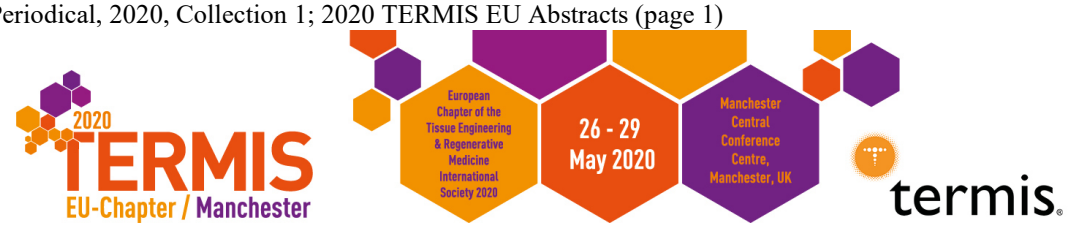

High Throughput Screening of Polymeric Biomaterials for Personalised 3D Printed Treatments

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INTRODUCTION:Three-Dimensional (3D) printing is an established manufacturing method(1). However, the use of 3D printing is limited in industry due to the few materials available and the long development and optimisation process. A high throughput methodology has been developed allowing to quickly asses the printability and biocompatibility of a large library of materials(2,3). This method is adapted and used here, to screen a library of 64 PCL-based hyper-branched polymers and determine their suitability for a use as a gradient osteochondral interface.

METHODS:To screen the synthesised polymer library, their printability (Z parameter) for inkjet printing is first assessed using a liquid handler, able to measure viscosity and surface tension (in DMF). The high-throughput assessment of mechanical and biocompatible properties is enabled by the use of a microarray strategy. Surface chemical characterisation is carried out using time-of-flight secondary ion mass spectrometry (ToF-SIMS), while localised mechanical properties (elastic moduli) are determined using atomic force microscopy (AFM). Combination of these techniques with cytotoxicity screening leads to the selection of three suitable polymers for the targeted application.

RESULTS:Solubility in DMF and printability determination led to the selection of around 60% of the tested library. Subsequently, the polymer arrays were fabricated by contact printing using polymer solutions in DMF and glass slides with a super hydrophilic/hydrophobic coating as substrate. ToF-Sims surface analysis ruled out any chemical contaminations and confirmed the deposition of the desired polymers. Assessment of the mechanical properties and cytotoxicity screening led to the selection of three materials used for preliminary printing essays.

DISCUSSION & CONCLUSIONS:The polymer library showed interesting mechanical properties with elastic moduli matching that of human bones and demonstrated the feasibility of manufacturing such scaffolds. Three selected materials for the library are currently being printed to create gradient scaffolds. The use of this high-throughput strategy allowed the rapid and accurate assessment of the polymers.

ACKNOWLEDGEMENTS:The author would like to thank Laurence Burroughs, Xinyong Chen, Nichola Starr and Gustavo Ferraz Trindade for their scientific and technical help.

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Keywords: Additive manufacturing, Personalised medicine.

Silk-fibroin based scaffold 3D bioprinting deposition: mathematical modeling and experimental characterization

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INTRODUCTION:Robotic dispensing-based 3D bioprinting strategies are widely used to print cell-laden hydrogel constructs. This enables the fabrication of neo-engineered constructs able to maintain cells alive and to regenerate tissues. Nowadays, efforts in this field are mainly driven by experiments and trial-and-error approaches. In order to predict the deposited filament width through extrusion-based 3D bioprinting, a mathematical model[1] was applied considering different printing parameters such as pressure and printing speed. The printing parameters were chosen to print silk fibroin(SF)-gelatin(G) based scaffolds laden with human-derived mesenchymal stromal cells (hMSCs).

METHODS:The rheological characterization of cell-friendly SF-G bioink used in our previous study[2] was used to define the viscosity and the power law index. A mathematical model of deposition was formulated including the following parameters: printing pressure (0-3 bar), speed (0.5-3 mm/s) and cylindrical stainless-steel needle features (diameters: 0.2, 0.41 and 0.5 mm and lengths: 6.35, 12.7 and 25.4 mm). Then, an experimental characterization was carried out to validate the model monitoring filament width as the output. Shear stress values were also estimated to ensure cell viability during the process. Printing parameters were chosen to print 3D cell-embedded construct with open and interconnected pores to guarantee nutrients permeability. Cell viability and cartilage genes expression were investigated at different time points (days 14 and 28).

RESULTS:The mathematical model was successfully validated by experimental data: the smallest discrepancy (1.5%) was found with needle diameter of 0.2 mm and length of 6.35 mm, whereas, the largest discrepancy (65.8%) with needle diameter of 0.5 mm and length of 25.4 mm. The model helped to define the best printing parameters to achieve a filament width of 200 µm using a pressure of 1.2 bar and a printing speed of 1.5 mm/s. The selected printing parameters and SF-G bioink ensured open pores formation and good cell viability until day 28. Moreover, we confirmed an increase expression of typical chondrogenic markers (SOX9, COLL2), at day 28 by both gene expression and immunofluorescence analyses.

DISCUSSION & CONCLUSIONS:This study evidenced that the proposed model can be used to predict the features of the silk-based hydrogels permitting to save time and resources by avoiding trial-and-error approaches. Moreover, we confirmed that SF-G bioink enhanced cartilaginous matrix formation.
ACKNOWLEDGEMENTS:This work was founded by Italian Ministry of Foreign Affairs and International Cooperation, Rizzoli fund "5x1000" and by EU Project Horizon 2020, ADMAIORA, grant N.814413.
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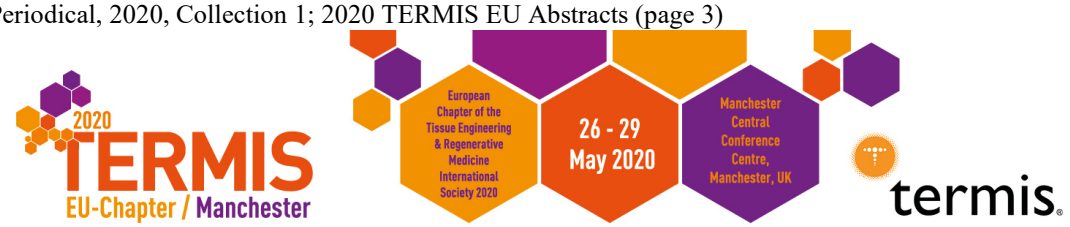
Keywords: 3D printing and bioprinting, Biomaterials



Robust Human Organoid Printing and Culture in an Integrated Plate System for Predictive Compound Screening

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INTRODUCTION:There have been several three-dimensional (3D) cell culture platforms developed, including ultra-low attachment well plates, Transwell inserts, hanging droplet plates, and microfluidic plates, but these platforms are relatively low throughput and/or unsuitable for high-throughput organoid culture and analysis in situ.

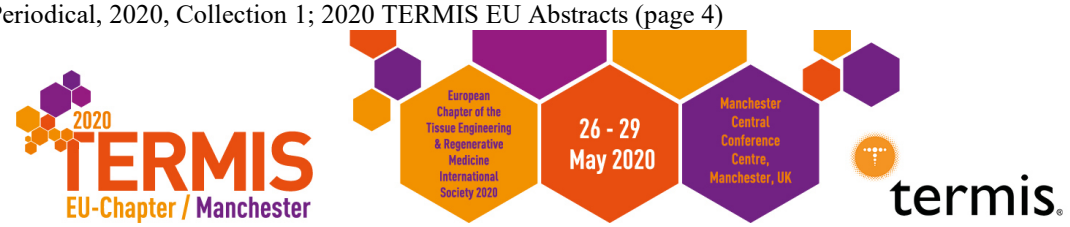
METHODS:To facilitate robust organoid culture in a high-throughput screening (HTS) system, we have developed miniature “3D bioprinting” technology and an integrated plate system consisting of a pillar plate and a complementary perfusion well plate, which is highly flexible and easily combined with conventional 384-well plates to support organotypic cell cultures and multiplexed high-content imaging assays.

RESULTS:We have demonstrated that our miniature 3D bioprinting platform can be used for optimizing organoid culture conditions that are critical to successfully create human tissue replicas. Several human organoids including brain, liver, intestine, and pancreas have been successfully printed, encapsulated in biomimetic hydrogels including Matrigel, differentiated, and imaged on the pillar plate platform for high-throughput compound screening. The optically clear pillar/perfusion well plates allowed direct visualization of organoids on the pillars for predictive cell-based assays. The entire organoids on the pillar plate were permeabilized, fixed, stained with primary and secondary antibodies, and cleared with tissue clearing solutions simultaneously for in situ whole organoid imaging without the need for cryosectioning. The flexible pillar and perfusion well format connected by microchannels and reservoirs made it easy to change growth media for organoid culture without the use of bulky pumps and tubes. It is compatible with standard 384-well plates and existing HTS equipment including fluorescent cell imagers and microtiter well plate readers, which is an important feature for developing HTS assays. It is easy to connect different types of organoids cultured on the pillars by using the perfusion well plate, which is critical to simulate human diseases.

DISCUSSION & CONCLUSIONS:Thus, our miniature 3D bioprinting technology and the novel plate platforms could offer new opportunities for creating highly organized tissue replicas by dispensing human stem cell types in hydrogels precisely with printing robots and mimicking the microenvironment of tissues in vivo, thereby potentially revolutionizing regenerative medicine, oncology, and drug discovery.

Acknowledgements:This study is supported by the National Institutes of Health (NIEHS R01ES025779 and NIDDK UG3DK119982) and institutional grants from Cleveland State University (Faculty Research Development and Faculty Innovation Fund). We appreciate Drs. Takanori Takebe and James Wells at Cincinnati Children's Hospital Medical Center for providing human organoids.

References:Lee, M.Y., Microarray Bioprinting Technology: Fundamentals and Practices (2016)**Keywords:** 3D printing and bioprinting, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models



Cyclic Mechanical Loading Enhances Bone-like Tissue Formation and Compressive Modulus in 3D Bioprinted Cell-laden Scaffolds

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INTRODUCTION:There is growing evidence that mechanical signals play a critical role in the regulation of human mesenchymal stem cells (hMSCs) osteogenesis and in bone development [1]. However, the influence of mechanical loading on mineral formation for engineering bone tissue is still unclear. Here, we investigated the effect of mechanical loading and pre-culture duration on compressive modulus and mineral formation of 3D bioprinted hMSCs-laden graphene oxide (GO) composite scaffolds.

METHODS:Bioink was prepared by mixing GO/alginate/gelatin (0.1%/0.8%/4.1% w/v) solution with hMSCs. 3D cell-laden scaffolds were bioprinted layer-by-layer on the platform with double-sided tape using a INKREDIBLE+ cell bioprinter. Scaffolds (n=13) were cultured in compression bioreactors with osteogenic media for up to 56 days. Cyclic mechanical loading (0.07 N preload, 1% strain, 5 Hz frequency) was applied for 5 min/day, 5 days per week. The influence of pre-culture duration was investigated by starting the mechanical loading at day 1 (ML1) or at day 21 (ML21). Non-mechanically loaded scaffolds were set as control group. Mineral volume was assessed non-invasively by micro-computed tomography imaging weekly and was calculated by different threshold values to clarify the different maturation rates of mineral. Compressive modulus was monitored weekly using the mechanical stimulation unit at day 56.

RESULTS:ML1 exhibited significant increases in mineral volume (> 83.34 mg/cm3 hydroxyapatite) compared to control group from day 21 to day 42, while no statistical differences were detected thereafter. Mineral volume was not statistically different in ML21 from day 21 to day 35, but was significant higher than control group from day 42 to day 56. The hard mineral volume (> 178.47 mg/cm3 hydroxyapatite), and scaffold mineral density (SMD) of ML1 was significantly higher than ML21 and control group at day 49 and 56. More interestingly, the compressive modulus of ML1 was 57.4 ± 8.4 kPa, which was significantly higher than ML21 (22.4 ± 2.7 kPa) and control group (9.7 ± 3.7 kPa) at day 56. Meanwhile, the compressive modulus of ML21 was significantly higher than control group at day 56.

DISCUSSION & CONCLUSIONS:Our findings demonstrated that cyclic loading lead to higher mineralization and drastically improved mechanical competence of the scaffolds. It is therefore recommended to add cyclic mechanical loading as a normal part of the protocol in bone tissue engineering, similar to the development in human and animal bone.
Acknowledgements:J. Zhang gratefully acknowledges financial support from the China Scholarship Council (CSC).

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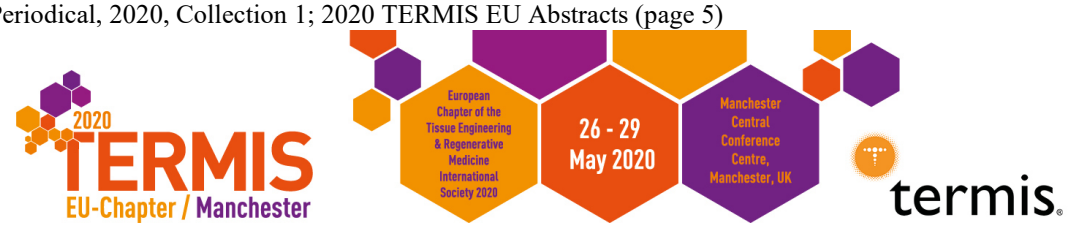
Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models



Development of Osteoconductive Bioink for 3D Bioprinting of Bone Cells

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INTRODUCTION:Bioprinting enables designed cell placement in tissue engineering. In extrusion-based bioprinting, hydrogels are used as cell carriers, called bioinks, and commonly combinations of different biomaterials. Alginate is a favoured biomaterial in bioprinting because of its fast gelation properties. However, in order to hold the integrity of the printed structure until the cross-linking of alginate, gelatin is used as a sacrificial material. The two, however are not enough to perform as a stable ink and, therefore, cellulose nanofiber (CNF) was used to improve printability. To develop the bioink further, nano-hydroxyapatite (nHA), an inorganic component of natural bone and a known osteoconductive component in bone tissue engineering applications, was used. The aim of this study was to investigate the influence of addition of both CNF and nHA to alginate-gelatin ink in terms of printability and cell viability.

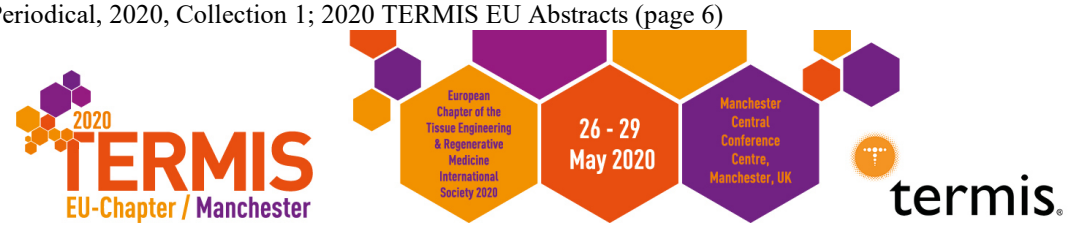
METHODS:To investigate the influence of CNF, first generation alginate-gelatin –based inks were prepared with different concentrations of CNF. To bioprint, Saos-2 cells (1.5×10^6/ml) were mixed with the inks and printed using a 3D-Bioplotter (EnvisionTEC). Through Live/Dead staining (L/D), the viability of the cells was assessed directly after printing and cross-linking to detect cell death caused by either step. L/D was also assessed at day 7. Second generation inks were prepared by adding nHA (1%) and CNF (0.75% w/v). To bioprint, Saos-2 or bone marrow stromal cells (BMSCs) were mixed with the inks and printed. Cell proliferation was measured with PicoGreen assay up to 14 days. Early osteogenic differentiation was evaluated by ALP-kit.

RESULTS:In the 1st generation bioinks, the addition of CNF to alginate-gelatin system increased printability and maintained high cell viability for 7 days. However, the structures disintegrated in 7 days. Next, addition of nHA to the alginate-gelatin system in the 2nd generation bioinks maintained the stability achieved with CNF and supported the viability and proliferation of both Saos-2 and BMSCs. The 2nd generation bioinks maintained their structure over 14 days.

DISCUSSION & CONCLUSIONS:The results of the current study show that both 1st and 2nd generation bioinks support viability and proliferation. In addition, the structural integrity was improved with 2nd generation bioink. Moreover, due to its stability, the nHA-CNF containing ink demonstrates accurate 3D printed structures with high fidelity. Thus, this ink has a promising potential in bioprinting applications and bone tissue engineering.

Acknowledgements:This work has been funded by Trond Mohn Foundation (project no. BFS2018TMT10)
References:

Keywords: Biofabrication,



A tuneable 3D printed cochlea model for cochlear implant studies

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INTRODUCTION:Cochlear implant brings sound to millions with profound hearing loss by electrically stimulating the auditory nerve directly. Today's cochlear implants are still far from perfect, attributed to the frequency distortion in the perceived sound resulted from the undesired current spread within cochleae. In addition, the success rate of cochlear implantation is controversial among individuals, partly due to the fact that human cochlea is individually shaped, like a fingerprint. Hence, using animal models in pre-clinical research cannot represent the anatomical features and the individual variability of human cochleae. Since there is no representative model for cochlear implant testing, we developed an *in vitro* cochlea model with embedded 3D printing technology

METHODS:To replicate the geometry of human cochlea, a fugitive template with the shape of human cochlea was printed inside a bath of polymer gel with conductive elements embedded. The composition of the gel bath was precisely tuned to match the conductivity of human cochleae. After printing, the matrix was crosslinked and subsequently the fugitive ink was removed, leaving a hollow structure with the shape of cochlea inside the matrix. To evaluate its capability of replicating the clinical response in patients, electric field imaging (EFI) profiles were performed to examine the intra-cochlear voltage distribution evoked by cochlear implants within the models.

RESULTS:The model that we fabricated exhibits the distinctive physiological current spread that happens in patients. By fabricating models with different cochlear geometries and conductivities, we found that the current spread pattern is highly dependent on the shape and conductivity of cochlea. Personalised model was also produced to match the patient-specific current distribution resulted from individual cochlear geometry.
DISCUSSION & CONCLUSIONS:Our 3D printed model is representative of the cochlear geometry and conductivity. It is able to replicate the intra-cochlear current spread, therefore capable of predicting the clinical electrical performance of cochlear implants in patients. This model can be potentially used as a pre-clinical model for developing new cochlear implants, or a tool to predict patient-specific clinical outcome after cochlear implantation. We anticipate that our 3D printed model can accelerate the advancement of personalised cochlear implants.
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References:

Keywords: Biofabrication, Personalised medicine

Magnetic levitation as a novel method for 3D biofabrication of scaffolds based on calcium phosphate particles

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INTRODUCTION: Calcium phosphates (CP) are widely used bioceramics with excellent biodegradability, biocompatibility and osteoconductive properties [1]. Synthetic CP granules can be considered as building blocks for 3D biofabrication of engineered bone scaffolds. The magnetic levitational assembly of single CP diamagnetic granules is a novel approach to produce biomaterials and scaffolds for bone defects replacement [2-3].

METHODS:We used α-tricalcium phosphate particles (α-TCP) of equal size and certain porosity, which undergo the process of recrystallization in the special buffer solution under the levitation conditions to provide 3D scaffold fabrication. To perform the assembly, we used a custom-designed magnetic setup, which provides non-homogeneous magnetic field in the working area. We carried out mathematical modeling and computer simulations to predict magnetic field and kinetics of particles assembly into 3D tissue scaffolds. To allow the levitation of calcium phosphate particles, we paramagnetised the buffer solution by adding 3M gadolinium salt. We evaluated the cytotoxicity of 3D scaffolds by the extract-based assay and estimated their surface properties in regards to mesenchymal stem cells colonization by fluorescence microscopy and scanning electron microscopy.

RESULTS:For the first time, we demonstrated that α-TCP particles can be assembled in 3D scaffolds via levitational formative method by using non-homogeneous magnetic field in the presence of gadolinium salts. We performed a chemical synthesis of octacalcium phosphate (OCP) through recrystallization of TCP into the OCP under the condition of magnetic levitation in non-homogeneous magnetic field. We confirmed high biocompatibility of the obtained CP-based 3D scaffolds.

DISCUSSION & CONCLUSIONS:Thus, in our study we showed that magnetic levitation of calcium phosphate particles is a promising approach for rapid 3D fabrication and attractive alternative to standard methods of chemical synthesis. Taking into account the good surface properties of the obtained CP-based constructs, these data demonstrate the fundamental feasibility to biofabricate tissue-engineered scaffolds based on calcium phosphate and living cells. Further development of magnetic levitation technology involving the combination with acoustic or electric fields will allow to produce 3D scaffolds with complex shape and specific macro- and microstructure.

Acknowledgements:The reported study was funded by RFBR according to the research project № 18-29-11076.
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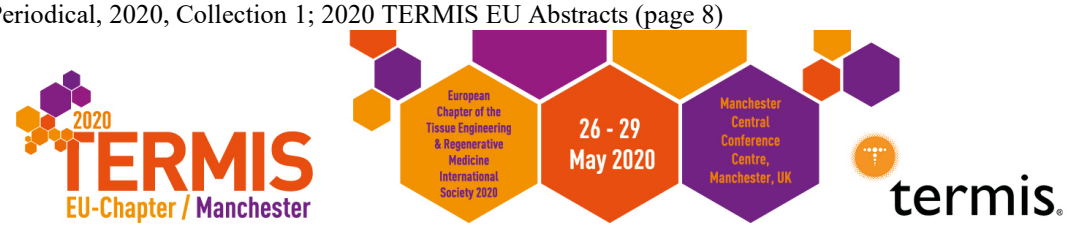
Keywords: Biomaterials, Biofabrication



Using the Reactive Jet Impingement Process to print cells for osteoarthritis models

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INTRODUCTION:3D bioprinting allows for the production of living tissue through the printing of cells and supporting materials into complex 3D structures. The bioinks used for this application generally comprise of cells suspended in either liquid media or a gel. When compared to similar, manually produced gels and tissue structures, bioprinting offers the additional benefit of a more controlled and even cell distribution allowing for the production of organized layered tissue structures. The applications for this are far reaching including the production of microtissues and organs for restorative treatments and transplants, as well as drug testing and toxicology models.

METHODS:The focus of this research has been the production of a stratified osteochondral interface co-culture model. Newcastle University have developed a new method of inkjet bioprinting known as the Reactive Jet Impingement (ReJI) method. This method allows for deposition of the desired high viscosity, high cellular density gel at a high rate on a drop on demand basis. Using this method, two opposing valves simultaneously eject droplets of gel precursor and a crosslinking agent containing cells, these droplets impinge in the air, instantly crosslinking to form a gel before landing on the target substrate. The ReJI technique has been used to create layered MSC/osteoblast/chondrocyte co-cultures as precursor osteoarthritis models. The printed tissue structures have been evaluated to assess cell viability, and stained for collagen II and aggrecan distribution, known indicators of cartilage extracellular matrix production.

RESULTS:It is possible to print both chondrocytes and mesenchymal stem cells in densities of up to 40 million cells per ml of bioink with a high cell viability. Results show rapid ECM production, especially at high cell densities. Ongoing work includes quantification of distributed collagen and aggrecan as well as analysis of metabolic activity.

DISCUSSION & CONCLUSIONS:The rapid ECM production indicates that producing tissue models using the ReJI technique could significantly reduce the culture time needed to produce mature tissue models.

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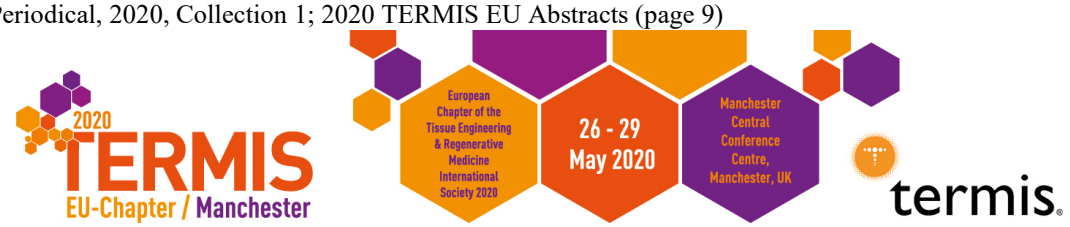
Keywords: Biofabrication, Biomaterials



High-speed volumetric bioprinting approach for biofabrication of complex living tissue structures

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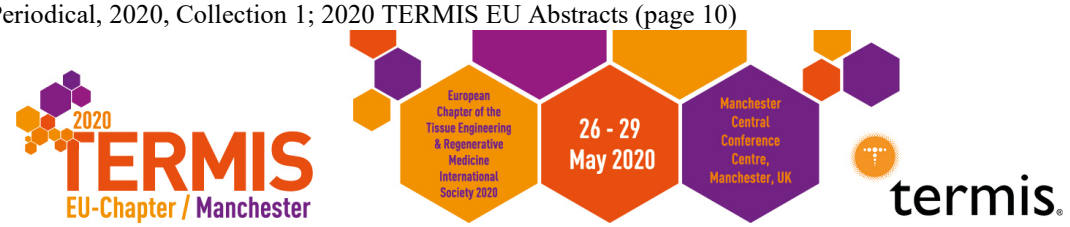
INTRODUCTION:The generation of complex, tissue-mimetic living structures of clinically-relevant size remains an unsolved challenge in tissue engineering. 3D bioprinting is a promising approach to shape cell-laden biomaterials into native-like constructs. Widely used bioprinting techniques like extrusion bioprinting (EB) and digital light processing (DLP) employ a layer-by-layer fabrication strategy. This results in extended printing times for large structures and difficulty in capturing the convoluted porosity typical of many native tissues necessary for patient-specific grafts. Novel optical tomography-inspired printing approaches in which visible-light projections of a 3D object are used to rapidly fabricate large-scale structures in a single step overcome these challenges. Herein, the concept of volumetric bioprinting (VBP) is introduced, demonstrating the fabrication of complex, cell-laden biological structures within seconds.

METHODS:A photosensitive gelatin methacryloyl formulation supplemented with the visible-light photoinitiator lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate (LAP) was developed for VBP. Printing time of centimeter-scale constructs was compared with conventional bioprinting strategies (EB and DLP). Viability and metabolic activity of bioprinted cells was assessed. Functionality post-printing was evaluated through the fabrication of a cell-laden trabecular bone model subsequently seeded with endothelial cells to assess neo-vascularization in vitro and a meniscus model to evaluate long-term biochemical and mechanical maturation.

RESULTS:Gelatin-based bio-resins were printed into human auricle constructs in 22.7s with high volume accuracy (5.71±2.31% mismatch vs. the CAD design). Printing time remained constant for printing samples scaled to 1.23 and 4.14cm3, while it increased considerably for EB (~30-90min) and DLP (~20-30min). Cells printed via VBP maintained high viability (>80%) comparable to EB and DLP-prints and cast samples. The trabecular bone model presented the smallest resolved feature measuring 144.69±13.55µm and exhibited a complex porous network. After endothelial cell seeding, these constructs showed enhanced neo-vessel formation. Finally, meniscus constructs cultured for 28 days produced fibrocartilage-like matrix and exhibited increasing compressive properties over time, approaching values comparable to native meniscal fibrocartilage (~300kPa).

DISCUSSION & CONCLUSIONS:This study established a novel approach for shaping hydrogels into complex, tissue-like architectures within seconds. Short printing times and freedom of design shown by VBP make the technique appealing for biomedical applications like creating patient-specific grafts and in vitro disease models. The use of this technique did not affect cell viability and complex biological structures were successfully printed. Cells in these printed constructs exhibited salient features post-printing and long-term biochemical and mechanical maturation. These findings open new avenues for designing the next generation of biomaterial-based bioprinted constructs of clinically-relevant size, a

Keywords: Biofabrication, Biomaterials



Composites of calcium phosphate cements and mesoporous bioactive glasses – a versatile material system for 3D plotting of functionalized bone tissue implants

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INTRODUCTION:Hydroxyapatite-forming calcium phosphate cements (CPC) are established for treatment of bone defects possessing excellent osteoconductivity and bioactivity. Due to their nanocrystallinity, they are bioresorbable. However, their degradation rate in vivo is low. Mesoporous bioactive glasses (MBG) are bone replacement materials characterized by a high degradation rate and an osteostimulatory effect based on the released ions; via a polymer-template method a highly ordered mesoporous structure is formed. Therapeutically active metal ions can be integrated and the mesopores can be loaded with drugs and proteins. Using a conventional powder/liquid CPC, we have recently demonstrated that incorporation of MBG microparticles into the CPC matrix enhances its degradation and provides a promising protein delivery strategy[1]. Herein, we developed an injectable CPC-MBG material system suitable for extrusion.

METHODS:Silicate-based MBG microparticles, with/without Sr2+ modification, were prepared as described2, paste CPC3 was provided by INNOTERE (Germany). CPC-MBG scaffolds were plotted using a Bioscaffolder 2.1 (GeSiM, Germany), hardened in water-saturated atmosphere and physicochemically characterized. Ion release was quantified with ICP-OES. Released proteins were quantified using Bradford assay (lysozyme) or ELISA (VEGF); biological activity was studied using enzyme activity (lysozyme) or endothelial cell proliferation assays (VEGF).

RESULTS:By adjusting the ratio of solid and non-aqueous carrier liquid2, MBG microparticles up to 13 wt-% can be integrated into the CPC paste while retaining its excellent printing properties. The mass loss over 28 days of incubation was significantly higher for CPC-MBG composites compared to pure CPC indicating enhanced degradation. The ion release is customizable using different MBG compositions. As demonstrated for Sr2+, therapeutically effective concentrations were released from the composites; Sr2+ release was significantly higher from 3D plotted compared to bulk samples of same mass. In order to prevent setting of the CPC starting before extrusion, a freeze-drying-based protocol for loading of the MBG with aqueous protein solutions was developed. The respective composites showed no altered printing properties while biological activity of the model proteins lysozyme and VEGF was maintained; long-term release of the proteins from the loaded composites was observed.

DISCUSSION & CONCLUSIONS:A highly customizable calcium phosphate material system was developed that could be functionalized and tailored to indication- and patient-specific requirements, thus leading to fabrication of individualized and complex implants.

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Keywords: Composite materials, Drug delivery

Bioprinting of Zonal Cartilage Scaffolds Using Different Cell Densities: A Biomimetic Approach for Cartilage Regeneration

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INTRODUCTION:Articular cartilage (AC) is a functionally graded tissue with three distinct layers, each exhibiting differences in cell density: the superficial zone having the highest cell density and the deep zone, the lowest [1]. Nevertheless, only a few studies have introduced a cell density gradient into scaffolds [2] and none of them have optimized the scaffold mechanical properties, hence failing to provide a more biomimetic environment for the embedded cells. In this study, we aimed to fabricate bioprinted scaffolds with different zonal cell densities, assess their stiffness and the influence of the cell density on the cell-mediated extracellular matrix deposition.

METHODS:The scaffolds were bioprinted using poly-ε-caprolactone (PCL) as support and a mix of human chondrocytes embedded in an alginate-bioink (Cellink-bioink). The design of the scaffolds included two cell densities: 2.5x106 cells/ml (high) and 1.25x106 cells/ml (low). The layer height was set to 0.2 mm and each scaffold consisted of 15 layers. Eight different PCL designs with various framework thicknesses and number of square unit cells were assessed by compressive tests. To create the zonal cell density, in each scaffold, the 8 bottom layers were printed with the lower cell density and the 7 top layers, with the higher. The scaffolds were cultured in DMEM+10% FBS+1%P/S (Gibco) for 14 days. Live-dead and histological staining (Alcian blue, Hematoxilin-Eosin, and Picrosirius-red) were performed at days 0,7,14.

RESULTS:The PCL design which showed the closest stiffness to the native AC was further used for bioprinting of the zonal scaffolds. The results of the live-dead at day 14 revealed the ability to efficiently generate a defined zonal cell density keeping the chondrocyte viable in the two zones of the scaffolds. Furthermore, the images of the sagittal plane showed a smooth transition between the zones with low and high cell density. Ongoing histological analyses will evaluate the effect of cell density on matrix deposition.

DISCUSSION & CONCLUSIONS:This qualitative data demonstrate the generation of different zonal cell densities within bioprinted scaffolds, maintained for 14 days. A fusion of the layers at the interface of high and low cell densities zones created a smooth gradient. Follow-up experiments are planned to fabricate scaffolds with three zones with different cell densities to recapitulate the tri-phasic structure of the AC.

ACKNOWLEDGEMENTS:Performed as part of Dutch Medical Delta project: RegMed4D.
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Keywords: 3D printing and bioprinting, Cartilage / joint and arthritic conditions

3D printing of collagen fibrils with controlled orientation within a hyaluronan matrix as biomimetic cartilage implant

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INTRODUCTION:Cartilage, like most tissues, presents microarchitectural features fundamental to determine its structure and properties. We developed a bioink and workflow to control anisotropic features at the microstructural level inducing collagen (col) orientation within a hyaluronan viscoelastic matrix via 3D printing. In this study we investigated shear induced anisotropy and chondrogenic properties of this bioink.

METHODS:Tyrosine modified hyaluronic acid (THA)[1] was mixed with acidic col1 to prepare a biomimetic ink containing fibrillar col1 in a HA-based matrix. To closer mimic ECM composition of articular cartilage, a second composite consisting of THA and was investigated consisting of THA and col1/2 like hydrogel (Jellagen) at same weight. Shear induced microstructure of the composite upon 3D printing (3D discovery, RegenHU) was analysed by visualization of col fibrils using confocal and second harmonic generation microscopy.

