OF MASS AND MOMENTUM TRANSPORT IN CON-
HFMBS FOR LONG BONE TISSUE ENGINEERING
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effects of convection and a distributed and delocal-
ollow fibre membrane bioreactors(HFMBs) have been
the culture of cm-scale BMSC aggregates, pos-
limitations typical of other bioreactors for bone tis-
hematical modelling of mass transport, cells growth
particularly interesting given the difficulty to monitor
oncentration in the presence of a closed shell, to opti-
nd operation. Most proposed models consider cells
e extracapillary space, in contrast with experimental
ctive flows, and are then inadequate for this purpose.
hematical models of nutrients profiles inside HFMBs
mode from diffusion-limited to convection-dominant
for both uniform cell distribution and the actual non-
served in experiments with BMSCs.
sed on a multi-compartment description of HFMBs
ter assumption, and on a quasi-steady state analysis
cell concentration profiles. Relevant non-dimensional
ed, and governing momentum and mass transport
y solved with a finite element commercial code. Meta-
ary and immortalized cell were used, proliferation and
ere assessed from culture experiments.
etabolic requirement cell types, like immortalized ones
actors for 3D constructs culture easier, being diffusion-
models inadequate only for cell density close to natural
sults demonstrate the importance of convective nutrient
meability and packing density in the cell compartment
in the ECS when primary cells are used.
ction-dominant nutrients transport is necessary to over-
mitation when culturing cells types with physiological
r 3D cm-scale constructs.

ON DEVELOPMENT OF THE SERIES OF THE HOL-
E BIOREACTORS (DEVOTED TO DIFFERENT CELL
HS)
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bioreactors have broad spectrum of applications in-
and tissue engineering studies. Structure of the mem-
change of the biochemical compounds with required
the inner and outer bioreactor compartments. In our
hod of modification of the standard polysulfone mem-
ming at a controllable increase of the membranes cut
e of this preliminary study was to evaluate filtration
he prototype, modified, polysulfone membranes de-
in the cell bioreactor.
of membranes: A, B, C (subgroups: C1, C2, C3) and
actor casings were tested under *in vitro* conditions.
rane structure was performed based on change of the
ological procedure. Membranes were evaluated with
studies (quantitative assessment) and in the separa-
sma (qualitative assessment). Sieving coefficients for
DL, IGM, and LDL were calculated and then MWCO

the ultrafiltration coefficient were as follows: 6, 28, 13,
mHg·m²), for membrane type A, B, C1, C2, C3 and D,
value of MWCO = 80kDa was obtained for bioreactor
in the remaining groups, the following MWCO values
a, C1 – 110kDa, C2 – 150kDa, C3 – 2000kDa and for
was greater than 2700kDa.
demonstrated that MWCO increased as a response
membrane structure (MWCO ≥ 80 kDa) in comparison
polysulfone membrane characterized by the MWCO
the developed method makes it possible to prepare
the membranes with required permeability appropriate for specific bioreactor applications.

P181 (EI0411)

PLATELET LYSATES AS A SCAFFOLD COMPLEMENT PROMOTING HASCS PROLIFERATION AND OSTEOGENIC DIFFERENTIATION

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Objectives: This work aims to establish Platelet Lysates (PL) as optimal source of growth factors and other molecules that are vital for promoting cell proliferation and differentiation pathways, eventually allowing the substitution FBS and/or osteogenic supplements in culture media in bone tissue engineering strategies. Furthermore we intend to design new approaches to incorporate PLs in a scaffold material, as a hydrogel encapsulating the cells or as a coating for 3D porous structures, thus developing a tissue engineered construct with enhanced/multiple functionalities.
Methods: Starch–polycaprolactone (SPCL) meshes were obtained by a fiber bonding method as previously described. PL gels were obtained by activation of platelets coagulation cascade using thrombin dissolved in a calcium chloride solution. Human adipose stem cells (hASCs) were obtained by enzymatic digestion of lipoaspirates samples. hASCs were either seeded directly into the SPCL scaffolds (control group) or into the scaffolds previously coated with PL gels or suspended in the PL and then seeded in the scaffold and gellified. hASCs proliferation and differentiation was assessed after different culturing time points of the constructs, by DNA and ALP quantification and by RT-PCR and immunohystological analysis.
Results: The preliminary results obtained sustain the hypothesis that growth factors and other signaling molecules present in PL groups are actually active and vital to initiate proliferation and osteogenic differentiation of hASCs. DNA quantification and cell viability were similar and even higher in PL groups, as well as early markers of osteogenic differentiation, such as ALP activity. Latest time-points revealed less noteworthy differences especially due to the progressive degradation of the PL gel.
Conclusions: PL represents a substrate and a delivery system of important growth factors and other signaling molecules, and therefore making these molecules available for cells within a tissue engineering construct provides an important enhancement of autologous bone tissue engineering strategies.

