. The ASC-scaffold constructs were cultured in media supporting either adipogenic or osteogenic differentiation of ASCs for 21 days. 3D differentiation of the cells was examined using qRT-PCR for differentiation specific markers. Interestingely, C2C12 cells differentiated in the myogenic lineage as well as ASCs treated with adipogenic differentiation medium showed increased marker expression in 3D compared to 2D, suggesting that the thermoresponsive PCL-PEGMA scaffold qualifies for 3D differentiation. Conclusion: The thermoresponsive scaffold presented in this study is a suitable material for 3D soft tissue engineering. The properties of the material show limited potential to support osteogenic differentiation of C2C12 or ASCs. In contrast, the matrix allows the attachment of C2C12 cells as well as ASCs and was able to support 3D myogenic differentiation of C2C12 cells and 3D adipogenic differentiation of ASCs.

TS024
Intracellular delivery of methylprednisolone by dendrimer-based nanoparticles improves locomotor outcomes after spinal cord injury

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Spinal cord injuries (SCI) still remain a major challenge in current biomedical research. In spite of several advances in the understanding of its mechanisms there has not been an equal significant translation into the clinics. As a result, there is no effective treatment that can overcome the biochemical and cellular adverse reactions that lead to a chronic severely impaired condition. One of the first opportunities to minimize these drastic consequences is to control the secondary events that follow the trauma. We are proposing the local delivery of an anti-inflammatory corticosteroid - methylprednisolone (MP) – in an attempt to modulate the noxious effects of the inflammation in the acute SCI. A sustained delivery as the one provided by these nanoparticles (NP) can be highly advantageous, maximizing the drug's potency in the target site. Therefore, we synthesized MP-loaded NPs composed of an inner poly/(amido)amine (PAMAM) dendrimeric core and grafted with carboxymethylchitosan (CMCht). Chemical and biological characterization studies were carried out showing that the NPs are stable in acidic and neutral buffer solutions. Also, the viability of primary glial cultures was not compromised by the presence of 200 μg/mL of NP. In turn, an MP action in microglial cultures was observed in dosages above 1 mg/mL showing that MP is being released from the NPs inside the cells. The uptake profile of these NPs is time dependent and reaches its maximum 24 h after incubation with astrocytes, oligodendrocytes and microglia. In a preview of a possible therapeutic effect, the NPs were administered in hemisected spinal cord injured rats. To assess the efficacy of local injections around the lesion site the animals were sacrificed 3 h after surgery and frozen sections were observed. The fluorescently labeled-NPs were detected in the injury and in the surrounding spinal tissue indicating a successful delivery of the NP to the spinal tissue. The local injections were repeated in hemisected rats that were kept for 1 month, performing the BBB locomotory test weekly. Significant differences in the BBB test were found between the MP-loaded NPs injected rats and the sham group as well as the ones injected with MP, demonstrating a favorable action of the MP-NPs in the acute phase of the injury. This work revealed that sustained delivery of MP via a NP system can be highly beneficial in the management of the secondary injury that follows SCI improving the overall functional outcome of the injured animals.

TS025
Testing and FEA modelling of a modified suture technique to accommodate a tissue engineered tendon *in vivo*

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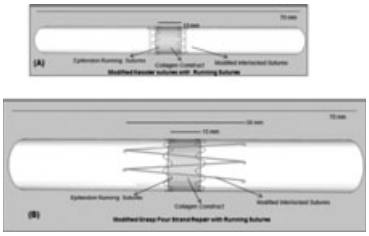
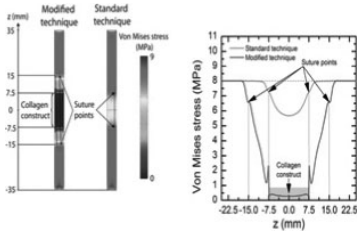
Introduction: Tendon repair is surgically challenging as the tendon often retracts resulting in a gap between the torn end and its bony insertion. Tendon auto- or allografts are currently used to fill this deficit, but both are associated with potential complications. We have developed a highly reproducible, rapid process technique to manufacture compressed cell seeded type I collagen tissue engineered constructs to replace tendon grafts (1).However, the material properties of the engineered constructs are currently unsuitable to complete load bearing *in vivo*. A modified suture technique has been developed to withstand physiological loading and off load the artificial construct whilst integration occurs.
Methods: Lapine tendons were used to test the strength of different suture techniques with different sizes of prolene sutures and tissue engineered collagen constructs in situ.

Figure 1 (A) Standard repair for tendon with modified Kessler sutures, (B) Modified repair Grasp four strand sutures with tissue engineered collagen construct The data was compared to standard modified Kessler suture using a standard tendon graft. Mechanical testing was carried out and a FEA stress distribution model constructed using COMSOL 3.5 software.



Results: The break point for modified suture technique with tissue engineered collagen construct was significantly higher (50.62 ± 1.62 N) compared to standard modified Kessler suture [12.49 ± 8.17N ($P < 0.05$)].

Figure 2 Stress distribution finite element analysis model for modified suture technique and standard technique, Von Mises stress across suture points and collagen construct. In FEA modelling Von Mises stress in the middle of the geometry, i.e. in the middle of the collagen construct (for the modified technique) or at the point of the running sutures (for the standard technique) are as follows (at a load of 8×10^6 Pa): standard technique 5.6×10^6 Pa; modified technique 2.8×10^5 Pa. Hence, it is evident that, stress is 20 times less if the modified technique is applied.



Discussion & Conclusions: Distributing suture tension further proximally and distally from the tendon ends increased the mechanical strength of the repairs. Using this proof of concept data, we will now test this modified suture technique *in vivo* to test integration of bone marrow stem cell seeded collagen construct and it is function in a lapine model.
Acknowledgement: I would like to thank government of India for funding this project.
Reference:
1. V Mudera, M Morgan, U Cheema, S Nazhat, R Brown, Ultra-rapid engineered collagen constructs tested in an *in vivo* nursery site, *J Tissue Eng Regen M*. 2007;1(3):192–8.

TS026
Ccbe1: a secreted protein with cardiomyogenic potential

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Cardiovascular diseases are one of the leading causes of death and disability in the world. The interruption of blood supply to a part of the heart during myocardial infarction leads to injury and eventually death of the cardiac tissue. Since adult cardiomyocytes have very limited regenerative capacity, the muscle tissue is replaced by other cells types in a process called cardiac fibrosis or scarring. The routine usage of embryonic stem cells (ESCs), and the promise that lies on its pluripotency to differentiate into any cellular type has encouraged many researchers to derive cardiomyocytes and try to use them to improve the remodeling of the tissue. Concurrently, others have been trying to find factors that facilitate the cardiac commitment from ESCs and/or that may stimulate putative adult heart stem cells to form new muscle tissue. There are several studies unraveling the function of such factors, but only very few examples of extracellular proteins with such cardiomyogenic capacity. The function and molecular mechanism of the secreted protein Collagen and Calcium Binding EGF domains 1 (Ccbe1) remains largely unclear. Recent reports, however, demonstrate that Ccbe1 has a role in lymphangiogenesis and ovary cancer metastasis, potentially trough a role in regulating migration. Furthermore, we have recently reported that *Ccbe1* is expressed in murine heart progenitor regions suggesting a role during heart development. Interestingly, according to our present data, Ccbe1 potentiates the differentiation of mouse ESCs towards cardiomyocytes, as seen by an increase in the number of beating foci and expression of definitive cardiomyocyte gene(s). In addition, *Ccbe1* is expressed in undifferentiated ESCs but, during differentiation, the peak of *Ccbe1* expression accompanies the increase in the expression of heart-progenitor markers *Nkx2.5* and *Isl1*. Taken together, our data suggests that Ccbe1 may have a role during cardiogenesis. To understand this further we are currently performing several experiments where we are testing the (inducible) gain- and loss-of-function of *Ccbe1*. This involves, respectively, the generation and transduction of a polycistronic lentiviral vector containing a tetracycline-inducible promoter to co-(over)express *Ccbe1* and *cyan fluorescent protein* (*CFP*), and the transduction of lentiviral vectors expressing siRNAs to downregulate the expression of *Ccbe1*. Overexpression is induced with doxycycline during different stages of differentiation. This will allow us to dissect when, where and how Ccbe1 is relevant during cardiac differentiation from mouse ESCs. Nonetheless, it is becoming safer to infer that Ccbe1 may potentially be used in non-transgenic cardiac regeneration/treatment.

TS027
Sulfation degree of glycosaminoglycans triggers distinct cytoskeleton organisation in mesenchymal stem cells

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Glycosaminoglycans (GAGs) comprise the closest cellular environment: they are building elements of ECM and can be also found on cells surface. Their biological activity depends on several parameters among which the negative charge is of prime importance[1]. This charge is generally associated with the presence of sulfate groups (-OSO_3H). Sulfation is a dynamic modification: it can occur at various positions within the glycan and different sulfation patterns have been identified for the same organs and cells during their development. However, the mechanisms of coding and transferring information by these functionalities are not yet complete understood, mainly because of (i) the complex physiological microenvironment in which GAGs interactions occur and (ii) the inability to access homogeneous GAGs[2]. In this work, we propose model surfaces bearing GAGs with different sulfation degree as platform to investigate the pathways by which mesenchymal stem cells (MSCs) sense and respond to this peculiar functionality: the -OSO_3H. We have selected two natural GAGs for this study: hyaluronic acid (HA) because it is the only non-sulfated glycan and heparin (HEP) as the GAG with the highest degree of sulfation. To obtain a larger range of sulfation degrees, we also prepared a synthetic analogue of HA with a sulfation degree of 1.4 (sHA). All these GAGs were covalently bonded to aminothiols deposited on gold surfaces. MSCs, both from bone marrow and adipose tissue, adhered well to all surfaces. Formation of focal adhesions was observed after only 1h of culture for bone marrow-derived MSCs regardless the used substrate. The presence of -OSO_3H groups induced different morphology and cytoskeleton organisation: formation of longer filopodia and well pronounced actin fibers were visible for MSCs from both sources. Moreover, cells were more spread after 24 h in contact with -OSO_3H-containing surfaces. Cells behaved similarly on both sulfated surfaces (sHA and HEP) and differences in cell morphology were less obvious: higher sulfation degree induced less lamellipodia formation while filopodia number and length increased. In summary, the present study provides evidence that sulfation degree of GAGs triggers distinct cytoskeleton organisation in mesenchymal stem cells that may be related with the differentiation of those cells. However, further studies at the molecular level about the exact mechanism of these processes need to be carried out.

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References

1. Capila I. and Linhardt R.J., A. Chemie-International Edition, 2002. **41**(3):391–412.
2. Soares da Costa D. et al., J. Materials Chemistry, 2012. **22**(15):7172–7178.