To study the effect of col origin on cell differentiation, hMSC spheroids were embedded into composite materials and cultured for 21 days in chondrogenic media containing TGF-β1 (10ng/ml). Chondrogenic differentiations was analysed by gene expression (Col1, 2 and 10, aggrecan, RunX2), histological staining (Safranin-O/Fast-Green) and quantification of glycosaminoglycans. hMSC pellet culture was chosen as gold standard for chondrogenic differentiation.

RESULTS:Anisotropic alignment of col1 fibrils was achieved through 3D printing that guided cell migration along fiber orientation shown by F-actin staining. Cell migration of hMSC spheroids showed similar behavior comparing isotropic THA-col1 and col1 after 3 days and overcame shrinkage present for col1 only. Chondrogenic differentiation for isotropic THA-col1 and MSC pellet culture resulted in a strong increase in cartilage related genes (col 2, aggrecan) with low tendency of hypertrophy (col1 and 10, RunX2). Col2/Co1 ratio was higher for THA-col than in pellet culture on day14 and 21. Cartilaginous matrix deposition was further corroborated by Safranin-O staining and quantification of GAG/DNA resulting in an increase within 21 days of culture for THA-col1 (7.85 ± 5.8ug/ug) similar to hMSC pellet control (7.32 ± 3.9ug/ug).

DISCUSSION & CONCLUSIONS:THA-col1 bioink showed excellent potential for cartilage tissue engineering with hMSC undergoing chondrogenic differentiation comparable to pellet culture. Extrusion-based printing was investigated as promising tool to introduce anisotropic properties on the microscale exploiting the shear forces inducing alignment of col fibres within a shear thinning HA matrix.
The THA-col1/2 like composite mimics articular cartilage composition more closely than THA-col1. If the col type can further stimulate chondrogenic differentiation is under investigation.

ACKNOWLEDGEMENTS:AO foundation and Graubünden Innovationsstiftung for their financial support.

REFERENCES:(1) Petta et al, Biofabrication, 2018

Keywords: Hydrogels and injectable systems, Biofabrication

Pre-Vascularized Bio-inks for the 3D Bioprinting of Functional Human Heart Tissues

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INTRODUCTION:Despite the latest developments in 3D bioprinting for cardiac applications, optimal vascularization remains one of the biggest challenges for proper tissue viability and function [1]. To overcome this, our group has developed pre-vascularised bio-inks to prevent cell death while promoting tissue function. This approach is based on our previous studies demonstrating that pre-vascularised microtissues together with VEGF and tissue-tailored hydrogels are optimal tools for the biofabrication of vascularized tissues [1,2]. Our recent studies demonstrated that co-cultures of cardiac myocytes, fibroblasts and endothelial cells at ratios approximating the ones found in the human heart generate cardiac microtissues that better recapitulate the in vivo human heart biochemistry, physiology and pharmacology [3,4,5]. These microtissues are called “vascularized cardiac spheroids” (“VCSs”) and can be generated from either primary or stem cell-derived cells, depending on their applications. In this study we evaluated the potential use of VCSs as pre-vascularized bio-inks for 3D bioprinting of functional human heart tissues.

METHODS:Prevascularized bio-inks were generated by suspending VCSs generated as previously described [5] in alginate/gelatin-based hydrogels. Pre-vascularised bio-inks were extruded in rods or squares. Viability was evaluated using calcein-AM/ethidium homodimer to stain for live/dead cells, respectively. Vascularization was evaluated using antibodies against CD31. VCS contractile function was evaluated using field stimulation with an IonOptix system.

RESULTS:Bioprinted pre-vascularized cardiac tissues are viable for at least 30 days and CD-31 staining demonstrated a complex vascular network throughout the whole tissue. VEGF treatment not only induced angiogenesis within bioprinted cardiac tissues, but also increased tissue fusion compared to control, untreated tissues. Synchronous contractile activity at physiological frequencies was measured before and after VEGF-mediated fusion of bioprinted VCSs.

DISCUSSION & CONCLUSIONS:Our results strongly suggest that pre-vascularized cardiac bio-inks can be used for the biofabrication of functional heart tissues. Current studies are evaluating their use to study human heart biology and pharmacology in vitro and to promote heart regeneration in cardiovascular disease patients.

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Keywords: 3D printing and bioprinting, Cardiovascular

Robotics-aided biomedical in situ bioprinting for restoration of full-thickness skin defects

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INTRODUCTION:Currently, in situ 3D bioprinting is considered as an extremely promising novel area in medicine. In situ technology could restore impaired tissue and organs directly on the patient's body. However, in most of the current approaches, in situ 3D bioprinting is carried out on the fixed and immobile parts of the patient's body (i.e. cranial scalp, limb skin) [1,2]. Our robotic system allows in situ bioprinting in mobile due to the respiratory excursion regions.

METHODS:The developed robotic system consisted of a collaborative robot manipulator, equipped with force / torque sensors to protect live objects from damaging in case of contact. A printing head with technical vision system for evaluation of the object's size and orientation was installed at the working arm. A round shape full-thickness skin defects with a diameter of 2 cm were formed in dorsal surfaces of 60 young male Wistar rats. A 40C collagen hydrogel [3] with the addition of growth factors and / or rat skin fibroblasts was used for bioprinting. The resulted structure had the form of a lattice with a pore size of 700 μm and circular enlargements on the borders with intact skin. The healing activity was evaluated by measuring the changes in wound diameters, followed by histological morphometry. The biomechanical properties of skin flaps were studied by tensile strength estimation.

RESULTS:The robotics installation determined the location of the defect and calculated the pattern of the manipulator movement according to assumed multilayer 3D model. The feedback system allowed to correct the trajectory in accordance with the breathing movements of the animal, which made it possible to print a complex structure without significant deviations from the original model and damages to the skin. In all experimental groups, the defects healed within 4 weeks: meanwhile, improved re-epithelialization, dermal cell repopulation, and restoration of the hair-covering could be observed. The best parameters of the mechanical strength were obtained in cases of using collagen solution and skin fibroblasts combinations.

DISCUSSION & CONCLUSIONS:A novel sophisticated system based on a collaborative robot for in situ bioprinting in moving body parts was developed and tested. The effectiveness of the approach in preclinical studies on the model of a skin defect restoration confirmed the feasibility of its subsequent translation into clinical practice.

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Keywords: Wound healing, Advanced therapy medicinal products

Mimicking natural gradients in 3D printed constructs for osteochondral regeneration

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INTRODUCTION:Damaged articular cartilage is unable to regenerate itself due to its avascular and aneural nature. Although tissue engineers are already designing osteochondral (OC) constructs with a stratified structure, it remains challenging to regenerate OC lesions. Whereas most constructs are designed to mimic specific zones, in reality the cells and tissue gradually transform from cartilage into bone across the OC region. Therefore, we hypothesized that for regeneration of functional cartilage tissue we need to develop an implant in which a smooth transition of cells from the chondrogenic to the osteogenic phenotype is mimicked. To this end, we aim to design a 3D printed (3DP) construct, which gradually controls human mesenchymal stromal differentiation (hMSCs) across the scaffold. The fiber surfaces were modified with peptides in countercurrent gradients to achieve this goal. Here, we show the fabrication process, in-vitro and in-vivo rat studies.

METHODS:We designed a versatile material system by synthesizing polycaprolactone (PCL) with either terminal azide or maleimide moieties to selectively click peptides with complementary alkyne or thiol groups on fiber surfaces. In this study, we used TGF-β and BMP-2 derived peptides to direct hMSC differentiation selectively towards either chondrogenic or osteogenic lineages. The countercurrent polymer gradients are extruded by a custom-made print head. Fluorescent molecules were used as model agents to assess functional group availability and printed gradients. In-vitro studies with single material, biphasic and gradient scaffolds were completed to assess the effect of the peptides on hMSC differentiation. Finally, the scaffolds were implanted in a rat subcutaneous model to assess biocompatibility and cellular infiltration.

RESULTS:Availability of the functional groups on the fiber surface was verified with spectrophotometric read-out and fluorescent microscopy. We validated the print head's ability to create gradients with spectrophotometry and 1H NMR. Furthermore, we observed with immunohistochemistry and biochemical assays the potential of our peptides to induce selectively hMSC differentiation. We observed high cellular infiltration and no toxicity in our animal model with or without attached peptides.
DISCUSSION & CONCLUSIONS:We have created a versatile system to attach peptides on 3DP fibers surface. We were able to print gradients within a construct. Additionally, we show selective control of hMSC differentiation in biphasic scaffolds in-vitro, as well as biocompatibility in a rat model. Our findings demonstrate that we are able to create countercurrent peptide gradients in a scaffold, which influences cell behavior locally.

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References:

Keywords: Additive manufacturing, Cartilage / joint and arthritic conditions

Engineered cell seeding media density and viscosity for homogeneous cell distribution on 3D additive manufactured scaffolds

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INTRODUCTION:3D polymeric additive manufactured (AM) scaffolds provide an idoneous structural support for bone tissue regeneration. However, the macroporosity and lack of cell-material interaction sites on these scaffolds, together with the gravity force, impede the achievement of sufficient initial cell numbers and adequate cell distribution upon static seeding. Here, we demonstrate a simple and reliable method to statically seed 3D AM scaffolds, regardless of their architecture and surface chemistry. By the independent addition of two biocompatible macromolecules, dextran and Ficoll (Ficoll Paque), we adjust the viscosity and density of seeding media, respectively, to tune the cell settling velocity, reduce sedimentation and allow for cell attachment and improved distribution along the scaffolds' cross section.

METHODS:The copolymer PEOT/PBT was used to fabricate 3D scaffolds using a melt extrusion based AM technique. Human mesenchymal stromal cells (hMSCs) distribution and viability was compared among conventional static seeded scaffolds (CS) and scaffolds seeded with 10 wt% dextran (MS-Dextran) or 60 vol% Ficoll-Paque (MS-Ficoll-Pq). Macromolecules removal was evaluated by using FITC-labeled macromolecules. Scaffolds were cultured for 28 days in basic and mineralization media. During this period, DNA content, alkaline phosphatase activity (ALP), matrix production, calcium deposition and the expression of the osteogenic related genes RUNX2, collagen type I, osteocalcin and osteonectin were evaluated.

RESULTS:10 wt% dextran increased the media viscosity ~ 25-fold without significantly affecting the media density and resulted in homogeneous hMSCs distribution across the scaffold cross-section. Similarly, seeding with a 60 vol% Ficoll-Pq solution distributed cells homogeneously throughout the scaffold by keeping cells in suspension due to matching densities, without significantly affecting media viscosity. Cell viability was preserved upon macromolecules based seeding (MS). Importantly, macromolecules removal was confirmed after 24 h of culture. Gene expression analysis confirmed that the use of dextran or Ficoll-Pq did not affect the osteogenic differentiation potential of hMSCs. Interestingly, extracellular matrix production and matrix mineralization was confirmed on MS scaffolds, when compared to CS scaffolds.

DISCUSSION & CONCLUSIONS:The presented technique offers a simple, unique and universal approach to statically seed 3D AM scaffolds in a precise and reproducible manner. We demonstrated beneficial effects of the improved seeding for bone tissue engineering and envision that its applicability could go beyond bone tissue regeneration. We believe our method could help to maximize the efficiency of scaffolds fabricated with any newly developed synthetic biomaterials, which often lack cell adhesion sites.

Acknowledgements:H2020-NMP-PILOTS-2015 (GA n. 685825).
References:Cámara-Torres, M., et al., Acta Biomaterialia, 2020. 101: p.183-195

Keywords: Biomaterials, Differentiation

Proteomics Characterization of hMSC chondrogenic and osteogenic differentiation in 3D printed scaffolds

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INTRODUCTION:The proteome, the entire protein complement expressed in a tissue, contains information about cell function, differentiation and tissue homeostasis. Here, we present a proteomics characterization of human mesenchymal stromal cells (hMSCs) cultured on three additive manufactured (AM) polymers, commonly used in skeletal tissue regeneration: polylactic acid (PLA), polyactive (PA) and polycaprolactone (PCL), to understand which better suits orthopedic applications.

METHODS:AM scaffolds were fabricated with the same structural characteristics (fiber-spacing 0,55 mm and layer thickness 0,2 mm). Mechanical testing, gel permeation chromatography, and contact angle measurements were performed. hMSCs chondrogenic and osteogenic differentiation was induced on PCL, PA and PLA scaffolds for 35 days of culture. Proteins were extracted and digested into tryptic peptides and analyzed using liquid chromatography– label free- mass spectrometry (LC-MS). Principal component analysis (PCA) was used to find linear combinations of protein levels and culture conditions. Significantly differentially expressed proteins (ratio $\geq$ 1.3, adj. p-value $\leq$ 0.05) were subjected to pathway enrichment analysis using Reactome Software.

RESULTS:The biomaterials displayed different properties: PCL resulted to be the stiffest biomaterial under compression with 70 MPa and a contact angle of 79,4° ± 1,3°, while PA was less stiff with 40 MPa and more hydrophilic with a contact angle of 67° ± 1,15°. PLA was extremely brittle and with an intermediate contact angle of 74,3° ± 1,3°. GAG and ALP assay, immunofluorescence and histology confirmed the differentiation of hMSCs. The proteomics analysis revealed a higher difference among different media compared to the scaffold type. In all three materials, chondrogenesis was characterized by a lower but more diverse amount of proteins. The biggest variance (first principal component) was observed between different cell media, in particular between chondrogenic medium and the other media. Less variance was caused by the different biomaterials but some clustering per biomaterial was still visible. PCL induced ECM production in both differentiation media, but it led to GAG degradation in the chondrogenic medium. During chondrogenesis in PA and PLA, cell differentiation resulted in the activation of collagen formation and ECM remodeling.

DISCUSSION & CONCLUSIONS:This work focuses on hMSC proteome composition during proliferation and differentiation on scaffolds. Different AM scaffolds exerted different ECM organization and protein abundance. Analyzing the pathways behind the ECM production could improve the fabrication of AM scaffolds for hMSCs differentiation.

Keywords: Additive manufacturing, Differentiation

A scaffold-free graft for large critical size bone defect: preclinical evidence to clinical proof of concept

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INTRODUCTION:Large critical-sized bone defect remais a challenging pathology in orthopaedics. The direct application of adipose stem cells (ASCs) remains limited by a low homing efficiency associated to a low survival rate. This study aims to demonstrate the osteogenic role of ASCs in a scaffold-free approach.

METHODS:3D scaffold-free grafts were characterized by q-RT-PCR (for skeletal development/angiogenesis). The bioactivity of the scaffold-free graft was studied in 2 nude rat models: (i) the comparison of fresh and decellularized grafts in term of angiogenesis promotion up to 1 month post-transplantation in a fibrotic tissue (in a cauterized muscular pocket,n=20); (ii) the osteogenicity of the scaffold-free graft (in comparison to HA/bTCP bone substitute) at 1/2/3 months post-implantation, in an irreversible femoral critical-sized bone defect (n=28). Angiogenesis was investigated by histomorphometry, cellular engraftment by HLA-I staining, mineralization by micro-CTscan and osteogenic genes expression by q-RT-PCR. A 5-year-old boy with congenital pseudarthrosis of the tibia (previously treated by nailing and grafting without success) was proposed for the scaffold-free graft approach (made of autologous ASCs) in combination with the induced membrane technique. The pseudarthrosis area (fibula and tibia) was firstly resected and filled by a cement spacer. Then, the adipose tissue (AT) was procured in view to isolate ASCs and to produce the 3D scaffold-free graft. At 3 months post-AT procurement, the cement was removed, and the 3D-graft was placed into the defect to be followed clinically and radiologically.

RESULTS:After intra-muscular transplantation in nude rats, cellular survival (with major osteogenic genes expression) and the promotion of angiogenesis (in a fibrotic/hypoxic site) was found. A complete integration and bone fusion were found for the 3D-graft in comparison to the bone substitute which revealed a lack of tissue remodelling and osteogenesis. Specific osteogenic genes were overexpressed in the bone defect treated with the 3D-grafts (at 4 weeks post-implantation). A large volume (>15cm3) of the 3D-graft was manufactured in GMP conditions and then implanted without any modification of the surgical procedure. The graft was easily handled and implanted after cement removal. The graft demonstrated a continuous remodelling (with bone formation) during the first 2 years post-implantation to obtain a sufficient bone fusion (allowing walk without pain) and no recurrence of the disease.

DISCUSSION & CONCLUSIONS:In conclusion, the scaffold-free 3D-graft (made of ASCs) plays a major role to promote ASCs engraftment and consequence to induce osteogenesis in a fibrotic environment and to recover a bone fusion in a critical-sized bone defect.

Keywords: Advanced therapy medicinal products, Cell therapy

Mussel-Inspired Injectable Hydrogel Adhesive Formed under Mild Conditions Features Near-Native Tissue Properties

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INTRODUCTION:Injectable hydrogel adhesives, especially those that can strongly adhere to tissues and feature near-native tissue mechanical properties, are desirable biomaterials for tissue repair.(1) However, regardless of recent advances, an ideal injectable hydrogel adhesive with both proper adhesion and mechanical matching between hydrogels and tissues is yet to be demonstrated with cytocompatible and efficient in situ curing methods. Inspired by marine mussels, where different mussel foot proteins (Mfps) function cooperatively to achieve excellent wet adhesion,(2) we herein report a dual-mode mimicking strategy by modifying gelatin (Gel) biopolymers with a single type thiourea-catechol (TU-Cat) functionality to mimic two types of Mfps and their mode of action. This strategy features a minor, yet impactful modification of biopolymers, which gives access to collective properties of an ideal injectable hydrogel adhesive. (3)

METHODS:The hydrogel adhesive was prepared by a dual-syringe injection method. Hydrogel mechanical properties were characterized by rheometric measurements and compressive tests. The tissue adhesion performance was evaluated by stardard T-peeling tests and bursting tests on porcine heart tissues. Cytocompatibility was assesed by 3D culturing of fibroblasts within the hydrogels.

RESULTS:With TU-Cat functionalization of only ~0.4 - 1.2 mol% of total amino acid residues, the Mfp-mimetic gelatin biopolymer (Gel-TU-Cat) can be injected and cured rapidly under mild and cytocompatible conditions, giving rise to tissue adhesive hydrogels with excellent matrix ductility, proper wet adhesion and native tissue-like stress relaxation behaviors.

DISCUSSION & CONCLUSIONS:Unlike other mussel-inspired polymers with high catechol contents and non-reactive linkages that form hydrogels within minutes, Gel-TU-Cat polymers with much lower catechol contents can be crosslinked rapidly (within 10 seconds) under mild conditions, thanks to the Mfp-6-mimicking nucleophilic thiourea (TU) linkages. While minor modification of the cost-effective gelatin biopolymer imparts a collective of versatile properties to the tissue adhesive hydrogels, it barely interferes with the cell-binding motifs allowing extensive spreading of the encapsulated cells. These properties of Gel-TU-Cat hydrogel make it amenable to a diversity of applications in regenerative medicine and warranting further in vivo evaluation as a logical next step towards future clinical application.

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Keywords: Biomaterials, Hydrogels and injectable systems

π-SACS: pH Induced Self-Assembled Cell Sheets Without the Need for Modified Surfaces

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INTRODUCTION:Cell sheet engineering is a more recent approach in tissue engineering that allows formation of structures based on self-assembly of cells that preserves secreted extracellular matrix and cell-cell junctions. Cell sheets have been formed by using temperature responsive [1] or polyelectrolyte deposited surfaces that facilitate delamination of the sheets by changing temperature or inducing dissolution, respectively [2].

METHODS:A new technique that uses a simple pH trigger to rapidly detach layers of cells formed on traditional cell culture plates has been developed for cells capable of syncytialization and fusion such as placenta cells and skeletal muscle cells (BeWo and C2C12 cell lines). After culturing cells in their culture media, they were treated with the appropriate differentiation/fusion media. Before confluence and fusion, the cells were trypsinized and replated at 90% confluency (0.235×106 C2C12s and 0.2×106 BeWos in 24 well plates). This procedure resulted in fusion as well as slow proliferation which caused traction on the edges. The confluent cell layer was then treated with a slightly acidic medium that initiated rapid and instantaneous delamination and formation of a cell sheet. Subsequent treatment with a slightly basic medium arrested the traction force and maintained the cell sheets flat. Eventually sheets were transferred to a neutral medium as single-layers and were assembled into multilayer constructs.

RESULTS:Live/dead staining of sheets after delamination showed very few dead cells present indicating that the delamination process didn't adversely affect cell viability. Single and multiple-layer constructs shrunk but the extent of shrinkage was higher when number of layers was lower (single-layer constructs showed 91.94±1.11% shrinkage while quadruple constructs showed 87.06±0.81% shrinkage). Sheets with a coculture of endothelial, fibroblast, and neuronal cells with the fusogenic cells were also formed. BeWo cells formed robust cell sheets but they were not homogeneous and had small holes in them.

DISCUSSION & CONCLUSIONS:The new method demonstrated here is the simplest and most robust technique for formation of cell sheets. Unlike other methods, it does not require use of modified surfaces or electrical/mechanical stimuli to form the sheets. The sheets formed with this method can be stacked to form thicker structures. Co-culture of other cell types with fusogenic cells extend the applicability of this method. This technique can be further improved by aligning cells in the wells before delamination is performed.

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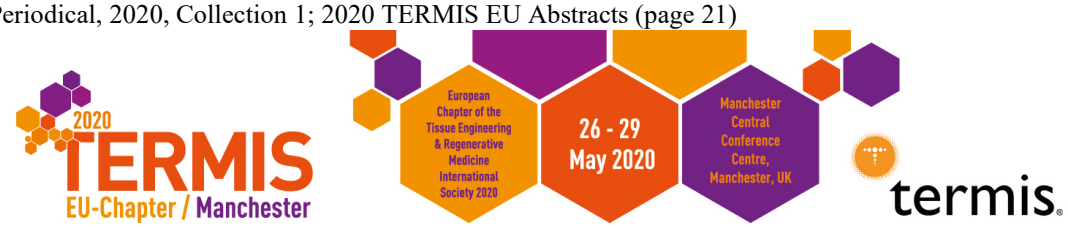
Keywords: In vitro microenvironments, Musculoskeletal (inc ligament / tendon / muscle / etc)



Magnetically-driven assembly and control of 3D multi-cellular structures

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INTRODUCTION:3D multi-cellular constructs are valuable as in vitro mimics of natural tissues and as building blocks for tissue engineering [1,2]. Gentle mechanical forces applied to cells can influence their function and be used to enhance cell-cell interactions to create aggregates simply and rapidly [3]. One attractive way to achieve this is using magnetic labelling of cells placed in magnetic fields to remotely manipulate cells and create complex structures.

METHODS:We have fabricated biodegradable polymer composite microspheres containing iron oxide nanoparticles for magnetic labeling of cells. The effects of magnetic forces on NIH 3T3 fibroblasts and 3T3-L1 pre-adipocytes were studied in culture as these cells are important for adipose tissue engineering. Cells were labelled with magnetic particles of ~5 μm diameter and magnetic fields were generated using various configurations of permanent neodymium magnets. Magnetic fields were simulated using finite element methods to estimate the forces exerted on the cells.

RESULTS:Biodegradable magnetic microspheres of controlled sizes were produced. Cells were labelled by incubation with magnetic particles at doses found not to cause cytotoxic effects (<30 μg/mL). Relatively large particles were used to minimize their internalization, and their attachment to the cell membranes was confirmed by confocal microscopy.
Magnetic forces were used to facilitate assembly of 2D and 3D structures with different spatial arrangements of cells in culture. Compact cell spheroids could be produced within 8 h under the combined influences of gravitational and magnetic forces, significantly faster than conventional hanging drop and centrifugation methods. Live/dead staining indicated that > 90% cells in cell spheroids produced through magnetic forces remained viable in culture for at least 10 days.

DISCUSSION & CONCLUSIONS:An external magnetic field can be used to create multi-cellular assemblies whilst avoiding the high shear conditions that may be experienced through methods such as 3D-printing. The magnetically labeled constructs can be manipulated to control their shape, delay tissue remodeling and retain desired tissue structures over time in culture, as well as allowing desired forces to be imparted to the cells subsequent to the construct fabrication.

Acknowledgements:The authors thank the Particulate Fluids Processing Centre (PFPC), Bio21 Advanced Microscopy Platform, O`Brien Institute, St Vincent's Institute and the Australian Government for International Postgraduate Research Scholarships (IPRS) and Australian Postgraduate Award (APA).

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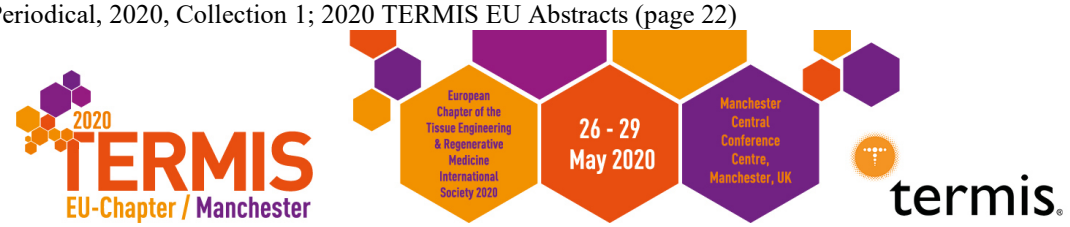
Keywords: Biofabrication, Biomechanics / biophysical stimuli and mechanotransduction



In vivo engineering of stable bizonal cartilage with a calcified bottom layer

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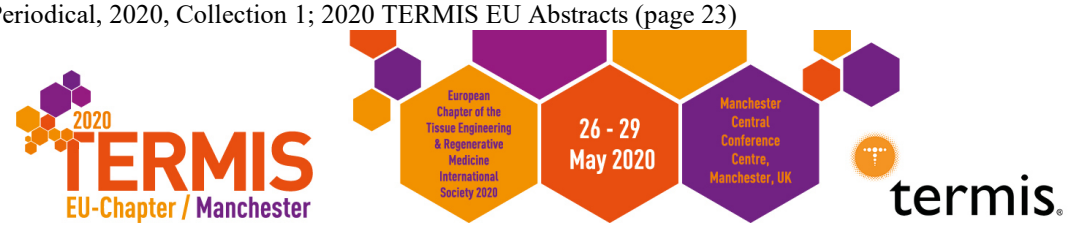
INTRODUCTION:Repaired cartilage tissue lacks the typical zonal structure of healthy native cartilage needed for appropriate function. Current grafts for treating full-thickness cartilage defects focus on a non-zonal design and this may be a reason why inferior non-zonal regeneration tissue develops *in vivo*. No biomaterial-based solutions have been developed so far to induce a proper zonal architecture into a non-mineralized and a calcified cartilage layer. The objective was to grow bizonal cartilage with a calcified cartilage bottom zone wherein main tissue development is occurring *in vivo*. We hypothesized that starPEG/heparin-hydrogel owing to the glycosaminoglycan heparin contained as a building-block would be superior to fibrin-hydrogel in preventing mineralization of the upper cartilage zone and in inhibiting long-term progression of calcified cartilage into bone.

METHODS:To obtain a thin mineralized bottom layer like in native articular cartilage, biomaterial-free self-assembly of porcine mesenchymal stromal cells (MSCs) was realized in isotropic transwell culture supporting self-condensation. After three weeks of chondrogenic pre-culture, the upper layer (articular chondrocytes, ACs, embedded in starPEG/heparin or fibrin-hydrogel) was cast onto the discs. Obtained constructs were immediately implanted subcutaneously into immune-deficient mice for *in vivo* tissue development.

RESULTS:Constructs developed *in vivo* into bizonal-organized cartilage tissue in which mineralization remained restricted to the bottom zone. The stability of tissue organization, however, depended on the type of hydrogel. Although fibrin supported ectopic development of a collagen II and proteoglycan-rich upper part, the zonal fibrin constructs lost volume and allowed expansion of collagen X, ALP activity and mineralization from the bottom layer into upper regions. In contrast, in starPEG/heparin constructs, the zonal architecture remained stable. The collagen II and proteoglycan-positive calcified cartilage bottom layer persisted over 12 weeks, while non-zonal MSC-derived control discs formed bone. This indicated that the starPEG/heparin-chondrocyte layer actively prevented further progression of calcified cartilage into bone.

DISCUSSION & CONCLUSIONS:We here provide the first strategy which allows *in vivo* growth of zonal cartilage with long-term organizational stability between non-mineralized and calcified layers without progression to bone formation. This was possible without the need for growth factor immobilization and is applicable also for a one-step joint intervention surgery. Key were the special features of starPEG/heparin-hydrogel, selection of ACs *versus* MSCs for top and bottom zone and the spatiotemporal design of our approach. Altogether, our work is an important milestone encouraging direct in vivo growth of organized cartilage after biofabrication and could greatly accelerate development of new treatment modalities for cartilage repair.

Keywords: Cartilage / joint and arthritic conditions, Microenvironment and niche engineering



Magnetic levitational bioassembly in Space

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INTRODUCTION:Tissue engineering promises to solve an acute clinical problem – shortage of human tissues and organs for transplantation. Existing biofabrication methods offer various solutions [1] to assemble cells to construct tissue models, however, they all rely on the gravitational force for objects to be placed onto a dish. No method for biofabrication of tissue constructs in the absence of gravity in Space has been demonstrated so far. Magnetic levitational bioassembly of 3D tissue constructs represents a novel rapidly emerging scaffold- and label-free approach and alternative conceptual and experimental advance in tissue engineering [1,2]. However, implementation of magnetic levitation on Earth requires the use of relatively toxic paramagnetic medium, in most cases containing gadolinium salts. We hypothesize that magnetic levitational bioassembly under the conditions of Space microgravity can be implemented at non-toxic low concentrations of gadolinium salts.

METHODS:The magnetic levitational bioassembly of 3D tissue construct from tissue spheroids consists of directional levitational assembly under the action of magnetic forces of the construct from tissue spheroids, followed by fusion of tissue spheroids.

RESULTS:New magnetic bioprinter has been designed, developed and certified for life space research. Tissue spheroids and magnetic bioprinter were delivered on the «Soyuz MS-11», «Soyuz MS-13» and «Soyuz MS-14» ship to the Russian segment of the International Space Station in a temperature-sensitive non-adhesive hydrogel. 6 constructs from thyrospheres and chondrospheres and 8 constructs from myospheres were fabricated in a magnetic bioprinter. The constructs fused within 24 hours and 48 hours in a magnetic field at of 37°C. Then, the fused constructs were removed from the magnetic bioprinter and fixed with formalin and glutaraldehyde. Finally, the cuvettes with the constructs were sent to Earth where histology, immunohistochemistry, scanning and transmission electron microscopy of the constructs were performed.

DISCUSSION & CONCLUSIONS:Thus, our data strongly suggest that scaffold-free formative biofabrication using magnetic fields is a feasible alternative to traditional scaffold-based approaches hinting a new perspective avenue of research which could significantly advance tissue engineering. Magnetic levitational bioassembly of 3D tissue constructs in Space can advance Space life science. Acknowledgements:The project was implemented with the assistance of the «Roscosmos» State Corporation, Russia.

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Keywords: 3D printing and bioprinting, Other

Integrating molecular self-assembly and additive manufacturing for the biofabrication of fluidic in vitro models with biologically relevant properties

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INTRODUCTION:Organ-on-chip offer the opportunity to recreate a variety of biological niches1. However, most materials used to fabricate these systems do not replicate the physical and chemical properties of the native environment. In contrast, self-assembly represents an attractive route to develop more complex materials2 with enhanced modularity, tuneability, and structural hierarchy3. Here, we report an elastin-like protein (ELP)-graphene oxide (GO) self-assembling and biofabricated fluidic device with biologically relevant properties for organ-on-chip applications.

METHODS:An interfacial self-assembling system was designed based on GO sheets ELPs by hydrophobic and electrostatic interactions. The system could be manipulated to fabricate tubular structures when a drop of ELP (2 wt%, 18 μL) solution was slowly injected into the suspension of GO (0.1 wt%, 100 μL) to trigger a diffusion-reaction mechanism leading to a well-defined multilayer tubular wall. The process was recreated by liquid-on-liquid 3D printing of the ELP solution into the GO solution, which could be carried out in presence of hUVECs, generating complex and perfusable tubular networks down to 50 μm in internal diameter, 10 μm in wall thickness.

RESULTS:This ELP-GO co-assembling system integrates advantages of both self-assembly and additive manufacturing to develop capillary-based functional fluidic devices exhibiting a series of physiological chemical and physical properties. The process is cell-friendly and facilitates bioprinting, resulting in the fabrication of biocompatible fluidic structures (assessed via in vitro and ex vivo experiments) that can withstand flow immediately after bioprinting. Taking advantage of the self-assembling process, we demonstrate the capacity to tune capillary porosity and permeability by varying the content of GO percentage and cell density and demonstrate that the capillaries exhibit a Young Moduli ~500 kPa. We have also standardized the 3D printing process by optimizing the nozzle speed and release pressure.

DISCUSSION & CONCLUSIONS:The study introduces an innovative way to biofabricate complex and functional fluidic devices by self-assembly. This functional fluidic device may offer potential applications in the field of biomedicine such as tissue engineering scaffolds, microfluidic systems, and organ-on-chip devices.