P182 (EI0370)

BUILDING THE BASIS FOR HUMAN MENISCUS REGENERATION

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Objectives: Total or partial meniscectomy has been the gold standard for the treatment of degenerated/diseased menisci. Despite meniscal regeneration represents a recent trend in tissue engineering, fundamental studies related to human meniscus biochemistry and biomechanics are still scarce. This work aims to contribute in the knowledge of this tissue aiming at future clinical applications, namely the aspects dealing with the cellular phenotypes and density, biomechanics and extracellular matrix composition.
Methods: Human tissue was obtained from local hospitals by means of surgery or biopsy, in accordance with local ethical committee guidelines. The HMC's were isolated from different donor (sex and age) explants or using an enzymatic standard protocol. Micro-computed tomography (Micro-CT) of freeze-dried meniscus was carried out. Histological (haematoxylin and eosin - H&E, trichrome stain and toluidine blue stainings) analysis was performed for segmental characterization of ECM and cells density. Dynamic mechanical analysis was carried out for medial, anterior and posterior segments of meniscus (in PBS at pH 7.4).
Results and Discussion: Micro-CT analysis revealed that meniscus (freeze-dried) possessed a mean porosity of 53%, a mean pore size and trabeculae thickness of 85μm and 80μm, respectively. The cells isolated from meniscus are

a mixed population of cells, *i.e.* fibrochondrocyte-like and MSCs. The histological evaluation has shown that meniscus ECM is composed of collagen-type I. This tissue is fibrocartilagineous in nature and presented a higher cell density in the periphery as compared to meniscus core. Cellular density among the different segments (anterior, medial, posterior) of meniscus was quantified using the H&E 2-D histological images.
Conclusions: This study has contributed to improve the knowledge on meniscus biology and mechanical properties. It is believed that these important issues should be considered to develop adequate acellular and cellular strategies for tissue engineer meniscus.

P183 (EI0079)
COMBINING OPTICS AND ULTRASOUND TO IMAGE 3D TISSUE CONSTRUCTS
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Objectives: Tissue scaffolds are an integral part of the tissue engineering process, assisting in the culturing of cells in three dimensions. It is important to understand both the properties of the scaffold and the growth of cells within the scaffold. This paper describes a system to characterise scaffolds using acoustic techniques alone and the development of an ultrasound-modulated optical tomography system to study the growth of cells within the scaffolds. The ultrasound modulated system allows the effects of light scattering in relatively thick tissue constructs (several mm) to be reduced.
Methods: Acoustic techniques alone have been applied to characterise foamed scaffolds manufactured from synthetic polyesters poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) via a supercritical fluid process. An ultrasound modulated optical tomography system has been used to image absorbing and fluorescent objects in gel scaffolds.
Results: Although foamed scaffolds are porous and therefore highly scattering to sound waves, results demonstrate that acoustic signals are detectable through a 6mm thick foamed scaffold. Images of optically-absorbing materials embedded in gel-based tissue phantoms will be presented demonstrating that a lateral resolution of 250μm and an axial resolution of ~90μm can be achieved in scattering samples. Preliminary results of non-linear acousto-optic modulation will also be presented.
Conclusions: Combining optics and ultrasound can be used to obtain high-resolution optical images of highly scattering, thick tissue constructs.