TS028
Organ printing and stem cells as future of regenerative medicine: influence of the cell encapsulation system on cell behavior

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Introduction: Many techniques were developed in the area of regenerative medicine, however they are still imperfect and generally based on the creation of tissue replacement scaffolds, while organ printing tends to build an organ de novo as closer as possible to the origin organ using stem cells as a source of regenerative cells. This can potentially eliminate needs in organ transplants, and therefore, is very promising approach in regenerative medicine. Cell encapsulation by electro-hydro dynamic jetting is a perspective technique for organ printing. However, during the encapsulation process, as well as during the further cell growth inside beads, several changes in cell proliferation, activity, and protein expression can happen due to the complexity of the cell encapsulation system. Thereby, before starting the process of organ printing, it is important to make sure that encapsulated cells are not harmed. Thus, the main aim of the initial project is to characterize cell behavior during and after the cell encapsulation process. Glyceraldehide-3-phosphate dehydrogenase (GAPDH), the housekeeping protein that interacts with telomeric DNA and participates in glycolisis, apoptosis, cell aging, cell proliferation, and other vitally important processes was selected for checking genes activity of encapsulated cells.

Materials and methods: B50 rat neuroblastoma cell line was selected for the experiment.

1.2×10^6 cells were seeded in a tissue culture plate ($S = 12.56\ cm^2$);

2.4×10^6 cells were seeded on the top of 2% alginate coating ($S = 12.56\ cm^2$);

5×10^6 cells were microencapsulated in 1 mL of 2% alginate beads, 200 μm in size;

10^5 cells released from the beads were reseeded ($S = 4.5\ cm^2$).

Cross-linking of alginate occured with the presence of Ca^{2+} cations. GAPDH expression was analysed by SDS-PAGE and Western blotting analysis, whole cell lyses were collected on days 0, 1 and 3; reseeded cells were lysed on the day 5. Cell parameterses were checked with Alamar Blue test, and with cell viability test (Calcein/PropidiumIodide staining followed by confocal microscopy visualization).

Results and conclusions: Cell viability analysis showed that cells remain alive inside the beads even after encapsulation for a long time (D7), however, the activity of entrapped cells slightly decreased. The WB results showed a strong increase in GAPDH expression, comparing to preceding days of the experiment. Analyzing the results, the studying of production of the heat shock protein (Hsp70) that is only expressed in response to cells stress, appeared to be essential, as well as the proliferation of entrapped cells.

TS029
Novel approach to create hybrid and hierarchical scaffolds aimed for tissue regeneration

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Tissue Engineering scaffolds with a wide range of properties and using several types of materials have been produced using different processing techniques. Among those, hybrid scaffolds, made of synthetic biodegradable and natural-origin polysaccharides, have been arising as the most adequate 3D structures to support the mechanical solicitations once implanted as well as cell adhesion, proliferation and differentiation. A well-known methodology to combine micro/nanofibers with/ within scaffolds is the combination of electrospinning and bare scaffold. However, in the common approach, fibers are not homogeneously distributed along the 3D scaffold, being limited to its surface; to achieve deposition on the interior of the scaffold, the electrospinning has to be used during bare scaffolds preparation in a complex process that may lead to structure delamination. Herein, we present a novel approach to hybridize and introduce fibrillar structures and coatings inside 3D scaffolds, rendering truly hierarchical systems. The structures were created combining an unconventional layer-by-layer (LbL) electrostatic self-assembly technology with physical crosslinking by freeze-drying. LbL is based on a simple alternated deposition of polyanions and polycations, i.e. polyelectrolytes (PEs), and the introduction of such materials inside the scaffolds creates a new environment which allows to control cell behavior, by enhancing surface area available for cells attachment and the similarity to extracellular matrix composition and structure, without damaging the mechanical integrity and properties of the bare scaffold. Alginate and chitosan were used as polyanion and polycation, respectively, and polycaprolactone bare scaffolds were produced by rapid prototyping. Scaffolds were modified with the PEs using a home-made dipping robot to study the effect of several LbL assembling parameters on the final structure. Characterization of the structures revealed that one can obtain nanocoatings or nanocoatings plus fibrillar structures inside the scaffolds, homogeneously distributed and linked to the wall of the bare scaffold in a controlled manner. SaOs-2 osteoblastic-like cells were used to assess the cytocompatibility of the hybrid scaffolds quantifying dsDNA, ALP activity and observing cell distribution. After 7 days in culture, cells were able to colonize whole longitudinal section of the scaffolds, being able to adhere to the fibrillar structures and showing similar or higher ALP activity and dsDNA content comparing with the unmodified PCL scaffolds. In conclusion, this new methodology virtually allows the modification of any 3D structure with the introduction of a new hierarchical level in tissue engineering scaffolds, as coatings or fibrillar structures, which acts as systems to control cell adhesion, proliferation and differentiation.

TS030
Differentiation of rat bone marrow mesenchymal stem cells into insulin producing cells – impact of extracellular matrix proteins

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Introduction: Pancreatic islet transplantation is a promising approach for type 1 diabetes treatment, however it is limited by a shortage of pancreas donors. This study was performed to investigate whether bone marrow mesenchymal stem cells (MSCs) could be transdifferentiated into insulin producing cells *in vitro* and evaluate the role of collagen, laminin and vitronectin in this process.
Methods: MSCs were isolated from 8-week Wistar rats and cultivated under standard conditions. MSCs phenotype was confirmed by flow cytometry. The cells were induced to differentiate into a pancreatic endocrine phenotype by defined culture conditions within 22 days with or without extracellular matrix proteins (collagen, laminin and vitronectin). Pancreatic characteristics were evaluated by real time reverse transcription polymerase chain reaction (qRT-PCR) and immunofluorescence analysis. Insulin content and release in response to glucose stimulation were tested with radioimmunoassay.
Results: Flow cytometry confirmed the homogeneous MSCs phenotype. MSCs derived from 3rd passage were positive for the CD44 (99.5% of cells) and CD90 (99.7% of cells) markers and negative for typical endothelial and hematopoietic markers CD34 (99.6% of cells) and CD45 (99.2% of cells). The induced cells expressed pancreatic endocrine cell related genes. Collagen and laminin increased the expression of Nkx6.1, NeuroD1, Pax4 and PCSK. Laminin induced formation of the islet-like structures and increased expression of Ins1 and Ins2. However combining collagen, laminin and vitronectin did not heighten the expression of endocrine cell markers. Immunofluorescence analysis revealed the expression of insulin. Insulin secreted from differentiated cells was much higher than that from pre-differentiated cells.
Conclusions: Rat bone marrow MSCs can differentiate into insulin producing cells *in vitro*. Laminin plays an important role in formation of three dimensional structure and pancreatic endocrine cell maturation

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TS031
Urinary conduit construction using tissue engineering

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Introduction: Standard procedure after bladder resection is use of ileal segment for urine transport outside the body. This procedure require additional chirurgical method on ileum and is associated with many side effects. Aim of our study was to construct artificial conduit that replace ileal fragment.
Material and Methods: Fourteen Wistar rats were used in experiment. Decelluralized aortic arch or policaprolactone scaffold were used for artificial conduit construction. Scaffold was anastomosed end to end with ureter on one side, and with skin layer on other side. Second ureter was undisturbed. Follow-up was to 3 weeks. After that time rats were sacrificed and artificial conduits were analyzed using hematoxylin-eosin staining. Results Decelluralized aortic arch was used in 12 rats, in four cases follw-up was under 1 week because of rats death. Policaprolactone was used in two rats with 3 week follow-up. In all cases outflow of urine was stopped, also presence of pus was observed. Hematoxylin-eosin staining showed lack of cell layers regeneration on decelluralized aortic arch. One policaprolacton scaffold showed lack of integration with ureter, in second scaffold regeneration of cell layers was observed.
Discussion: Our results showed that unseeded aortic scaffolds are not suitable for ureter conduit construction. Results obtained using policaprolacton scaffolds are promising, but outflow of urine must be kept, and more experiments have to be performed to confirm promising properties of this scaffold.

TS032
Electron beam cross linking of hydrogels as a novel and versatile technique of scaffold fabrication in tissue engineering strategies

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Radiation induced processes have many advantages over other conventional methods [1]. Electron Beam processing of thermoplastic polymers results in an array of enhancements, such as an increase in tensile strength, and resistance to abrasions, stress cracking and solvents [2–4]. Indeed, applications of electron beam processing is not limited to these areas but modification of biomaterials [5–7]. Hydrogels, on the other hand are important candidates in fabrication of suitable scaffolds for tissue engineering purposes. Radiation techniques have proved to be particularly suitable for producing hydrogels for biomedical use. In the present research, novel hydrogels were prepared using Poly vinyl Pyrolydone (PVP), Poly ethylene glycole (PEG) and Water soluble chitosan [8]. Homogeneous mixture of hydrogel constituents were irradiated by 10Mev electron beam in the dose range of 15–40 kGy. According to the results, the gel fraction of hydrogels increased with the increasing of the dose and decreased with the increasing of the relative content of PEG in hydrogel. The results show that the maximum swelling increases with increasing of the PEG concentration, which is due to a decrease of the crosslink density. The hydrogels containing PVP and PEG (without chitosan) were brittle and showed poor mechanical properties. With addition of chitosan to hydrogels formulation, the mechanical properties increased. The hydrogel containing 6%PVP, 6%PEG and 3%chitosan showed the best mechanical properties in 25 kGy.
References:

1. *Shamekhi MA*, Jafari SH, Khonakdar HA, Ehsani M," Preparation and Characterization of UV Irradiation Cross-linked LDPE/EVA Blends". *Plastics, Rubber and Composites, 39, 10,(2010),431–436.*
2. Goodarzian N, *Shamekhi MA*, "Chemical Cross linking Versus High Energy Electron Beam Cross linking of HDPE: Electrical Properties Study". *J. Iran. Chem. Res. 2 (2009) 189–194*.
3. Ziaie F, Borhani M, *Shamekhi MA*, "Storing Effects on Electrical Properties of High Energy Electron Beam Irradiated LDPE and HDPE". *International Journal of Polymeric Materials, 60:939–946, 2011*.
4. *Shamekhi MA* et al., "Novel Polymer Blends Based on Electron Beam Crosslinked TPU/EVA", IAU, Sarvestan Branch, research Grant.
5. Yamato M, Shimizu T, Kikuchi A, Okano T. 'Radiation-Initiated Nano Surface Modification for Tissue Engineering', IAEA-CT, Seattle, USA, (2002).
6. Yamato M. et al., Thermally Responsive Polymer-Grafted Surfaces Facilitate Patterned Cell Seeding and Co-culture, Biomaterials 23 (2002) 561–567.
7. Gao C, Mao Z, Ma Z, "Surface Modification and Property Analysis of Biomedical Polymers Used for Tissue Engineering" Colloids and Surfaces B: Biointerfaces 60 (2007) 137–157.
8. Lu SH et al., Preparation of Water-Soluble Chitosan, J Appl Polym Sci 91: 3497–3503, 2004

TS033
Integrative studies on the role of Ccbe1 in cardiogenesis: from the embryo to ES cell derived cardiac tissue

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A central challenge for the future perspective of cardiac regenerative medicine is the generation of large numbers of patient-specific cardiac myocytes. *Ccbe1* encodes a secreted molecule that was firstly identified using an Affymetrix GeneChip differential screen for chick heart precursor cells expressed genes (Bento et al., 2011). In mouse and chick, *Ccbe1* is expressed in major cardiac progenitor lineages that contribute to distinct heart structures during heart organogenesis (Facucho-Oliveira et al., 2011). Moreover, analysis of gain and loss of function performed in both mouse and chick embryos showed abnormal cardiac morphogenesis and aberrant chamber formation further elucidating the role of Ccbe1 for cardiac development. Similarly, in mouse and human ES cells, increased levels of *Ccbe1* expression were detected after cardiac lineage commitment demonstrating well-coordinated expression of various early and late cardiac specific markers and *Ccbe1*. Knock-Down in mouse and human ES cells demonstrated the requirement of Ccbe1 for proper cardiogenesis. Modulation of mCcbe1 activity in differentiating mES cells using media supplemented with mCcbe1 recombinant protein has demonstrated a remarkable inductive potential of mCcbe1 to enhance cardiogenesis. Taken together, this data strongly suggest that Ccbe1 has the ability to direct the expression of cardiac inducers and to control cardiac progenitor expansion *in vitro* and *in vivo,* allowing the generation of non-genetically manipulated cardiac cells from a renewable cell source for regenerative cardiovascular medicine.