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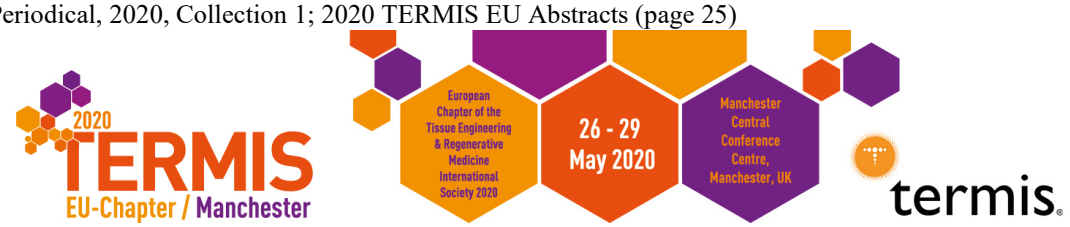
Keywords: Biomaterials, Additive manufacturing



Fabrication of Scaffold-free All-Cellular Living Fibers

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INTRODUCTION:Scaffold-free microtissues have found interest both as tissue models and high retention cell delivery/pro-regenerative devices. Usually, microtissues are assembled as standalone cell sheets and spheroid-shaped aggregates. Both types of tissues are incompatible with free-form deposition and difficult to upscale into high-order 3D structures. Fiber-shaped constructs enable omnidirectional positioning. The conventional fabrication of scaffold-free cellular fibers has required exogenous proteins and sacrificial biomaterials templates. We here report the fabrication of centimeter-long cell-only monotypic and heterotypic fibers, using human adipose-derived mesenchymal stem cell (hASCs) and their combinations with endothelial cells.

METHODS:All-cell fibers comprised hASCs seeded onto wettable channels on superhydrophobic surfaces, generating a hanging column setup after upside-down rotation. The viability and morphometric features of cell fibers were evaluated, as well as their free-form positioning and ability to fill complex-shaped Gel-MA defects. Fibers were also tested for tissue invasion capability and secretory profile of pro-regenerative factors, including HGF, VEGF, Osteopontin, IL-6 and IL-10. In this scope, cellular fibers were either embedded within a liquid Gel-MA followed by UV-mediated solidification, or added to a pre-solidified Gel-MA hydrogel. Monotypic (hASCs) and heterotypic (hASCs+HUVECs) fibers were added separately to a chorioallantoic membrane (CAM) *ex ovo* model, to test their potential for tissue invasion and neo-angiogenesis.

RESULTS:The self-assembly of cells into fiber-shaped constructs rendered highly malleable structures, able to adapt to complex-shaped defects. Those exhibited high viability and homogenous thickness distribution. After 7 days of culture into a Gel-MA hydrogel – here used as a simple model of tissue extracellular matrix -, hASCs fibers were able to integrate the surrounding structure, to sprout and secrete pro-angiogenic (VEGF) and trophic factors (HGF, Osteopontin), as well as immunomodulatory interleukines (IL6 and IL 10). The integration of cell fibroids into an *ex ovo* matrix was confirmed, and heterotypic fibers (hASCs+HUVECs) also showed pro-angiogenic properties.

DISCUSSION & CONCLUSIONS:We report the rapid fabrication of all-cellular centimetric fibers in the absence of any externally provided supplement or biomaterial. These micro-to-macrotissues showcasing high viability, tissue integration ability and pro-regenerative secretory profile may find future application in regenerative medicine, or as *in vitro* screening devices.

ACKNOWLEDGEMENTS:This work was financed by the Programa Operacional Competitividade e Internacionalização, in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the project “TranSphera” (PTDC/BTM-ORG/30770/2017). Ana Rita Sousa acknowledges the FCT grant (POCI-01-0145-FEDER-030770). M. B. Oliveira acknowledges the individual contract CEECIND/03605/2017.

Keywords: Cell therapy, Hydrogels and injectable systems

3D-Bioprinted Aptamer-Functionalized Bio-inks for Spatiotemporally Controlled Growth Factor Delivery

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INTRODUCTION:Spatiotemporally controlled growth factors delivering systems are crucial for tissue engineering. However, most of the current strategies for growth factors delivery often focuses on the immobilization or coupling of growth factors within the engineered matrices (hydrogel) via various linker proteins or peptides. These systems provide passive release rates and growth factor delivery on demand, but fail to adapt their release rates in accordance with the tissue development. To overcome this limitation, the present study employed nucleic acid based aptamers for achieving spatiotemporally controlled growth factor delivery. Aptamers are affinity ligands selected from DNA/RNA libraries to recognize proteins with high affinity and specificity.1 Aptamer based growth factor delivery systems are able to load/release multiple growth factors on demand with high specificity. In the present study, the authors have 3D-bioprinted aptamer-functionalized bio-inks to evaluate their potential for growth factor sequestering, programmable release and for studying their effect on vascular network formation.

METHODS:The aptamer-functionalized hydrogels were prepared via photo-polymerization of gelatin methacryloyl (GelMA) and acrydite functionalized aptamers having sequence specific for binding to vascular endothelial growth factor (VEGF165). Visible light photoinitiator, tris(2,2′-bipyridyl)dichloro-ruthenium(II) hexahydrate with sodium persulfate was used. The 3D-bioprinting experiments were carried out using Rokit Invivo 3D printer. The viscoelastic properties of the bio-inks were evaluated and compared with control GelMA bio-ink. To study the programmable growth factor release efficiency, VEGF antibody immunostaining was used. For studying the effect of triggered growth factor release on vascular network formation, human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) were encapsulated within the bio-inks.

RESULTS:The results obtained from VEGF antibody immunostainings confirmed the sequestration and triggered release of VEGF in response to complementary sequence addition from the 3D bioprinted construct after 5 days of culture. The bioprinted construct showed high cellular viability. The F-Actin/DAPI staining showed cellular sprouting and vascular network formation within the 3D printing aptamer functionalized bio-ink regions. In addition, the endothelial cells showed variations in cellular organization based on the VEGF bound aptamer availability within the bioprinted construct. These observations altogether confirms the bioactivity of VEGF bound aptamers within the printed constructs.

DISCUSSION & CONCLUSIONS:The present study shows the vasculogenic potential of 3D bioprinted aptamer-functionalized bio-inks via spatiotemporally controlling VEGF availability within the hydrogel system.

Acknowledgements:This work is supported by an ERC Consolidator Grant under grant agreement no 724469.

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Keywords: 3D printing and bioprinting, Vascular systems / vascularisation and heart

Platelet-rich plasma does not inhibit inflammation or promote regeneration in human osteoarthritic chondrocytes

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INTRODUCTION:Platelet-rich plasma (PRP) is a concentrated blood product, rich in growth factors and anti-inflammatory components. Through activation of PRP by thrombin and calcium, a growth factor-loaded gel forms which can be used as a 3D substrate to culture cells. We hypothesise that PRP gel stimulates cartilage regeneration, superior to commercially available fibrin gel. The aim of the study was to assess the anti-inflammatory properties of PRP and investigate the regenerative potential of PRP gel on cartilage extracellular matrix (ECM) production by primary chondrocytes.

METHODS:PRP was prepared from blood derived from healthy volunteers by double-spin centrifugation. Primary human chondrocytes (passage 2) derived from osteoarthritic knees were cultured in monolayers, after which inflammation was mimicked by stimulation with TNF-α. Next, the cells were treated for 48 h with 0-, 2-, 5-, 10-, or 20% (v/v) PRP. Expression of genes involved in inflammation and chondrogenesis was measured by real-time PCR. In addition, chondrocytes were cultured in PRP gels and fibrin gels consisting of increasing concentrations of PRP. After 28 days of culture without any additional growth factors, production of cartilage extracellular matrix (ECM) was assessed. Deposition and release of glycosaminoglycans (GAG) and collagen was quantitatively determined and visualized by (immuno)histochemistry. Proliferation of chondrocytes was assessed by quantitative measurement of DNA.

RESULTS:The inflammatory response of TNF-α-stimulated chondrocytes could not be suppressed by PRP, as determined by gene expression of inflammatory markers COX2 and IL1B. Expression of chondrogenic markers COL2A1 and ACAN was downregulated by PRP, independent of PRP concentration. Chondrocytes cultured in PRP gel for 28 days proliferated significantly more when compared to chondrocytes cultured in fibrin gels. This effect was dose-dependent. Significantly less GAGs and collagen was produced by chondrocytes cultured in PRP gels when compared to fibrin gels. This was qualitatively confirmed by histology.

DISCUSSION & CONCLUSIONS:Platelet-rich plasma stimulated chondrocyte proliferation in a dose-dependent manner. However, production of cartilage ECM was strongly downregulated by PRP. Furthermore, PRP did not act anti-inflammatory on chondrocytes in an in vitro inflammation model.

Keywords: Cartilage / joint and arthritic conditions, Biologics and growth factors

Bioactive glass ceramic scaffolds filled with pNIPAM-co-DMAc hydrogel for large bone defects

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INTRODUCTION:To date, a variety of bioactive glass, ceramics, and organic scaffolds such as hydrogels and their composites have been developed to facilitate regeneration of lost bone tissue resulted from trauma or degenerative diseases. Biocompatibility, appropriate degradation rate and mechanical integrity are some of the key requirements for suitable candidates. We have previously developed a non-biodegradable Laponite® crosslinked pNIPAM-co-DMAc (L-pNIPAM-co-DMAc) hydrogel which promotes osteogenic differentiation of mesenchymal stem cells (MSCs) when loaded with hydroxyapatite nanoparticles[1]. In addition, due to the low viscosity of this hydrogel at elevated temperatures (>37°C), it can be used to fill irregular cavities within the defected tissue. However, for large and load-bearing bone defects this hydrogel is not mechanically suitable and requires an additional supporting scaffold. This study explores the suitability of a bioactive glass ceramic material as the supporting scaffold for the developed hydrogel and investigates the effects associated with incorporation of the hydrogel into the bioactive scaffolds on the degradation rate, bioactivity and cell viability.

METHODS:Bioactive glass (13-93) was synthesised using a sol-gel method and porous scaffolds were prepared using the foam replication technique. For the degradation study, scaffolds with and without hydrogel were immersed in PBS (0.002 g/mL) and incubated at 37°C for up to 14 days. Apatite mineralisation was characterised as a function of time using XRD. Furthermore, to evaluate the influence of the hydrogel presence on hMSCs attachment, growth and osteogenic differentiation on the porous scaffolds, SEM and EDX techniques were used.

RESULTS:The degradation study indicated that presence of hydrogel does not affect the ion exchange rate between the scaffold and the surrounding environment. The XRD data confirmed in vitro apatite mineralisation after 14 days of aging in PBS for scaffolds with and without hydrogel. In addition, it was shown that the cells' shape and composition were maintained in the hydrogel-glass ceramic system and calcium-rich extracellular matrices were deposited on the cells after 21 days. We are currently evaluating cell viability using a series of histological and immunohistochemical characterisations. DISCUSSION & CONCLUSIONS:We hypothesize that the non-biodegradable nature of the hydrogel will provide long-term support whilst the bioactive ceramic network degrades, and a new tissue is generated. This could help with the issue associated with matching scaffolds degeneration with matrix regeneration.

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Keywords: Biomaterials, Hydrogels and injectable systems

Silk Fibroin/Poly-3-Hydroxybutyrate Aligned Nanofibrous Matrix for Tendon Regeneration

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INTRODUCTION:Injuries such as rupture, laceration and stretching in tissues such as tendons and ligaments due to sports injuries and traumas are important because they restricts daily movements and to avoid scar tissue formation they must be treated quickly. In many cases, tissue loss occurs in the region and tissue engineering products are needed due to lack of autologous grafts. In this study, silk fibroin/poly-3-hydroxybutyrate (SF/PHB) nano-fibrous matrices consisting of aligned nano-fibers were developed using electrospinning method since tendons and ligaments consist of dense and parallel aligned collagen fibers. PHB is a biocompatible, biodegradable, highly hydrophobic polymer with superior mechanical strength and is frequently used in tissue constructs. Silk fibroin is a water soluble polymer that promotes cell adhesion and has been used to enhance the adverse effects of PHB.

METHODS:The SF and PHB polymers were dissolved in HFIP at a concentration of 5% (w/v) which SF:PHB ratio is 3:1. The electrospinning parameters were optimized as 14 kV applied voltage, 1.0 mL/h feeding rate, 25 cm collector distance and 2,000 rpm mandrel rotation speed. To prevent scaffolds from shrinking SF/PHB nanofibrous matrices were stabilized by soaking in methanol.

RESULTS:According to scanning electron microscope images, the average diameter of fibers was found to be 698 ± 203 nm and approximately 80% of the nanofibers were aligned within +/- 15° of the mean orientation. SF/PHB scaffolds showed an ultimate tensile strength of 7.7±0.4 MPa and elastic modulus of 197±7.7 MPa with an average elongation value of 52±3% in tensile analysis. Fourier transform infrared spectroscopy analysis (FT-IR) showed that the main structure of SF in the nano-fibrous scaffold was β-sheet. Autoclave (A-SF/PHB) and ethylene oxide (EO-SF/PHB) sterilization were applied to find the appropriate sterilization method for the use of SF/PHB scaffolds in cell culture studies. The water contact angle on SF/PHB was found to be 113±10°, while EO-SF/PHB and A-SF/PHB was 122±6° and 97±12°, respectively. X-ray diffraction (XRD) analysis showed that crystallinity percentage for PHB, SF/PHB, EO-SF/PHB and A-SF/PHB samples are 47.0%, 14.4%, 16.5% and 14.3%, respectively. Adhesion and proliferation of rat adipose-derived mesenchymal stem cells on A-SF/PHB and EO-SF/PHB scaffolds were examined and quantified at 1, 3, 5 and 7 days. The results showed that the cells adhered more to the A-SF/PHB than EO-SF/PHB scaffold.

DISCUSSION & CONCLUSIONS:Our results indicated that the SF/PHB scaffold can be considered as a candidate matrix for tendon or ligament tissue engineering.

Keywords: Polymers - natural / synthetic / responsive, Nanomaterials (inc graphene)

"In vivo" studies of additive manufactured polycaprolactone-hydroxyapatite interconnected porous bone scaffolds

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INTRODUCTION:Conventional treatments for bone regeneration are based on the use of autografts and allografts and present several limitations, such as lack of availability, risk of rejection the need for secondary surgery, disease transmission and costs. The use of synthetic grafts (scaffolds) produced by additive manufacturing technologies represents an alternative and viable approach for bone tissue engineering. One of the most commonly used materials for bone scaffolds is poly(ε-caprolactone) (PCL) scaffold for bone regeneration, however it presents poor bioactivity and long degradation time. To overcome some of PCL limitations, it can be combined with hydroxyapatite (HA), an inorganic material, presenting a similar mineral structure to natural bone and osteoconductive properties. Moreover, as bone is a piezoelectric tissue, the application of electrical stimulation (ES) at physiological levels plays an important role in both cellular and molecular signalling pathways. The main objective of this research work was to evaluate the use of polycaprolactone/hydroxyapatite scaffolds and electrical stimulation therapy for bone regeneration in an animal model.

METHODS:The experimental protocol was approved by animal ethical committee (075/2017). PCL and PCL+20% of HA interconnected porous scaffolds were printed (0/90°_lay-down-pattern) using and a screw-assisted extrusion-based additive manufacturing system (RegenHu, Switzerland). Wistar rats were submitted to a 5mm×5mm critical-sized defect on the calvary bone. Different testing groups were considered: SHAM (no scaffold), SHAM+ES (no scaffold and electrical stimulation 10µA/5min, twice a week), PCL, PCL+ES, PCL/HA and PCL/HA+ES. Samples underwent histomorphometric evaluation (blood vessels, connective and mineralized tissues) using ImageJ and GraphPad_Prism softwares (Two-way ANOVA and Bonferroni's test, α=5% significance level).

RESULTS:Although the number of blood vessels was similar to all groups on the 30th day, PCL/HA and PCL/HA+ES groups presented higher vascular area (approximately 4.5% of area) compared to SHAM (1.8%), SHAM+ES (2%) and PCL (1.7%) groups. On the 60th day all scaffolds-treated groups presented higher connective tissue formation (around 90%) compared to SHAM (76%) and SHAM+ES (68%). On the 30th day, PCL/HA and PCL/HA+ES groups presented higher mineralized tissue (16% and 19%, respectively) compared to SHAM(0%), SHAM+ES(1.5%), PCL(5%) and PCL+ES(0.5%).

DISCUSSION & CONCLUSIONS:The use of PCL interconnected porous scaffold produced with 20% hydroxyapatite and electrostimulation therapy shows to be a promising approach for bone tissue engineering, since it presented higher vascular area formation and stimulated higher mineralized tissue, as a positive indicator of bone formation.

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Keywords: Additive manufacturing, In vivo and animal models

Development of scaffold-based system for co-delivery of therapeutic microRNAs for treatment of large volume bone defects

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INTRODUCTION:The treatment of large volume bone defects, which frequently result in delayed or non-union of tissue, remains one of the biggest challenges of modern tissue engineering. The use of growth factors such as bone morphogenetic protein-2 has yielded some success but is expensive and associated with numerous side effects. The delivery of microRNAs (miRs) potentially offers a more attractive alternative as it regulates gene expression thereby inducing the endogenous cells to produce multiple therapeutic proteins at physiological levels. However, the effective delivery of miRs is frequently jeopardised by their poor stability. The aim of this study was to investigate the potential of using scaffold-based approach for sustained delivery of therapeutic miRs. The collagen-nanohydroxyapatite (coll-nHA) scaffolds[1], which have been optimised for bone repair within our lab previously, were coupled with novel, cell-penetrating RALA vector[2] in order to establish dual microRNA-activated scaffold system, which co-delivers miR-mimic (m-26a[3]) and miR-inhibitor (a-133a[1]), for bone tissue engineering applications.

METHODS:Collagen-hydroxyapatite scaffolds (coll-nHA) were fabricated using freeze-drying techniques[1] and cross-linked with EDAC/NHS solution. miRs were complexed with cell-penetrating peptide, lyophilised[2] and incorporated into coll-nHA scaffolds. 3×10^5 of human mesenchymal stem cells (hMSC) were seeded and evaluated in terms of m-26a and a-133a expression, metabolic activity, DNA content, ALP activity and calcium content. Staining of calcium deposition and H&E were performed at day 28.

RESULTS:The miR-activated scaffold effectively transfected the hMSCs and increased the expression of studied miRs up to 14 days. Importantly, the dual m-26a/a-133a scaffold system effectively deliver both cargoes to hMSCs. The miR-activated scaffold enhanced proliferation of hMSCs of approx. 40% and stimulated their ALP activity and mineralization compared to miR-free coll-nHA scaffold. The co-delivery of m-26a and a-133a stimulated to greater extent the osteogenesis of hMSCs compared to m-26a and a-133a alone.

DISCUSSION & CONCLUSIONS:This study describes the development of a miR-activated scaffold system with a cell-penetrating peptide for controlled and sustained delivery of therapeutic miRs for orthopaedic applications. The incorporation of a new miR-approaches into biomaterials allows transient gene editing and induces host cells to produce targeted proteins at physiological levels with minimal immunogenicity. Thus, this cell-free system has potential to become a new 'off-the-shelf' product capable of stimulating bone healing. Moreover, a novel system for co-delivery of miRs could be tailored for a myriad of applications beyond bone repair. Acknowledgements:NSF-SFI US-Ireland R&D Partnership Programme (NSF_ 17_US_3437)

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Keywords: Gene therapy, Bone and bone disorders (osteoporosis etc)

Towards the Biohybrid Lung: Optimisation of endothelial cell seeding density on hollow fibres oxygenators

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INTRODUCTION:Chronic obstructive pulmonary disease is one of the main causes of death [1]. Lung transplantation is the only currently available treatment; however, donor shortage and organ rejection represent major limitations. The current short-term alternative is extracorporeal membrane oxygenators (ECMO) that comprise highly-hydrophobic hollow fibbers (HFs) made of polymethylpentene. Thrombogenicity and associated clotting of the ECMO limits its operation to 30 days, requiring multiple replacements during treatment. Endothelialisation of the blood contacting surfaces of the ECMO has been proposed to enhance the hemocompatibility and decrease the thrombogenicity of the device. The aim of this study was to optimise the seeding of the HF membranes of the oxygenator with endothelial cells (ECs).

METHODS:Human cord blood endothelial cell (HCBEC) adhesion was assessed on rheoparin®-coated HF membrane samples measuring 2.6×4 cm that were isolated from commercially-available oxygenators (Xenios, Hilite 2400 LT). EC adhesion was also assessed on HF membrane samples coated with both rheoparin® and fibronectin. Initially, the cell number required to cover the outer surface of the HFs ($1.69x10^4$ cells/cm²) was estimated from the HF surface area (8 cm²) of the HF membrane samples and the cell number per surface area obtained from confluent HCBEC monolayers in culture flasks. The HF membrane samples were mounted in custom-made frames and placed in falcon tubes filled with cell suspension at defined cell densities ($1.69x10^4$, $3.44x10^4$, $6.88x10^4$, $8.56x10^4$, $1.03x10^5$ and $1.20x10^5$ cells/cm²). Seeding was carried out dynamically for 4 h under rotation (1 rpm), and for two different cell passages (P7 and P11). Subsequently, the samples were cultured statically for 2 days in petri dishes prior to assessing cell adhesion and attached cell numbers via live/dead staining.

RESULTS:Rheoparin alone did not allow HCBEC adhesion. HF membrane samples coated with rheoparin and fibronectin demonstrated improved cell adhesion. Confluent cell monolayers were observed at cell densities of $1.03x10^5$ and $1.20x10^5$ cells/cm² (6x and 7x the initial estimation). Cell adhesion was improved for the P7 cells.

DISCUSSION & CONCLUSIONS:Confluent HCBEC coverage was achieved around the HFs coated with rheoparin and fibronectin at an optimum seeding density of $1.03x10^5$ cells/cm². These results provide the starting point for scaling up the seeding strategy for the endothelialisation of the whole HF membrane stack in commercially available oxygenators.

Acknowledgements:German Research Foundation SPP2014 (348028075) and Cluster of Excellence REBIRTH (EXC 62).

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Keywords: Biofabrication,

Development of tissue adhesion barrier sheet functionalized with cell-selective adhesion peptides

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INTRODUCTION:It is known that adhesions in the surrounding tissue frequently occur in the process of wound healing after surgery. Adhesions after surgery rise risks in organ dysfunction and can trigger life-threatening troubles in the second surgery. The formation of such tissue adhesions is a part of the repair mechanism; therefore, it is difficult to simply inhibit them by drug administration. Medical devices play an important role to rescue such tissue adhesion formation as a supportive device or with itself. Anti-adhesion sheets are one of the effective medical devices used in thoracic surgeries. However, conventional polymers used in the present anti-adhesion sheets have low bio-compatibility or regenerative performance.Our research group has been reporting c functional peptides to provide the biomaterial surface “cell-selective performances”, such as cell adhesion or proliferation to enhance the regeneration of implanted area with the combination of reducing negative effects triggered by unwanted cells [1]. In this study, we report the effect of cell-selective peptides to functionalize the polymer surfaces for anti-adhesion medical devices.

METHODS:Cell-selective adhesion peptides for controlling anti-adhesion effect were screened by SPOT peptide array technique which is synthesized on cellulose membrane by conventional Fmoc chemistry [2]. The cell-selective effects were analyzed directly on the SPOT peptide array or on the objective polymers. After 2 hours of cell adhesion, the adhesive cells were measured by fluorescence plate reader in high throughput manner.

RESULTS:As a result, we found several new sequences and their common physicochemical properties to enhance the adhesion of objective cells together with their inhibiting effect on un-wanted cells. The relative adhesion of objective and unwanted cells on our new peptide indicated that we can form the cell-selective peptide functionalized surface on medical device composing polymers.

DISCUSSION & CONCLUSIONS:Our functional peptides to function as cell-selective surface functionalization peptides was confirmed to preserve their function on practically used medical device polymers. The peptides property rules and its surface modification methods should contribute to new biopolymer functionalization for anti-adhesion medical implants.

Acknowledgements:This work is partially supported by JSPS KAKENHI Grant Number 16K15630, 18K14061, 19H03737.

References:[1] Biotechnol Bioeng. 2012 Jul;109(7):1808-16.
[2] Tetrahedron. 1992 48(42):9217-32.

Keywords: Interfaces - biological, Composite materials

Fabrication of a Polycaprolactone Electrospun Patch for Knee Ligament Repair

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INTRODUCTION:Surgical repair of the anterior cruciate ligament (ACL) still yields unsatisfactory clinical outcomes. With the failure rate of primary ACL reconstruction surgery as high as 25% (Kim et al., 2018), there is need to develop new ligament replacement strategies. The use of bioresorbable scaffolds is a promising strategy to improve ACL repair. Ideally, a scaffold for ACL reconstruction should have a structure mimicking that of the natural ligament, while providing adequate mechanical strength to support physiological loading. With this in mind, we have developed a robust electrospun patch prototype made of polycaprolactone (PCL), and have characterised it against a current market competitor – FiberWire®.

METHODS:The prototype scaffold was produced by electrospinning of continuous PCL filaments (Mouthuy et al., 2015), and by subsequently weaving these into a 10 mm wide fabric. The mechanical properties of the woven patch were measured by tensile testing. Scaffold morphology was assessed via SEM. Finally, cells derived from primary human ACLs were cultured on scaffolds for 14 days and viability was monitored using PrestoBlue™ assay.

RESULTS:The tensile strength of the patch was 272 ± 13 N (n = 10), comparable to hamstring tendon allograft, the current gold standard for repair, which has a reported tensile load of 406 ± 203 N (n=12, Mustamsir et al., 2017) The SEM images demonstrated a biomimetic morphology of the patch, with aligned nanofibers, similar to the ligament microstructure. ACL cells attached to the prototype scaffolds and to FiberWire®. Cells on prototype scaffolds demonstrated an elongated morphology with long filopodia protrusions whereas cells cultured on FiberWire® showed more rounded morphology, with some cells presenting disrupted cell membrane with characteristic apoptotic blebs. Human ACL cells seeded on both scaffolds had increased metabolic activity from day 7 of culture.

DISCUSSION & CONCLUSIONS:This study supports the suitability of a novel PCL electrospun scaffold for ACL reconstruction.

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Keywords: Polymers - natural / synthetic / responsive, Biomaterials

Harnessing electrical stimulation for neural stem cells culture using polyaniline-based coaxial electrospun fibers

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INTRODUCTION:Chronic diseases will have a prominent impact on the general population in this century. Among those, neurodegenerative diseases remain incompletely understood and ineffectively treated. Replenishing neurons in the brain may represent the best therapy for these progressive and often fatal diseases. However, clinical trials show that implanted cell integration into patient's brain tissue is limited by poor cell survival. A challenge that can be addressed by the development of new scaffolds, with enhanced biomimetic properties using mechanical/electrical cues that promote stem cell differentiation into neurons.

METHODS:The present study aimed at developing biocompatible polyaniline (PANI) -based coaxial electrospun fibers for neural tissue engineering. The work was developed in three stages: (1) optimization of the best polycaprolactone (PCL) to PANI ratio for an optimal electroconductivity vs biocompatibility [1]; (2) optimization of the solvent system to enhance electroconductivity; and (3) production of co-axial fibers, composed by an external conductive layer of PCL-PANI and an internal layer of poly(glycerolsebacate) (PGS). The fibers physico-chemical properties were then investigated using scanning electron microscopy (SEM), Fourier transform infrared analysis (FTIR) and differential scanning calorimetry (DSC), followed by electrical and mechanical characterization. In vitro stability and biodegradation of the fibermats was assessed using Thermomyces lanuginosus lipase. Biocompatibility was evaluated with and without electrical stimulation, and changes in protein and gene expression were evaluated.

RESULTS:We successfully produced co-axial fibers with average diameter of 951 ± 465 nm. The presence of a co-axial structure was confirmed by SEM, FTIR and DSC. Electroconductive ((6,3 ± 2,9) x 10-2 S cm-1) and mechanical (ε = 13 kPa) measurements indicate that the fibers obtained are promising for neural stem cell culture and differentiation, according with protein and gene expression assays. Proliferation and differentiation of ReN-VM cells was successful. Finally, with the aim of neural tissue engineering applications, iPSC differentiation towards the neural lineage was also evaluated.

DISCUSSION & CONCLUSIONS:Our results support the notion that the fibers developed have potential applications in neural tissue engineering applications: (1) to build reliable in-vitro platforms for drug screening; (2) interfaces for deep-brain electrodes; and (3) for direct transplantation of fully grown and functional neurons into patients' brains.

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References:[1] F.F.F. Garrudo et al, Eur Polym J 117 (2019) 28–37.

Keywords: Electroactive materials, Nervous system (brain-central-peripheral / disorders)

Fabrication of a 3D bilayer tubular in vitro model to study angiogenesis under physiologically more relevant conditions: A platform to evaluate pro-angiogenic agents and flow

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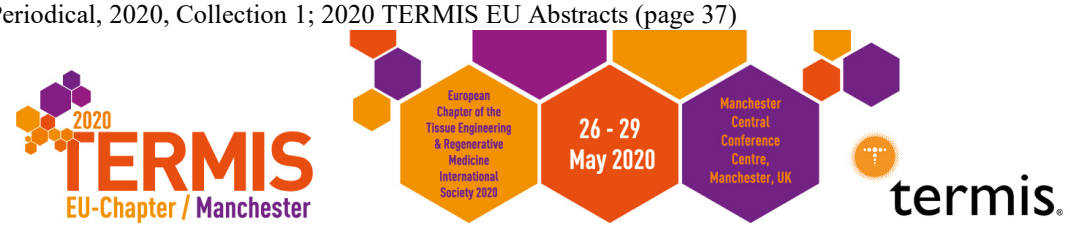
INTRODUCTION:Angiogenesis is the formation of new blood vessels from existing vasculature, and it is a complex process involving the relationship of endothelial cells (ECs) with their extracellular environment. There are several factors which regulate angiogenesis. They include but not limited to growth factors, flow, and the surrounding environment. Current in vitro angiogenesis assays mostly focus on proliferation, migration, and differentiation of ECs in 2D. While these systems provide a general understanding of potential angiogenic factors such as drugs, flow, and cell-cell interactions, they do not fully represent the 3D native environment of the blood vessels. Accordingly, we developed a physiologically relevant in vitro model to reduce the use of animals in angiogenesis research. The developed model enables the study of the multiple features of angiogenesis such as drugs, flow, and cell-cell interactions.

METHODS:3D model was fabricated combining electrospinning and emulsion templating. Inner PHBV tubes were electrospun using a hexagonal key attached to a rotator. Outer PCL PolyHIPE tubes were manufactured via emulsion templating. Both tubes were then combined by inserting one to another. First human aortic endothelial cells (HAECs) were seeded into the electrospun layer to create an endothelium-like layer. A flow chamber was designed and fabricated via 3D printing, and using the developed angiogenesis model, the effect of shear stress either in the presence or absence of two angiogenic agents, vascular endothelial growth factor (VEGF) and 2-deoxy-D-ribose (2dDR), on promoting growth and migration of HAECs was investigated.

RESULTS:The results showed that low shear stress stimulated the outgrowth of HAECs while no outgrowth was observed in the static culture (no flow) conditions. The introduction of the both angiogenic agents further promoted the outgrowth of HAECs with a slightly better response when VEGF was used.

DISCUSSION & CONCLUSIONS:We concluded that the developed model has a potential to be used in the evaluation of angiogenesis in vitro, and could offer a great alternative to the use of animals in angiogenesis research.

Keywords: Biomaterials, Biologics and growth factors



2-deoxy-D-ribose as an alternative to the use of exogenous VEGF to induce angiogenesis in tissue-engineered constructs

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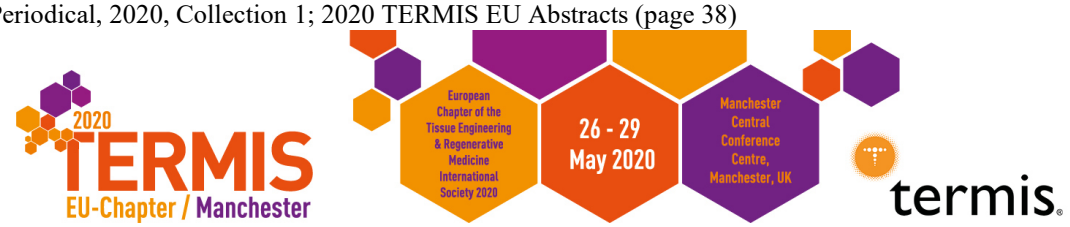
INTRODUCTION:Failure of tissue engineered (TE) constructs following implantation is one of the most challenging problems of tissue engineering. Thus, ensuring rapid vascularisation is crucial for translation of TE constructs to the clinic. Use of proangiogenic factors is a promising approach to overcome slow neovascularisation. VEGF is recognised as the gold standard for promoting angiogenesis but it is also unstable, expensive and the use of high doses of VEGF can result in leaky immature vessels in vivo. The aim of this study is to develop E2 and 2dDR releasing scaffolds and evaluate their potential for promoting angiogenesis.