P184 (EI0086)
REGULATION AND CHARACTERISATION OF CORNEAL STROMAL CELL CONTRACTION
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Objectives: Collagen hydrogels have been extensively used as scaffolds for corneal tissue engineering. However, corneal stromal cells differentiate into contractile fibroblasts in the hydrogel *in vitro* culture, rather than keratocytes. The aim of this study is to develop techniques to regulate the contraction by either chemical or topographical cues which mimic the native corneal environment, and characterize the cellular feedback in prolonged culture periods via novel, non-destructive monitoring protocols.
Methods: $5x10^5$ human corneal stromal cells were seeded in collagen hydrogels with and without the incorporation of poly-lactic acid aligned nanofibers. A non-destructive spherical indentation technique was used to examine the alteration of the mechanical properties of the individual collagen hydrogel specimens under different media respectively up to 28 days. The dimensional change of the specimens caused by the cells' contraction was measured by optical coherence tomography in parallel. The quantitative-PCR with respect to the expression of keratocytic and fibroblastic markers was conducted to cross-validate the observed physical properties. It was revealed that stromal cells cultured under media with insulin and without serum exhibited constant elastic modulus and gel dimension, indicating that contraction was suppressed, which was cross-validated by the expression of keratocan and ALDH3; whilst stromal cells cultured with serum demonstrated continuously increased modulus and reduction of thickness, typical of contraction process. The presence of aligned nanofibers reduced the degree to which the cells were able to contract the hydrogel constructs in a vertical direction, thus encouraging the cells cultured in fibroblastic media to behave more like non-contractile keratocytes.
Conclusions: The alteration of culture conditions and the addition of topographical cues can regulate corneal stromal cell differentiation. This can potentially enhance the field of corneal tissue engineering using collagen hydrogel models. The non-destructive monitoring protocols provide convenient tools for observing biological phenomenon for prolonged culture periods in the same specimen.

Tissue Engineering of Skin

P185 (EI0366)
ATTACHMENT AND SPREADING OF FIBROBLASTS ON SELF-ASSEMBLING BIOACTIVE MATRICES
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Objectives: The primary objective of this work was to investigate the potential of 2D biodegradable membranes as supportive bioactive matrix for wound healing by studying the behavior of human fibroblasts on these membranes. Towards this goal, we developed a biomimetic matrix that incorporates structural components of skin extracellular matrix (hyaluronan) and biochemical signals (RGDS epitope) to recreate some aspects of skin tissue niche. The RGDS sequence is present in cell binding domains of extracellular proteins (such as fibronectin) and is known to promote integrin-mediated cell adhesion.
Methods: The proposed bioactive matrices result from the self-assembly between peptide amphiphiles and hyaluronic acid (HA), a major component of skin ECM. The RGDS sequence was incorporated in the peptide structure to provide the matrices with cell-adhesive properties. Cell culture was then performed and the effect of the RGDS epitope on the adhesion, morphology and proliferation of primary human dermal fibroblasts was followed respectively by, scanning electron microscopy, immunostaining and DNA quantification.
Results: Cell responses to RGDS matrices were compared to matrices containing DGSR (scrambled sequence that does not promote cell adhesion). When cultured on membranes without the cell recognition epitope RGDS, human dermal fibroblasts showed lower adhesion to the matrices when compared to the ones containing RGDS. SEM analysis showed adherent cells on the RGDS matrices and the presence of filopodia which are known to be involved in the regulation of cell migration.
Conclusions: We expect that the proposed biodegradable bioactive matrices could offer significant potential in skin regeneration strategies and also as model systems for fundamental mechanistic studies in wound remodeling.
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P186 (EI0307)
AN ARGININE INCORPORATED NANOCOMPOSITE POLYMER FOR CARDIOVASCULAR IMPLANTS
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Objectives: Cardiovascular implants must resist thrombosis. Polyhedral- o
gomeric-silsesquioxane-poly(carbonate-urea)urethane (POSS-PCU) nanocon
posite polymer has demonstrated suitable properties for cardiovascular app
cations. L-arginine is recognised as a significant amino acid with anticoagula
properties with a link to nitric oxide synthesis. Water soluble arginine is immo
lized within the polymer via nanoparticles thus presenting a novel surface mo
fication method for blood contacting polymers. The study aims to determine t
antithrombogenic properties of Arginine-POSS-PCU.
Methods: Arginine was reacted with amine functionalized fumed silica nanop
ticles using fmoc chemistry and incorporated into POSS-PCU at 5-8%W/W. S
face properties of Arginine-POSS-PCU samples were determined using FTIR
XPS. A thorough investigation of whole blood kinetics on Arginine-POSS-P
was performed with Thromboelastography polymer coated cups. Platelets w
introduced onto Arginine-POSS-PCU samples and incubated for 90mins at 3
on a shaker before platelet adhesion morphology with SEM and the change
platelet activated factors were determined with ELISA. Plasma from whole bl
was also introduced onto Arginine-POSS-PCU and the changes in protein