TS034
Development of a bilayered scaffold based on silk fibroin and silk fibroin/nano-calcium phosphate for osteochondral regeneration

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Objectives: Osteochondral defect is a common condtion in clinic. Satisfactory outcomes are rarely achieved by traditional methods. Tissue engineering might be a promising strategy for this hinder. The aim of this study is to mimick the stratified structure of osteochondral tissue, by developing a bilayered scaffold for osteochondral regeneration. The developed bilayered scaffold is composed of a porous silk fibroin scaffold as the cartilage-like layer and a porous silk fibroin/nano-calcium phosphate (CaP) scaffold as the bone-like layer.

Methods: The silk and silk/nano-CaP bilayered scaffolds were prepared by a combination of salt-leaching and freeze-drying approaches, as previously reported [1,2]. Briefly, the concentrated silk fibroion aqueous solution (16%) was mixed with calcium chloride and ammonium phosphate dibasic solution to generate the silk/nano-CaP suspension. The bottom layer was prepared by adding the sodium chloride particles (500–1000 μm) into the suspension. A 16% silk fibroin solution was then added onto the top of the silk/nano-CaP layer. Sodium chloride particles of the same size were added into the silk solution to produce the top layer. After 48 h, sodium chloride was leached out from the layered scaffold and the final bilayered scaffolds were lyophilized. The generated scaffolds were characterized by SEM, micro-CT, and EDX.

Results and Discussion: SEM images showed that a macro/micro porous structure was observed in both layers. These two layers integrate well, without the formation of a clear interface. Micro-CT analysis allowed observing that the layered scaffolds were of porous structure, with homogeneous porosity distribution in each layer. The CaP was homogeneously distributed in the bottom layer, while there was no CaP detected in the top layer. By EDX analysis, the amount of CaP from the bottom to the top layer was mapped, which presented a gradient decrease in the interface region, indicating a good integration between both layers.

Conclusions: Silk fibroin and silk fibroin/nano-CaP bilayered scaffolds were successfully generated. The porous structure was maintained in each layer. The CaP was homogeneously distributed only in the bottom layer, and presented a gradient decrease at the interface. The most important features of this bilayered construct is the good integration between the two layers at the interface region. This is important since when implanted this region can be particularly sensitive to mechanical stresses. Therefore, this bilayered scaffold may be useful for an osteochondral regeneration approach.

References:

1. Yan LP, et al. *Acta Biomater, 2012,* 8(1), 289–301. 2. Yan LP, et al. Nanomedicine, 2012, accepted.

TS035
Superhydrophobic patterned chips for the combinatorial and rapid study of 3D biomaterials-cells interactions and protein delivery systems

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The development of optimized products in the tissue engineering (TE) field is a time and resource consuming process due to the unpredictable influence of the combination of variables such as biomaterials, cells and soluble factors. As multiple combinations can be considered high-throughput (HT) methods were suggested as a way to master complexity in TE. Usually, HT systems test cell-2D biomaterials interactions and, more recently, cell-3D hydrogels interactions. Using a chip consisting of superhydrophobic surfaces patterned with wettable regions we tested cells-hydrogels interactions in three-dimensional environment [1]. The versatility of the chip allowed its use for the first time on-chip combinatorial study of 3D miniaturized porous scaffolds. Arrays of biomaterials were dispensed and processed in situ as porous scaffolds with distinct composition, surface characteristics, porosity/pore size and mechanical properties. Those characteristics were assessed by adapting microcomputed tomography equipment and a dynamic mechanical analyzer. The interactions between cell types of two distinct origins – osteoblast-like and fibroblasts - and the scaffolds modified with distinct amounts of fibronectin were studied by image-based methods and validated by comparison with conventional destructive methods. Physical and biological on-chip results were coherent with conventional measures, and conclusions about the most favorable media for the growth of both cell types were taken. Growth factors (GF) proved to play an important role in TE approaches, mainly for determining cell fate in applications containing stem cells. We developed a chip based on wettability contrast with torus-shaped hydrophilic transparent regions disposed in an array matrix. Concentrically to these wettable regions a superhydrophobic circle was maintained, so the hydrogels could be processed as protein-loaded spheres with minimum protein loss [2] and fixed with an indentation. A combinatorial system of BSA-FITC – a commonly used GF model – encapsulated in alginate hydrogels was designed. The protein release from the hydrogels could be studied by image analysis, avoiding manipulation and protein loss. The results were compared with conventional protein release tests and similar tendencies were observed. We believe that the proposed innovative uses for the superhydrophobic chip and their upgrade in future applications may constitute a promising breakthrough in integrated technologies for the rapid development of TE systems.

References:

1. Salgado CL, Oliveira MB, Mano JF. Combinatorial cell-3D biomaterials cytocompatibility screening for tissue engineering using bioinspired superhydrophobic substrates. Integrative Biology. 2012;4:318–27.
2. Song WL, Lima AC, Mano JF. Bioinspired methodology to fabricate hydrogel spheres for multi-applications using superhydrophobic substrates. Soft Matter. 2010;6:5868–71.

TS036
Nanostructured Multilayer compartments: towards multifunctionality and "cell-like" hierarchical complexity

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In living organisms, there are phenomena that require the presence of specific biomolecules with distinct function and in variable concentrations at a given time, such as the healing and regeneration of tissue and organ lesions. In this work, we propose the use of a compartmented drug delivery device for the multiple release of bioactive agents. It consists of nanostructured microcapsules confined within a millimetric container that can be easily handled, mimicking the concept of cells which possess organelles with specialized functions. Each hierarchical structure was conceived using the layer-by-layer (LbL) method to form micro and macrocapsules that could individually carry either molecules and release them with distinct kinetics or magnetic nanoparticles (MNPs) to be used in targeted therapies. Furthermore, the internal microcontainers were constructed with a temperature-responsive elastin-like recombinamer (ELR) to further add smart properties to the proposed system. Sacrificial $CaCO_3$ microparticles empty or entrapping either rhodamine or Fe_3O_4 MNPs were incubated in chitosan and ELR solutions using LbL for the conception of the microcapsules. Then, the microcapsules were suspended in alginate which was ionically cross-linked in $CaCl_2$ drop-wise. Rhodamine could be encapsulated at this point in the alginate. The bead was coated with chitosan and alginate to build the external macrocapsule compartment. All structures were coated with 3 bilayers. The $CaCO_3$ cores were chelated and the alginate beads liquefied using EDTA. Fluorescence microscopy using FITC and rhodamine markers showed a uniform distribution of the microcapsules within the macroreservoir. The release of rhodamine from either in the micro or macrocapsule was assessed at 25 and 37 °C in PBS. While the release from the macrocapsule follows a profile similar to that of traditional drug delivery systems, it is more sustained and delayed when released from the internal compartments. Such retention is more pronounced at 37 °C (65% of release in comparison to 90%). This is due to the temperature responsive behavior of ELRs, which undergo a phase transition and make the LbL shell less permeable. For the magnetic response, the incorporation of the MNPs was observed by transmitted light microscopy. The attraction of the devices was observed by applying an external magnetic field along a defined trajectory. The results let foresee the use of such multilayer devices as compartmented structures to encapsulate growth factors, MNPs and stem cells for their controlled differentiation and maintenance or for guided regeneration of tissues and organs.

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TS037
Surface modification of tissue engineering scaffolds by UV laser

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Tissue engineering is a promising area of research in which efforts are being made to generate human tissue or organs substitutes in case of their failure. The need for substitutes to replace or repair tissues or organs is overwhelming. Scaffold preparation for tissue substrates is a very important issue in tissue engineering. In this study, we used laser energy to modify the scaffold's surface according to tissue engineering needs. UV laser energy was applied to the surface of the scaffold in a trial to get a surface texturing that allow the most reliable cell adhesion and proliferation. Modification of polycaprolacton (PCL) scaffold surfaces using specific excimer laser beam parameter produced distributed regular ripples on their surfaces that influence the surface roughness, surface wettability which in turn enhance cell attachment, proliferation and the degradation rate of the scaffold. The differential scanning calorimetry (DSC) results showed a significant shift in the endothermic peak of PCL (the peak shifted from 60 °C to 63 °C), which is an indication of lack of significant changes in the crystalline state of PCL. The SEM showed very smooth morphology with lack of any rough or ripple or pores of the surface of unmodified scaffold. On the other hand, the exposed surface of PCL to laser exciter with E = 31 m j, N = 100 pulses and p.r.f = 5 Hz showed formation of highly distributed, regular ripple on the scaffold surface with size range from 200–600 nm. The Water Contact angle measurement indicated that the modified scaffold yields much higher surface energy than unmodified scaffold. Roughness results showed that the modified PCL scaffold revealed higher surface roughness than the unmodified PCL scaffold. The degradation test revealed that the modified PCL scaffold, have good degradability *in vitro* bioactivity, more than the PCL unmodified scaffolds and it possess the ability to make a direct bond with living cells when implanted in the body. From *In vitro* biological testing imaging, the SEM revealed good adhesion of cells over the modified scaffold in comparison to the unmodified scaffold. MTT cell viability indicated that the modified PCL scaffold showed excellent cell viability relative to control rather than the unmodified scaffold. This is due to lower cell adhesion on the unmodified scaffold due to lack of ripple structure on its surface. These ripples enhanced the cell adhesion and cell growth on the surface of the modified PCL scaffold.