METHODS:We first assessed the angiogenic potency of 2dDR versus VEGF in vitro and in an ex-ovo chick chorioallantoic membrane (CAM) assay. 2dDR was then electrospun into PHBV fibres. Releases of both agents were observed over 30 days from these scaffolds using a spectrophotometric method. The proangiogenic activities of the drug releasing scaffolds were assessed using ex-ovo CAM assay. Finally, a rhodamine-labelled lens culinaris agglutinin (LCA) solution was injected into the circulation of CAM to visualize the microvasculature. Then, a diabetic rat model was used to evaluate the potential of 2dDR for stimulating wound healing when released from commercially available alginate dressings.

RESULTS:2dDR was almost 90% as effective as VEGF in terms of stimulating endothelial cell proliferation, migration and tube formation in vitro. In ex ovo CAM assay, 2dDR was found approximately 80% as potent as VEGF in stimulating new blood vessels in CAM assay when applied directly onto CAM. It was very effective at stimulating neovascularisation over 7 days when released from PHBV fibres. The structure of microvasculature of CAMs and the vascular areas showed that an endothelial cell hypertrophy together with smaller lacunae compared to PBS can be observed for VEGF and 2dDR applied groups. The macroscopic and histological evaluation of the diabetic wounds showed that 2dDR did not only induced angiogenesis but also stimulated the healing of diabetic wounds in 20 days.

DISCUSSION & CONCLUSIONS:We conclude that 2dDR offers attractive alternative to VEGF for the functionalisation of TE scaffolds to promote angiogenesis. The gradual release of 2dDR from fibres stimulated the formation of new blood vessels in the ex-ovo CAM assay. The release of 2dDR from alginate dressings stimulated angiogenesis and wound healing simultaneously.
Acknowledgements:
References:

Keywords: Biomaterials, Wound healing



Liquid Substrates for Stem Cell Expansion – Design of Ultra-Soft Interfaces and Implications for Stem Cell Technologies and Tissue Engineering

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INTRODUCTION:The mechanical behaviour of the extracellular matrix has an important impact on cell phenotype. Despite the importance of mechanotransduction in regulating a wide range of phenotypes, we recently reported the surprising observation that cells (keratinocytes and mesenchymal stem cells) can adhere, spread and proliferate at the surface of liquids [1-3]. This observation is particularly surprising as the reinforcement of cell adhesion is thought to require a solid elastic or viscoelastic substrate that can resist cell-mediated contractile forces. Our work has evidenced the formation of protein nanosheets, self-assembled at the liquid-liquid interface, displaying strong mechanical properties that can provide a sufficient mechanical scaffold to promote cell adhesion and expansion. We showed that this is sufficient to regulate stem cell phenotype. However, the parameters controlling the self-assembly and the mechanical properties of protein nanosheets remain poorly understood. In this work we investigate the assembly of polymers and proteins at liquid-liquid interfaces, and the impact of pro-surfactants with a wide range of chemistries. We identify structural features that control the visco-elastic properties of the resulting nanosheets and regulate associated cell phenotype. Finally, we explore how associated liquid interfaces may be harnessed for the development of novel stem cell technologies, for expansion in emulsion-based bioreactors and cell delivery for tissue engineering.

METHODS:Assembly at liquid-liquid interfaces is studied using interfacial rheology. Protein nanosheets are characterised by scanning electron microscopy, atomic force microscopy and XPS. Cell adhesion and phenotype was characterised by fluorescence microscopy and qPCR.

RESULTS:In this work, we show the importance of parameters such interactions with pro-surfactants on protein self-assembly and interfacial mechanics. We show how these parameters regulate interfacial viscoelasticity over a wide range, and ultimately regulate cell adhesion and proliferation. Finally, we demonstrate the proof-of-concept of using such liquid substrates, in the form of emulsions, for stem cell culture in 3D bioreactors and delivery for tissue engineering.

DISCUSSION & CONCLUSIONS:Overall, our results suggest that nanoscale mechanical properties of biomaterials may dominate over bulk physical properties. This concept has important implications for the design of biomaterials in the field of regenerative medicine and allow the rational design of liquid substrates for stem cell technologies, such as the design of emulsion-based bioreactors, and for tissue engineering.

Acknowledgements:Funding from the Leverhulme Trust (RPG-2017-229-Grant-69241) and the ERC (ProLiCell-772462) is acknowledged.

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Keywords: Interfaces - biological, Nanomaterials (inc graphene)

Fabrication of 3D-Printed Scaffolds for Tracheal Replacement

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INTRODUCTION:Attempted tracheal replacement efforts thus far have had very little success. Major limiting factors have been the inability of the transplanted scaffolds to regenerate a protective layer of respiratory epithelium and to efficiently re-vascularise, thus succumbing to bacterial infections, poor tissue growth and stenosis [1, 2]. The major objective of this study was to optimise a previously developed collagen-hyaluronic acid scaffold (CHyA-B), which has shown to facilitate the growth of respiratory epithelial cells and fibroblast cells in distinct regions [3], as a potential tracheal replacement device. The scaffold will be optimised to provide sufficient mechanical properties to replace tracheal cartilage and then assessed for its ability to support blood vessel growth.

METHODS:A biodegradable thermoplastic polymer was used to 3D-print (3DP) (Allevi II, USA) two main scaffold designs – a complete and partial ring. The mechanical properties of the scaffolds were assessed using compression, three-point bending and fatigue testing (Zwick Roell, Z005, Germany). The 3DP constructs were incorporated into the CHyA-B scaffolds and Human Vein Endothelial Cells (HUVECs) and Human Mesenchymal Stem Cells (hMSCs) were seeded in mono- and co-culture conditions to assess the influence of the polymer fibres on vessel formation. Cell metabolic activity, Vascular Endothelial Growth Factor (VEGF) & Platelet Derived Growth Factor (PDGF-BB) (ELISA, R&D Systems, USA) production were assessed during days 6, 10 & 14.

RESULTS:The polymeric backbone provided sufficient strength to the CHyA-B scaffold, compressive moduli ranged from 0.09 to 0.19 MPa, and the flexural moduli ranged from 0.13 to 0.29 MPa. The reinforced scaffolds supported endothelial cell growth in mono and co-culture, with cell metabolism increasing from day 6 to day 10 in both the reinforced and non-reinforced scaffolds. Furthermore, VEGF production also increased from day 6 to day 10.

DISCUSSION & CONCLUSIONS:By addressing both the mechanical and physiological requirements of a tracheal scaffold, this work has begun to pave the way for a new therapeutic option to resolve the shortcomings of current treatment options, potentially improving patient outcomes. Future experimental work will validate the scaffold's ability to support the growth & differentiation of respiratory epithelial cells in a tri-culture model with hMSCs and HUVECs.

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Keywords: 3D printing and bioprinting,

Biomaterial biocompatibility: the correlation between the adsorption of complement proteins and macrophage polarization

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INTRODUCTION:The first event after biomaterial implantation is the protein adsorption onto its surface, which can regulate cell responses. Due to the attachment of complement proteins, biocompatibility problems might arise from the activation of complement pathways, resulting in a foreign body reaction. Hence, a deeper understanding of the interaction between biomaterials, proteins and cells could minimize these biocompatibility-related issues. 3-glycidoxypropyl- trimethoxysilane (GPTMS), precursor employed to develop sol-gel materials for bone regeneration, was associated with biocompatibility problems in past studies [1]. Its use could allow correlating different levels of immune responses with the adsorption of immune-associated proteins on the biomaterial.

METHODS:The sol-gel route was used to synthetize materials with distinct amounts of GPTMS. These compositions were applied as coatings onto titanium discs. *In vitro* testing was made using two distinct cell lines: MC3T3-E1 osteoblasts and RAW 264.7 macrophages. The gene expression of osteogenic markers (ALP, IL-6, COL I, TGF-β) was measured using qRT-PCR. Liberation of pro-inflammatory (TNF-α, IL1β) and anti-inflammatory markers (IL-10, TGF-β) from RAW 264.7 was measured by ELISA. Macrophage polarization was tested through immunostaining using IL7-R (M1) and CD206 (M2). For proteomic analyses samples were incubated in human serum. Proteins adsorbed onto the materials were analyzed through LC-MS/MS. Tibia rabbit model was used *in vivo* and histological analysis was made.

RESULTS:Materials with GPTMS displayed an upregulation of TGF-β gene expression on osteoblasts. Macrophages increased the pro-inflammatory TNF-α and IL-10 release and the IL7-R fluorescence on the GPTMS-doped materials. *In vivo* showed a higher density of multinucleated giant cells surrounding the coatings with more GPTMS. Proteomic studies revealed that complement system activators FCN2, CRP, C3 and C5 increased their adsorption on the GPTMS-coatings on a dose-dependent manner.

DISCUSSION & CONCLUSIONS:Higher immune response was observed as more GPTMS was incorporate in the material both *in vitro* and *in vivo*. In addition, attached complement-related proteins could promote the macrophage switching to M1 pro-inflammatory phenotype. It was found a correlation between the complement protein adsorption patterns and this increasing immune reaction. This correlation highlights the importance of controlling protein adsorption onto biomaterials and the potential of proteomics as a tool to assess their biocompatibility.

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Keywords: Interfaces - biological, Immunity / immunomodulation / macrophage

Xeno-free culture systems for human pluripotent stem cell-cardiomyocytes discovered by high-throughput screening

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INTRODUCTION:Cardiovascular disease (CVD) remains one of the leading causes of ill-health and mortality. The ability to derive human induced pluripotent stem cells (hiPSCs) from patients and healthy individuals, and then differentiate these cells into cardiomyocytes (hiPSC-CMs) provides new opportunities to model and understand human diseases, including CVDs. However, current approaches to produce hiPSC-CMs results in cells that lack maturity relative to the counterparts within the adult human heart. One approach to overcome this limitation is the use of biomaterials, which can improve hiPSC-CM functionality by altering biophysical and biomechanical properties including surface chemistry and topography to better represent the *in vivo* environment (A.K.Patel et al., Current Opinion in Solid State and Materials Science, 2016).

METHODS:In this study, high-throughput micro-array screening approaches were used to assess 24,924 cell-polymer interactions (previously described in A.K.Patel et al., Biomaterials, 2015) and >2000 unique topographies (previously described in Unadkat et al., PNAS, 2011) in individual assays. A third micro-array platform, ChemoTopoChip: combining polymer and topography interactions (L.Burroughs et al., under review) has been subsequently used to identify topographically enhanced cell culture systems for improved maturity of hiPSC-CMs. Experiments at scale, focussed on assessing hPSC-CM attachment using high-content imaging (Operetta, Perkin Elmer) and functionality by measuring contractility (Cell Optiq, Clyde Biosciences) to polymer substrates.

RESULTS:From a first-generation ~280 homopolymer screen, high attachment of hiPSC-CMs was achieved with amine-containing polymers which were previously identified to improve hiPSC-CM functionality (M. Alvarez-Paino et al., ACS Appl. Mater. Interfaces, 2019). From this group, 24 polymers that supported high hiPSC-CM attachment were combined to produce a combinatorial array of 576 co-polymers. Of 20 co-polymers selected for scale-up based on supporting attachment on arrays, only 4 supported high attachment of both hiPSCs as well as hiPSC-CMs. These were co-polymers consisting of amine-containing polyacrylates which showed improved hiPSC-CM contractility. Preliminary screening of hiPSCs and hiPSC-CMs with these diverse topographies have identified how feature size and frequency influences hiPSC pluripotency and colony expansion as well as structural maturation respectively. Mathematical algorithms were applied to identify surfaces that can support both cell types (hiPSCs and hiPSC-CMs) to identify surfaces that support both hiPSC expansion and differentiations.

DISCUSSION & CONCLUSIONS:Topo-chemical combinations are now being tested to investigate the interplay between surface chemistry and topography. The aim being that this will identify xeno-free topographically enhanced culture systems that mediate both hiPSC expansion and *in situ* differentiation of hiPSC-CMs with improved maturity and functionality for better CVD modelling.

Keywords: Cardiovascular, Induced pluripotent stem cells

Bioactive NCO-sP(EO-stat-PO)/gelatin bi-component fibrous hydrogel network as versatile cellular scaffolds

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INTRODUCTION:Gelatin is widely studied natural material mainly due to its excellent biocompatibility and accessibility. Here in, we used versatile isocyanate chemistry within a six arm star-shaped polymer (NCO-sP(EO-stat-PO) to produce fibrous crosslinked functional hydrogel networks via co-electrospinning with gelatin.

METHODS:Varying amount gelatin and NCO-sP(EO-stat-PO) were dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol(HFIP) and tetrahydrofuran, respectively and the two solutions were mixed to obtain homogenous spinning solutions. Electrospinability and the morphology of the fibers were visualized with SEM. Contact angle measurements highlighted the hydrophilicity changes on the scaffolds. Uniaxial tensile tests were applied on the scaffolds to observe mechanical features. Bi-component interactions, network and water stability were analysed via SEM, FTIR, DSC, TG and weight loss analysis. Raw 264.7 cells and human macrophages were used in the in vitro studies.

RESULTS:Gelatin solution, prepared in HFIP, can be directly electrospun into fibrous meshes. Electrospinning of NCO-sP(EO-stat-PO)/gelatin blend (weight ratios ranging from 2:1 to 1:10) also resulted in fibrous morphology with only minor changes in fiber diameters. With higher NCO-sP(EO-stat-PO) content, the hydrophilicity and the maximum tensile strength of the scaffolds increased as well as the stability of the fibrous morphology in aqueous conditions. Weight loss studies further indicated a greater mass loss with decreasing amount of NCO-sP(EO-stat-PO) whereas FTIR and TG studies suggested that this mass loss is mainly due to the washing away of the gelatin. Changes is the DSC profiles pointed out the interaction between NCO-sP(EO-stat-PO) and gelatin which facilitated more stable scaffolds. Regardless of the NCO-sP(EO-stat-PO)/gelatin ratio, the scaffolds were found biocompatible, evidenced by increasing DNA content and high metabolic activity of Raw 264.7 cells and human macrophages. The varying amount of NCO-sP(EO-stat-PO) and gelatin influenced cell morphology e.g. spreading and pro/anti-inflammatory phenotype of human macrophages.

DISCUSSION & CONCLUSIONS:Herein, we demonstrated the facile use of NCO-sP(EO-stat-PO) as a functional component and a crosslinker for gelatin. Stability of fibrous hydrogel scaffolds correlated with NCO-sP(EO-stat-PO) content, although all scaffolds supported the cell adhesion and proliferation. Further in-depth studies on the use of gelatin/NCO-sP(EO-stat-PO) fibers as a multifunctional cell scaffold for the tuning of inflammatory responses of macrophages are ongoing.

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Keywords: Immunity / immunomodulation / macrophage, Hydrogels and injectable systems

Influence of hydroxypropyl methacrylate on poly (N-isopropylacrylamide) based Laponite® hydrogels for tissue cell differentiation

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INTRODUCTION:Hydrogels have become very popula and numerous attempts have been made to develop these materials to be used in tissue engineering applications, due to their high water content and biocompatibility [1]. The chemical composition of hydrogels and the subsequent effect on mechanical properties, swelling and deswelling behaviour and structural properties has been studied intensively. In this study poly (N-isopropylacrylamide) co poly(hydroxypropyl methacrylate) Laponite® hydrogels were synthesised and the influence of incorporation of hydroxypropyl methacrylate (HPMA) on physical, mechanical and cell behaviour within these materials were investigated.

METHODS:All samples were synthesised in accordance with previous research [2] using different HPMA concentrations: 5, 10%wt. Synthesized hydrogels were characterized using scanning electron microscopy (SEM) to interrogate the internal morphology and matrix deposition produced by human mesenchymal stem cells (hMSCs). Fourier transform infrared (FTIR) spectroscopy was used to monitor the dehydration and rehydration properties in situ. Dynamic mechanical analysis (DMA) was used to explore mechanical properties of the hydrogels. Swelling and deswelling behaviour were investigated and mesenchymal stem cells viability and phenotype within the hydrogel investigated for up to 4 weeks.

RESULTS:SEM results showed changes in hydrogel morphology where pore sizes within hydrogel structure increased dramatically as HPMA was incorporated. However, when the HPMA concentration was doubled pore size was slightly decreased when compared to 5 wt. % hydrogel. Adding HPMA resulted in softer hydrogel materials, where the storage modulus (G') decreased significantly. The dehydration rate decreased when HPMA was added. However, rehydration rate was increased. Cell viability was increased significantly by adding HPMA, and histological showed an increase in proteoglycans, calcium and collagen deposition when HPMA was incorporated. Immunohistochemistry revealed significant increases in collagen type I and osteopontin, which confirm histological findings that HPMA enhance the ability of this hydrogel to induce osteogenic differentiation of hMSCs.

DISCUSSION & CONCLUSIONS:We have demonstrated the influence of using HPMA as comonomer on physical and mechanical properties of poly (N-isopropylacrylamide) co poly (hydroxypropyl methacrylate) based Laponite® hydrogel and how this improve cell viability and phenotype within the hydrogels.

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Keywords: Biomaterials, Hydrogels and injectable systems

Emulsion Templated Porous Polymers and Fibrin Based Scaffolds for the Formation of a Tissue Engineered Bone-Ligament-Bone Construct

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INTRODUCTION:Injuries to the scapholunate interosseous ligament of the wrist often occur due to a fall on an outstretched hand. Damage to this ligament can progress to debilitating pain, carpal instability, and severe arthritis of the wrist [1]. While treatments involve autografts from the forearm, they do not replicate the soft-to-hard transition of the native scapholunate interface. In this study we explore the formation of a biocompatible and biodegradable in-vitro bone-ligament-bone construct (BLB).

METHODS:Emulsion templated porous polymers (polyHIPE) were synthesised according to a previous protocol [2]. PolyHIPEs were cut to 3mm cubes and pinned onto polydimethylsiloxane (PDMS) coated 6-well tissue culture plates via stainless steel minutien pins (two PolyHIPE cubes per well). Fibrin scaffolds were formed around the polyHIPE anchors and 100K human foreskin fibroblast cells (HFFs) were seeded per well [3]. Constructs were maintained for a period of up to 60 days. Constructs were analysed via histology, LIVE/DEAD staining, polarised light microscopy, scanning electron microscopy, and cell viability assays (resazurin sodium salt).

RESULTS:Cell mediated contraction of fibrin scaffolds occurred over a period of 14 days. Constructs were also self-supporting and opaque after this culture period, an indication of ECM formation. BLB constructs remained viable in cell culture conditions for extended time periods as indicated by LIVE/DEAD assays and cell viability assays. Histological staining of BLB scaffolds revealed a dense network of HFF cells aligned parallel to PolyHIPE anchors and the formation of parallel collagen fibers across the construct. Low fibroblast cell migration were also observed in PolyHIPEs anchors.

DISCUSSION & CONCLUSIONS:Bone-ligament-bone constructs with a hard to soft tissue interface can be synthesised from the use of emulsion templated porous polymers and fibrin scaffolds.

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Keywords: Polymers - natural / synthetic / responsive.

Engineering miniaturized high-throughput freestanding multilayered membranes for bottom-up tissue engineering strategies

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INTRODUCTION:Layer-by-layer technology (LbL) has emerged as a powerful strategy for engineering flat and patterned freestanding (FS) multilayered membranes to be applied in wound healing, drug delivery and tissue regeneration.[1] However, FS membranes still rely on macroscale multilayered systems without spatial control over cell distribution and organization to mimic native tissue architectures. In this work, a pioneering concept of miniaturized FS membranes with specific shape and size was exploited for high-throughput fabrication of cell-seeding building blocks with different cell phenotypes that could be further assembled into a single complex geometry for microscale tissue engineering.

METHODS:FS multilayered films with different geometries were build-up via LbL assembly of poly-L-lisine and alginate polymers onto superhydrophobic-superhydrophilic microarrays, later prepared as previously reported elsewhere.[1] After detachment, poly-L-lysine outer layers of FS micro-membranes were chemically immobilized via EDC/NHS coupling chemistry. Different cell phenotypes were seeded on membranes and cultured up to 21 days. At specific time-points, cell viability, DNA quantification, and nuclei and cytoskeleton fluorescent staining were performed. Finally, as a proof-of-concept, FS micro-membranes units with different cells were spatially arranged into highly hierarchical 3D microscale tissues.

RESULTS:After detachment from microarray platforms, multilayered micro-membranes have shown the same geometrical features of the underlying substrate. Such micro-membranes have still exhibited homogeneous size distribution across the different geometries. Membrane geometrical features (e.g. shape, size and curvature) have enhanced cell-surface membrane interactions triggering cellular adhesion and proliferation. Different shapes of cell-seeding micro-membranes were also assembled, demonstrating the ability to achieve physical and biological heterogeneities.

DISCUSSION & CONCLUSIONS:High-throughput fabrication of freestanding micro-membranes with specific sizes and geometries were successfully developed by spatially confined layer-by-layer assembly onto the superhydrophilic areas of microarrays platforms, holding great potential as heterogeneous building blocks for creating functional 3D tissue-mimetic constructs.

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Keywords: Polymers - natural / synthetic / responsive, Interfaces – engineered

Layer-by-layer building of hybrid vascularized tissues by microscale assembly of cell/µ-scaffolds modules

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INTRODUCTION:The bottom-up realization of three-dimensional (3D) bio-hybrid structures composed of both biomaterials and cells are one of the most interesting approaches for in vitro and in vivo tissue regeneration [1]. µ-scaffolds made of biocompatible and biodegradable polymers are essential elements of these bottom-up approaches. They serve as template structures for cells adhesion, proliferation and 3D assembly [2]. Such 3D bio-hybrids have been used as possible models to study cells differentiation and tissue vascularization [3]. Nevertheless, there are limited studies regarding the microscale assembly of cell/µ-scaffolds modules to enhance the control of the final tissue architecture. This study focuses on the development of a bottom-up approach enabling the layer-by-layer building of highly complex and precisely designed bio-hybrids by the ordered assembling of cell/µ-scaffolds modules.

METHODS:Porous biodegradable polymeric µ-scaffolds were fabricated by fluidic emulsion/solvent evaporation technique. The morphological and structural properties of µ-scaffolds were characterized by scanning electron microscopy (SEM) and Micro-CT analysis. A soft lithography approach using µ milling technique and replica molding process were used to prepare patterned molds for hybrid tissue layers growth. This was achieved by patterning µ-scaffolds into the molds and carrying out co-culture of human dermal fibroblasts (hHDFs) and human umbilical vein endothelial cells (HUVECs). The morphology and cell/ECM composition of bio-hybrids were assessed by immunofluorescence analysis and histology.

RESULTS:The results of µ-scaffolds evidenced a porous morphology on both surface and inner part suitable for cells adhesion, colonization and proliferation. Furthermore, the µ-scaffolds are characterized by 70% overall porosity and 100% pores interconnectivity (Micro-CT analysis results). µ-scaffolds biocompatibility was validated with hHDFs in vitro culture tests. The tests demonstrated the capability of the µ-scaffolds to support cells adhesion, proliferation and extracellular matrix biosynthesis into the entire porosity. Hybrid layered structures with pre-defined porosity and structure were fabricated by soft lithography strategy. The layers were overlapped and assembled together to build 3D bio-hybrids with pre-defined composition, high cells density and synthesized collagen as well as architecture of blood vessels network.

DISCUSSION & CONCLUSIONS:This study demonstrated that the proposed approach enabled the design and building cell/µ-scaffold hybrid systems with microscale control over system composition and architecture suitable to be tested for tissue engineering purposes.

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Keywords: Vascular systems / vascularisation and heart

Growth-factor free multicomponent nanocomposite hydrogels that stimulate bone formation

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INTRODUCTION:Synthetic osteo-promoting materials that are able to stimulate and accelerate bone formation without the addition of exogenous cells or growth factors would represent a major opportunity for an aging world population. We report on a co-assembling system that integrates hyaluronic acid tyramine (HA-Tyr), bioactive peptide amphiphiles (GHK-Cu2+), and Laponite® (Lap) to engineer hydrogels with physical, mechanical, and biomolecular signals that can be tuned to enhance bone regeneration.

METHODS:The central design element of the multicomponent hydrogels was the integration of self-assembly and enzyme-mediated oxidative coupling to optimize structure and mechanical properties in combination with the incorporation of an osteo- and angio-promoting segments to facilitate signaling.

RESULTS:Spectroscopic techniques were used to confirm the interplay of orthogonal covalent and supramolecular interactions in the multicomponent hydrogels formation. Electron microscopy and analytical techniques were used to confirm co-assembly by assessing changes in the nanostructures and elemental compositions of the multicomponent hydrogels. Furthermore, physico-mechanical characterizations revealed that the multicomponent hydrogels exhibited improved compressive strength, stress relaxation profile, low swelling ratio, and retarded enzymatic degradation compared to the single component hydrogels. Applicability was validated in vitro using human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (hUVECs), and in vivo using a rabbit maxillary sinus floor reconstruction model. Animals treated with the HA-Tyr-HA-Tyr-GHK-Cu2+ hydrogels exhibited significantly enhanced bone formation relative to controls including the commercially available Bio-Oss®

DISCUSSION & CONCLUSIONS:We have developed a practical and multifunctional self-assembling hydrogel biomaterial for bone regeneration applications. The material takes advantage of both covalent and non-covalent interactions to integrate hyaluronic acid (HA), peptide amphiphiles (PAs), and Laponite® (Lap) into a bioactive hydrogel with a spectrum of molecular, physical, and mechanical properties designed to promote bone regeneration as well as minimally invasive implantation. We demonstrate the capacity of the hydrogels to support cell growth and stimulate both osteoblastic differentiation and angiogenic sprouting of hUVECs in vitro as well as promote faster bone regeneration in a rabbit model compared to a commercially available gold- standard material. The current study introduces a new molecularly designed self-assembling material that stimulates bone formation without the use of exogenous growth factors and demonstrated its potential use in maxillary sinus reconstruction and other bone tissue regeneration procedures.

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Keywords: Bone and bone disorders (osteoporosis etc), Other

Decellularized human liver incorporated into electrospun scaffolds for in-vitro liver tissue models

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INTRODUCTION:Liver disease is on the rise around the world and the only current treatment option available is a liver transplant, however transplant availability currently only meets 10% of demand [1]. This study investigates the potential for donor Human Liver Extracellular matrix (ECM), discarded as clinical waste, to be used in electrospun in-vitro liver tissue models. Previously, Human ECM has been shown to elicit unique responses from hepatic cells in comparison to individual ECM proteins [2]. Herein we investigate donor-to-donor responses from HepG2 cells for five different donor ECMs electrospun in our hybrid PCL-ECM scaffolds.

METHODS:Liver tissue from five separate human donors has been decellularised through adaptation of an established method using a custom in-house perfusion device [2]. Briefly, 35mm diameter by 4mm thick liver tissue discs were perfused with 0.5% Sodium dodecyl Sulphate (SDS) at 30 mmHg for 24 Hrs before washing under dH2O perfusion for a further 24 Hrs. The resulting decellularised discs were then lyophilised and ball-milled to a powder. These powders were each subsequently incorporated into 5 separate electrospinning solutions with Poly-caprolactone (PCL) and electrospun into fibres of consistent morphology. A cellular activity study was conducted using the HepG2 cell line; collecting viability, DNA quantitation, immunohistochemistry (IHC) and RT-qPCR results. ECM and scaffold composition was analysed by standard histology, IHC, and FTIR spectroscopy for each donor tissue.

RESULTS:SEM imaging of the fibre structures demonstrated the production of consistent micro-fibre scaffolds containing different donor ECM. Differences between each liver donor were characterised both visually and through histological analyses, with confirmation of ECM protein presence within the scaffolds also shown via FTIR and IHC staining. Each scaffold group supported the proliferation of HepG2 cells. RT-qPCR data shows the trends in genes associated with liver function, differentiation and ECM production.

DISCUSSION & CONCLUSIONS:This study confirms that our method is appropriate for the production of ECM-PCL scaffolds with different human liver donor tissues. HepG2 cells survive on the scaffolds with key genes maintained. Therefore, there remains scope for utilising waste donor tissue for controllable electrospun liver tissue models.

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Keywords: Decellularised matrices, Disease models

Hierarchically mineralizing 3D printed scaffolds for hard tissue regeneration

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INTRODUCTION:Hard tissue disorders such as osteoporosis, maxillofacial and cranial defects, oral defects affecting enamel, dentine, and periodontal tissues affect more than half of the world population [1, 2]. Here we report an integrated approach combining additive manufacturing with supramolecular chemistry to develop acellular hierarchically mineralizing scaffolds for hard-tissue regeneration. Our approach uses the interplay between protein's order and disorder to generate a supramolecular framework capable of triggering hierarchical apatite growth within 3D-printed scaffolds guiding mineralization from the nanoscale upto complex macroscopic structures. The scaffolds can exhibit surfaces with designed bio-mimetic chemical composition, structural hierarchy, and mechanical properties [3].

METHODS:Polymeric scaffolds of desired structural parameters such as porosity, pore size, and pore connectivity were 3D printed using computer-controlled rapid prototyping technology. Scaffolds were coated with elastin like protein (ELP) to form a uniform ~10µm thick layer. Our approach enabled modulation of protein's order and disorder to generate a supramolecular framework with the capacity to nucleate and grow hierarchically mineralized structures on large and uneven surface. Mineralization efficiency of these scaffolds was tested *in vitro* and characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD). Bioactivity of the mineralized scaffolds in terms of osteogenic potential was analyzed using alkaline phosphatase (ALP) activity on human bone marrow cells (hBMCs).

RESULTS:The supramolecular ELP framework enabled us to generate hierarchically mineralized structures comprising crystallographically aligned 50nm thick apatite nanocrystals bundled into 5µm wide prisms to generate well-defined 200µm diameter macroscopic structures. These structures were successfully grown within printed scaffolds exhibiting 300µm thick strands and 200µm pore size. In this manner organic-inorganic interactions were guided from the nanoscale to the macroscale within complex geometries. These acellularly mineralized scaffolds were acid resistant and exhibited surface mechanical properties which were tuneable and comparable to hard tissues. Furthermore, scaffolds supported hBMCs adhesion and proliferation as well as enhanced expression of ALP.

DISCUSSION & CONCLUSIONS:Overall, this approach integrates advantages of supramolecular chemistry, tunable organic-inorganic interactions, additive manufacturing, and commemorates ease of design and fabrication of a novel platform to enhance osteo-inductivity and osteo-conductivity in bone regeneration. We envisage that this approach can have important implications for the design of smart-biomaterials which not only can acellularly self-mineralize by drawing ions from the implant site but also exhibit capacity to infiltrate and integrate with the underlying native tissues.

ACKNOWLEDGEMENTS:ERC grant (STROFUNSCAFF) AO Foundation

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Keywords: 3D printing and bioprinting, Bone and bone disorders (osteoporosis etc)

Blended PCL/PLA/GO grooved scaffolds:Nerve guidance conduit with improved properties for peripheral nerve repair

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INTRODUCTION:Peripheral nerve repair has been a longstanding problem despite developments in microsurgery and artificial nerve scaffolds. Synthetic nerve guidance conduits are promising due to their multifunctional polymer composition, engineered surface morphology and bespoke design for individual patients. The demand for high-performance nerve tissue engineering scaffolds is projected to increase, but economic effectiveness, such as easy fabrication and longer shelf life after gamma sterilisation need to be addressed.

To help overcome these problems, surface structure and morphology are key elements to control cell-material interactions and also polymer degradation processes. Graphene oxide (GO), have shown to be potential candidates for neural regeneration due to intrinsic benefits and reported ability of supporting neuro-reconstruction[1]. Ideally, GO could facilitate beneficial cell-material interactions through abundant surface functional groups. The fabrication of GO-modified PCL/PLA films and also effect of sterilisation (UV and gamma irradiation) were investigated.

METHODS:Solvent casted polymeric films were fabricated by blending polymer solutions of PCL and PLA (in dichloromethane at a concentration of 7% w/v) with GO dispersion (0.1% w/w of GO powder in tetrahydrofuran). Material properties were characterised, including chemical composition (FTIR), surface wettability (WCA), surface morphology (SEM), GO distribution (Raman), thermal properties (DCS and TGA), mechanical properties and cell-material interactions. The degradation study was conducted according to the ASTM 1635 11 standard. Mass loss and molecular weight were studied following degradation experiments. RESULTS:SEM and confocal microscopy indicated the grooved pattern was successfully copied after adding GO nanomaterials. Wettability of PCL/PLA/GO (73.62±1.81) was significantly improved compared to PCL/PLA (77.03±0.52). Raman spectroscopy mapping confirmed GO was well dispersed with polymer. Regarding thermal properties, Tg of modified films (-57.82±0.92oC) increased from -61.49±0.14oC and also Tm (56.64±0.091oC) were increased from 55.96±0.035oC. The presence of GO also improved mechanical properties, for instance, the young's modulus of modified films increased (2.54±0.1546MPa) in comparison to PCL/PLA (1.98±0.0727MPa). An *in vitro* study using RT4 SCs suggested improved cell attachment and proliferation.

DISCUSSION & CONCLUSIONS:PCL/PLA scaffolds modified with GO were produced, and their properties evaluated following degradation experiments after irradiation. The grooved PCL/PLA/GO films improved wettability, mechanical properties and neural cell behaviour, in addition to maintaining mechanical properties after irradiation. These results indicated that the modified films could be effective nerve tissue engineering scaffolds with prolonged shelf life and improved cell behaviour.

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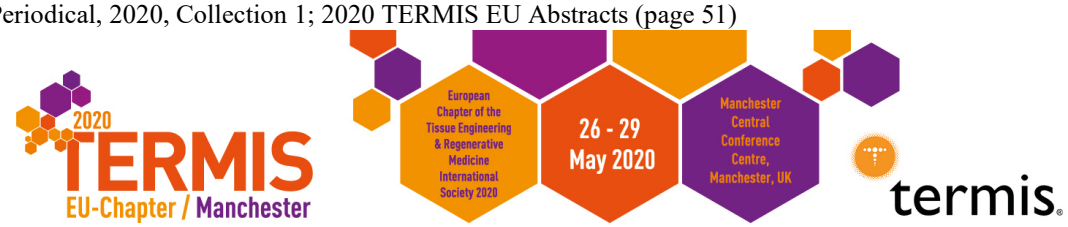
Keywords: Polymers - natural / synthetic / responsive, Nervous system (brain-central-peripheral / disorders)



Molecular biological and biotribological properties of osteochondral grafts treated with proinflammatory cytokines

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INTRODUCTION:Osteoarthritis leads to an imbalance between anabolic and catabolic factors. This can result in the release of proinflammatory cytokines and degradative enzymes, leading to inflammation of the joint. During the disease, cartilage degradation occurs, which influences the biomechanical and tribological properties of the cartilage. Also, the synthesis of ECM components by chondrocytes is reduced and apoptosis of the cells occurs. This study investigates the effects of inflammation on cartilage and chondrocytes under biomechanical stress in a tribological test system.

METHODS:Bovine osteochondral grafts from four different animals were punched out from the medial condyle and treated with proinflammatory cytokines (IL-1β, TNF-α, IL-6) for two weeks. Untreated samples were used for control purposes. Post incubation tribological tests were performed in a cartilage-on-cartilage test system for 2 hours (alternating 10 minutes test and pause; 39°C, 180 N, 1 Hz, 2mm stroke). Prior to and after testing the cartilage surface was imaged with an optical microscope. The coefficient of friction was measured during testing, while gene expression analysis and metabolic activity of chondrocytes were investigated after testing. Also, histological sections of the tissue and abrasion products from the test fluid were analyzed.

RESULTS:After the tribological tests, cracks on the cartilage surface were found in both treated and untreated osteochondral grafts. In treated grafts, the coefficient of friction was increased and the proteoglycan content in the cartilage tissue was reduced, with structural changes in the tissue occurring after the tests. Chondrocytes of treated grafts showed increased expression of genes for degradative enzymes, while cartilage-specific gene expression and metabolic activity showed no changes between treatment and non-treatment. The measurement of abrasion products in the test medium also showed no significant difference.

DISCUSSION & CONCLUSIONS:Our study shows that the treatment of osteochondral grafts with cytokines leads to significant changes in proteoglycan content of the cartilage and compression of the cartilage matrix during biotribological tests. Treatment with cytokines also resulted in a significantly increased coefficient of friction, with surface cracks being detected in both treated and untreated samples. The approach allows testing of currently used viscosupplements and brings prospective on development of new formulations.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Cartilage / joint and arthritic conditions

Shear stress enhances stem cell characteristics, stratification and barrier function in human corneal epithelial cells

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INTRODUCTION:The corneal epithelium is the outermost layer of the cornea containing a limbal stem cell niche that replenishes this layer differentiating into epithelial cells as they do so. When these cells are lost, transplantation of donor or autologous tissue may be required. Due to donor shortage of corneal tissue, alternative therapies are required[1].Shear stress is imparted on the corneal epithelium during blinking, the effect of this is poorly understood[2].Therefore, the aim of this study was to examine how shear stress affects differentiation and barrier function of limbal stem cells.

METHODS:A fluidic unit and pump system (ibidi) was used. Shear stress was applied for 1 and 3 days at 0.122Pa (low shear) and 0.243Pa (high shear). A static control with daily media exchange was used. Gene expression of mature epithelial markers cytokeratin 3 and 12 (CK3 and CK12) and stem cell markers cytokeratin 15 (CK15) and ATP binding cassette subfamily G member 2 (ABCG2) were examined. Zonula occludens 1 (ZO-1) and integrin β1 was examined using immunocytochemistry.

RESULTS:Stem cell markers ABCG2 and CK15 were the most significantly upregulated compared to static cultures after 1 day low shear. Mature markers were also significantly up-regulated at both time points in the presence of shear. Barrier function, integrin β1 expression and stratification of cells were all increased after 3 days high shear compared to static culture controls.

DISCUSSION & CONCLUSIONS:A significant upregulation of stem cell markers after 1 day low shear and reduced expression of mature markers suggests that this shear stress rate could be used in the ex vivo cell culture of limbal stem cells, enhancing their use in transplantation by upregulating stem cell marker genes. Longer term 3 day high shear stress increases ZO-1 tight junction formation, integrin β1- an immature marker and stratification as well as increasing mature marker gene expression which may be used as an in vitro model of the corneal epithelium. This work will aim to elucidate how corneal epithelial cells react to shear stress in a time and shear dependent manner as well as aiming to determine how this is regulated. This information will enhance our understanding of corneal epithelial biology as well as a possible novel culture technique for limbal epithelial cells that are to be transplanted.

Acknowledgements:This research has been supported by the European Research Council starting grant (EyeRegen 637460).

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Keywords: Eye, In vitro microenvironments

Modulating mechanical and biological parameters to investigate phenotypic changes in human liver sinusoidal endothelial cells

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INTRODUCTION:Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells forming the barrier between blood cells on one side and hepatocytes and stellate cells on the other side within the liver blood microcirculation. In a healthy liver, LSECs are exposed to various mechanical constraints (very weak stiffness from the subendothelial space of Disse and shear stress from the blood flow). LSECs display a discontinuous architecture due to the fusion of the luminal and abluminal plasma membrane in areas called “fenestrae”.
Capillarization is an early event in chronic liver pathologies such as cirrhosis or hepatocellular carcinoma. At the LSECs’ level, this event translates into the development of a basal membrane, appearance of specific markers and disappearance of fenestrae. The same phenomenon occurs in isolated LSEC within 48 hours when cultured in vitro.
This project aims at identifying the experimental parameters required to ensure the long-term maintaining of fenestrae.

METHODS:UV-crosslinked chemically modified gelatin-based hydrogels (Gelatin Methacryloyl or GelMA) were synthetized and characterized for their mechanical properties both at the millimetric and micrometric scale, in wet conditions, using compression tests and microindentation. Sk-Hep1 cell line (human LSEC) were cultured with VEGF onto the various hydrogels and the cell phenotype was investigated (adhesion, spreading, proliferation, viability). The presence of fenestrae and their characteristics (diameter, density, absence of diaphragm…) was assessed by scanning electron microscopy and LSEC specific functions (such as endocytosis) were also investigated. Lastly, hepatocyte-conditioned media were also applied on LSEC.

RESULTS:GelMA hydrogels with Young’s modulus of 2 and 20 kPa (well suited to mimic respectively a healthy and a fibrotic liver environment) were synthesized by using gelatins of different Bloom numbers and different experimental parameters. Sk-Hep1 cells displayed good spreading and proliferation on these gels, suggesting their good biocompatibility for cell culture. LSECs formed an endothelium currently under investigation as a functional barrier.
Hepatocyte-conditioned media (HepaRG) improved LSEC proliferation and viability, underlining the importance of LSEC and hepatocyte crosstalk for the cell phenotype.

DISCUSSION & CONCLUSIONS:One of the short-term perspectives is to perform co-cultures of functional LSECs with hepatocytes. Thus, identifying the most important factor(s) modulating the LSEC phenotype would be of great interest for the liver tissue engineering.
Moreover, it would enable the development of more relevant liver on chip models that could be used as drug screening platforms in order to assess human hepatotoxicity in the early stages of drug development.

Keywords: Biomaterials, Hydrogels and injectable systems.

Influence of peracetic acid sterilisation on the biomechanical properties of human tendons

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INTRODUCTION:Human allograft tendons are used in a variety of indications in the fields of sports medicine, foot and ankle as well as trauma surgery. In Germany, allograft sterilisation is still mandatory. While some sterilisation procedures affect tissue quality (McGilvray et al. 2011), peracetic acid (PAA) sterilisation is well-established and was shown to preserve tissue biomechanics of bone-patella tendon-bone constructs (Scheffler et al. 2005). This study aims to evaluate the influence of PAA-sterilisation on the biomechanical properties of human tendons.

METHODS:Pairs of tibialis anterior (TA) and posterior (TP) tendons were explanted and frozen according to tissue banking standards. For each pair, one tendon was sterilised in PAA leaving the contralateral tendon frozen. An a priori power analysis ($\beta = 0.8$) for paired comparisons revealed a necessary sample size of 9 in both groups. Tensile tests were performed on an InspektTableBlue (H&P, Germany). Samples were looped around bolts and cryoclamped on both ends while the midsubstance of the tendons remained free, adhering to material testing standards. Tendons received a 10-cycle preconditioning between 50 N and 250 N, a 5-minute load relaxation and a failure test with 50 mm/s speed.

RESULTS:Out of 27 donors tested, tendons of nine pairs (TP: 5; TA:4) exhibited a valid failure mode of rupture at the midsubstance and hence were included in the analysis. There was no significant difference between PAA treated and fresh frozen (FF) TA and TP tendons in terms of failure load (PAA: 2557 ± 401 N vs. FF: 2394 ± 614 N). Additionally, both types of treatment revealed similar load relaxation behaviour (PAA: 17.0 ± 4.2 % vs. 18.4 ± 5.9 %).

DISCUSSION & CONCLUSIONS:PAA-sterilisation does not negatively influence biomechanical properties of human tendons in terms of relaxation and failure loads allowing for a safe and biomechanically reliable graft. The observed failure loads are comparable to the current literature.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Musculoskeletal (inc ligament / tendon / muscle / etc)

Specific microtopographies enhance mesenchymal stem cell osteogenesis via changes to cell and nuclear architecture and mechanotransduction

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INTRODUCTION:Mesenchymal stem cells (MSCs) are sensitive to their physical environment through a process known as mechanotransduction. Our understanding of these processes has evolved alongside our ability to create culture conditions that specifically modulate different aspects of the extracellular environment. Recent developments in micro and nano-fabrication mean that it is now possible to determine the impact of precisely designed substrate topographies on MSC fate. In this study, we screen a library of microstructured surfaces for their impact on MSC morphology and osteogenesis and provide novel insights into how substrate-induced changes to MSC nuclear architecture leads to changes in osteogenic capacity.

METHODS:Microstructured surfaces were fabricated in Ormocomp® using soft lithographic methods. Surfaces were plasma treated and human mesenchymal stem cells (hMSCs) seeded at 5000 cells/cm². To induce osteogenesis, medium containing osteogenic supplements was added at 24 h post-seeding. Effects on cell and nuclear morphology was determined by FIB/SEM and immunofluorescence. Osteogenesis at day 21 was assessed by Osteoimage staining and qPCR. Cells were also treated with drugs to disrupt both the actin and microtubule networks to determine the influence of mechanotransduction on the observed effects.

RESULTS:Quantitative analysis of immunofluorescent staining showed significant changes to MSC spreading, shape, cytoskeletal and nuclear architecture in response to different microstructures. At 21 days differences in MSC osteogenesis were also observed, with a 3-fold increase in osteogenesis on the optimum pattern compared to flat controls. Enhanced differentiation was correlated with specific patterns' of nuclear deformation and chromatin reorganisation. The extent of both nuclear changes and osteogenesis could be blocked by treating the cells with drugs to disrupt the cytoskeleton.

DISCUSSION & CONCLUSIONS:Overall, the data suggest that specific microstructures can be used to enhance the osteogenesis of MSCs. The correlation of stark changes in nuclear morphology in the samples with optimum osteogenesis also suggests that nuclear mechanotransduction may play a role in this process and is supported by the fact that disruption of cytoskeletal tension abolishes these effects. Overall this work provides exciting potential as a means to pattern the surface of orthopaedic implants for better integration and stability and also illustrates new understanding of the mechanisms by which the cells respond to microstructured substrates.

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Keywords: In vitro microenvironments, Multipotent (mesenchymal) stem cells

Stem cell morphology controls protein nucelar import within a bioengineered 3D niche

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INTRODUCTION:Smart biomaterials are increasingly being used to control stem cell fate in vitro by the recapitulation of the native niche microenvironment. The environment produced by these biomaterials transmits forces through the cell cytoskeleton, activating the mechanotrasduction pathway, guiding the cell fate [1]. We developed an innovative microstructured 3D culture scaffold, named "nichoid" [2,3], that provides a geometric constraint to adhering cells, inducing nuclear deformation and modulating the nuclear import of fluorescent transcription factor Myod-GFP and the inert fluorescent protein GFP in mesenchymal stem cells (MSC).

METHODS:3D microstructure scaffolds were produced by two photon polymerization technique. 10^4 rats bone marrow MSC were seeded in the nichoid and on glass coverslip as 2D flat control. Cells were processed for immunofluorescence analysis to characterize focal adhesion localization, actin cytoskeleton organization and the nuclear deformation. To calculate the global influx of transcription factors into the cell nucleus, the MyoD-GFP fluorescent transcription factor and the inert GFP protein were transiently transfected to perform fluorescent recovery after photobleaching (FRAP) assay. A finite element computational model integrating the experimental results was performed to describe the nuclear permeability as function of the nuclear geometry.

RESULTS:By integrating experimental measurements with numerical models, we show that in mesenchymal stem cells grown inside a 3D synthetic niche both facilitated nuclear transport of the myogenic differentiation factor and the passive nuclear diffusion of a smaller inert protein are reduced. We also demonstrate that cell morphology modulates nuclear import through a partition of the nuclear envelope surface exposed to flows, which becomes a thin but extremely permeable annular portion in cells cultured on 2D glass substrates, in which the MSC nucleus has a morphology similar to a thin, stretched disk.

DISCUSSION & CONCLUSIONS:Our results demonstrate that external forces acting on cell nuclei change the permeability of the nuclear envelope both in the case of facilitated transport and passive diffusion.
Our results support the new emerging hypothesis that in stem cell differentiation, the nuclear import of gene-regulating transcription factors is controlled by a strain-dependent nuclear envelope permeability [4,5]. The outcomes represent a breakthrough in biomimetic approaches to engineering cell fate.

ACKNOWLEDGEMENTS:ERC grant NICHOID, Grant Agreement n. 646990
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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Stem cell niche

The interaction between SUN1 and Nup153 may result in the transmission of external forces to the nuclear pore complex

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INTRODUCTION:Stem cell fate strongly correlates to nuclear morphology. In previous work, we have shown that stem cells cultured in a 3D artificial "nichoid" microscaffold maintain a roundish configuration, a higher level of stemness and a reduced molecular exchange with the nucleus when compared with cells spread on 2D controls[1]. To move from correlation to causation, we hypothesize that forces transmitted to the nucleus via integrins, actins, nesprins and SUN proteins affects the nuclear pore complex (NPC) architecture and, therefore, transcription factor import/export through the nuclear envelope[2]. Here, we investigate the interaction of the nuclear envelope protein SUN1 with nucleoporin Nup153 which is found in the nucleoplasmic portion of the NPC called the nuclear basket. METHODS:We designed several protein constructs of Nup153 that spanned the entire protein sequence. Each construct was designed with both GST and AVI tags, for protein purification and bio-layer interferometry (BLI) analysis, respectively. The constructs were produced in E. coli cells and purified by affinity and size exclusion chromatography. We verified protein purity with SDS-PAGE analysis and subsequently removed the GST tag. For SUN1, we identified its nucleoplasmic domain as the region of interest for interaction with Nup153, designing two subdomains fused with either HGB1 or BDsumo tags for protein solubility. We produced the proteins in E. coli cells, purified using Ni-affinity and size exclusion chromatography, and tested Nup153-SUN1 protein interactions using BLI. RESULTS:BLI results suggest that the SUN1 N-terminal domain interacts with an N-terminal region of Nup153. By testing the different constructs of Nup153 N-terminal region we detect at least 2 different binding sites with SUN1. Neither the C-terminal nor the Zinc-finger domain of Nup153 interacts with SUN1.

DISCUSSION & CONCLUSIONS:We were able to identify a N-terminal binding domain within Nup153 that interacts, with the nucleoplasmic domain of SUN1. This result suggests that SUN1 could transmit external forces directly to the nuclear basket at Nup153. These forces may be responsible, at least in part, in a rearrangement of the nuclear basket affecting the molecular flux through the nuclear envelope shown in literature. Our next steps will be to crystallize the SUN1-Nup153 complex to solve its structure and to implement a molecular dynamics simulation able to predict how the force transmission affects the basket architecture.

ACKNOWLEDGEMENTS:ERC grant NICHOID, Grant Agreement n. 646990

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Keywords: Other

Mechanical stimulation and biomechanical characterization of cartilage micropellets with a single and new custom-made device

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INTRODUCTION:Articular cartilage is a tissue with poor self-repair capacity, which is prone to progressive destruction after injuries. In cartilage, the chondrocytes surrounded by their extracellular matrix are organized in a complex structure that plays an essential weight-bearing role in the joint and can both sense and respond to various mechanical stimuli. Cartilage micropellet is a relevant and widely used in vitro model to study cartilage growth but poorly investigated in terms of mechanical characterization because of its small size and imperfect round shape. The objective of the study was to develop an original custom-made device allowing both the mechanical stimulation and characterization of mesenchymal stromal cells (MSCs)-derived cartilage micropellets.

METHODS:Human bone marrow-derived MSCs were differentiated into chondrocytes by culture in micropellets with 10 ng/mL TGFβ3-containing inductive medium for 21 days. The fluidic-based device was designed for the concomitant culture of six micropellets placed into the conical wells of a chamber where they were stimulated by a positive pressure (sinusoidal, square or constant). The sinking of each micropellet into the cone and its deformation were recorded by a camera. Expression of the chondrocyte markers SOX9, AGG and COL2B were quantified 24 hours after stimulation by RT-qPCR. The Young modulus were determined using a finite element model employing a neo-Hookean hyperelastic law.

RESULTS:Alginate- and collagen-based microspheres were first used to validate the reliability of the device. Repeatability and reproducibility of pressure signals used for mechanical stimulation were demonstrated. The mechanical properties of the microspheres were equivalent to those determined by a conventional compression test and shown to be reproducible.

MSC-derived cartilage micropellets were stimulated with sinusoidal, square or constant pressure. Different parameters (amplitude, frequency, duration) of the square pressure signal were tested on the expression of chondrocyte genes. A stimulation of 1 Hz at 0.07 bar during 30 min induced a significant increase of chondrocyte markers. The mechanical properties of the micropellets were measured and a Young's modulus of 77.8 ± 47.8 kPa was determined.

DISCUSSION & CONCLUSIONS:The interest of this new device lies in the reliability to mechanically stimulate and characterize microspheres with radius in range of 600 to 1300 µm. Of importance in case of MSC-based cartilage micropellets, mechanical stimulation can be performed in parallel on six microspheres allowing the molecular and mechanical characterization on the same group of samples. In the future, the device will be useful to evaluate the growth of cartilage micropellets under mechanical stimuli in a longitudinal study.

Keywords: Cartilage / joint and arthritic conditions, Differentiation

In Vitro Electrical Stimulation Model System for Neural Tissue Engineering

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INTRODUCTION:The application of electrical stimulation (ES) is escalating in the field of tissue engineering (TE) and regenerative medicine. ES induces regenerative responses in cells in vitro [1] and promotes healing of defected tissues, such as bone, skin, muscle, and nerve, in vivo [2]. However, plenty of therapeutics based on ES failed clinical translation. Therefore, yet significant optimisation is needed to uncover effective ES protocols for each particular tissue in different diseased or healthy conditions, considering their unique biological complexities, and corresponding biophysical and molecular mechanisms [3]. In this particular study, we focused on the use of ES to induce neural plasticity and regeneration in brain cortical cells after stroke. Our approach is intended to develop a model system combining a relevant biological diseased model, a computational predictive model, and an effective ES device.

METHODS:Stroke model: SD-rat embryonic (E17) brain cortex cells are isolated and cultured for 10 days and were incubated in a glucose/oxygen deprivation condition for 3 hours.
Enhanced electrodes: Metallic nano-structured electrodes are fabricated using glancing angle deposition with magnetron sputtering magnetron sputtering. Their electrical properties are characterised through electrical impedance spectroscopy and cyclic voltammetry to define the electrical impedance and charge injection capacity as well as the effective surface area of electrodes.
Computational model: a computational model was built to predict the changes in the function applied to the system and the signal received by cells during ES experiment.

RESULTS:We validated our in vitro stroke model through MTT, LDH and Live/Dead/Apoptosis assays: 20 to 30% cell death and apoptosis resulted from stroke insult. Regarding the electrodes electrical properties, they showed significant decrease in the impedance of the system and increase in charge injection capacity due to the higher effective surface area of nano-structured coatings compared to thin films. Finally, our computational model allows us to obtain the actual electrical conditions within the culture medium as a function of the applied electrical parameters.

DISCUSSION & CONCLUSIONS:Standard testing platforms for ES application in TE are needed. We reflect our approach toward a well-defined in vitro ES model system and implement it for inducing neural plasticity after stroke that aims to uncover underlying mechanisms of ES and improve development of ES-based therapeutics.

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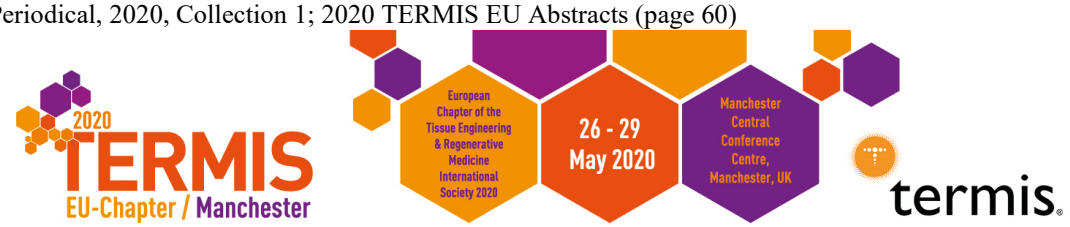
Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Nervous system (brain-central-peripheral / disorders)



The effect of miR-99a in extracellular matrix components

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INTRODUCTION:Osteoporosis is a chronic skeletal disorder characterized by an unbalance between bone formation and bone resorption. Modulation of Mesenchymal Stem/stromal Cells (MSC) towards a pro-osteogenic profile is a potential strategy to induce bone regeneration of osteoporotic fractures. miRNA has been shown to regulate several cellular processes including differentiation, proliferation, angiogenesis and inflammation [1,2]. In this context, miRNA delivery to MSC or pre-osteoblasts may promote bone regeneration/repair. Moreover, combination of miRNA-engineered cells with biomaterials might led to a synergistic effect on bone regeneration. The aim of this study is 1) to explore the impact of miR-99a in osteogenic differentiation and 2) to address the effect of miR-99a on extracellular matrix proteins.

METHODS:Human MSCs and MC3T3 cells were differentiated to osteoblasts and miR-99a expression was quantified by RT-qPCR. miR-99a levels were modulated by transfection of miR-99a mimics, inhibitor, or the respective controls. The effect on osteogenic differentiation was evaluated by ALP and calcium deposits histochemical staining. Cell proliferation/metabolic activity and cell apoptosis were evaluated by resazurin assay and flow cytometry, respectively. Novel miR-99-5p downstream targets were analyzed by mass spectrometry-based proteomics. Protein identification was performed with Sequest HT search engine against entries from the UniProt database. Clustering of cell differentiation and extracellular matrix proteins were performed according to Gene Ontology (GO) annotations.

RESULTS:miR-99a-5p expression was significantly down-regulated during early stages of hMSCs osteogenic differentiation and during MC3T3 differentiation. miR-99a overexpression in MC3T3 led to a decrease of osteogenic differentiation markers, including RUNX2 and ALP gene expression and staining, whereas its inhibition had the opposite effect. Moreover, mineralization was decreased in miR-99a-overexpressing cells. Modulation of miR-99a levels had no effect on proliferation or apoptosis of MC3T3 cells. High-throughput analysis showed that distinct intracellular proteins related with osteogenic differentiation, as well as extracellular matrix proteins, were modulated by miR-99a. These include 2 proteoglycans, namely Fibromodulin (Fmod) and Lumican (Lum), which participate in the assembly of the collagen fibers of the extracellular matrix [3].

DISCUSSION & CONCLUSIONS:miR-99a-5p acts as a negative regulator of osteogenic differentiation. Simultaneously, it can program pre-osteoblasts to produce specific extracellular matrix components.

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Keywords: Differentiation, Gene therapy

New Bone Formation in Whole Decellularized Bone Scaffold Assisted with a Vascular Pedicle

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INTRODUCTION:Decellularized allogeneic/xenogeneic bone chips primarily act as a scaffold for the growth of bone tissue from the adjacent bone by allowing for creeping substitution. Due to their lack of the osteoinductive and osteogenic potentials in comparison with autologous bone graft, decellularized bone scaffolds (DBSs) have limited application and are typically applied only for small partial bone defects in clinical settings. Herein, we aimed to generate new bone formation within a whole cortical DBS via vascularization using a vascular pedicle as an in vivo bioreactor.

METHODS:A total of 40 decellularized bone scaffolds were divided into four groups (A; DBS + DBM, B; DBS + DBM + MSCs, C; DBS + DBM + vascular pedicle, D; DBS + DBM + vascular pedicle + MSCs) and implanted into the back of five rabbits. Half of the DBSs from each group were explanted at 8 weeks after implanting, and the other half were removed at 16 weeks to determine the level of vascularization and osteogenesis within the differentially treated DBSs.

RESULTS:New bone formation and bone-forming cells related to osteogenesis were observed via histological staining (H&E, Masson's trichrome, and PAS staining). The inclusion of the vascular pedicle (group C and D) resulted in larger areas of bone regeneration (proportion rate of osteogenesis thickness to entire DBS thickness at 16 weeks; A:B:C:D =30.3%: 32.0%:61.2%:68%). With time, osteon structures became prominent in the osteocalcin immunofluorescent stain in all groups, but more prominent structures were seen in groups containing the vascular pedicle.

DISCUSSION & CONCLUSIONS:Multiple trials to vascularize tissue-engineered bone substitutes currently exist. To supplement the viability of these tissue substitutes, artificial vessels in tissue-engineered substitutes have been created by using 3D bioprinters. However, high levels of vascularization have yet to be achieved. Vessels in our bodies can act as perfect micro-bioreactors supplying nutrients to tissue substitutes, as well as decellularized bone scaffold. Herein, we sought to combine these two elements to make a vascularized DBS.Preparation of vascularized DBS may prove beneficial in overcoming the size limitations for DBSs in clinical use by providing osteoinductive and osteogenic properties for better tissue ingrowth. Furthermore, the bone regeneration time can be reduced, and the amount of avascular area within DBS can be decreased. Therefore, vascularized DBS can be used as a bone substitute for large significant bone defects.

Keywords: Biomaterials, Bioreactors

Development of Innovative Osteo-Regenerative Collagen-Bioceramic Composites for Bone Repair

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INTRODUCTION:Bone remodelling is mediated through the coordination of bone formation (anabolism) by osteoblasts and bone resorption (catabolism) by osteoclasts (1). Imbalances in the bone remodelling cycle is an underling cause of metabolic bone diseases such as osteoporosis, where bone resorption exceeds formation. Current therapeutic strategies to repair osteoporotic bone fractures focus solely in targeting anabolism or supressing catabolism (2). However, these therapeutics do not reverse the structural damage present at the defect site, ultimately leading to impaired fracture healing. Herein, we focus on investigating a combined versatile pro-anabolic and anti-catabolic effect of Magnesium (Mg^{2+}) to modulate bone cell behaviour (3), to develop a biomimetic bioactive biomaterial technology structurally designed to enhance bone formation while impeding pathological osteoclast resorption activities to facilitate better bone healing and promote repair.

METHODS:Pre-osteoblasts MC3T3-E1 (OBs) and the osteoclasts progenitors RAW 264.7 (OCs) cell lines were cultured in growth media exposed to increasing concentrations of $MgCl_2$ (0, 0.5, 1, 10, 25 and 50mM) and the optimal concentration to concurrently promote the differentiation of OBs and inhibit the differentiation or funtion of RANKL-induced OCs was assessed. We used Fluorescence Lifetime Imaging Microscopy to investigate changes in the metabolic pathways during OBs and OCs differentiation when exposed to increasing $MgCl_2$ concentrations. We developed a range of magnesium-incorporated collagen scaffolds to investigate the behaviour of bone cells in a 3D environment.

RESULTS:We reported an increase in the expression of the bone formation markers osteocalcin and osteopontin for OBs exposed to 10mM $MgCl_2$, and a significant downregulation of the osteoclast-specific markers TRAP and cathepsin K in RANKL-induced OCs differentiation when exposed to 25mM $MgCl_2$. Moreover, 25mM $MgCl_2$ induced changes in the energy metabolism of OCs from a predominantly oxidative phosphorylation towards a more glycolytic pathway, suggesting a regulatory effect of Mg^{2+} in the underlying mechanisms of osteoclasts formation and function. The developed porous collagen-magnesium scaffolds significantly reduced the expression of early osteoclastogenic markers RANK and NFkB, and an elevated expression of the osteogenic markers Runx2 and Col1A1 was reported over 7 days.

DISCUSSION & CONCLUSIONS:Our research to date has provided evidences to demonstrate the potential of Mg^{2+} to concurrently enhance osteogenesis while inhibiting osteoclastogenesis in vitro, potentially introducing new targets for developing therapies to repair osteoporotic bone fractures.

Acknowledgements:This project has received financial support of Science Foundation Ireland, SFI/AMBER (17/RC-PhD/3477).

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Keywords: Bone and bone disorders (osteoporosis etc), Composite materials

Denosumab Induced RANK Ligand Inhibition within a 3D Biomimetic Model of Ameloblastoma

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INTRODUCTION:Rare diseases such as ameloblastoma have limited research interest and therefore treatment options are not explored extensively. Currently, treatment options for this benign cancer of the jaw include radical, surgical resection of the mandible bone. Investigating whether the novel RANK Ligand (RANKL) inhibitor denosumab could actively stop bone resorption caused by ameloblastoma, would provide a less invasive therapeutic alternative (Stoppacciaro et al 2012). Within the field of tissue-engineering cancer, the utilisation of 3D high density collagen models has become extremely useful in recapitulating the cancer microenvironment within the tumour stroma.

METHODS:We utilised a well established 3D model of cancer (Pape et al 2019) and incorporated ameloblastoma cell lines AM-1 and AM-3 within a biomimetic stromal compartment. Monomeric collagen density was increased utilising the RAFT plastic compression system (Magdeldin et al 2017). Firstly, AM-1 and AM-3 cells were assessed for RANKL expression within 2D and 3D and when in co-culture with osteoblast and osteoclast cells. RANKL inhibition was then tested through the establishment of IC50 values within a 14 drug assay set up. Finally, cells were assessed for morphology and rate of invasion with LIVE/DEAD and Phalloidin/DAPI staining.

RESULTS:AM-1 and AM-3 cell lines expressed high levels of RANKL at the gene and protein levels. Other genes involved within the RANKL pathway were also investigated and high levels of RANKL upstream gene regulator AKAP11 was expressed. Both cell lines were then used within 2D and 3D to ascertain IC50 values in response to being exposed to denosumab. RANKL expression and drug response was altered within co-culture.

DISCUSSION & CONCLUSIONS:These preliminary results indicate that AM-1 and AM-3 cells can be used to establish a complex 3D model of ameloblastoma. The set up can also be used to establish preliminary drug assays in response to the RANKL inhibitor denosumab. This lays the groundwork for future knockout work in order to investigate the exact pathways involved within this response.

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Keywords: In vitro microenvironments, Drug delivery

Development of a multicellular scaffold based pancreatic ductal adenocarcinoma model

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INTRODUCTION:Scaffold assisted tumour models are considered to have better niche mimicking capability in comparison to 2D systems, due to their tuneable mechanical properties and provision of better cell-cell and cell-extracellular matrix (ECM) interactions [1].They are also easier to use as compared to animals. We previously reported a poly urethane (PU) scaffold based robust mono-cellular (cancer cells) pancreatic cancer model appropriate for short and long term chemo-radiotherapy screening [2,3]. However, the tumour niche consists of other cell types, e.g., stellate and endothelial cells, which contribute to the tumour formation, metastasis and treatment response. Thus, recent studies have focused on the generation of multicellular models [4]. However, a scaffold assisted multicellular model of pancreatic cancer is currently not available.
Herein we developed for the first time, a PU scaffold based, hybrid, multicellular 3D pancreatic tumour model using cancer, stellate and endothelial cells.

METHODS:PU scaffolds prepared using Thermal Induced Phase Separation were surface modified via absorption of fibronectin or collagen I [2]. Following initial results showing different ECM preferences for different cell types, a hybrid scaffold was fabricated. Specifically, a zonal structure with (i) endothelial and stellate cells on the collagen coated outer cylinder and (ii) cancer cells in the fibronectin coated inner scaffold was designed, followed by long term culture (4 weeks). Perfusion was established to study shear stress effects. In situ assays for monitoring cell viability, spatial organisation, ECM production were carried out.

RESULTS:Our data show that endothelial and stellate cells are able to attach and proliferate on coated and uncoated PU scaffolds for 4 weeks but prefer collagen I coating. We show the feasibility of maintaining long term a tri-culture system on both uncoated and coated PU scaffolds and have established a zonal multicellular 3D model showing extensive desmoplastic reaction in the presence of stellate cells, mimicking a key in vivo characteristic of pancreatic cancer.