TS038
Scaffold free bone tissue engineering using multilayer cell sheet technology

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The restoration of bone loss due to trauma and congenital problems is a major clinical need. The use of stem/osteoprogenitor cells in combination with scaffolds has been the gold standard in bone tissue engineering. However, the most of the materials used are either non-biocompatible or the degraded materials cause side effect. In addition, the stem cells have to be detached by chemical methods which will damage the cells and remove the extracellular matrix. Cell sheet technology use thermal sensitive polymer coated dishes (UPCell™) to harvest cells as an intact monolayer without using routine chemical methods. This method reduces the cell loss and retains extracellular matrix so that it can be transplanted to the host tissues (as autograft) without sutures and any scaffold. The aim of this study was to investigate the potential of using multilayer cell sheet technology and HDPSCs for bone tissue engineering. Briefly, human Dental Pulp Stem cells (HDPSCs) were isolated from wisdom teeth and cultured in 35mm UPCell™ temperature responsive culture dishes (Passage 3; 5×10^5 cells per dish) in basal (1 week) and osteogenic media (2 weeks) at 37 °C and 5% CO_2. Cells were harvested from dishes by reducing the temperature form 37 °C to 5% CO_2. Then three layers of HDPSCs were assembled using CellShifter™. Half of the multilayer cells sheet were fixed in 98% ethanol for alkaline phosphatises (ALP) staining. Half were fixed in 10% neutral buffered formalin and paraffin sections were stained by Hematoxylin and Eosin (H&E) as well as Alcian Blue/Sirius Red (A/S). H&E staining confirmed the formation of a three layer cell sheet. Positive ALP staining showed that the HDPSCs maintained their osteogenic phenotype within the cell sheets. A/S Red staining revealed collagen matrix formation within the cell layers which was confirmed by birefringence. The mineral nodule formation was also observed. To date, the most of methods for bone tissue engineering required 3D scaffold to support the cells growth. In this study, a scaffold free multilayer cell sheet technique has been evaluated. Unlike the other methods, this technology preserves the extracellular matrix, cell-cell junctions, and cell surface proteins. It can assemble a functional 3D tissue without any scaffold. The cells within the multilayer were remaining osteogenic and produced mineralized collagen matrix. In conclusion, this study indicated the potential of using multilayer cell sheet technology in combination with HDPSCs for bone tissue engineering which may lead to translational research for bone augmentation.

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TS039
Cardiogenic differentiation of stem cells on poly(glycerol sebacate)(Pgs)/gelatin/vegf loaded nanofibrous constructs for treatment of myocardial infarction

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Heart failure due to myocardial infarction (MI) remains the leading cause of death in many industrial nations, mainly due to the lack of ability of the myocardial tissue to regenerate. Current therapeutic approaches used to treat patients with chronic heart disease include left ventricle assist devices, pharmacologic therapies and surgery, but still heart transplantation remains the only possible solution for end-stage heart failure. The recent advances in the field of tissue engineering and stem cell biology have paved way for new strategies for myocardial regeneration. The main objective of this study was to develop a cardiac patch that is biocompatible, has mechanical properties similar to that of the native tissue and is capable of inducing cardiogenic differentiation of mesenchymal stem cells (MSCs) for MI regeneration. PGS/gelatin fibers were fabricated by core/shell electrospinning with gelatin as the shell material, responsible for biological interactions and core material as PGS for favorable mechanical properties. VEGF growth factor was also incorporated into the core material to favor its control release, thereby providing angiogenic properties and anti-apoptotic properties at the infarcted site. The fabricated fibers were characterized by scanning electron microscopy (SEM), contact angle, Fourier transform infrared spectroscopy, and tensile testing to analyze the morphology, wettability of the membrane, surface functional groups and the mechanical properties of the material. The cell–scaffold interactions between MSCs and PGS/gelatin nanofibers were analyzed by cell proliferation, confocal analysis to confirm the cardiogenic differentiation of MSCs by employing MSC specific marker protein CD 90 and cardiac-specific marker protein troponin, and SEM to analyze cell morphology. This novel construct comprising of PGS/gelatin and VEGF can provide the required tensile strength to support seeded MSCs differentiation into cardiomyocytes for tissue regeneration to repair the infarcted myocardium and improve cardiac functions. Such an elastomeric material can resist the heart wall pressure while providing a structure that enhances the potential for cell proliferation and differentiation of MSCs into cardiomyocytes. This combinatorial epitome might eventually bring cardiac tissue engineering into the clinical forefront.

TS040
Differentiation of embryonic stem cells can be influenced by different processing of gelatin/silk fibroin hydrogel

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Introduction: ESCs (Embryonic Stem Cells) are Pluripotent cells capable of differentiating into any cell type of the body, which hold great promise in treating diseases by replacement of body parts and organs through the generation of new tissues in tissue engineering. However the greatest challenge of using ESCs in clinical applications is the lack of knowledge in directing their differentiation fate. Directing the controlled differentiation of stem cells into a lineage-specific manner requires a controlled biophysical and biochemical microenvironment and signals. Here we present how different processing of Gelatin/Silk Fibroin hydrogels can influence ESCs differentiation fates. Gelatin is the denature form of collagen which is the major constituent of all extracellular matrix in animals. Silk Fibroin (SF) has been explored for many biomedical applications due to its impressive biocompatibility and biodegradability. We created the Gelatin/SF hydrogel by the chemical crosslinker genipin and by physical sonication crosslinking. The ESCs cells cultured on these hydrogel surfaces showed distinct ectoderm differentiation fates.
Materials and Methods: E14tg.2a cells were dissociated in single-cell suspension and 1000 cells/cm^2 were plated on gelatin-coated 12-well plates and on different hydrogel surfaces. Cells were cultured in Knockout serum medium that was replaced every 2 days.
Results: The protocol is a single-step tool to convert ES cells into neurons on gelatin-coated plate, we initially analyzed the expression profiles of genes of neuroectodermal lineage: Nestin, BIIITublin, NCAM. The expression of the three genes was greatly decreased in the cells cultured on the G-SF and Gel5SF compared to the control and sonication crosslinked SF based hydrogels which indicated that on genipin crosslinked hydrogels the neural differentiation was blocked. We then analyzed the expression of K18(non-neural ectoderm), GATA4(mesoderm), Sox17(endoderm) in the cells of Genipin crosslinked samples. mESCs cultured on the the G-SF and Gel5SF showed greatly increased expression of K18, while mesodermal and endodermal genes expression levels comparable to the control and undifferentiated ES. This suggests that the Genipin crosslinked Gelatin/SF hydrogel can change the cell differentiation fate from neuroectodermal lineage to epithelial ectoderm lineage.
Conclusions: All these indicated that the different processing of Gelatin/SF hydrogels can influence the differentiation fate of ESCs. This may depend on the fact that the genipin crosslinked Gelatin/SF could activate the JNK/BMP4 signal pathways to suppress neural differentiation and enhance epithelial differentiation.

Name	Sample ratio	crosslink
G-SF	100(SF)	Genipin
Gel	100(Gelatin)	Genipin
Gel/5SF	95/5(Gelatin/SF)	Genipin
SF	100(SF)	Sonication
SF/5Gel	95/5(SF/Gelatin)	Sonication

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TS041
Decellularized porcine liver scaffolds for stem cell cultivation and differentiation

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Decellularized scaffolds are getting more important in TE. Currently, there is no efficient system to expand the functions of human adult hepatocytes *in vitro*. A three-dimensional structure could present a supportive environment for the cells to facilitate the viability and functionality of liver cells in culture. The extracellular matrix (ECM) and it compartments can enhance the cell proliferation and differentiation *in vitro*. Especially the use of an ECM derived from the same tissue origin should provide an adequate structure for the cells. One main focus of this project is the reduction of the need for animal drug testing models by developing new *in vitro* alternative techniques. Our experiment was divided into three parts. Cell free biological scaffolds were prepared during the pre-cell culture step. 400 μm thick tissues slices (Ø 8 mm) were cut, serving as scaffold. For an effective cell removal the slices were treated with a 3% Triton X-100 solution for 1.5 h. Afterwards the slices were rinsed with PBS over night to wash out the agent. HE and TMRM staining revealed cell-free conditions after treatment. In the cell culture step, the scaffolds were recellularized with endothelial cells (ECs), human adipose-derived stem cells (hASCs, p0-p6) and immortalized adipose-derived stem cells (hTERT ASCs). Calcein AM staining, HE staining and scanning electron microscopy (SEM) analysis were performed to determine the recellularisation rate. To compare cell qualities hASCs were cultivated on fibronectin (FN) coated tissue culture plates (TCPs) in a parallel approach. Following the cell seeding a hepatocytic differentiation was induced using human hepatocytic growth factor (HGF) and oncostatin M (OSM) in serum free media. After up to 28 days of differentiation intracellular and extracellular changes were determined using light microscopy and urea quantification assay. Quantitative PCR was performed to detect the amount of albumin and α-fetoprotein expression levels. Decellularisation, as well as recellularisation of porcine liver scaffolds was successfully performed using different cell types. We observed that the cell source seems to be critical for hepatocytic differentiation. All in all our system showed promising results to develop new methods for human *in vitro* drug screening/testing systems.

TS042
Platelet lysate – a substitute for fetal calf serum

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Fetal calf serum (FCS) is the most common supplement in media used for cell expansion. Due to its animal origin, it bears a potential risk to trigger immune reactions and to transmit xenogenic proteins, prions and viruses. This is a major concern in case of cells intended for human use in cell therapy and regenerative approaches. In order to determine alternatives, several studies were already performed comparing FCS to human serum or defined media in combination with recombinant growth factors (GF) Human platelet lysate (hPL) is known for its high concentrations in GF representing another potential alternative for FCS. Several cell lines and especially stem cells have already been successfully cultivated in the presence of hPL. For example, human adipose-derived stem cells (ASC) and human articular chondrocytes (HAC) were expanded with 10% FCS compared to 5% hPL. We could show that the proliferation potential as well as the chondrogenic differentiation potential could be enhanced by using hPL compared to FCS. Still the use of hPL is not very common. The main reason might be the production itself. So far, no standardized procedure for large scale production could be established. An overview on different production processes will be given. Additionally, we will present results using hPL on cultivation of HAC, ASC and amnion derived cell lines as well as data of our attempts on product characterization.

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TS043
Modulation of adipose tissue derived stem cells encapsulated in injectable multi-functional hydrogels

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Objective: The cellular microenvironment plays an important role in controlling the cellular behavior. Therefore, in Tissue Engineering strategies, systems have been designed so that by combining cells, signaling molecules and engineered substrates, one can mimic as closely as possible the native microenvironment of the extracellular matrix. In this context, injectable hydrogels have been developed that allow controlling the cellular spatial distribution and that provide a 3D support. However, many of these systems fail to replicate the mechanical properties of tissues and more significantly to provide a cell-friendly environment. Therefore, the aim of this study is to evaluate the cellular response of human adipose stem cells (hACS) within a growth factor-enriched injectable hydrogel.
Methods: Hydrogels were formed by combining platelet lysates (PLs) with a methacrylated gellan gum (MeGG) solution at different ratios. The hydrogels were further stabilized by photopolymerization. The parameters of photocrosslinking were varied and the dynamical mechanical properties evaluated for all the conditions. Total protein content released from the hydrogels was quantified for all the conditions using micro-BCA assay. Human ASCs were both seeded on the surface of the hydrogels for 7 days and encapsulated within the materials for 14 days.
Results and Discussion: Hydrogels with tunable mechanical properties were fabricated by changing the volume ratio of PLs and MeGG. The highest elastic modulus (nearly 500 kPa) was achieved for the condition with the lowest volume of PLs (2MeGG:1PLs), being significantly more elastic than MeGG alone. The rate of release of proteins present in the PLs from the PLs-MeGG hydrogels was higher for the condition with equal volume of MeGG and PLs (1MeGG:1PLs), as a result of the lower crosslinked polymer network. Human ASCs cultured onto the engineered surfaces showed a good metabolic activity and proliferation. Immunostaining revealed that MeGG:PL combinations fostered the attachment and spreading of cells. A strikingly improved cellular behavior was observed for the formulations with PLs, when compared to MeGG alone. This behavior was further confirmed at a 3D scale, demonstrated by a significantly higher cellular metabolic activity on the hydrogels with PLs.
Conclusion: Our system combines the mechanical support from MeGG with the biological cues from PLs to engineer a cell-friendly injectable hydrogel with tunable mechanical properties. Given the simplicity in producing these rather enriched hydrogels, we envision that it may be beneficial for various tissue engineering and regenerative medicine applications.

TS044
Cartilage regeneration approach based on squid chitosan scaffolds: *in-vitro* assessment

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During the past decades, marine organisms have been the focus of considerable attention as potential source of valuable materials. For instance, chitosan is a biopolymer with high potential in the biomedical field and can be produced from crustacean shells and squid pens [1]. In this sense, we propose the use of chitosan to produce scaffolds for regenerative medicine purposes. An alkaline solution was used to deproteinize squid pens and isolate β-chitin (Chaussard 2004), which was further converted into chitosan through a deacetylation reaction. Chitosan was then processed into porous structures by freeze-drying [3], where chitosan solutions (4%) were submitted to different freezing temperature of -80 °C and -196 °C. The produced structures were further submitted to neutralization methods with 4% NaHO, including in some cases a pre-washing step using ethanol/water solutions (100:0; 90:10, 80:20; 70:30 and 50:50) [4]. The morphology of scaffolds produced using either squid or commercial chitosan revealed a lamellar structure, independent of the source and/or freezing temperature. All chitosan scaffolds produced exhibited no-cytotoxic behaviour over L929 cells. To test the *in vitro* functionality of the scaffolds, cells from the mouse chondrogenic cell line ATDC-5 were seeded in the scaffolds and cultured for different time periods. Scaffolds made from squid chitosan were shown to promote better cell adhesion than commercial chitosan scaffolds and comparable or better cell proliferation. This demonstrates that squid chitosan is a valuable alternative to produce scaffolds for different applications in regenerative medicine, namely the regeneration of cartilage.
References:

1. Tiago H. Silva, A.A., Bruno M. Ferreira, Joaquim M. Oliveira, Lara L. Reys, Ricarte J.F. Ferreira, Rui A. Sousa, Simone S. Silva, João F. Mano, Rui L. Reis International Materials Reviews DOI: 10.1179/ 1743280412Y.0000000002, 2011.
2. G. Chaussard, Domard, A., *Biomacromolecules,* vol. 5, pp. 559–564, (2004).
3. L.L. Reys, S.S. Silva, J.M. Oliveira, S.G. Caridade, J.F. Mano, T.H. Silva, R.L. Reis, *"Unraveling the potential of squid chitosan based structures for biomedical applications"* (submited 2012) [4] V. M. Madihally, Mathee, H.W.T., *Biomaterials,* vol. 20, pp. 1133–1142, (1999).

TS045
Tenogenic potential of human stem cells from the amniotic fluid and adipose tissue

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Introduction: Tendons are highly prone to injury and the intrinsic hypocellularity and hypovascularity make their natural healing extremely slow and inefficient when severed damaged. Surgical repair with grafts is common but unsuccessful in a long term basis. The development of tissue engineering strategies based on stem cells explores a natural endogenous system of regeneration with potential for tendon application. We propose to establish biochemical culturing conditions to assess the tenogenic potential of human adipose stem cells (hASCs) and amniotic fluid-derived stem cells (hAFSCs), known for their proliferative and differentiation capacities. Since several growth factors (GFs) participate in tendon formation and ECM synthesis, these GFs were added to the culture medium to stimulate tenogenic differentiation of these cells(1, 2). This study also envisions the application of hASCs and/or hAFSCs in cell-based strategies for tendon repair.
Materials and Methods: hASCs were enzymatically isolated from lipoaspirates and expanded in basic medium(α-MEM, 10% FBS, 1% antibiotic) before being cultured in (i) basic medium, (ii) basic medium with glutamine(2 mM) and ascorbic acid(0.2 mM) plus (iii) EGF(10 ng/mL), (iv) FGF(10 ng/mL), (v) PDGF(10 ng/mL) or (vi) TGF-β(10 ng/mL). hAFSCs were obtained from amniocentesis procedures and expanded in α-MEM medium plus 15% embryonic screened FBS, 1% glutamine, 1% antibiotic, 18% Chang B and 2% Chang C. Similarly to hASCs, hAFSCs were cultured in media (i), (iii), (iv), (v) and (vi) but (ii) corresponded to hAFSCs expansion medium. Tenogenic differentiation was weekly evaluated up to 28 days based on cell morphology and Tenascin-C and Collagen3 protein expression, as well as on PCR analysis for tendon related markers (scleraxis, tenascin C, decorin and collagen 1 and 3. Primary tenoblasts obtained from surgery surplus were used as cellular control of the experiment.
Results: hAFSCs and hASCs showed a tenocyte-like aligned distribution in the different culture media by 14 and 21 days, respectively. But hAFSCs tend to lose the typical alignment by growing confluent. Also, hASCs expressed higher levels of Tenascin-C protein than hAFSCs.
Conclusions: Preliminary data on the aligned morphology and tenascin-C expression shows that both cell sources can be biochemically induced towards tenogenic features. Overall, and despite variations found, selected GFs do not actively participate in the tenogenic process of these cells. Ongoing studies on molecular biology and other tendon matrix related markers will clarify which stem cell source has more potential for tendon regeneration strategies.
References:

1. Rodrigues, MT et al. (2012) TERM, online published;
2. Liu, CF *et al*.(2011) Tissue Eng Part B Rev. 17: 165

TS046
Facs-purified human motor neurons from amplified human stem cell-derived cultures generate robust survival assays

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Human Embryonic Stem Cells (hESCs) and Induced Pluripotent Stem Cells (hiPSC) can virtually give rise to all cell types in the body, constituting an inexhaustible source of relevant cell types. Motor neurons derived from hESCs and hiPSCs are a potentially important tool to model and to unravel mechanisms of pathological motor neuron cell death. The culturing conditions to specify motor neurons (MNs) from human pluripotent stem cells have been extensively studied and in general work robustly. However, the production of human motor neurons on a scale compatible with high-throughput studies remains impractical due to the relatively low yield of current *in vitro* differentiation protocols. On the other hand, robust survival assays based on purified human motor neurons have not been developed. Here, we demonstrate for the first time the presence of ongoing neurogenesis in human stem cell-derived cultures of mixed spinal cord identity, which leads to the continuous generation of new-born motor neurons for several weeks. We initially exploited this as a means to increase motor neuron yields and screened a collection of bioactive molecules. The Rho-associated kinase (ROCK) inhibitor Y-27632 was identified as a compound that amplifies motor neuron yields up to 4-fold in differentiated hESC and hiPSC cultures. This likely occurs in a ROCK-independent manner, through the promotion of proliferation of Olig2-expressing motor neuron progenitors. Since ongoing neurogenesis constitutes a major potential confound for survival assays, amplified motor neurons expressing the HB9::GFP reporter were therefore FACS-purified and employed to develop a reproducible and robust survival assay for human motor neurons. Using this assay, we were capable to demonstrate for the first time in human motor neurons a significant survival-promoting activity of an array of known neurotrophic factors and also showed that Y-27632 itself supports motor neuron survival. Together, our results highlight Y-27632 as a useful tool to increase yields of human motor neurons from pluripotent stem cells for cell-based screening and biochemical applications.

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TS047
Bioprocess engineering human pluripotent stem cells for clinical application

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Objectives: Human pluripotent stem cells (hPSCs), with their ability for extensive proliferation and pluripotency, are renewable cellular resources with outstanding potential for Cell Therapy and Tissue Engineering applications. An imperative pre-requisite for the transition of hPSCs to these fields is the establishment flexible platforms for the large-scale production and storage of hPSC-based products in tightly controlled conditions, to deliver high quality cells in relevant quantities to satisfy clinical demands. The laborious and time consuming 2D-cultures are difficult to control, present poor yields and often lack the required cell functionality. These characteristics can have severe consequences on robustness, reproducibility, scalability and relevance of the cell systems, hampering their possible application in the clinic.
Methods: Our work has been focused on the development of integrated bioprocesses capable to guarantee efficient cell expansion, differentiation, purification and cryopreservation of clinically relevant hPSCs (embryonic and induced) and/or their derivatives (cardiomyocytes). Different 3D setting have been explored and combined with stirred tank bioreactor technology aiming at controlling hPSC's fate and culture outcome.
Results and Discussion: By combining microcarrier technology with environmentally controlled stirred tank bioreactors and further on with cell microencapsulation technology, we were able to improve the final yield of hPSCs in 15-fold when compared to the standard 2D cultures. This strategy ensured high expansion ratios (20-fold increase in cell concentration) and high cell recovery yields after cryopreservation (>70%). Alginate was the encapsulation material used due to its intrinsic properties including biocompatibility, biosafety and permeability and due to its potential in cell transplantation. For the cardiac differentiation of induced PSCs, different bioprocessing parameters were evaluated and the results showed the importance of controlling pH, pO_2 and stirring profile to improve the final yields of functional cardiomyocytes. The incorporation of a perfusion system in the bioreactor is being evaluated, providing a promising tool to facilitate and enhance the purification of induced PSC-derived cardiomyocytes.
Conclusion: The knowledge of hPSC bioprocessing gained from our work provides important insights for the establishment of more robust production platforms, hopefully potentiating the implementation of novel hPSC-based therapies.