DISCUSSION & CONCLUSIONS:Our data show, for the first time, the feasibility of PU scaffolds to support a dynamic multicellular pancreatic tumour niche growth along with the possibility for ECM mimicry and recapitulation of desmoplasia.

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Keywords: Cancer, In vitro microenvironments

Biomimicry of the hypoxic tumour microenvironment of a pancreatic ductal adenocarcinoma – towards hypoxia associated radio-resistance profiling

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INTRODUCTION:The interdisciplinary field of tissue engineering is emerging to bridge the gap between simplistic 2D cell culture models and complex animal xenografts, more readily emulating tumour architecture, porosity, cell-cell and cell-matrix interactions, as well as hypoxic regions [1]. Non-specific symptoms and high metastatic occurrence of pancreatic ductal adenocarcinoma (PDAC) results in devastatingly low patient survival rates [2]. The PDAC tumour microenvironment (TME) is extremely complex and hypoxic [3]. PDAC radio-resistance and treatment failure is largely associated with this dense hypoxia. We have recently developed a 3D porous polymeric scaffolding system to support long term PDAC cell growth and *in vivo* properties [1]. Furthermore, we have shown that this system enables realistic short-term and long-term chemoradiotherapy screening [4]. Utilising this system, the aim of this work is to incorporate long-term hypoxic exposure, in order to investigate the potential hypoxia induced radio-resistance for the clinical application and optimisation of radiotherapy.

METHODS:3D polymeric scaffolds were fabricated via TIPS [5]. PANC-1 cells were seeded and cultured for 4 weeks before being placed at 5% oxygen in a hypoxic chamber. Thereafter, radiotherapy was performed in the scaffolds. Scanning electron microscopy and confocal laser Scanning microscopy (CLSM) enabled scaffold characterization, allowing analysis of cellular organisation and viability. More specially, live/dead analysis and hypoxic biomarkers permit quantification and spatial mapping of radio-resistance.

RESULTS:The 3D PU scaffold sustained prolonged PANC-1 cell growth for several weeks. ICLSM revealed the spatial distribution of cell viability. Hypoxia levels similar to PDAC tissue were observed via oxidative stress markers up-regulation. A positive correlation was drawn between oxidative stress and the presence of radio-resistance.

DISCUSSION & CONCLUSIONS:This research provides a platform for studying the complex and hypoxic PDAC TME. Our data show for the first time, a 3D scaffold supporting long term hypoxic PDAC cell culture. This system enables hypoxia-associated radio-resistance profiling of PDAC.

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Keywords: Cancer, Disease models

Investigation on the effect of matrix adhesion strength in the metastatic pattern of tumor spheroid invasion

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INTRODUCTION:The main threat and the reason for most cancer deaths are metastases. During the metastasis, invasion of cancer cells plays an important role that leads to the development of malignancy. To understand the specific patterns of tumor invasion and migration, as well as the relationship between cells, extracellular matrix (ECM) and substrate. Engineered surfaces that not only can control the mechanical properties of the ECM structural but also can regulate cell adhesion, morphology, migration and other processes germane to cancer metastasis.

METHODS:T24 bladder cancer cells were grown into multicellular tumor spheroid (MTS) prior to embedding in to collagen type I. The MTS-collagen mixture were then put onto the surface of either glass or polydimethylsiloxane (PDMS) membrane, where the surface modification was performed using Poly-D-Lysine (PDL), glutaraldehyde (GA) and pluronic. For PDL surface modification, the PDMS substrate was treated with 1 mg/mL PDL solution for 4 h, followed by DI water rinsing. For glutaraldehyde coating, the substrate was treated with 10% v/v APTES for 1 h at 60°C, followed by 1 h at room temperature of 0.5% v/v glutaraldehyde. Pluronic was directly coated on the substrate for 30 min incubation at room temperature. Migration of T24 MTS invading ECM at matrix-substrate interface was observed for 24 h through time-lapse imaging.

RESULTS:The matrix-substrate adhesion force was altered based on the surface modification performed on the surface. Given that GA coating is known to provide covalent crosslinking with collagen, and PDL could enhance physio-electrostatic interaction, the matrix-substrate interface was adhered better as compared to hydrophilic modification by pluronic that prevented matrix-substrate binding. Results showed that cells preferentially migrated more on the glass substrate than that of the PDMS due to durotaxis. Furthermore, MTS was found to preferentially migrate collectively in PDL and GA coated conditions at 5 h. However, due to different interlayer strength, MTS invasion was more amplified in the PDL condition compared to the GA. Interestingly, MTS was shown to leverage individual migration in pluronic coated condition.

DISCUSSION & CONCLUSIONS:Our results indicated that the behavior of the MTS invasion could not only affected through local substrate stiffness but also the different matrix-substrate adhesion strength. It is worth noting the current setup could be further explored on the early metastatic pattern for better cancer research.

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Keywords: Cancer, In vitro microenvironments

Inhibiting cancer metabolism with aromatic N-glucosides that act as GLUT1 antagonists and substrates for biocatalytic self-assembly

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INTRODUCTION:Cancer cells are distinguished by accelerated metabolism, the associated high energy demand and increased glucose uptake known as Warburg effect.[1] Herein, we designed an approach that targets selectively the accelerated cancer metabolism by two synergistic pathways: by formation of a supramolecular net around the cancer cells via biocatalytic self-assembly (BSA)[2] and by the inhibition of the glucose transporter GLUT1 that is overexpressed in most cancers. We tested this approach in 2D cell cultures but also in 3D spheroids[3] to recapitulate the complexity and heterogeneity of the tumour microenvironment.

METHODS:We designed and synthesized two aromatic glucose derivatives, namely fluorenylmethoxycarbonyl-glucosamine-6-phosphate(FmocGlcP) and fluorenylmethoxycarbonyl-glucosamine(FmocGlc). These amphiphiles can self-assemble into a nanofibrous network.4 The glucose unit allows selective targeting of GLUT1 pocket that was confirmed by in silico studies. In vitro tests were performed using 2D cultures and 3D spheroids of osteosarcoma(SaOs2) and breast cancer(HS587T) cells that overexpress GLUT1 but have different expression of alkaline phosphatase(ALP) – the enzyme that triggers the self-assembly. Knockdown of GLUT1 and ALP inhibition was also carried out to confirm the involvement of these two proteins in the selective cancer cell death.

RESULTS:We observed that the cell death is both concentration and time dependent: at shorter time (30 min) and lower concentration (0.5 mM) of amphiphile the GLUT1 antagonist effect is predominant. Decrease in glycolysis was confirmed by a reduction of lactate production. BSA was observed(SEM) only for cells that overexpress ALP and for longer (more than 7h) culture time. In these cultures, inhibition of ALP rescues cells from death, demonstrating the involvement of this enzyme in activating apoptotic/necrotic pathways. In both cultures (SaOs2 and HS5887T), GLUT1 depletion significantly reversed the toxicity of FmocGlcP. GLUT1 overexpression was much pronounced in 3D spheroids when compared to 2D cultures. This difference explains the higher efficiency of our treatment under a 3D environment. Finally, the efficacy of the treatment was also proven by re-plating the spheroids after treatment. We did not observe any relapse, i.e. de novo formation of a spheroid.

DISCUSSION & CONCLUSIONS:Our results demonstrate that FmocGlcP is a good candidate for selective and efficient cancer therapy, as it acts via a synergetic mechanism consolidating its role as GLUT1 antagonist and as a substrate for BSA.

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Keywords: Cancer, Biomaterials

Evaluation of different hydrogels for a 3D biofabricated cancer model

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INTRODUCTION:There is a high demand for reliable in vitro and in vivo cancer models to understand and study tumor pathophysiology and investigate potential new therapeutic approaches. Here, the emerging technology of biofabrication offers novel possibilities to simulate heterogenic tumor-type-specific microenvironments as closely as possible.

METHODS:The behavior of different breast cancer (MCF-7, MDA-MB-231) and melanoma (Mel Im, MV3) cell lines was studied in vitro in different hydrogels (alginate, alginate dialdehyde crosslinked with gelatin (ADA-GEL), thiol-modified hyaluronan (HA-SH crosslinked with PEGDA)) using viability assays and microscopy over 14 days. The viscoelastic properties of the applied hydrogels were characterized using dynamic mechanical analysis. For testing printability of melanoma cells, we further used two commercially available bioinks (alginate-based/gelatin-methacrylate-based) with and without RGD- and laminin-modification. Matrigel served as established control material for the printing experiments.

RESULTS:In low percentage alginate and HA-SH, all cell lines showed a significant increase in cell number over culture time while only Mel Im increased in viability in ADA-Gel. Overall, melanoma cell lines preferred ADA-GEL and HA-SH compared to breast cancer cells. When increasing the polymer concentration, these effects also became significant for HA-SH. In 1% alginate cell lines showed similar behavior. Higher concentrated alginate-gels resulted in stiffer gels and led to a reduction in cell proliferation. The printability of melanoma cells could be proven in all biomaterials with significant differences in the subsequent cellular behavior over 14 days. While cells proliferated and spread strongly in the control (Matrigel), alginate-based bioinks showed no cell proliferation at all and in gelatin-methacrylate-based bioinks a strong cell cluster formation was visible. RGD- and laminin-modification of bioinks did not result in an improved cellular behavior[1].

DISCUSSION & CONCLUSIONS:With this study, we could demonstrate that different cancer cell lines show typical characteristics in vitro that could also be observed in vivo. In particular, in 1% alginate and HA-SH all cell lines showed good proliferation while maintaining the expected tumor heterogeneity. Printing of melanoma cells indicated the importance of precise bioink adaption according to the demands of the applied tumor cells. By demonstrating a successful printing of melanoma cells, we paved the way to establish a biofabricated in vitro tumor model that is currently transferred into an in vivo model to study tumor heterogeneity and progression.

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Keywords: Biofabrication, 3D printing and bioprinting

Silk biomaterials for 3D tissue engineered tumor models for drug delivery

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INTRODUCTION:Cancer is considered a complex disease where multiple factors interact together to remodel the tumor niche and drive the malignant progression. Three-dimensional cancer models are gaining attention because they are able to recapitulate the interplay among cancer and stroma cells together with the extracellular matrix. [1] Biomaterials such as silk fibroin offer biochemical and mechanical cues to mimic the cancer niche. It has already been demonstrated how silk fibroin is able to copycat hepatocarcinoma and breast adenocarcinoma niches. [2] [3] In this work, silk protein fibroin from Bombyx mori silkworm is used alone and is blended with gellan gum to recapitulate the native cancer microenvironment. The developed 3D cancer models are also suitable as tools for drug testing.

METHODS:Freeze-dried scaffold with 2 wt% silk fibroin are prepared. Breast cancer cells and normal fibroblasts are seeded on the freeze-dried silk fibroin scaffolds and are cultured for 14 days. At day 14, 3D bioengineered breast cancer models are treated with doxorubicin. The cell growth inhibition is assessed at 24, 48 and 72h post-treatment. Silk fibroin is also blended with gellan gum at different concentrations [4] to improve the mechanical properties. Saos2 and human adipose stem cells are seeded together in order to develop a 3D osteosarcoma model. In both models, the cell proliferation is evaluated by mean of Alamar blue assay, while cell morphology and distribution are observed by mean of confocal microscopy after staining with DAPI/Phalloidin.

RESULTS:The 3D osteosarcoma model thus established indicates the formation of cancer spheroid in a typical mechanical stiffness of the hydrogel. The other experimental results demonstrate that a heterotypic breast cancer model, made of fibroblasts and cancer cells, is more resistant to doxorubicin treatment in comparison to monoculture.

DISCUSSION & CONCLUSIONS:In this work, we present two different bioengineered 3D cancer models that can recapitulate cancer cells migration as well as fibroblasts role in drug response. The platforms designed may represent promising model for understanding the crosstalk between cancer cells, fibroblasts and extracellular matrix.

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Keywords: Disease models, Biomaterials

Friend or foe? Transglutaminase-2 and a complex role in the tumour microenvironment

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INTRODUCTION:Development and progression of cancer is dependent on complex interactions between cancer cells and the tumour microenvironment (TME), which lead to extensive tissue remodelling. Excessive deposition and enzymatic modification of matrix components create a stiff, fibrous microenvironment, driving cancer progression through biomechanical signalling. Transglutaminase-2 (TG2) is a protein cross-linking enzyme prominently expressed in the TME and linked to progression of a range of cancers. Paradoxically, TG2 appears to restrict tumour spheroid growth in vitro through matrix cross-linking - yet TG2 in patients is linked to poorer disease outcome. To investigate the complex role of TG2, this work examined how cellular communication within the TME determines TG2 expression in colorectal cancer (CRC), and how this links to invasive behaviour and treatment response in a range of in vitro TME models.

METHODS:3D culture models were prepared using primary colorectal fibroblasts or human foreskin fibroblasts (HFF2), embedded in Type I collagen/Geltrex matrix and seeded with SW480 primary CRC cells. After 7 days of culture, gels were fixed, paraffin embedded and sectioned for analysis. Haemotoxylin/eosin and sirius red staining was performed, and immunofluorescent microscopy used to localise TG2 expression. For co-culture experiments, fibroblasts and CRC cells were stained with PKH dyes, and TG2 expression analysed by flow cytometry. Finally, the TG2 inhibitor LDN-27219 was used to probe how TG2 activity influences uptake of doxorubicin in vitro using fluorescence monitoring. Protein expression analysis was performed by Western blotting for TG2 and smooth muscle actin (SMA) normalised to F-actin.

RESULTS:Immunofluorescent imaging data indicated that TG2 expression was prominently expressed around the invasive edge of tumour spheroids - however distinct regions of lower expression were observed, associated with regions of localised CRC invasion. Flow cytometry analysis demonstrated that co-culture of labelled CRC cells with fibroblasts led to decreased expression levels of TG2 in the fibroblasts, and comparison of paired cancer-associated and normal fibroblasts suggested that expression of TG2 was reduced in cancer-associated lines derived from patients. Finally, inhibiting TG2 led to increased uptake of doxorubicin in CRC spheroids.

DISCUSSION & CONCLUSIONS:Our data indicates that TG2 restricts CRC growth and invasion, localised loss of TG2 promotes invasive behaviour, and CRC cells can down-regulate fibroblast TG2 to facilitate this. However, TG2 also appears to restrict bioavailability of chemotherapy agents in spheroids. We propose that short-term restriction of tumour growth may come at the cost of reduced treatment efficiency with implications for patient treatment and outcome.

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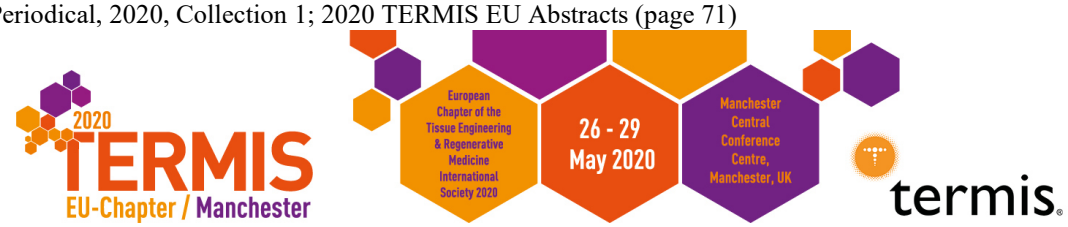
Keywords: Disease models, Microenvironment and niche engineering



Building of human cardiac composite engineered systems with Melt Electrowriting

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INTRODUCTION:The successful generation of bioengineered tissues stands on the cornerstone of the precise reproduction of the natural properties of that tissue, biological and physical. Cardiac tissue is characterised by its relatively low Young´s Modulus (~10s kPa) whilst being capable of sustaining the almost infinite strain-relaxation cycles of cardiac pumping. Although hydrogels have proven capable of reproducing the former, they usual fail to maintain their integrity under strain. Fibre-reinforcing technologies as Melt Electrowriting (MEW) constitute a game changer in this area.

METHODS:Medical grade poly-ε-caprolactone (PCL) was employed to fabricate arrays of MEW fibres for hydrogel reinforcement. A conventional hydrogel, Matrigel, was employed throughout the work, and the system mechanically characterized. Human induced pluripotent stem cells (hiPSC) were differentiated to cardiomyocytes (CMs) by means of chemical biphasic modulation of the Wnt-pathway, followed by metabolic enrichment in glucose-depleted lactate-supplemented medium. hiPSC-CMs were embedded in matrigel, casted on MEW-PCL fibres and cultured for 28 days. Gene expression (RT-qPCR), structure (IF) and functionality (optical mapping) were assessed and compared with conventionally-cultured hiPSC-CMs in 2D.

RESULTS:Scaffolds were MEW-printed in medical grade PCL, with fibres 10 µm in diameter. Fibre reinforcement resulted in a significant upgrade of mechanical properties measured by rheometry. Differentiation of 3 lines of hiPSCs yielded cultures of >80% cTnT+ cells which were successfully incorporated to the scaffolds. hiPSC-CMs formed microtissues and were able to survive and to contract macroscopically for the duration of the experiments (4 weeks). Gene expression showed a significant downregulation of the foetal-expressed isoform of MYH, MYH6, and of the pacemaker-related gene HCN4, and a concomitant uregulation of the ventricular isoform of MYL, MYL2. Although no difference was found in the Z-band span versus 2D-cultured hiPSC-CMs, the composite system was able to align the cells in the direction of the fibres, and direct contraction. Analysis showed a more mature functionality as compared with 2D controls on matrigel. This was related to an increased expression of the fast conducting gap junction Cx40 (GJA5) and an increase in the sodium channel SCN5A. Surprisingly, no difference was found on the expression of calcium-cycle regulators as CACNA1C, RYR2 or ATP2A2.

DISCUSSION & CONCLUSIONS:MEW fibres were able to strengthen a hydrogel matrix, resulting in the generation of more mature hiPSC-CM-only microtissues at the functional, structural and gene expression levels. All in all we have planted the seeds for the generation of advanced cardiac engineered tissues.
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Keywords: Induced pluripotent stem cells, Biofabrication

In Situ Tissue Engineering Vascular Access Grafts in a Large Animal Model

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INTRODUCTION:Vascular access is considered the Achilles' heel of hemodialysis, resulting in frequent and recurring inverventions[1]. Autologous vascular grafts offer the best patency rates when creating vascular access, usually by means of an arteriovenous(AV) shunt. However, pre-existing disease, previous harvesting, and/or unsuitability limits their employability. Synthetic alternatives like ePTFE are currently used, however, these are susceptible to infection, intimal hyperplasia, and/or thrombosis[2]. We aimed to create an off-the-shelf, porous, biodegradable self-healing vascular access graft by means of *in situ* tissue engineering that *in vivo* gradually transforms into a living vascular graft with improved long-term functionality. We applied our biodegradable supramolecular elastomeric materials platform, which enabled us to modulate the mechanical properties of the graft material and biofunctionalize our graft with selective cell adhesive molecules to maximise cellular integration. We investigated the remodelling and functional capacity of these AV-grafts and the added benefit of bio-functionalisation with an SDF1α-derived peptide.

METHODS:We created an electrospun porous, biodegradable, vascular scaffold (7cm length- 6mm diameter) made of a supramolecular polymer, reinforced with a PCL 3D-printed coil to prevent kinking. 7 of a total of 15 graft have been implanted so far, in combination with ePTFe controls. A subset of grafts were functionalized with a bioactive SDF1α-derived peptide additive that was supramolecularly linked to the polymer material[5]. Grafts were implanted bilaterally in Dutch milk goats (ca.60kg) as an AV shunt with end-to-side anastomoses connecting the carotid artery and jugular vein in a straight configuration. Patency and flow was monitored monthly by ultrasound and grafts were explanted after 3 months. Explants were analyzed for mechanical properties, polymer material breakdown, cellular infiltration, extracellular matrix deposition and tissue architecture.

RESULTS:After 3 months most grafts were still patent, showed remodeling, and bore no signs of aneurysm, or other deformations. The majority of the synthetic material has been resorbed and replaced by autologous tissue consisting of vascular cells (endothelial and smooth muscle cells) and extracellular matrix. Our experimental grafts and control ePTFE grafts showed equal rates of stenosis. No endothelial layer was found in the ePTFE control grafts.

DISCUSSION & CONCLUSIONS:Our first implantations of an off-the-shelf, biodegradable, porous, non-kinking vascular scaffold in a goat model shows a clear remodeling response which gradually replaced the synthetic material by autologous vascular tissue. Our results show great promise for *in situ* tissue engineering of vascular access grafts in hemodialysis patients.

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Keywords: Cardiovascular, In vivo and animal models

Thymosin β4 leads the adult epicardium to embryonic phenotype and promotes EMT in *ex-vivo* organotypic culture

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INTRODUCTION:The epicardium is the most external layer of the heart. Following cardiac injury, the epicardium contributes to the repair both by differentiating into fibroblasts and vascular cells, and by releasing paracrine factors [1]. This remarkable plasticity of the epicardium represents a unique target to induce a coordinated and efficient repair of the whole ischaemic area, reducing pathological remodelling. In this project, we developed a robust ex-vivo 3D organotypic model to study the role of the epicardium in heart regeneration.

METHODS:Thin epicardial/cardiac tissue slices (EpCardio-TS) are cut from porcine hearts using a high precision vibratome [2]. Slices are cultured for up to 72h in a bioreactor based system on a 3D printed chamber connected to a peristaltic pump and a feedback control system, ensuring stable and tuneable culture conditions (pH, pO2, Temperature). Epicardial cells are tracked by localised intracellular delivery of fluorescent quantum-dots using nanoneedle-mediated transfer [3]. Slices morphology is visualised in 3D by immunofluorescence on decolourised slices.

RESULTS:EpCardio-TS can be obtained consistently, presenting both a healthy epicardium and an electrically connected myocardium. The optimized culture conditions preserve the viability of the epicardium upon culture as indicated by calcein staining (T0: 65.6±19.1%; 24h: 60.2±26.1% 48h 72.9±26.9%) and confirmed by tunel assay (<9% WT1+ apoptotic cells). The addition of Thymosin β4 (Tβ4) reactivated the epicardial cells within EpCardio-TS yielding a significant increase of the WT1+ cell number and a boost of proliferative PCNA+ epicardial cells. Moreover, the gene expression analysis confirms the regenerative activity of Tβ4 showing an upregulation of embryonic epicardial markers (WT1, Tbx18 and Epicardin), as well as the triggering of epithelial to mesenchymal transition related genes (Snai1, Snai2 and Twist).

DISCUSSION & CONCLUSIONS:Our 3D organotypic culture enables to investigate *in vitro* the interactions between epicardial cells with myocardial and non-myocardial cells of the heart during tissue remodelling. In this project, we also assessed the regenerative effect of Tβ4 on adult epicardial cells. These data confirm the prominent role of the epicardial cells in the myocardial repair and proposes a new tool to explore the complex interactions in the heart environment helping to reduce the use of animal models.

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Keywords: Cardiovascular, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Lactate: a novel signal for cardiac reprogramming

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INTRODUCTION:Lactate is a typical metabolite of glycolysis, commonly produced by cells. It is also used as a substrate for rapidly dividing cells, and growing evidence suggests that lactate can act as a signaling molecule and key factor in many processes. Here we explored the potential role of lactate on cardiac dedifferentiation and cellular reprogramming for tissue regeneration applications.

METHODS:Neonatal mice cardiac cells were isolated and incubated with L-lactate solution. Human iPSCs were differentiated to cardiomyocytes by GSK3 and Wnt inhibition. Proliferation was assessed by ki67 and AuroraB immunofluorescence and confocal microscopy. The expression of different genes was evaluated by RT-qPCR and whole transcriptome sequencing. Whole neonatal mice hearts were also maintained ex vivo in Matrigel and cultured with lactate.

RESULTS:Immunostaining analysis with ki67 revealed an increase in proliferating mice cardiomyocytes in the presence of lactate. Moreover, different transcription factors involved in stem and progenitor cell regulation, such as p63, were upregulated in the presence of lactate as revealed by RT-qPCR. Ex vivo culture of neonatal mice hearts showed a better maintenance of the integrity of the heart tissue up to 8 days in lactate, as well as an enhanced beating ability compared to control without lactate. This result was correlated with a higher sarcomere width in cardiac tissue from hearts incubated in lactate, as confirmed by TEM image analysis.
Therefore, the effect of lactate on human iPSCs-derived cardiomyocytes was also evaluated. MTS assay was used to assess the viability of these cells with different concentrations of lactate. The results obtained on mice were confirmed on human iPSCs-derived cardiomyocytes, both the enhancement in proliferation by immunofluorescence of AuroraB and the increase in the expression of cardiac progenitor genes. Furthermore, whole transcriptome sequencing of these cells shed light on the molecular mechanisms behind the cardiac reprogramming of lactate. These results suggest that lactate-based biomaterials can be used to modify the microenvironment of cardiac tissue and promote regeneration.

DISCUSSION & CONCLUSIONS:These data support the use of lactate, a simple metabolic molecule, as a modulating signal for reprogramming of cardiomyocytes towards dedifferentiation and thus its potential use for cardiac tissue engineering.

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Keywords: Microenvironment and niche engineering, Induced pluripotent stem cells

Biohybrid cardiovascular implants based on elastin-like recombinamers

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INTRODUCTION:One of the main challenges in cardiovascular tissue engineering consists in developing implants able to function under the demanding conditions of the cardiovascular system. To achieve this, we propose a biohybrid approach in which we combine the long-term performance and stability of textile technical components with the bioactivity, hemocompatibility and elasticity of an advanced class of bioinspired recombinant polymers known as elastin-like recombinamers [1].

METHODS:Two ELR modified with the azide and cyclooctines groups for click-chemistry [2] were used as the bio-based component of the biohybrid devices. Technical components included warp-knitted polyvinylidene fluoride (PVDF), electrospun polycaprolactone meshes and Co-Cr-alloy coronary stents. The materials were processed by injection-molding, salt-leaching/gas foaming, electrospinning and layer-by-layer dip coating to fabricate cardiovascular implants such as vascular grafts and coronary stents. The microstructure was investigated by scanning electron microscopy. The thrombogenicity was assessed by platelet adhesion studies. The mechanical characterization included burst strength, suture retention and compliance tests. Cellular studies were carried out with smooth muscle cells and endothelial progenitor cells, and subsequently analyzed by immunohistochemistry and confocal microscopy.

RESULTS:The biohybrid vascular grafts featured elastic properties and hemocompatibility provided by the ELRs, besides suturability, long-term stability, burst strength and tunable compliance provided by the technical textile components. Cellular studies showed cell infiltration, extracellular matrix production, and endothelialization, which make these grafts of high interest for endogenous tissue regeneration [3]. The ELR-covered coronary stents withstood an in vitro simulated implantation procedure by balloon dilatation without structural damage [4], which evidenced the elastic performance of the membrane. Additionally, the ELR-membrane showed minimal platelet adhesion and enabled endothelialization.

DISCUSSION & CONCLUSIONS:Overall, the elastic performance and hemocompatibility provided by the ELR together with the stability of the technical components has resulted in promising off-the-shelf cardiovascular implants to treat the narrowing or blockage of small-caliber arteries.

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Keywords: Biomaterials, Biofabrication

Transient Reprogramming of Cardiomyocytes to a Proliferative De-Differentiated State

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INTRODUCTION:Transient dedifferentiation of cardiomyocytes has been established as a key mechanism behind the enhanced proliferation and cardiac regenerative capacity observed in model organisms such as the zebrafish[1]. We have previously demonstrated that somatic cells in mouse liver and skeletal muscle can be reprogrammed temporarily to a proliferative, dedifferentiated state in vivo through the forced expression of Oct3/4, Sox2, Klf4 and c-Myc (OSKM)[2 3].

METHODS:Here, we used a non-integrating adenoviral vector encoding OSKM to investigate transient reprogramming in postnatal cardiomyocytes in vitro and in the adult mammalian heart in vivo.

RESULTS:Forced expression of OSKM in cultures of postnatal rat and mouse cardiomyocytes induced rapid dedifferentiation characterised by decreased cardiac gene expression, sarcomere dis-assembly and increased cell cycle activity. The generation of cells positive for epithelial markers further indicated the initiation of cell reprogramming. However, this response appeared to be partial and no pluripotent-like cell colonies were generated. Instead, pluripotency and cardiac related gene expression spontaneously returned to baseline levels coinciding with the restoration of normal autorhythmic contractile activity. OSKM expression in the myocardium of adult mice in vivo was achieved by a single intra-myocardial injection of the same vector. Consistent with the response in vitro, upregulation of endogenous pluripotency genes (Oct3/4 and Gdf3) was observed suggesting dedifferentiation of resident cells. The increased expression of such genes was maintained only temporarily consistent with a transient and partial reprogramming response.

DISCUSSION & CONCLUSIONS:Further investigations are ongoing to elucidate the differentiation state and fate of reprogrammed cardiac cells. Our results to date suggest that transient in vivo reprogramming in the heart is possible and could provide a novel gene therapy approach to enable tissue rejuvenation and regeneration.

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Keywords: Gene therapy, Induced pluripotent stem cells

Bioengineered Transmural Cardiac Constructs

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INTRODUCTION:Human induced pluripotent stem cells (hiPSs) are the most promising source for generating *in vitro* 3D cardiac tissue models, but currently do not fully replicate mature myocardium and lack 3D macroscopic tissue organization. Previously, we fabricated melt electrowritten (MEW) microfiber scaffolds with hexagonal 3D microstructures with similar biomechanical properties to the native myocardium that support contracting hiPS-derived cardiomyocytes (hiPS-CMs) and drive tissue organization and maturation[1]. However, these relatively thin microfiber scaffolds cannot (yet) mimic the specific transmural 3D arrangement and thickness of the cardiac muscle. We hypothesized that by incorporating other cardiac cell lineages, and by providing contact guidance, and reinforcement through strategically stacked multi-layered MEW meshes, will enable us to better replicate the native cardiac muscle structure and function. In this study, we present the generation of a human bioengineered transmural cardiac construct (TmC).

METHODS:Hexagonal microarchitectures (side length=400µm, thickness=420µm) were fabricated with a 3DDiscovery printer (RegenHU). Three candidate hydrogels for cardiac-cell seeding were employed: 0.2%/0.5% collagen/agarose, 5 wt% GelMA and 5 wt% once-autoclaved GelMA and evaluated for their effect on tissue functionality (contraction) and morphology (immunostainings). TmCs were generated by stacking multiple MEW meshes and were seeded with hiPS-CMs and hiPS cardiac fibroblasts (hiPS-cFBs)[2] (9:1) in 5% GelMA and cultured for four weeks. TmC function, morphology, and maturation were evaluated using beating rate, cell-specific immunofluorescent staining, histology, and qRT-PCR.

RESULTS:Cardiac cell seeding in 5% GelMA demonstrated increased rhythmic beating (26.7±2.3 bpm), and improved tissue-like alignment compared to the other two hydrogels. The manufactured TmCs were 8mm in diameter and 10mm in thickness. Beating cell clusters were observed 3-4 days after TmC seeding, proceeded by whole construct synchronized beating (40bpm) after 10-14 days, including construct radial deformation of approx. 1-2mm. Histology sections highlighted a clear multi-layered structure with a uniform cellular distribution. Immunofluorescent staining showed hiPS-CMs and hiPS-cFBs co-organized in tissue-like structures, aligned with the elongated longest direction. An increased expression of cardiomyocyte maturation-related genes (RYR2/TNNI3/CPT1B) was observed in the TmCs compared to single-mesh constructs.

DISCUSSION & CONCLUSIONS:Through combining hiPS-CMs and hiPS-cFBs in a 5% GelMA, multi-layered MEW construct further enhanced cardiac tissue organisation, maturation, and functionality. Our study provides an important step towards the generation of 3D *in vitro* cardiac models of relevant dimensions with native-like tissue architecture and function.
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Keywords: Vascular systems / vascularisation and heart, Biofabrication

Treatment of osteochondral defects of the knee using bilayered scaffold-free constructs in rats

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INTRODUCTION:Restoration of deep osteochondral defects represents a significant unmet clinical need. Moreover, untreated lesions lead to a high rate of osteoarthritis[1]. Current strategies to repair osteochondral defects such as osteochondral grafting or “sandwich” strategies (e.g. bone autografts plus ACI) fail to generate long-lasting osteochondral interfaces. This study aimed at providing proof-of-principle of the increased capacity of immature Osteochondral Grafts (OCGs) to repair deep osteochondral defects of the knee, and generating biomimetic TE constructs inspired by the immature OCGs.

METHODS:Dermal punches were used to transplant cylindrical OCGs from the knee of skeletally immature rats into osteochondral defects created in skeletally mature rats. To create bilayered TE constructs, micromasses of human periosteum-derived progenitor cells (hPDCs) and human articular chondrocytes (hACs) were produced in vitro using chemically defined medium formulations. These constructs were subsequently implanted in osteochondral defects created in the knees of nude rats.

RESULTS:Our results showed that the osteochondral unit of the knee in 5 weeks old rats exhibit an immature phenotype, displaying active subchondral bone formation through endochondral ossification and the absence of a tidemark. When transplanted into skeletally mature animals, the immature OCGs resumed their maturation process, i.e., formed new subchondral bone, partially established the tidemark, and maintained their Safranin O-positive hyaline cartilage at 16 weeks after transplantation. The bilayered TE constructs (hPDCs + hACs) could partially recapitulate the cascade of events as seen with the immature OCGs, i.e., the regeneration of the subchondral bone and the formation of the typical joint surface architecture, ranging from non-mineralized hyaline cartilage in the superficial layers to a progressively mineralized matrix at the interface with a new subchondral bone plate.