TS048
Canine adipose stem cells: the influence of the anatomy and passaging on the stemness and osteogenic differentiation potential

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Periodontal disease is an inflammatory pathology highly prevalent both in humans and in dogs, which is characterized by destruction of the periodontal ligament (PDL), cementum and alveolar bone. The development of new therapies, such as Tissue Engineering strategies, is mandatory due to the inefficacy of conventional therapies currently used. Dog is a very relevant animal model to study several human diseases, such as PD and simultaneously an important subject in veterinary medicine. Adipose derived stem cells have a great potential for application in cell based therapies, such as tissue engineering. The aim of this study was to assess the potential of the canine adipose tissue derived stem cells (cASCs) to differentiate into osteoblasts and chondroblasts and study the anatomical origin and cell passaging effect on the cASCs stemness and osteogenic potential. In addition, we aimed at assessing the behavior of cASCs when cultured onto a newly developed double-layer scaffold, specifically designed for the regeneration of periodontal defects. The adipose tissue was harvested from the abdominal subcutaneous layer and from the greater omentum from adult healthy dogs, according to the animal welfare Portuguese legislation. cASCs were isolated by an enzymatic method and expanded along 4 passages in basal medium. cASCs were cultured using either osteogenic medium or chondrogenic medium. The stemness and osteogenic differentiation was followed by real time RT-PCR analysis of typical markers of MSCs, namely CD73, CD90 and CD105, and osteoblasts, like COLIA1, RUNX2 and Osteocalcin. The behavior of the cASCs was then evaluated on a previously developed double layer scaffold based on a starch and polycaprolactone (SPCL) blend that comprises a functionalized 3D fiber mesh to promote osteogenesis (an thus support alveolar bone regeneration) combined with a membrane aiming at acts as a physical barrier and promote PDL regeneration. The cellular proliferation was assessed by dsDNA quantification and SEM observation. The gene expression of MSCs/osteogenic typical markers was assessed by real time RT-PCR. The obtained data revealed that cASCs exhibit a progressively decreased expression of the MSCs markers along passages and also a decreased osteogenic differentiation potential. Moreover, the results showed that the anatomical origin of the adipose tissue has an evident effect in the differentiation potential of the cASCs. The resemblances with the human ASCs make the cASCs a suitable cell model for study new cell therapies for humans. Additionally, we reported the high potential of a newly developed scaffold combined with ASCs for periodontal tissue engineering.

TS049
Platelet lysates scaffolds with tunable gradients of mineral microparticles guide osteogenic differentiation of human adipose derived stem cells

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Objectives: Platelet lysates (PLs) are an enriched pool of growth factors (GFs) obtained from activation of platelets, promoting the recruitment, growth and morphogenesis of stem cells. PLs can be used as either a GFs source or as a 3D hydrogel for simultaneous GFs and cell delivery. However, most of current PLs-based hydrogels lack stability, exhibiting constant shrinking in culture and need to be activated with animal-derived thrombin. In this work we report the development of stable and bioactive 3D PLs-based scaffolds, by crosslinking with genipin and incorporating a gradient of Bioglass® microparticles under supercritical fluid conditions. It is hypothesized that the structure itself is capable of providing the adequate support and biological cues to promote osteogenic differentiation of seeded human adipose derived stem cells (hASCs) envisioning applications in bone tissue engineering.
Methods: Briefly, 1.5 mL of lysates solution mixed with genipin (crosslinking agent, 0.18% w/v) and Bioglass® microparticles (2.5% w/v) were placed inside the high-pressure vessel and underwent phase inversion. Morphological characterization of the scaffolds was performed by scanning electron microscopy and micro-computed tomography. In vitro release quantification of specific GFs (Platelet Derived GF and Transforming GF – PDGF and TGF, respectively) was also performed. Human ASCs were seeded onto the PLs-based hydrogels and cultured *in vitro* using either basal or osteogenic media. Deposition of mineralized extracellular matrix (ECM) and expression of osteogenic markers were characterized by histological, immunohistochemical and real time RT-PCR analysis.
Results and Discussion: Morphological analysis of the stable PLs-based scaffolds crosslinked with genipin indicated adequate levels of porosity and interconnectivity. In vitro protein release assays showed a controlled profile up to 28 days. When the scaffolds were seeded with hASCs, deposition of osteogenic ECM could be detected in constructs cultured in both basal and osteogenic supplemented media, as shown by the alizarin red staining and through the detection of collagen type I and osteocalcin through immunohistochemical and gene expression analysis.
Conclusions: The developed PLs-based scaffolds can simultaneously act as a template, with a well-defined gradient of a mineral component, and as a multiple GFs release system. The combination of PLs scaffolds with hASCs represents a feasible strategy for an autologous-oriented bone engineering approach. The possibility to fine tune the properties of the PLs scaffolds has demonstrated, in a previous work, its potential to be used for cartilage regeneration. Coupling these two systems may offer significant advantages in osteochondral regeneration strategies.

TS050
Translating human adipose-derived stem cells (hASCs) to clinical practice requires standardization of isolation and culture protocols

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Adipose-derived stromal/stem cells (ASCs) have been explored in recent pre-clinical trials to treat diseases in a broad range of tissues. The ultimate goal is to translate these findings to clinical trials to test safety and efficacy in human subjects. However, multiple steps are needed to make ASC clinically available: adipose tissue must be stable for up to 24 h during transit to cGMP laboratories[1] for further processing. Second, isolation and expansion protocols must eliminate exposure to animal proteins[2]. This will require the development of animal-free products suitable for the culturing of the cells. Current protocols[3,4] employ several animal-derived reagents, such as collagenase for the enzymatic digestion of adipose tissue, FBS for cell culture and expansion, trypsin derived from porcine tissue (stomach) for cell detaching and passaging, etc. Likewise, the length of time between adipose tissue harvest and processing will need to be systematically evaluated with respect to cell yield, viability, and function. The current study explores these technical challenges exploring alternative, non-animal sources of collagenase and trypsin-like enzymes for the isolation and passage of ASCs and assessing the effect of time delays on the yield and function of ASCs after collagenase digestion. The results obtained demonstrated that it's possible to use purified or animal-free enzymes for digestion of adipose tissue without decreasing the yield of stromal/stem cells nor affecting their surface markers[5] and differentiation potential. Cell yield and viability prove to be similar amongst any of the products (for both SVF and ASCs). The differentiation potential was not affected and ASCs were easily induced to adipogenic, osteogenic and chondrogenic lineages. Cell surface markers analysis showed no significant differences amongst any of the different grades of collagenase and trypsin-like products. These outcomes have practical implications with respect to the development of Standard Operating Procedures for cGMP manufacture of clinical grade human ASCs, which are essential for allowing their future use in the clinical practice.
References:
1. Carvalho, P.P., et al., Cells Tissues Organs, 2011. **194**(6): p. 494–500.
2. Carvalho, P.P., et al., Cytotherapy, 2011. **13**(5): p. 594–7.
3. Dubois, S.G., et al., Methods Mol Biol, 2008. **449**: p. 69–79.
4. Bunnell, B.A., et al., Methods, 2008. **45**(2): p. 115–20.
5. Dominici, M., et al., Cytotherapy, 2006. **8**(4): p. 315–7.

TS051
Exploring the osteoconductive effect of silanol groups by using human adipose stem cells

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Current methods for bone tissue engineering are concentrated in the *in vitro* proliferation and osteogenic differentiation of stem cells seeded onto 3D scaffolds, as these are key elements to achieve tissue regeneration. One critical strategy in bone tissue engineering is to develop 3D environments that can provide the necessary cues for guiding cell recruitment and driving tissue regeneration by mimicking the bone microenvironment and eventually eliminate the need to seed cells previously to implantation. Silicon is known to have an influence on calcium phosphate deposition and on the differentiation of bone precursor cells[1,2]. In previous work[3] we demonstrated that a wet spun fiber mesh based on a blend of corn-starch with polycaprolactone (30/70 wt.%, SPCL) with silanol (Si-OH) groups sustained human adipose stem cells (hASC) proliferation and osteogenic differentiation when cultured under dynamic conditions. The present study attempts to analyze in more detail the role of Si-OH groups on the osteogenic gene expression of hASC. Thus the *in vitro* evaluation of SPCL with and without Si-OH groups was done with human ASCs cultured in either α-MEM or osteogenic medium for up to 21 days. Results showed that hASC readily proliferated and migrated into the interior of the wet-spun fibre mesh scaffolds, and calcium and phosphate on the cell surfaces were detected, within only 14 days of culture. Moreover, the studies *in vitro* indicated an enhancement of osteogenic gene expression of hASC when cultured in the presence of Si-OH groups. The present work shows that combining wet-spinning technology with a calcium silicate solution as a non-solvent allows designing scaffolds with key cues to render an osteoconductive behavior as the classic ceramic materials for bone regeneration. Additionally, these results suggests that silicon and calcium either individually or in combination may relevantly contribute to properly drive hASC towards the osteoblastic phenotype and highlight the potential of those ions in the control of cellular response such as cell differentiation and/or in stem cells recruitment upon implantation of a cell-free scaffold.

References:

1. Hoppe A., et al., Biomaterials 32: 2757–2774, 2011.
2. Beck Jr G.R., et al., Nanomedicine: Nanotechnology, Biology, and Medicine, 1–11, 2011.
3. Rodrigues A.I., et al., Acta Biomaterialia, 2012, DOI: 10.1016/j.actbio.2012.05.025.

TS052
Induction of human mesenchymal stem cells osteogenesis by bioactive agent-releasing liposomes

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Stem cell therapy is a rapidly evolving area of research in regenerative medicine. Mesenchymal stem cells (MSCs) have received considerable attention by the scientific community because of their potential of expansion and the ability to differentiate into various mesodermal tissues. Liposomes are well-established non-viral carrier systems, presenting significant advantages over other nanoparticle-based drug delivery systems, namely a high load carrying capacity, a relative safety and an ease of large-scale production, as well as a versatile nature in terms of possible formulation and functionalization. The objectives of the present study were to evaluate the efficacy of growth differentiation factor-releasing liposomes on the induction of MSCs osteogenesis. For that, dexamethasone (Dex) was encapsulated within the liposome bilayer at different lipid formulations. The obtained liposomes showed a monodisperse distribution of particles size, and an increased ζ-potential for the PEGylated liposomes. Dex encapsulation studies demonstrate that the presence of cholesterol (Chol) decreases the Dex loading capacity of the liposome bilayer. The different stabilizing effect of Chol and Dex on the liposomes is due to differences in their interaction with phospholipid molecules. Highly lipophilic Chol gets incorporated between the acyl chains and reduces chain movement increasing rigidity and stabilization the membrane. Dex, being more hydrophilic, interacts differently with phospholipid acyl chains and head groups and destabilizes the membrane. In vitro release study demonstrated an initial burst release within an initial timeframe of 24 h. Following the initial release, a slower release was observed until 6 days. Afterwards, Dex continues to be released at a slower but steady rate until day 21. The effect of Dex-loaded liposomes on viability, proliferation and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs) was assessed. The results of the biological activity showed that the Dex-loaded liposomes do not have any cytotoxic effect and, more importantly, were able to promote an earlier induction of hBMSCs differentiation into the osteogenic lineage, as demonstrated by the expression of osteoblastic markers at the phenotypic and the genotypic levels. Concluding, Dex-loaded liposomes represent a novel biological or nature-inspired nanoparticle strategy for tissue engineering and regenerative medicine applications.

TS053
Suitability of silk-based 3D biotextiles seeded with human adipose-derived stem cells for a bone tissue engineering approach

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Human Adipose-derived Stem Cells (hASCs) became an emerging possibility for tissue replacement therapies, such as bone tissue regeneration. Due to their osteogenic differentiation potential, easy isolation, expansion and *in vitro* proliferation, they have become a highly potential source of seed cells to be seeded in bone tissue engineering (TE) constructs and have demonstrated promising prospects in bone regeneration [1, 2]. To date several strategies have been proposed with more or less success to prepare porous three-dimensional biodegradable scaffolds for bone TE. Among them, textile technologies are particularly interesting since they can allow for producing finely tuned, fibre-based complex structures, offering superior control over the design (ex: size, shape, porosity, fibre alignment), manufacturing and reproducibility. The aim of this work is to evaluate the potential of recently developed silk-based biotextile structures [3] to promote hASCs adhesion, proliferation and osteoblastic differentiation. Natural silk yarns were processed into different 3D structures using standard knitting or warp-knitting technologies to increase the scaffold's tridimensionality. In the latter case two knitted silk layers are assembled and spaced by a monofilament of polyethylene terephthalate (PET). These constructs were characterized in terms of their morphology by Microcomputed Tomography (μ-CT) and scanning electron microscopy (SEM). The mechanical properties were investigated through compressive tests and dynamic mechanical analysis (DMA). All constructs disclose a biocompatible behavior, assessed using a mouse fibroblastic cell line (L929; ECACC, UK). hASCs were seeded onto the scaffolds and cultured for 14, 21 and 28 days in osteogenic medium. All textile constructs were analysed in terms of cell adhesion, proliferation and differentiation potential influence through the biological assays, alkaline phosphatase (ALP), DNA and Ca^{2+} quantification and histological, confocal, SEM and Real-Time PCR analysis. The obtained constructs present very reproducible intra-architectural scaffold geometry with high surface area and exhibiting a wide range of porosities. By the above mentioned assays it was possible validate the developed constructs as suitable for hASCs adhesion, proliferation and differentiation into an osteoblastic lineage. The positive influence of the developed 2D/3D textile structures on the osteoblastic differentiation potential of hACSs is an important outcome that validates future bone tissue enginnering approaches using these fibre-based architectures.

References:

1. de Girolamo, L., et al., 2009. **11**(6): p. 793–803.
2. Correia, C., et al., Acta Biomater, 2012. **8**(7): p. 2483–92.
3. Almeida, L.R., et al., 2011. **34**(8): p. 663–663.

TS054
Time-regulated drug delivery system on coaxially incorporated platelet alphagranules for biomedical use

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Aims: Platelet derivatives serve as an efficient source of natural growth factors. In the current study, we incorporated alpha granules in coaxial nanofibers.

Materials and Methods: A nanofiber scaffold containing alpha granules was prepared by coaxial electrospinning. The biological potential of the nanofiber scaffold was evaluated in chondrocyte and mesenchymal stem cell cultivation studies; additionally, the concentration of transforming growth factor (TGF)-β1 was determined.

Results: Microscopy studies showed that intact alpha granules were incorporated into the coaxial nanofibers. The cultivation tests showed that the novel scaffold stimulated viability and extracellular matrix production of chondrocytes and mesenchymal stem cells (MSC). The concentration of growth factors necessary for induction of cell proliferation was significantly decreased as well.

Conclusions: The system preserved alpha granule bioactivity and stimulated cell viability and chondrogenic differentiation of mesenchymal stem cells. Core/shell nanofibers incorporating alpha granules are a promising system for tissue engineering, namely, cartilage engineering.

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TS055
Injectable hydrogel functionalized by nanofibres as a drug delivery system for skeletal tissues regeneration

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Hydrogels are favorite materials in tissue engineering for its big potential to bond water. It enables good transport of nutrients and waste product and also homogenous distribution of cells. In addition, hydrogels can be injected directly to the defect and fill in defects of different shapes and sizes and subsequently can be polymerized chemically and physically. This material can also be enriched by bioactive molecules or particles and used as a drug delivery system. In our study, injectable hydrogels based on fibrin, hyaluronic acid (HA) and type I collagen were seeded with mesenchymal stem cells (MSCs) and tested. Moreover, the scaffolds were enriched with thrombocytes, as a source of native growth factors, and/or capsules from polycaprolactone (PCL) loaded with dexamethasone and ascorbic acid. The hydrogels were two-component and solidified after mixing in 37 °C. The first component contained fibrin and type I collagen, the second one comprised of thrombin, MSCs, HA and thrombocytes and/or PCL capsules. The cells were viable for the whole 14 days of experiment. It was shown, that cell proliferation and viability was improved by thrombocytes, but not by PCL capsules. Neither combination of both thrombocytes and capsules was better than the control. On the other hand, the presence of capsules prolonged the disintegration of samples. This way the biomechanical properties of hydrogels can be improved. Hydrogels based on fibrin, hyaluronic acid and type I collagen enriched by thrombocytes showed improved cell proliferation and viability in comparison to control. Presence of capsules in hydrogel enhanced biomechanical properties and prolonged scaffold degradation. Moreover, these particles can serve as a drug delivery system for stimulation of cell proliferation and differentiation. Further experiments are essential for adjustment of drug dose and release kinetics. *This work was supported by the Grant Agency of the Charles University (grant No. 330611, 384311, 626012), The Grant Agency of the Czech Republic (grant No. P304/10/1307) and Internal Grant Agency of the Ministry of Health of the Czech Republic (grant no. NT12156).*

TS056
Core/shell nanofibers with embedded liposomes as a drug delivery system

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The broader application of liposomes in regenerative medicine is hampered by their short half-life and inefficient retention at the site of application. These disadvantages could be significantly reduced by their combination with nanofibers. We produced two different nanofiber-liposome systems in the present study, i.e., liposomes blended within nanofibers and core/shell nanofibers with embedded liposomes. Herein, we demonstrate that blend electrospinning does not conserve intact liposomes. In contrast, coaxial electrospinning enables the incorporation of liposomes into nanofibers. We report polyvinyl alcohol-core/poly-ϵ-caprolactone-shell nanofibers with embedded liposomes and show that they preserve the enzymatic activity of encapsulated horseradish peroxidase. To demonstrate the potential of the coaxial system with embedded liposomes for a drug delivery of growth factors, we examined the viability and proliferation of MSCs on prepared nanofibers. MSCs were seeded on polyvinyl alcohol-core/poly-ϵ-caprolactone-shell nanofibers with the addition of a mixture of growth factors (bFGF, TGF-β and IGF-I) either dispersed in the core solution (GF) or encapsulated in liposomes (LIP-GF). Concentrations of released growth factors were detected by ELISA. Finally, core/shell nanofibrous scaffolds containing embedded liposomes with encapsulated recombinant growth factors were more potent at stimulating MSC proliferation than coaxial nanofibers without liposomes. In conclusion, intact liposomes incorporated into nanofibers by coaxial electrospinning are very promising drug delivery systems in tissue engineering and regenerative medicine applications.

TS057
Hypoxic preconditioned mesenchymal stem cells induces elevated expression of cardioprotective cytokines and growth factors

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Objective: Mesenchymal Stem Cells (MSCs) have ability to differentiate into various types of lineages which make them attractive candidates in the field of regenerative medicine. Transdifferentiation process of MSCs is mainly influenced by hypoxic microenvironment. Hypoxic preconditioning play a very important role in myocardial regeneration as it enhances the differentiation potential of stem cells and also upregulate the expression of different cardioprotective cytokines and growth factors. The present study main objectives were:

Isolation of Mesenchymal cells from the bone marrow of rat.

Isolation of cardiomyocytes from 1 day old neonatal rat's pups.

Characterization of these cells based on the presence of cell surface markers by immunostaining, PCR, flowcytometry and Western blotting.

Hypoxia treatment of mesenchymal stem cells with 2, 4 dinitrophenol.

Determination of the expression of various cytokines and growth factors by RT-PCR.

Determination of myogenic and angiogenic potential of these stem cells after co-culturing them with hypoxic cardiomyocytes at different time periods.

Determination of myogenic and angiogenic potential of these stem cells after co-culturing them with cytokines transfected cardiomyocytes at different time periods.

Methods: Rat bone marrow derived MSCs were cultured and assessed for their responsiveness to anoxia by an optimized dose of 0.25 mM 2, 4, dinitrophenol (DNP) and then allowed to propagate under normal condition for 2 and 24 h. Analysis of various cytokines and growth factors were done by RT-PCR and Determination of myogenic and angiogenic potential of stem cells co-cultured with hypoxic cardiomyocytes and cytokines transfected MSCs co cultured with normal cardiomyocytes were analyzed by flow cytometry.

Results: The morphological examination of MSCs has indicated that the cells were slightly shrunken immediately after the anoxic insults. Analysis of various cytokines through RT-PCR has shown higher expression of IL-7, SCF, TGFß, VEGF, IGF, HIF and HGF. The expression increased exponentially with the duration of anoxic insult. The coculture studies of hypoxic cardiomyocytes with MSCs showed higher fusion in conditioned medium as compared to normal medium. The coculture studies of cytokines transfected MSCs with cardiomyocytes showed higher fusion.

Conclusions: The results indicate that during hypoxia and ischemia, MSCs secrete cell survival factors that may operate via various signaling pathways enhancing their regenerating potential.

TS058
The effect of ionizing radiation on differentiation of human embryonic stem cells

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Pluripotent human embryonic stem cells (hESC) are able to differentiate to various cell types found in human body, and, thus, carry great potential to regenerative cell replacement-based medicine. The full realization of the therapeutic potential of hESC in practice may require labeling these cells with radionuclides for *in vivo* analysis of their engrafting and subsequent tracking of their fate with imaging modalities using low levels of ionizing radiation. In addition, study the effect of ionizing radiation (IR) on hESCs may provide valuable information on responses to IR exposure of human cells in their most naive state, and on consequences of IR exposure on the development of human organism. We hypothesize that irradiation of hESC may affect the pathways of their spontaneous and induced differentiation, and change the patterns of gene expression within the resulting lineage-specific cell types. To test this hypothesis we irradiated cultured pluripotent hESC with low doses of ^{60}Co gamma-radiation. Next we induced differentiation of irradiated hESC with two different methods; Activin A treatment to definitive endoderm, and spontaneous embryoid bodies formation. We then analyzed time course of the expression of pluripotency markers (Oct4 and Nanog), and early differentiation markers for definitive endoderm (FOXA2, Sox17), mesoderm (Brachyury), and neuroectoderm (PAX6) by quantitative RT PCR. Our results will provide important insights into sensitivity of hESC to IR, and could potentially results in development of an assay for detection of the effects of very low doses of ionizing radiation on hESC.