DISCUSSION & CONCLUSIONS:Our results suggested that the immature OCGs hold the capacity to repair deep osteochondral defects successfully in skeletally mature animals. The bilayered TE constructs could partially recapitulate this regenerative process, including the formation of new subchondral bone and generation of the typical joint surface architecture, including similar tissue structure and zonation. In summary, while immature OCGs provide an attractive model to investigate osteochondral maturation and repair, cell-based TE constructs displaying a hierarchically organized structure comprising of stable and transient cartilage intermediates seem an attractive strategy to repair osteochondral defects of the knee.

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Keywords: Cartilage / joint and arthritic conditions, Stem cells - general

Regeneration of Articular Cartilage by Activated Skeletal Stem Cells in Mouse and Human

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INTRODUCTION:The ability to regenerate cartilage following a diagnosis of Osteoarthritis (OA) has eluded many plastic and orthopedic surgeons. While there are preliminary pilot studies attempting to assess functional outcomes with “stem cell” therapies, there is no evidence at a cellular level of their efficacy. We have recently identified both mouse and human Skeletal Stem Cells (SSC) with the ability to form bone cartilage and stromal tissue.

METHODS:Mouse Data: Clonality was assessed using our Actin Cre ER mouse model. FACS analysis was performed using our published mSSC profile. SSC activation was performed using Microfracture surgery. Proliferation was assessed using EdU assays by IHC and intracellular FACS. Cellular origin was assessed with our parabiosis model. Gene expression was assessed using Microarray. Differentiation capacity was assessed in vitro and in vivo (both subcapsular and orthotopic). Biochemical manipulation used hydrogel and collagen scaffolds orthotopically. Outcomes were assessed by pentachrome staining and Atomic Force Microscopy.

Human Data: Freshly isolated cartilage was isolated from human fetal and adult articular cartilage. A novel xenograft model was used to assess MF surgery and fate skewing using hydrogel application. RESULTS:With skeletal maturity there is a significant reduction ($p<0.001$) in articular SSC, with a concomitant reduction in proteoglycan production at the articular surface in mouse and human. Following MF surgery there is a significant activation ($p<0.001$) of local SSC in mouse and human tissues. In mouse tissue, MF surgery leads to an intrinsic genetic "rejuvenation" leading to formation of fibrocartilage. With niche augmentation using BMP2 and VEGFr1 the regenerate forms articular cartilage (distinct from the fibrous scarred tissue) repeatedly which remains stable in both mouse and human tissues.

DISCUSSION & CONCLUSIONS:We are the first group to show that MF surgery has any significant effect on SSC in mouse and human tissue. We show that local application of BMP2 and VEGFr1 leads to cartilage differentiation of SSC in both species providing an exciting new clinically relevant approach for treating OA.

Acknowledgements:The Plastic Surgery Foundation- National Endowment to MPM.
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Keywords: Stem cells - general, Cartilage / joint and arthritic conditions

Development of siRNA-activated scaffold delivery system to ameliorate inflammatory responses during regeneration of osteoarthritic cartilage

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INTRODUCTION:In osteoarthritis (OA), chondrocytes propagate and experience an altered physiochemical microenvironment, including increased proinflammatory cytokine production, hindering the success of biomaterial guided regenerative strategies. Recent evidence links the Hippo (YAP/TAZ) pathway with IL1β and TNFα signalling (1). Meanwhile, mounting evidence indicates that connexon membrane channel proteins are involved in regulating gene/ protein expression and activity (2), including YAP (3) and NFκB (4) cellular localisation. Therefore, we aimed to investigate Cx43 activity in chondrocyte responses to proinflammatory cytokines and the link between Cx43 and the YAP-NFκB signalling axis. Then, we aimed to functionalize previously optimised chondrogenic collagen I-hyaluronic acid scaffolds (5) to develop a non-viral Cx43-siRNA biomaterial delivery platform targeting Cx43-mediated responses.

METHODS:In human articular chondrocytes (hACs), Cx43 and YAP-mediated responses to proinflammatory cytokines IL1B and TNFa were investigated using small molecule inhibitors and promotors (Cx43- GAP27; YAP-Verteporfin, VP; Lysophosphatidic Acid, LPA). The effects of Cx43 and YAP on cell viability, migration, proliferation and anabolic/ catabolic gene and protein expression in the presence of IL1B and TNFa were determined. For siRNA-activated scaffolds, Cx43 siRNA was complexed with the previously developed GET-(glycosaminoglycan-binding domain) peptide (6) for transfection. GET-siRNA complexes were incorporated into type I collagen-hyaluronic acid freeze-dried scaffolds. The effect of scaffold-delivered Cx43 knockdown in hAC responses to IL1B and TNFa stimulation in 3D OA-proinflammatory chondrogenic culture was assessed.

RESULTS:IL1B and TNFa decreased cell proliferation, migration and promoted catabolic gene and protein expression. Cx43-channel inhibition and YAP promotion were found to abrogate this effect. Catabolic gene and protein expression was significantly reduced in IL1B and TNFa samples treated with Cx43 siRNA, GAP27 or LPA. In scaffolds, GET-Cx43 complexes were successfully delivered by scaffolds, knocked down Cx43 expression in hACs and decreased catabolic responses to IL1B and TNFa.

DISCUSSION & CONCLUSIONS:This study highlights links between Cx43, YAP and proinflammatory signalling related to OA and demonstrates the potential for YAP1-Cx43 signalling as a therapeutic target. Get-siRNA complexes were successfully delivered in an advanced 3D scaffold delivery system, demonstrating the potential for this approach for controlled siRNA delivery in cartilage regeneration.

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Keywords: Cartilage / joint and arthritic conditions, Biomaterials

Biphasic extracellular matrix derived scaffolds with aligned pore architecture for osteochondral tissue engineering

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INTRODUCTION:Articular cartilage (AC) has an arcade-like collagen-fibre organisation, enabling it to withstand the high loads passing through joints[1]. Despite previous attempts to repair AC defects, recapitulation of this complex architecture remains an unmet challenge. It has been shown that the use of scaffolds with aligned pores improves cell infiltration, differentiation and zonally-organised matrix deposition[2]. The first objective of this study was to determine the effect of pore alignment in extracellular matrix (ECM) scaffolds[3,4] on chondrogenesis and osteogenesis of bone marrow stromal cells (MSCs) in vitro. Biphasic ECM scaffolds were then assessed preclinically in a large animal (caprine) model of osteochondral (OC) defect repair.

METHODS:Porcine AC and bone (BN) were pepsin-solubilised and freeze-dried to produce scaffolds (Φ5xh3mm) containing random or aligned pores. Pore morphology was characterised by SEM. Single phase scaffolds (AC) or biphasic scaffolds (AC-BN) were seeded with MSCs and cultured for 28 days in chondro- (CM) or osteogenic (OM) media. Biochemical and histological analysis was used to determined DNA, GAG, collagen, calcium levels. Off-the-shelf biphasic aligned scaffolds (Φ6xh6mm) were implanted in OC defects in goat knees. Defect repair was evaluated after 6 months by macroscopic, micro-CT and histological analysis.

RESULTS:AC scaffolds with aligned pores promoted oriented collagen matrix deposition in vitro. In biphasic scaffolds, the AC-ECM layer supported greater chondrogenesis than underlying BN-ECM layer, while the latter layer promoted higher levels of mineralization in vitro. After 6 months in vivo, macroscopic and micro-CT analysis showed a higher level of variability in the quality of repair in the empty group compared with defects treated with the biphasic ECM scaffold. Macroscopically, cartilage surface of scaffold treated groups appeared smooth and hyaline-like. Micro-CT analysis demonstrated abundant bone deposition in the osseous phase of the defect.

DISCUSSION & CONCLUSIONS:This study demonstrates that scaffold pore alignment can be tuned to engineer tissues in vitro that better recapitulate their native counterparts. In addition, the different tissue-specific layers (AC-BN) of the biphasic ECM scaffold promoted unique cellular phenotypes when seeded with MSCs. Preliminary analysis of the goat model revealed a more consistent repair in defects treated with the biphasic ECM derived scaffolds. Ongoing histological analysis will be used to assess the quality of the regenerated tissue.

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Keywords: Biomaterials, Cartilage / joint and arthritic conditions

Identification of an optimal xeno-free and cGMP compliant culture medium for maintenance of therapeutic potential of a corneal mesenchymal stem cell therapy

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INTRODUCTION:Corneal mesenchymal stem cells (C-MSC) can be extracted from the limbal stromal region of corneoscleral rims, a waste product of corneal transplants. C-MSC show regenerative properties similar to other MSC sources, and have been shown to release cytokines and growth factors that dampen inflammation and stimulate wound healing [1,2]. We are in the process of developing a topical stem cell therapy that applies the C-MSC to the injured ocular surface. In order to progress this project and validate the therapy, the culture medium needs to be transitioned from foetal bovine serum (FBS) to xeno-free and cGMP compliant without effecting phenotype or therapeutic potential.

METHODS:C-MSC were extracted from corneoscleral rims and cultured in 7 different media: M199 with 20% FBS; DMEM-F12 with 10% knock-out serum replacement (KSR); M199 with 10% human platelet lysate (HPL); and 4 commercially available mesenchymal stem cell media (StemXVivo, R&D Systems; StemProMSC, ThermoFisher; Mesenchymal XF, Sigma-Aldrich, StemMacsXF, Miltenyi Biotech). Effect on proliferation was assessed via prestoblue viability assays, effect on phenotype (MSC and corneal stem cells markers) assessed by flow cytometry, immunocytochemistry and RT-qPCR; and changes in uptake and release of pro- and anti- inflammatory cytokines (IL-6, IL-8, IL-10, TNF-α, IL-1, TSP-1, PTX-3) and growth factors (bFGF, EGF, HGF, PEDF, TGF-β1) assessed by ELISA.

RESULTS:The use of HPL or KSR as an alternative to FBS caused no significant changes in C-MSC phenotype or proliferation, and enhanced release of beneficial growth factors and cytokines. The 4 commercially available media had varying effects on cell viability and proliferation, some did not support growth of the C-MSC. Of these commercially available media StemXVivo (R&D Systems) produced cells with the most similar phenotype to the FBS-cultured cells but was no more successful than supplementing with either HPL or KSR.

DISCUSSION & CONCLUSIONS:This study showed that simple changes to the supplementation of basal media with defined and animal-free alternative to FBS can result in beneficial changes to the phenotype of C-MSC and maintain the ability to culture the cells in a cGMP environment. Although C-MSC are similar in phenotype to bone marrow MSC (BM-MSC), propagation in commercially available culture media designed for BM-MSC may not be suitable, with the reasons for this still to be elucidated.

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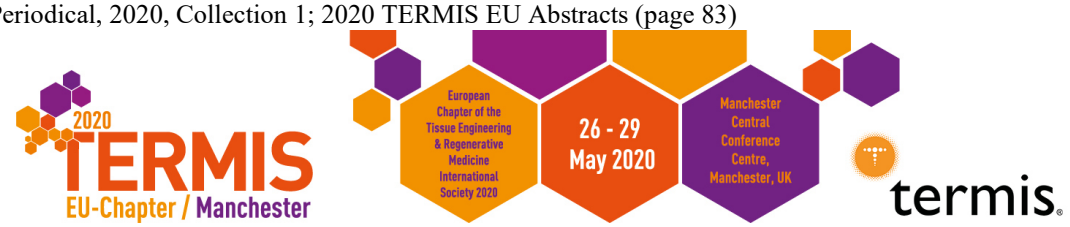
Keywords: Cell therapy, Multipotent (mesenchymal) stem cells



Freestanding magnetic microtissues for TE engineering applications

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INTRODUCTION:A main challenge in tissue engineering (TE) is to produce functional tissue-like constructs able to recapitulate the native tissue environment, composed by well-defined microscale subunits. “Bottom-up” approaches have been used to create large tissue-constructs using small tools to biomimicry such tissues subunits(1). Scaffold-free approaches, such as cell sheet (CS) engineering, have arisen as promising strategies able to maintain the intact cell matrix in tissue-constructs. Considering the tissues’ complexity, magnetic-force-based TE (Mag-TE) has been explored to developed hierarchal CSs using mild magnetic-force(2). In this sense, we herein propose the development of freestanding microtissues (Fs-CS) through Mag-TE as building blocks to the construction of hierarchal tissue-structures with several promising applications in TE.

METHODS:Fe3O4 nanoparticles were synthetized and incubated with a pre-osteoblastic cell line and human adipose-derived stromal cells. After confirming both cell viability and proliferation, magnetically-labelled cells were used to create Fs-CS with defined shapes using superhydrophobic surfaces patterned with wettable superhydrophilic domains. Briefly, the cells were seeded on the superhydrophilic domains, cultured for pre-determined periods and then detached with the aid of a magnet. The interaction between the microtissues of different cells phenotypes was also investigated. Lastly, the stemness and the ability of the Fs-CS to integrate and invade neighboring tissue models were evaluated along the time using platelet lysates and gelatin-methacrylate.

RESULTS:Robust microtissues with an enriched collagen-matrix and defined shapes were successfully engineered. The crosstalk between distinct microtissues was visualized by fluorescence microscopy and corroborated through the presence of vinculin. Additionally, the Fs-CS inserted in hydrogels-tissue models rapidly sprouted and invaded their structure, showing invasive properties while keeping the stemness features.

DISCUSSION & CONCLUSIONS:Mag-TE was used to demonstrate the ability to create robust microtissues with versatile shape. The magnetic-controllability was used to accelerate the directed assembly of individual microtissue units into larger tissue constructs under an external magnetic-force. Additionally, the ability of Fs-CS to invade the tissue models suggests that such structures could be used as delivery of platforms of stem cells, thus opening new insights for the fabrication and repositioning of complex and hierarchical 3D connected tissue that better resembles the in vivo environment.

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Keywords: Biomaterials, Cell therapy

Bone potential heterogeneity in hBMSCs is associated with their immunomodulatory capacity

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INTRODUCTION:Human bone marrow mesenchymal stromal cells (hBMSCs) associated to biomaterials are currently used in clinic for bone repair. However, their use may be hindered by a donor-dependent heterogeneity for bone formation.

METHODS:To evaluate this heterogeneity cells from 50 donors were amplified and grafted subcutaneously in a mouse model.

RESULTS:Our results confirmed that the bone potentials of the cells is donor-dependent with bone gradient going from no bone to high bone potential. The differences in bone potential was confirmed in an orthotopic model indicating that the ectopic model is relevant to study hBMSCs behaviour. We next wanted to understand the differences which underline this heterogeneity. To do so, we focused on the behaviour of hBMSCs after graft. We observed that hBMSCs with high bone potential were associated with higher cell survival in vivo. In order to understand how cell survival is controlled after graft, a transcriptomic analysis was performed. Our results indicated that 24h after graft the high bone cells are in high metabolic activity whereas low bone cells enter in a bioenergetic crisis and this is associated with a higher neutrophil activity. In order to evaluate whether inflammatory cells are involved in cells survival and cells energetic crisis, we evaluated the bone potential of hBMSCs in mice depleted or not in neutrophils. At 4 and 6 weeks post graft our results showed an increased bone formation by more than 3 times when low bone hBMSCs were graft in neutrophil-depleted mice. We then confirmed the enhancement of hBMSCs survival in absence of neutrophils. This was significant at 24h and still visible 2 weeks after graft.

DISCUSSION & CONCLUSIONS:In conclusion, our results show that part of hBMSCs heterogeneity is due to their ability to regulate the inflammatory response which has an impact on their survival and therefore their bone potential.

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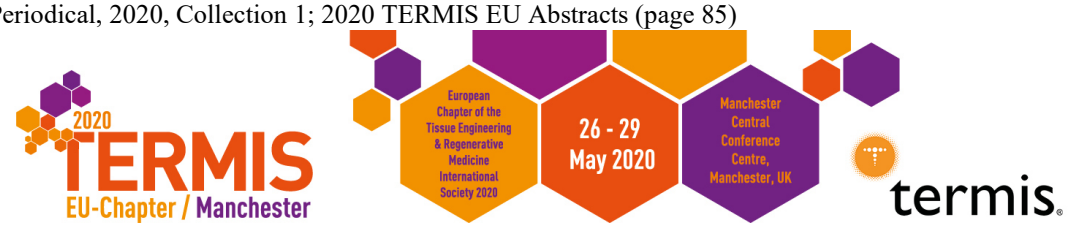
Keywords: Immunity / immunomodulation / macrophage, Bone and bone disorders (osteoporosis etc)



The potential of the mesenchymal stem cell secretome to promote spinal cord repair

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INTRODUCTION:It was documented in the Edwin Smith papyrus in 1700 BC that spinal cord injury was an “ailment not to be treated”. Today, nearly 4000 years later, treatment options remain very limited. WHO estimates there are up to 500,000 new cases of spinal cord injury every year and due to a lack of spontaneous recovery, 90% of patients are left with long-term disabilities including the loss of motor and sensory functions below the injury level. There are a number of obstacles to spinal cord injury repair including the poor intrinsic regenerative capacity of adult mammalian central nervous system (CNS) neurons, inhibitory lesion environment, glial scarring and the physical barrier of a cystic cavity. One promising approach for overcoming these obstacles are mesenchymal stem cells (MSCs). There is mounting evidence indicating that improvements in functional recovery in preclinical models of CNS injury can be mediated by the paracrine actions of MSCs. The secretome is a collective term for the vast array of chemokines, cytokines, growth factors and extracellular vesicles secreted by cells.

METHODS:This project aims to explore the potential of human bone marrow-derived MSC secretome in combination with an injectable self-assembling hydrogel to promote axonal regrowth in an *ex vivo* rat model of spinal cord injury, which mimics *in vivo* post-spinal cord injury environment. Both acute and delayed time points of gel/secretome treatment will be explored.

Keywords: Multipotent (mesenchymal) stem cells, Nervous system (brain-central-peripheral / disorders)

Hierarchical HRP-crosslinked silk fibroin/ZnSr-doped TCP nancocomposites towards osteochondral tissue regeneration: Biomechanical performance and in vivo assessment

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INTRODUCTION:Recent investigations highlight promising regenerative strategies for osteochondral (OC) tissue treatment, such as hierarchical nanocomposite scaffolds containing ionic dopants.1,2 They allow cell infiltration and ECM formation throughout the engineered cartilage and subchondral tissues. The biomechanical behavior, antibacterial properties, and in vivo performance of hierarchical nanostructures combining enzymatically crosslinked silk fibroin (SF) and ZnSr-doped β-tricalcium phosphate (ZnSrTCP) for OC tissue regeneration is herein assessed.

METHODS:Hierarchical scaffolds were fabricated using horseradish peroxidase (HRP) crosslinked SF (HRP-SF) as the articular cartilage-like layer, and HRP-SF/ZnSrTCP as subchondral bone-like layer, through salt-leaching/freeze-drying techniques. The failure behaviour of the scaffolds was evaluated under combined compression and shear loading. Antibacterial properties of the scaffolds were assessed by adhesion and biofilm formation of Escherichia coli (E. Coli) and Staphylococcus aureus (S. aureus) on its surface. In vivo OC regeneration potential of the scaffolds was evaluated in rabbit knee critical size OC defects implanted for 8 weeks. Then, explants were fixed in 10% formalin for 7 days at 4 °C and decalcified, and stained with H&E for histological and immunofluorescent analysis using transmitted and reflected light microscope.

RESULTS:The scaffolds showed capability to support tension and shear stress upon loading until 60% deformation, with a tendency of improved mechanical properties for the ZnSr-doped scaffolds. Limited E. coli and S. aureus adhesion and biofilm formation was observed on the scaffolds surface. After scaffolds implantation in knee OC defects, no evidence of adverse foreign body reactions and good integration into the host tissue was observed. Histological and immunofluorescence analysis showed positive collagen type-II and glycosaminoglycans' formation in the articular cartilage layer, and new bone ingrowth and blood vessels infiltration in the subchondral bone layer.

DISCUSSION & CONCLUSIONS:The ionic-doped scaffolds presented good mechanical properties to allow cellularity for ECM mineralization and ingrowth. The scaffolds have shown capability of preventing bacterial adhesion to its surfaces and biofilm formation. SF layer supported cartilage regeneration in knee OC defects, while the ionic incorporation into the subchondral bone-like layer favoured calcified tissue formation. Thus, the biomechanical performance together with the in vivo results shows the efficacy of these scaffolds of OC tissue regeneration.

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Keywords: Biomaterials, In vivo and animal models

Ex-Vivo Organ Culture Model for Elucidating Cell Interactions at the Decellularised Scaffold:Tissue Interface

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INTRODUCTION:Modulation of the cellular response to decellularised biological scaffolds towards an immuno-regulatory (M2) phenotype has been suggested to be a determinant of regenerative outcomes *in vivo*. A key role in recellularisation of functional decellularised heart valve scaffolds was revealed for macrophages in an *in vivo* ovine model, and CD163+ (M2) macrophages were shown to pioneer the initial cellular response to porcine decellularised bladder scaffold in a human tissue organ culture model. The aim of this study was to (1) develop a novel viable *ex vivo* organ culture model for decellularised cardiovascular scaffolds; (2) characterise the initial innate and stromal cell response at an ovine tissue: decellularised porcine scaffold interface.

METHODS:An interface was established between decellularised porcine pulmonary artery scaffolds and native ovine pulmonary artery. The constructs were maintained in culture for 0, 2, 6, and 11 days (n = 4 at each time point). Haematoxylin and eosin (H&E) staining, and immuno-histochemistry (IHC), were used to characterise the cellular response at the tissue:scaffold interface. Primary antibodies for the following markers were used: MRP14 (MAC387; pan macrophage), CD163 (M2 macrophage), CD80 (M1 macrophage), α-smooth muscle actin (smooth muscle cells), Ki67 (proliferating cells), low-affinity nerve growth factor receptor expression (CD271; progenitor cells), connective tissue growth factor (CTGF, fibrogenesis), von Willebrand Factor (endothelial cells) and CD34 (haematopoietic progenitor cells). Manipulation of the ovine *ex vivo* cellular response was investigated by exposing decellularised scaffolds to a carbodiimide cross-linker prior to organ culture.

RESULTS:Observation of H&E stained histological sections revealed a time dependent infiltration of cells into the non-crosslinked scaffolds. This was a heterogeneous population, expressing markers for stromal and progenitor cells. A striking number of cells within the scaffold and at the tissue: scaffold interface were CD163+. Analysis of the cellular response towards the cross-linker treated decellularised scaffold (H&E stained only) revealed that the ovine cellular response was attenuated in comparison to the non-crosslinked scaffolds. The cells appeared to collect at the interface, similar to an encapsulation response, rather than crossing into the scaffold.

DISCUSSION & CONCLUSIONS:The striking CD163+ cell presence, in combination with the recruitment of site specific stromal cells and progenitor cell types indicates that this *ex vivo* model may provide a valuable tool for investigating the mechanisms of an early regenerative cellular response. Further investigation of the cell infiltrate and mechanisms of recruitment and polarisation of macrophages at the scaffold interface is required.

Keywords: Decellularised matrices, Interfaces - biological

A novel dermal matrix scaffold developed for cardiac tissue engineering is remodeled by human resident cardiac progenitor cells

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INTRODUCTION:Cells *in vivo* are embedded in the extracellular compartment that consists mostly of the extracellular matrix (ECM). The advent of cardiac tissue engineering aroused considerable interest in the role of myocardial ECM in generating biochemical signals, providing mechanical features and determining cell behavior, such that the cardiac decellularized ECM (d-ECM) has rapidly emerged during the last decade has an attractive biomaterial for regenerative medicine applications. In the search for a biological and autologous substitute for the cardiac matrix, we have recently reported the suitability of the decellularized human skin (d-HuSk) as a scaffold material for cardiac tissue engineering [1]. However, despite the similarity found between dermal and cardiac d-ECM, any scaffold is to be intended as a temporary implant and, even more importantly, it should comply with resident cell requirements in terms of composition and signaling. Therefore, the scope of the present study is to evaluate whether d-HuSk can be remodeled and converted from a cardiac-like to a cardiac matrix by resident human cardiac progenitor cells (hCPCs) and differentiating cells *in vitro*.

METHODS:To this aim, we prepared three-dimensional scaffolds of d-HuSk from human abdominal skin, repopulated a subset of them with hCPCs isolated from explanted human hearts and cultured the recellularized scaffolds for two weeks. Afterwards, we induced the differentiation of seeded hCPCs towards cardiac myocytes and cultured them for two additional weeks. Then, we evaluated by histochemistry, immunohistochemistry, SEM analysis and quantitative assays the composition and organization of d-HuSk both before and after recellularization and culture.

RESULTS:The histological analysis revealed that repopulating d-HuSk with hCPCs and differentiating cardiac cells induces a reorganization of the dermal matrix involving mostly fibronectin, collagen, elastin and glycosaminoglycans (GAGs). Moreover, the amount of ECM components, like collagen, elastin and GAGs, resulted affected by seeded cells as well, as shown by quantitative dye binding assays. From SEM observation a network of tiny filaments resembling elastic fibers resulted more evident in repopulated d-HuSk scaffolds, whereas the thicker collagen fibers resulted less abundant.

DISCUSSION & CONCLUSIONS:The results emerging from our study support the hypothesis that d-HuSk serves as a platform for resident hCPCs and differentiating cells and is with them in a dynamic equilibrium. This evidence along with the fully biological nature and potentially autologous origin of d-HuSk strengthens its candidacy as the ideal biomaterial for cardiac tissue engineering applications.

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Keywords: Cardiovascular, Stem cells – general

Comparing heterogenous populations in high dimensional spaces: quantifying the effects of animal-derived extracellular matrices on macrophage morpholgy using machine learning

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INTRODUCTION:Guiding the behaviour of immune cells, such as macrophages, to promote regeneration and prevent fibrosis is an important goal of tissue engineering. Macrophages perform different functions during wound healing via polarisation into M1 (“acute inflammatory”) or M2 (“chronic inflammatory”) phenotypes. Macrophage polarisation is generally quantified by the expression of cell surface markers by FACS. However, these states are also characterized by dramatic changes in cell morphology: M1 cells tend to be large and well-spread with ruffling edges, whereas M2 cells tend to be small and/or spindle-shaped. Highly polarised M1 and M2 cells can be consistently distinguished by multi-parametric quantitative morphometry, but populations are extremely heterogeneous. The degree of heterogeneity and collinearity of features mean that comparing intermediate or mixed-state cell populations presents a statistical challenge. We set out to determine the impact of porcine decellularised extracellular matrices (dECMs) on macrophage polarisation in order to assess their utility as tissue regeneration adjuvants.

METHODS:Murine bone marrow monocytes were seeded on untreated or dECM-coated tissue culture plastic in differentiation medium for six days, then stimulated with soluble dECMs or cytokines (LPS/IFN-gamma or IL-4) to induce polarisation. Cells were fixed after 24 h, stained to label DNA, actin and microtubules, and imaged using high-content confocal microscopy. Quantitative morphometry was performed using automated segmentation and image analysis software. A machine learning method called Maximum Mean Discrepancy (MMD) was used to make multivariate comparisons across the matrix of treatment conditions. This method has previously been used for two-sample tests of high-dimensional data. We applied MMD as a series of three-sample tests to determine relative similarities in multivariate space, using the MMD statistics with estimated confidence intervals in place of p-values.

RESULTS:This method allowed us to meaningfully compare intermediate and mixed phenotypes against reference populations and quantify the degree of polarisation induced by dECMs alone or in combination with inflammatory cytokines. Soluble dECMs derived from cardiac and bladder tissues had significantly different effects on macrophage morphology than dECM derived from the small intestine. Plating cells on substrates coated with dECMs also had significant effects on M1 and M2 polarisation in response to cytokines, which were different from exposure to soluble matrices.

DISCUSSION & CONCLUSIONS:This method is useful not only for inferring macrophage function in culture, but could be applied to other morphologically diverse populations, such as tumour cells, as well as other types of noisy biological data, such as single cell sequencing.

Keywords: Differentiation, Immunity / immunomodulation / macrophage

Immune-perfusion of liver extracellular matrix-scaffolds in a custom-made bioreactor to explore cell-matrix interaction in liver disease

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INTRODUCTION:Liver fibrosis is driven by progressive accumulation of extracellular matrix (ECM), coupled with chronic inflammation. Traditional cell culture models lack the complexity to fully recapitulate disease and often lack immune cell and ECM components. Bioengineered models of liver disease using decellularised tissues present an appealing alternative. Here, we describe the generation of a bioengineered liver model which incorporates the dynamic culture of circulating immune cells with decellularised human liver scaffolds, supported in a custom-made bioreactor. This model allows us to explore interactions between the immune system and liver ECM proteins at different stages of fibrosis.

METHODS:Liver scaffolds were generated by decellularising liver tissue with/without underlying disease from human liver biopsies, or by perfusion decellularisation of whole rat liver using established protocols. PBMCS isolated from healthy donor whole-blood were cultured in the bioreactor under semi-continuous perfusion at high (4.22 Pa) or low (0.34 Pa) shear stress, or in static conditions. Longitudinal profiling of PBMC phenotype was determined by FACs for B, NK, T cells, T-regs and monocytes. Decellularised whole rat liver was perfused with PBMCs and cultured for 5 days. Immune-perfused tissue was stained for PBMC surface markers (T, B, NK cells and macrophages).

RESULTS:We designed a custom-made bioreactor which can house 6 decellularised human liver scaffolds and which supports dynamic culture of PBMCs with no significant loss in viability. We found that shear stress impacts PBMC viability and that PBMCs are better supported under low shear stress conditions, obtained by modulating flow rate or media viscosity with inert macromolecules. PBMC phenotype was analysed by FACs. Preliminary results show no significant changes in proportion of T cells or the ratio of CD4:CD8-positive cells between static and dynamic culture, while an increase in proportion of Tregs and a decrease in proportion of NK cells were detected in dynamic culture. Perfusion of PBMCs in decellularised scaffolds show homing of cells in different areas of the scaffold at all time points, and at 3 days 50% of cells are CD4+ T cells, with small clusters of macrophages and B cells.

DISCUSSION & CONCLUSIONS:Our bioengineered liver model can circulate PBMCs through decellularised scaffolds, with varying grades of fibrosis, allowing us to explore the interactions between the ECM and immune cells in liver disease in a dynamic perfusion system. A better understanding of ECM-immune interactions will aid our understanding of the driving factors behind fibrosis and could highlight new disease progression biomarkers and therapeutic targets to treat disease.

Keywords: Bioreactors, Immunity / immunomodulation / macrophage

Successful esophageal reconstruction by homologous acellular esophageal micro-perforated scaffold 3D seeded with autologous mesenchymal stromal cells

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INTRODUCTION:Tissue engineering techniques are interesting alternative for esophageal replacement avoiding hard surgery complications and promising good quality of life.
METHODS:We developed a full thickness cylindrical decellularized homologous esophageal scaffold as a porcine esophageal substitute that was micro-perforated by Quantum Molecular Resonance (QMR®) technology (Telea Electronic Engineering) to create a microporous network inside. The scaffold was seeded with autologous bone marrow-derived mesenchymal stromal cells (BM-MSCs) cultures previously prepared colonizing both outer and inner layers of the scaffolds in a 3D way.
The scaffold was then implanted in the thoracic esophagus in a pig by two termino-terminal anastomosis through a right thoracotomy and an extrapleural way substituting a full-thickness 4 cm long tract of the original organ. The animals were housed for a six months period of follow-up during which we performed clinical, endoscopic and radiographic controls with eventual endoscopic dilatation to allow normal oral feeding. After this period they were sacrificed and the esophagus removed and examined with immunoihistochemical coloration.

RESULTS:At the autopsy we demonstrated the regeneration of all the esophageal layers from one anastomosis to the other one, comprising the muscle layer, the submucosa and the mucosa with evidence of neo vascularization.

DISCUSSION & CONCLUSIONS:We were able to demonstrate that a decellularized micro perforated homologous esophageal scaffold allow 3D BM-MSCs seeding and promote vascular infiltration and tissue reconstruction from the anastomosis in a large animal based experimental model, even if the interposed tract needed endoscopic dilatation.
In conclusion, we developed a full thickness esophageal substitute starting from a porcine scaffold, able to recapitulate in shape and function the original esophageal layers. It represents a good chance to promote tissue engineering esophageal substitution. This is an ongoing experimental trial and further experimentations are needed.