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TS059
The use of embryos in stem cell research: scientific practices, political views and citizens' expectations in portugal

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The use of embryos in stem cell research depends on permissive policies, availability of stem cell research centers and citizens willing to donate embryos. This paper compares the views, actions and expectations mobilized by three different agents in Portugal: (i) *in vitro* fertilization (IVF) heterosexual couples in their decisions to donate embryos for scientific research; (ii) legislation and political debate about stem cell research; and (iii) scientific projects on embryonic stem cell research. The analysis of the interrelationships between science, politics and citizenship concerning the use of embryos in stem cell research was grounded on the content analysis of the following sources: (i) interviews conducted with 451 IVF patients (267 women and 184 men) at Hospital de São João (Porto, Portugal), between August 2011 and June 2012, about reasons underlying the donation of embryos for research; (ii) arguments used by the Portuguese Government to justify the approval of a new permissive regime for embryonic stem cell research on February 24, 2011 and the parliamentary discussions about assisted reproductive technologies conducted in January 2012; (iii) the scientific fields, topics and aims of research, as well as composition of the teams, in all embryo research projects funded by Foundation for Science and Technology (major research funding state agency in Portugal) between 2000 and 2009. Scientists, stakeholders and IVF patients were very receptive to medical and technological progress, revealing expectations concerning the regenerative and therapeutic power of embryonic stem cells that are founded on high levels of trust and hope on science and technology and on health professionals. While IVF patients justified the donation of embryos for research grounded on feelings of reciprocity towards science, responsibility on contributing to improve fertility treatments and altruism, stakeholders emphasized the human embryonic stem cell research contributions for economic activity and progress of the country, and scientists highlighted the human health as the justification for funding embryonic stem cell research projects. We conclude that it is needed to maximize the available information on the scientific projects using embryonic stem cells for dissemination among IVF patients and health professionals and to monitor stakeholders and citizens' knowledge and expectations about the upcoming therapies within regenerative medicine. The debates about research ethics and regulation of embryonic stem cell research should include the views and expectations of scientists, stakeholders and IVF couples, as well as other citizens, aiming to ensure wide participation and to achieve a consensus decision making process.

TS060
3D functionalized collagen/hydroxyapatite scaffold seeded with MSC for bone defect regeneration *in vivo*

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Objectives: The aim of this study was to develop the ideal scaffold for bone defect regeneration.
Materials and Methods: In our experiment was prepared a scaffold from Collagen type I (Col)/ Hydroxyapatite (HA) and combined with Poly-ϵ-caprolactone (PCL) nanofibres. Three variants of this scaffold were prepared. The scaffold seeded with autologous Mesenchymal Stem Cells (MSCs) in osteogenic differentiation media (Group1) or scaffold enriched with platelet rich plasma (PRP) (Group 2) or scaffold enriched with both MSCs and PRP (Group 3). All groups of scaffolds were implanted to the rabbit femur condyles where critical size defect 6 mm in diameter and 10 ± 0.5 mm in depth was made. Empty defects were used as a control (Group 4). 12 weeks later rabbits were sacrificed and the femoral condyles were examined by histological analysis. The aim was to analyze the volume fraction and distribution of bone within an experimental defect.
Results and Discussion: The highest bone volume fraction within the healing defect was found in samples with scaffolds enriched with both MSC and PRP. Bone volume fraction was comparable between the samples with PRP-enriched scaffolds, and MSC-enriched scaffolds, while samples without any scaffold contained the lowest bone volume fraction. Only samples containing both MSC and PRP showed uniform bone deposition in whole volume of defect. The moduli of elasticity under compressive test significant increased at the Col/ HA/ PCL scaffold compared to Col/ HA scaffold without PCL nanofibres.
Conclusion: This smart composite scaffold enriched with PCL nanofibres, MSCs and PRP present new possibilities for bone defect regeneration.
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TS061
3D cultures of human neural progenitor cells: dopaminergic differentiation and genetic modification

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Central nervous system (CNS) disorders remain a formidable challenge for the development of efficient therapies. Cell and gene therapy approaches are promising alternatives that can have a tremendous impact by treating the causes of the disease rather than the symptoms, providing specific targeting and prolonged duration of action. Hampering translation of these novel therapies from experimental to clinical settings is the lack of relevant *in vitro* and *in vivo* models that can be used in pre-clinical stages.

This work describes the development of 3D *in vitro* models of hCNS, using human midbrain-derived neural stem cells (hNSC) as a scalable supply of neural-subtype cells, in particular dopaminergic neurons. Following a systematic technological approach based on stirred-tank bioreactors, with control of chemical and physical environmental parameters, resulted in reproducible ratios of differentiated cells, as shown by detailed characterization using confocal microscopy, field emission scanning electron microscopy, transmission electron microscopy, qRT-PCR, Western Blot and metabolic profiling.

In order to establish methodologies for gene transfer and manipulation of gene expression in the 3D hCNS cell model, canine adenovirus type 2 (CAV-2) vectors were chosen Due to their characteristics: high cloning capacity, long-term transgene expression, low immunogenicity and neuronal tropism. CAV-2-GFP vector was used for optimization of transduction of differentiated neurospheres and assessment of vector entry and impact on neurosphere composition. The model system developed herein constitutes a practical and versatile new *in vitro* approach to study hCNS. Furthermore, this culture strategy may be extended to other sources of hNSC, such as human pluripotent stem cells, including patient-derived induced pluripotent stem cells.

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TS062
The potential of microtissues in osteogenic tissue engineering

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Large bone defects that do not heal spontaneously necessitate therapeutic measures for bone regeneration. As an alternative to classic therapies, osteogenic tissue engineering (TE) is regarded as a promising technology for bone regeneration. As a standard procedure multipotent stem cells are incubated in two-dimensional (2D) cell cultures before they are combined with scaffolds, to form a TE bone graft (TEBG) that is implanted. However, cells in a 3D-environment have more similar properties to cells *in vivo*. Multipotent human stem cells from umbilical cord blood (USSC) have been differentiated to spherical osteogenic Microtissues (MTs), which mineralized faster than 2D-cultures. Moreover, also control microspheres that have been treated without osteoinductive agents mineralized spontaneously (Langenbach *et al.*, Nat. Protoc. 2011). Prior to the use of MTs for TEBGs, it was ensured that cells are able to migrate out of osteogenic differentiated MTs to colonize spaces in scaffolds. It was found that the optimum duration for an osteogenic predifferentiation, while retaining the ability of cells to migrate out of the MT into extracellular matrix (ECM)-gel, is 3 days. For the detection of mineralization, a commercially available hydroxyapatit-specific fluorescent dye was adapted for histology, which provided higher sensitivity in mineral detection compared to standard histological stains. By further analysis of MT-minerals, it was found that minerals consisted of calcium-deficient hydroxyapatite (CDHA) amorphous-calciumphosphate (ACP), octa-calciumphosphate, (Mg)Whitlockit and hydroxyapatite. Moreover, dystrophic mineralization after apoptosis of cells could be excluded by TUNEL-assay. Best biocompatibility with USSC of several potential biomaterials for TEBGs was obtained for demineralized spongiosa (ICBM). In contrast to inoculation with cell suspensions a 40-fold increased cell number could be integrated in one scaffold by inoculating ICBM with MTs and a more precise administration of large cell numbers is possible. For the investigation of the osteogenic potential of USSC-ICBM- and MT-ICBM-scaffolds the constructs were implanted into dorsal muscle bags of immuno-compromised rats. It was found that ectopic bone formation was induced in USSC-ICBM- and MT-ICBM-scaffolds, whereas cell-free ICBM did not lead to bone formation. In conclusion, MTs offer improved *in vivo*-like conditions for stem cells compared to 2D-cultures, resulting in enhanced osteogenic differentiation. MT-technology can ameliorate seeding efficiency of biomaterials and can increase the cell load of a scaffold. Furthermore, microtissues reduce the risk of unwanted cellular distribution and wash out in tissue engineering and scaffold free cell therapy. With their capability of ectopic bone formation MT-ICBM-constructs hold promise for facilitated, accelerated and improved bone regeneration.

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TS063
Are statins able to improve EPCs response of diabetic patients with myocardial infarction?

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Aim: Our study aims to compare the response to statins of EPCs of diabetic vs. nondiabetic patients with myocardial infarction.
Methods: Ten patients admitted for acute myocardial infarction in a Coronary Care Unit, between January and March 2012, were included in this study. Levels of circulating EPCs were quantified from peripheral circulation by flow cytometry and cell culture was performed in order to evaluate their functional characteristics. To compare functional differences of EPCs, 5 diabetic and 5 nondiabetic patients' cells were isolated and cultivated under two different conditions: high glucose (25 mM), normal glucose (5.5 mM). Colony forming units (CFUs) were evaluated after 7 days of incubation as well as proliferation rate of EPCs, which were maintained in culture until the 15th day. To test the effects of statins on EPCs function of diabetics and non diabetics, cell culture was held under three conditions: control; atorvastatin (0.1 mM) and pravastatin (10 mM).
Results: We found that the rate of cell death was significantly higher in diabetic patients, achieving a significantly lower number of EPCs by field after 15 days in culture in control conditions (glucose concentration of 5.5 mM) (111.5 ± 4.2 vs. 160.4 ± 18.1 EPCs in non diabetic patients, $P < 0.05$). Furthermore, the ability to form CFUs was significantly reduced in diabetic patients by comparison with non diabetics (0.83 ± 0.44 vs 1.82 ± 0.082, $P < 0.05$). In nondiabetic patients we found no significant differences in proliferation rate of EPCs when cultured with atorvastatin or pravastatin compared with the control condition. However, in this group of patients the ability to form CFUs was significantly improved by atorvastatin. For diabetic patients we found that EPCs treated with atorvastatin slowed the progression of cell death, between the 6th and the 10th day of culture, when compared to control conditions. In addition, CFUs from diabetic patients only increased to numbers similar to non-diabetic patients when treated with atorvastatin.
Conclusions: These findings indicate a beneficial effect of atorvastatin in the function of EPCs from patients with myocardial infarction. Moreover, statins showed to improve the function of EPCs, even in diabetic patients with an endogenous impairment of EPCs.

TS064
Critical Assessment of rhBMP-2 Mediated Bone Induction: an *In Vitro* and *In Vivo* Evaluation

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Understanding the influence of formulation and storage conditions on rhBMP-2 bioactivity is extremely important for its clinical application. Reports in the literature show that different research groups employ different parameters such as formulation conditions, storage, doses for *in vivo* applications etc. that makes it difficult to correlate results from different experiments. We therefore decided to rationalize these anomalies by performing a basic study on such parameters using two commercially available BMPs. Our *in vitro* experiments suggest that BMPs from different sources have significant differences in their bioactivity. The clinically approved rhBMP-2 (InductOs®; BMP-P) showed superior stability, compared to rhBMP-2 from R&D Systems (BMP-R) at physiological pH (determined by ALP assay). This BMP-P also showed lower binding to polypropylene Eppendorf tubes. The BMP-R almost lost its bioactivity within 30 min at physiological pH and also shows more adhesion to plastic surfaces. The *in vitro* results were also reflected in the *in vivo* experiments, in a rat ectopic model with injectable hyaluronic acid (HA) hydrogel as BMP carrier. After 7 weeks post-implantation we observed larger bone volume with oriented collagen in the BMP-P group but a smaller bone with disoriented collagen in the BMP-R case. Our results highlight the large difference in activity between seemingly identical substances and also the importance of proper handling of such sensitive proteins.

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