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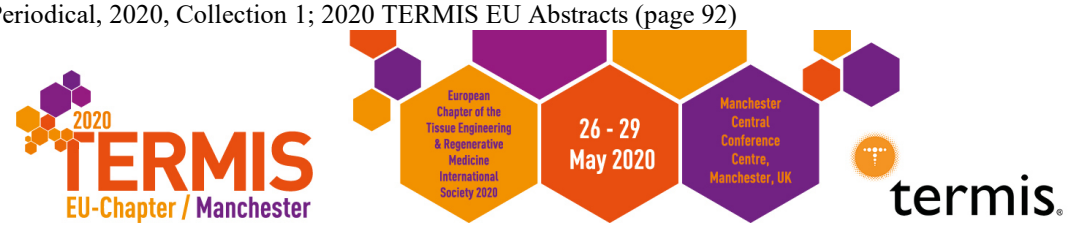
Keywords: Biomaterials, Stem cells – general



Hoxa3 functions as a therapeutic biologic in diabetic impaired wound healing by modulating epigenetic changes to inflammatory genes

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INTRODUCTION:Effective repair of cutaneous injury requires a transient inflammatory response during which macrophages are recruited from the periphery to the wound site. As healing progresses from the inflammatory phase to the tissue remodelling phase, macrophages switch from an M1-like, pro-inflammatory phenotype to an M2-like phenotype that promotes wound resolution. In the diabetic chronic wound, however, this switch is absent or delayed. Instead, myeloid cells continue to express M1-associated genes and do not transition to an M2-like pro-resolution state. Epigenetic changes to macrophages occur during ageing and in pathological conditions such as diabetes. These changes are associated with altered macrophage differentiation and function contributing to chronic inflammation, resulting in poor tissue repair and regeneration and delayed wound healing.

METHODS:We have used adoptive transfer of macrophages, transient gene therapy and protein transduction of the transcription factor Hoxa3 in mice in vivo and in human patient-derived macrophages in vitro to reprogramme cells from the diabetic environment to promote wound healing. In addition, we have used flow cytometry and next generation sequencing to identify intrinsic factors regulating macrophages during wound healing as well as identify factors modulated by Hoxa3 treatment.

RESULTS:We have identified several chromatin modifying enzymes that appear to play a role in the transition from the pro-inflammatory to pro-healing phenotype that may contribute to longer term changes in macrophages in pathological environments. Specifically, we have determined that these changes result in priming of macrophages and their progenitors to respond aberrantly to pro-inflammatory signals in the wound environment. Moreover, we have found that chromatin alterations are associated with aberrant differentiation of macrophages and their progenitors. We have also found that Hoxa3 can be used as an therapeutic factor and when provided exogenously can control macrophage differentiation and function through interactions with epigenetic regulators.

DISCUSSION & CONCLUSIONS:Therapeutic manipulation of macrophage epigenetics with biologics such as Hoxa3 can induce long-term changes to these cells and impact tissue repair and regeneration in diabetes and has significant relevance for people who have reversed their condition. Understanding how these mechanisms affect wound healing is critical to developing future therapies that work not just in diabetes but in ageing tissues as well.

Keywords: Immunity / immunomodulation / macrophage, Diabetic healing

Development of an in vitro three-dimensional colorectal tumor model for drug screening

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INTRODUCTION:Cell-derived matrices (CDM) are self-assembled structures composed of proteins, growth factors, and matrix macromolecules mimicking human extracellular matrix (ECM). Due to their bioactivity and biocompatibility, CDMs are a promising alternative to decellularized tissues/organs. Using 3D cell-cultured poly-lactic acid (PLA) microparticles combined with macromolecular crowding (MMC) effect, we show the possibility to tailor-make bioactive materials for tissue engineering and disease modelling applications. Here, we propose CDMs as potential in vitro colorectal tumor models for personalized medicine by mimicking tissue microenvironment properties [1].

METHODS:PLA microparticles jetted through a coaxial needle into a coagulation bath [2] were coated with human fibronectin to enhance cell adhesion.
Human adipose mesenchymal stem cells were seeded on PLA microparticles [2] and cultured for 10 days under stirring conditions. Obtained CDMs were characterized by quantifying total protein and DNA; gene and protein expression by qRT-PCR, semi-quantitative immunofluorescence staining and mass spectrometry; together with mechanical assessment using atomic force microscopy (AFM). Besides, decellularized and microcarriers-removed tissues were recellularized in a perfusion bioreactor with colon cancer cell lines and cancer associated fibroblasts (CAFs) [3] to further study cell-cell interactions, gene expression and cellular remodeling potential. Mechanical, biochemical and gene expression properties of colorectal cancer CDMs are currently being compared with human colorectal tumor biopsies from patients.

RESULTS:Addition of MMCs enhanced protein deposition in CDMs. Fibrillary proteins collagen types I, III and fibronectin, which are highly present in colon tumor ECM, were over expressed after 10 days of culture. Tissues density and size was increased, together with the final tissue stiffness. We established successful protocols for cell and microparticle removal without affecting structure and composition of CDMs. Their recellularization with colon cancer cells and CAFs, together with phenotypic and mechanical characterization is currently taking place to finally produce an in vitro tumor model to understand cancer promoting mechanisms, to develop a patient-specific drug screening platform and to identify potential therapeutic targets.

DISCUSSION & CONCLUSIONS:CDMs composition, like expression of fibrillar proteins of the ECM, and the tunable matrix stiffness provides reproducible tissue microenvironment. By repopulating the tissue microenvironment with cancer and stroma cells, we provide a platform to mimic native tissue structure and properties for in vitro tumor model generation.

Acknowledgements:Authors would like to thank MINECO (MAT2015-68906-R) and RTI2018-096320-B-C21 for funding.

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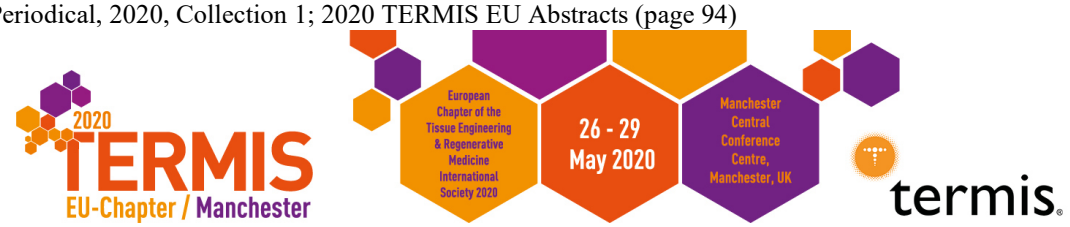
Keywords: Disease models, Microenvironment and niche engineering



Microfragmented human fat tissue is a natural scaffold for drug delivery: potential application for local cancer chemotherapy

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INTRODUCTION:Localization of chemotherapy at the tumor site can improve therapeutic efficacy and reduce systemic toxicity.. We here investigated the capacity of human micro-fragmented adipose tissue (MFAT), used as a natural scaffold of MSCs, to deliver PTX with the idea to improve local drug concentration and to prolong the therapeutic activity.

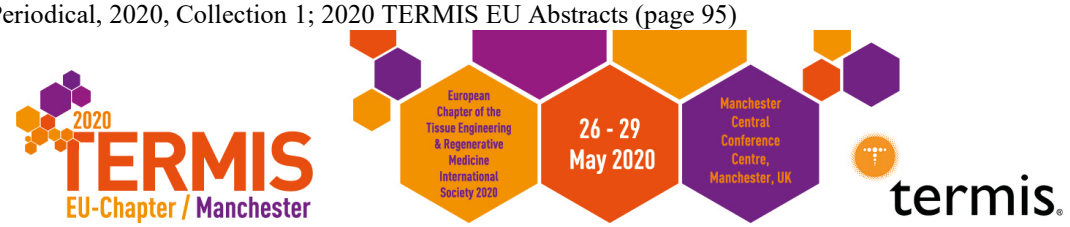
METHODS:Around 50/100 ml of LP was used for MFAT preparation by using a standard 225-ml Lipogems® device (provided by Lipogems® International, Milan, Italy). DMFAT was obtained following a previous published procedure. Aliquots of MFAT were kept at -20°C until use, others were analyzed to verify the absence of cell viability (DMFAT).
Based on our previous procedure used for PTX priming of MSCs, for each ml of both MFAT or DMFAT specimens, a PTX stock solution (6 mg/ml) diluted in Iscove complete medium was added to obtain the final concentration of 200 ug/200 ul.
The in vitro anti-cancer activity of PTX loaded into MFAT and DMFAT was evaluated against: i) the human Pancreatic Adenocarcinoma cell line CFPAC-1, ii) the human Glioblastoma cell line U87MG, iii) the human, wild type and luciferase (luc) transfected Neuroblastoma (NB) (IMR-32, SH-SY5Y, HTLA-230, NB1691) cell lines.
The in-vivo anti-cancer activity in an orthotopic nude mice model of NB transplant was studied.

RESULTS:Surprisingly, we found that both fresh but also devitalized MFAT (DMFAT) (by freezing/thawing procedure) were very effective to deliver and release significant amount of PTX, killing several human cancer cell lines in vitro with an impressive long lasting activity. In an orthotopic mice model of Neuroblastoma (NB) transplant, DMFAT loaded with PTX prevents or delay NB relapse when placed in the surgical area of tumor resection, without any collateral toxicity

DISCUSSION & CONCLUSIONS:We here, for the first time, clearly demonstrated that both MFAT and DMFAT work as natural biological scaffolds, able to adsorb and release a very significant amount of PTX and to efficiently kill cancer cells both in vitro and in vivo; in an orthotopic animal model of human NB the local administration of DMFAT-PTX at tumor site after its surgical resection blocked or delay NB relapse. We concluded that MFAT, but also DMFAT, may both represent very innovative natural biomaterials able to localize and release anti-cancer drugs at the tumor site, opening very interesting perspectives for their applications in human cancers

Keywords: Biomaterials, Innovation

An Efficient Delivery Platform to Modulate Fibrotic Response



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INTRODUCTION:An excessive matrix deposition following injury is a major issue for any implantation and the cause of fatal prognosis in chronic disorders. The inhibition of galectin-3 (Gal-3) have shown promising effects in reducing fibrosis in chronic disease models by blocking β-catenin nucleus translocation and myofibroblast differentiation. Novel Gal-3 inhibitor (TD139) is in clinical trials for the treatment of idiopathic pulmonary fibrosis and has rendered promise for the development of antifibrotic therapies. However; this inhibitor needs to be localised to avoid any off target effects. Hence the aim of this project is to conjugate TD139 to hyaluronic acid (HA) hydrogels through a biologically cleavable bond for its target delivery and balance the fibrotic response.

METHODS:Procedures were optimized with lactose a cost-effective analogue. Lactose was chemically modified at different degrees to allow its attachment to HA and tune its release. HA hydrogels were conformed using DMTMM and differing 4armPEGNH2. The hydrogel parameters based on degradability and drug release profiles were optimised using hyaluronidase/esterase. Enzymatic determination was used to quantify lactose loading. Mechanical and injectable properties were measured by rheology. Fibrotic β-catenin molecular pathway was studied *in vitro* by incubating primary human pulmonary fibroblasts (hPFs) with TGF-β1. Fibrotic markers were characterized by western blot.

RESULTS:1H NMR showed controllable amount of lactose modification and TLC suggested the presence of multiple products, although further LCMS characterization needs to be performed. Fluorescamine assay and enzymatic quantification displayed efficient lactose binding to HA and loading capacity up to 13% (w/w). Hyaluronidase degradability showed optimal HA (10 mg/mL) and cross-linker concentrations (6-10 µL/mL). Preliminary rheological tests exhibited hydrogel-like and injectable properties. TGF-β1 *in vitro* incubation of hPFs showed overexpression of smooth muscle actin and collagen type I fibrotic markers by β-catenin nucleus translocation.

DISCUSSION & CONCLUSIONS:The developed methodology permitted significant lactose attachment to HA hydrogels in a solubilized form. Control in lactose functionalization amount renders promise to tune release profile without altering drug structure. The aim is to translate developed technology to TD139 and measure enzymatic drug release by HPLC. Activation of β-catenin nucleus translocation and fibrotic markers by TGF-β1 incubation validated *in vitro* model for material testing. Inclusion of epithelial and endothelial cells as myofibroblasts source will be also tested. Controllable and targeted TD139 delivery renders promise to develop efficient antifibrotic treatments.

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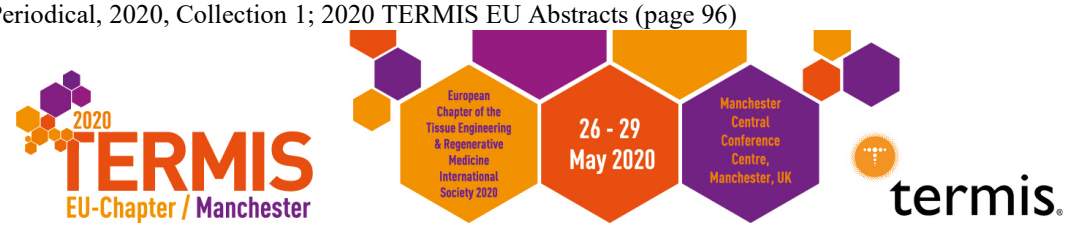
Keywords: Hydrogels and injectable systems, Advanced therapy medicinal products



Ultrasound responsive microbubbles for bone fracture repair

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INTRODUCTION:Impaired fracture healing has a significant physical and mental impact on patients, in addition to a financial burden for healthcare services. The aim of this study is to develop a targeted drug delivery system, activated by ultrasound, in order to overcome pharmakokinetic limitations which have halted progress of therapeutic agents. The development of acoustically-stimulated microbubbles (MBs) and nanodroplets (NDs) is investigated, as the effects of cavitation enhance the delivery, release and uptake of drugs by cells. Therefore, in this study, we tested the hypothesis that gas-filled MBs are non-toxic and promote osteoblastic differentiation. The bioactivity of osteogenic inducing agents, BIO and CHIR, are also tested, prior to incorporation with MBs and NDs.

METHODS:A MB suspension was prepared with a range of lipid compositions (Phospholipid/PEGylated lipid, 9:1 molar ratio). The lipid films were hydrated in PBS and sonicated to form gas-filled MBs (room air, oxygen, nitrogen). MG63 osteocarcinoma cells were used as a bone cell model, to assess MB cytotoxicity with exposure to varying concentrations for up to 72h. An Alamar Blue® assay was carried out as an indicator of cell viability. Bone Marrow Stromal cells (BMSCs) were exposed to gas-filled MBs, for 7-14 days, to test the osteogenic activity in the absence of ultrasound and osteoinductive agents using Alkaline Phosphatase assay (ALP). A cell luciferase assay using 3T3 cell lines was carried out to test the activation of an osteoinductive gene, Wnt, upon exposure to up to 10 μM and 20 μM of BIO and CHIR respectively.

RESULTS:MBs induced a dose-dependent decrease in metabolic activity at 72 hours exposure, with a significant reduction at 0.5 dilution. However, with exposures up to 24 hours, both formulations of MBs did not exhibit any cytotoxic effects. Gas-filled MBs did not induce any significant change in cell ALP activity relative to the control. Increasing concentration of free BIO and CHIR induced Wnt expression with a peak at 5 μM and 10 μM respectively. Concentrations greater than this, were cytotoxic.

DISCUSSION & CONCLUSIONS:Microbubbles have demonstrated no cytotoxic concerns when applied on a bone cell model, up to 3×10^7 MBs/ml for 24h, therefore, are a safe delivery method within clinically relevant time frames. MBs alone have not demonstrated any osteogenic activity, therefore, osteoinductive activity will next be tested with the incorporation of BIO and CHIR, in combination with ultrasound activation.

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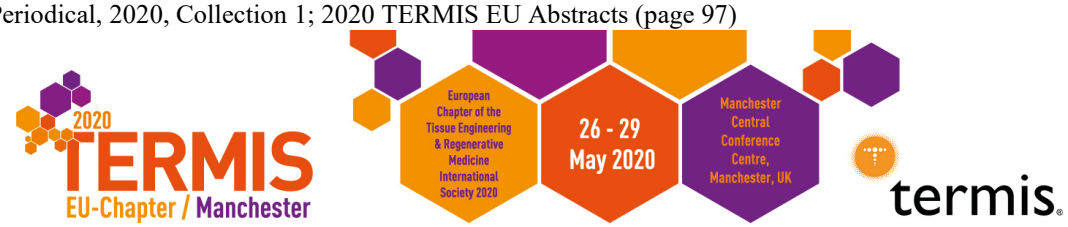
Keywords: Bone and bone disorders (osteoporosis etc), Stem cells – general



Silk based electroactive biomaterials for neural applications

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INTRODUCTION:Nerve injury is one of the most common causes of neurological disorders comprising 3% of the global disease burden, affecting circa one billion people according to the WHO. Despite the progress in understanding the pathophysiology of nerve injury and regeneration, as well as advancements in microsurgical techniques, peripheral nerve injury remains a major worldwide healthcare challenge due to minimal and slow regeneration capability. So, accelerating the rate of nerve regeneration can result in a better functional outcome for the denervated tissue. One of the potential methods for improving nerve regeneration and the restoration of function is electrical stimulation (ES). ES activates neurons through membrane depolarization which ultimately promote the speed and accuracy of motor and sensory axon regeneration both in-vitro and in- vivo. This initiated the idea of using electrically conductive neural scaffold to promote neurite outgrowth and thereby enhance nerve regeneration in culture.

METHODS:We have developed biocompatible and biodegradable 3D aligned electroconductive scaffolds with minimal immune response for possible use as smart nerve guidance channels (NGCs). The proposed smart NGCs were fabricated using natural fibroin protein of B. mori and A. assama silk (endemic to North-East India). To confer speedy axonal growth, we introduced electrically conducting polymer, polypyrrole (PPy), in the scaffold along with silk fibroin proteins. The hypothesis of speedy axonal growth in primary dorsal root ganglions (DRGs) and enhanced neural differentiation of adipose derived mesenchymal stem cells (ADMSCs) was tested under pulsed ES using a function generator at amplitude 0, 50, 100, 200, 300 and 500 mV/cm (500 Hz) for 2h/day (3 days).

RESULTS:Current-voltage and CV analysis demonstrated their voltage dependent conductive behaviour and redox stability in neutral electrolyte, including cell culture media. The ES test showed almost two-three fold increase in axonal growth in DRGs and enhanced neural differentiation of ADMSCs grown on the as-fabricated scaffolds. The materials showed minimal immune response when evaluated with murine macrophage cells for release of IL1-beta and nitric oxide (NO).

DISCUSSION & CONCLUSIONS:With the higher redox stability cell culture media and faster axonal growth as well as improved neural differentiation, our findings could be considered as a proof-of-concept for a new NGC for faster nerve repairing. Recently, we have fabricated a wearable/implantable triboelectric nanogenerator capable of harvesting biomechanical energy into electrical study to replace the conventional 220 V power source for ES.

Acknowledgements:The authors gratefully acknowledged Department of Science & Technology, Government of India, for the financial support.

Keywords: Biomaterials, Polymers - natural / synthetic / responsive

Pulsed Electromagnetic Field Therapy Improves Osseous Consolidation after High Tibial Osteotomy in Elderly Patients—A Randomized, Placebo-Controlled, Double-Blind Trial

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INTRODUCTION:Extremely low-frequency pulsed electromagnetic field (ELF-PEMF) therapy is proposed to support bone healing after injuries and surgical procedures, being of special interest for elderly patients. This study aimed at investigating the effect of a specific ELF-PEMF, recently identified to support osteoblast function in vitro, on bone healing after high tibial osteotomy (HTO).

METHODS:Patients who underwent HTO were randomized to ELF-PEMF or placebo treatment, both applied by optically identical external devices 7 min per day for 30 days following surgery. Osseous consolidation was evaluated by post-surgical X-rays (7 and 14 weeks). Serum markers were quantified by ELISA. Data were compared by a two-sided t-test ($\leq = 0.05$).

RESULTS:Device readouts showed excellent therapy compliance. Baseline parameters, including age, sex, body mass index, wedge height and blood cell count, were comparable between both groups. X-rays revealed faster osseous consolidation for ELF-PEMF compared to placebo treatment, which was significant in patients ≤50 years (Dmean = 0.68%/week; p = 0.003). Findings are supported by post-surgically increased bone-specific alkaline phosphatase serum levels following ELF-PEMF, compared to placebo (Dmean = 2.2 µg/L; p = 0.029) treatment. Adverse device effects were not reported. ELF-PEMF treatment showed a tendency to accelerate osseous consolidation after HTO. This effect was stronger and more significant for patients ≤50 years.

DISCUSSION & CONCLUSIONS:This ELF-PEMF treatment might represent a promising adjunct to conventional therapy supporting osseous consolidation in elderly patients.

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Keywords: Bone and bone disorders (osteoporosis etc), Translation and commercialisation (inc. clinical trials and regulatory approval)

Developing platforms for electrical stimulation –electroconductive materials for neural tissue engineering applications

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INTRODUCTION:Nanomedicine, material science and cells should be combined to support tissue engineering strategies on the develop successful regenerative medicine therapies for the treatment of progressive and yet incurable neurodegenerative diseases.
In vitro culture platforms hold the promise in increasing the safety and effectiveness of cell therapies. In fact, the use of scaffolds on those systems is based on the principle that in vivo cell proliferation, metabolism, migration and differentiation follow cues given by the extracellular matrix. Polymeric fibrous scaffolds obtained artificially by electrospinning present have large surface areas and porosity, adjustable mechanic properties and can be functionalized with adhesion motifs.
Electrical stimulation can also promote effective cell differentiation of neural and other cells, improving the safety of cell therapies while boosting their efficacy. Conductive polymers can been used for direct cell stimulation.
An interesting platform for tissue engineering applications should, therefore, be composed of electrospun fibers made of conductive polymers. The choice for the best conductive polymer will depend on various factors. Some of these include (1) ease of processing (electrospinnability), (2) physico-chemical properties (hydrophilicity, electroconductivity, mechanical properties) and (3) biocompatibility.

METHODS:Our group has developed new electroconductive substrates suitable for cell culture, mainly through electrospinning. Some examples include polybenzimidazole (PBI) monoaxial fibers, cross-linked polyacrylonitrile (PAN) fibers and blended polycaprolactone-polyaniline (PCL-PANi) monoaxial and co-axial fibers. Chemical doping with strong acids, cyclization and conductive to carrier polymers proportion were used to increase conductivity for, respectively, PBI, PAN and PCL-PANi fibers.
The physico-chemical properties for substrates were monitored using scanning electron microscopy (SEM), Fourier transform infrared analysis (FTIR), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), followed by electrical and mechanical characterization. Biocompatibility was evaluated, with and without electrical stimulation, and changes in protein and gene expression were evaluated.

RESULTS:Our materials show electroconductivity suitable for electrical stimulation of cells. These are accompanied by different changes in fibers diameter, mechanical properties and their respective FTIR/DSC/TGA profiles. The different treatments/strategies used do not compromise the materials biocompatibility. Neural stem cells (NSCs) were able to successfully proliferate and/or differentiate on all substrates tested.

DISCUSSION & CONCLUSIONS:Overall we highlight the use of electrospun electroconductive fibers as platforms for neural tissue engineering applications. This opens the possibility to tailor more reliable platforms for other electro-responsive cells such as muscle and cardiac cells.
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Keywords: In vitro microenvironments, Biomaterials

Mechano-electrically active microenvironments for skeletal muscle tissue regeneration

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INTRODUCTION:Piezoelectric polymers have attracted interest offering new opportunities for skeletal muscle tissue engineering (TE), since they are electromechanically active, allowing tissue mechano-electric and electro-mechanical stimulation[1]. Physical stimuli have demonstrated to be important parameters to be considered for TE, but poorly applied in muscle regeneration. In this context, this work reports on magnetoelectric biomaterials suitable for effective proliferation and differentiation of myoblast in a biomimetic microenvironment providing the electro-mechanical stimuli associated to this tissue in the human body.

METHODS:Magnetoelectric films were obtained by solvent casting through the combination of a piezoelectric polymer, poly(vinylidene fluoride-trifluoro-ethylene) (P(VDF-TrFE)), and magnetostrictive particles (CoFe2O4). The non-poled and poled (with negative and positive surface charge) magnetoelectric composites were used to investigate their effect on C2C12 myoblast adhesion, proliferation and differentiation. The nanocomposites characterization was done by SEM, AFM, VSM and WCA.
Cell cultures were performed under static and dynamic conditions though the use of two types of bioreactors (magnetic and mechanical).

RESULTS:The inclusion of CFO nanoparticles leads to samples with an average roughness of ≈83±19nm for CFO/P(VDF-TrFE) non-poled films, value which decrease to ≈75±20 and ≈78±21nm, after positive and negative poling.
The CFO/P(VDF-TrFE) samples wettability (≈87±8º) decreases to ≈65±2º for positive and ≈59±4º for negative poling.
The biomaterials allowed mechanical (0.013µm.mm-2) and electrical (up to 64µV) stimulation to the cells. Relatively to the stimulation by magnetic bioreactor, it was demonstrated that after 5 days of differentiation, the fusion and maturation index values were more significative in charged samples. Non-poled samples lead to higher myotubes diameters and the poled samples lead to higher myotubes length. Also, the poled samples present higher values of creatine kinase compared with the non-poled ones, under dynamic conditions.
Relatively to the stimulation through mechanical bioreactor, the same behavior was observed, where the electrically charged materials lead to higher fusion and maturation index values than non-poled samples.
DISCUSSION & CONCLUSIONS:This work demonstrates the relevance of electromechanically active microenvironments for muscle TE. The mechanical and electrical activation of the microenvironment has been performed either directly (direct application of mechanical solicitation to produce the piezoelectric response of the material) or indirectly (magnetically activated) by using magnetoelectric biomaterials - that allows triggering cellular response by non-contact external stimulation.
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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), In vitro microenvironments

Epigenetic regulation promotes the osteogenic potency of osteoblast-derived extracellular vesicles

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INTRODUCTION:For bone augmentation strategies, there is great precedence to develop instructive acellular technologies which circumnavigates the limitations associated with the translation of cell-based therapies. Previously our group demonstrated the osteoinductive capacity of extracellular vesicles (EVs) acquired from mineralising osteoblasts (1, 2). Numerous studies have shown that regulating the cell's epigenetics through the inhibition of histone deacetylase (HDAC) enhances their differentiation potential. Therefore, this study aimed to investigate the potential of using the HDAC inhibitor Trichostatin A (TSA) to enhance the osteogenic potency of osteoblast-derived EVs.

METHODS:Osteoblast viability following TSA treatment was assessed via AlamarBlue quantification. Epigenetic modifications was evaluated by assessing HDAC activity and histone acetylation. The effects of TSA on osteoblast mineralisation was determined by quantifying alkaline phosphatase specific activity (ALPSA) and calcium deposition. EVs were isolated from untreated/TSA treated mineralising osteoblasts over a 2 week period. Relative EV size and concentration was defined using dynamic light scattering, nanoparticle tracking analysis, transmission electron microscopy and CD63 ELISA. Osteogenic differentiation of human bone marrow stromal cells (hBMSCs) cultured with untreated/TSA treated osteoblast-derived EVs was evaluated by qPCR, biochemistry, In-cell western (ICW) and histological analysis.

RESULTS:A time-dose dependent reduction in osteoblast viability was observed following TSA treatment. TSA substantially altered epigenetic functionally with significantly reduced HDAC activity, and increased histone acetylation. Treatment with 5 nM TSA significantly enhanced osteoblast ALPSA and calcium deposition when compared to that of the untreated cells during osteogenic culture. The quantity of EVs generated, in addition to their protein content and size correlated with the degree of osteoblast differentiation. TSA induced EVs significantly upregulated hBMSCs gene expression for osteoblast-related markers (ALP, COL1A, BSP1, OCN) throughout 14 days osteogenic culture when compared to hBMSCs cultured with untreated EVs. Similarly, ICW showed a substantial increase in the intracellular expression of osteoblast-related proteins of hBMSCs treated with TSA induced EVs throughout 21 days osteogenic culture. Importantly, it was demonstrated that TSA induced EVs significantly enhanced hBMSCs extracellular matrix mineralisation after 28 days of osteoinductive culture.

DISCUSSION & CONCLUSIONS:Our data suggests that altering the epigenome of osteoblasts accelerates their mineralisation capacity and enhances the osteogenic potency of their secreted EVs. The regulation of osteoblast epigenome provides a novel approach to harness the regenerative capacity of EVs for bone augmentation strategies.

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Keywords: Bone and bone disorders (osteoporosis etc), Differentiation

Platelet Lysate-Derived Extracellular Vesicles for Tissue Engineering and Regenerative Medicine Applications

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INTRODUCTION:One of the most interesting and emerging trends in the field of regenerative medicine is the use of extracellular vesicles (EVs) from various sources, including from human platelets. Although platelets primary function is hemostasis, they orchestrate the highly complex microenvironment present in tissue healing, releasing a wide range of biochemical signals and structural elements in spatial-temporal controlled manner, that modulate inflammatory and repair/remodeling processes. Platelets are also the major source of circulating EVs in human body, which are one of the main players in cell paracrine communication. In this context, the aim of this study is to characterize different EVs subpopulations from platelet lysate (PL) and understanding their effects in different cells, envisioning their application in targeted tissue engineering and regenerative medicine applications.

METHODS:PL-derived EVs were isolated by differential centrifugation. Particle size and concentration was assessed by nanoparticle tracking analysis (NTA), surface markers by flow cytometry and protein profile through proteomic analysis. The role of EVs in human adipose derived stem cells (hASCs) differentiation was assessed by gene expression analysis using real time RT-PCR and EVs angiogenic potential was evaluated using human endothelial cells (HUVECs).

RESULTS:Our results showed that we were able to isolate two distinct EVs subpopulations, namely exosomes (EXs;157±70nm;17.66x108EXs/mL) and microvesicles (MVs;219±117nm;12.71x108MVs/mL), being these positively marked for the tetraspanins CD9, CD63 and for the integrin CD41, which is expressed by platelets, and also, they have residual expression of CD81. The proteomic analysis, identified a total of 1267 proteins in EVs, of which 107 were identified in EXs and 67 in MVs. Furthermore, 1093 of these proteins were shared by both populations. Confocal micrographs supported by flow cytometry analysis showed that EVs could be internalized by hASCs after 2h. In addition, gene expression analyses demonstrated that EVs at different concentrations significantly altered the expression profile of diverse osteogenic, chondrogenic and tenogenic genes after 1 and 5 days of culture. EVs were also uptaken by HUVECs, influencing the angiogenesis process after 24h, namely, it was observed that MVs promote higher tube formation than EXs, suggesting a greater angiogenic potential.

DISCUSSION & CONCLUSIONS:These results indicate that EVs have a functional impact in hASCs differentiation, while MVs act as angiogenesis stimulator, encouraging the further study and use of EVs as therapeutics tools in regenerative medicine approaches.

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Keywords: Stem cells – general

Extracellular nanovesicles as biological transporters and genetic controllers for cell reprogramming

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INTRODUCTION:An increasing number of studies revealed that exosomes with various forms of cargos have high potential for the treatment of many cardiovascular diseases, influencing the behaviour and modifying the metabolic and genetic profile of recipient cells. Different types of cells have been utilized to produce exosomes for therapeutic purposes, and recently for cell reprogramming [1]. Notably, exosomes delivered by cardiac primitive cells (Exo-CPCs) are emerging as vehicles for horizontal transfer of genetic material, proteins and growth factors boosting cell proliferation and protection from oxidative-stress-related apoptosis and, above all, their differentiation. Exo-CPCs also seem to affect the extracellular microenvironment by stimulating the synthesis of specific extracellular matrix proteins, like fibronectin and collagen IV by fibroblasts [2]. As exosomes from CPCs have yet shown their likely role as controllers of cell genetic expression, we speculate that their administration, in vitro, could also induce fibroblast genetic profile modifications, exploitable for their future direct reprogramming towards cardiac progenitor cells.

METHODS:To validate our hypothesis we isolated and cultured human CPCs from hearts of donors (CPC-N) in serum-free culture medium and isolated secreted exosomes precipitating them from the medium by the means of a specific reagent. Exosomes obtained were analysed to characterize their cargo, quantified and administrated to fibroblasts from explanted hearts (FC-P), by adding them to the culture medium at specific dose and time.

RESULTS:After seven days of culture, CF-P were collected and underwent several assays to check if any change in their genetic profile occurred. Real-time PCR showed that the expression of transcripts specific for cardiac differentiation, like NKX 2.5, Mef2C, Myosin and Actin was increased in FC-P treated with Exo-CPC with the respect to the control group. Immunofluorescence staining confirmed the presence of positivity for markers typical of progenitors of cardiac cell lineages, like cardiac myocytes and vascular smooth muscle cells.

DISCUSSION & CONCLUSIONS:Direct reprogramming of resident, mature and fully differentiated fibroblasts into cardiac progenitor cells represents an intriguing and minimally invasive approach for cardiac regeneration, avoiding concerns including teratoma formation or issues related to retrovirus usage. Nonetheless, we are still far from reaching this ambitious goal, and further investigations are needed to assess both the effectiveness and the safety of the use of exosomes for direct cellular reprogramming.

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Keywords: Cardiovascular, Stem cells – general

Alteration of Extracellular Vesicle Cargo after Pro-inflammatory Priming of Umbilical Cord Mesenchymal Stem Cells

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INTRODUCTION:Human Umbilical Cord Mesenchymal Stem Cells (UCMSCs) have been shown to suppress inflammatory responses in in vitro and animal studies of autoimmune diseases. These therapeutic effects can be attributed to paracrine signalling, by which extracellular vesicles (EVs) are one of the essential components. The anti-inflammatory properties of UCMSCs is dependent on the culture conditions they are grown. Therefore, this study aims to study the EVs from UCMSCs grown under different conditions: normal oxygen (21% O2), low oxygen (5%O2), primed with a pro-inflammatory cocktail. The aim of the study is to identify a population of EVs with an immunosuppressive potential that can supress activated T-cells from patients with rheumatoid arthritis.

METHODS:UCMSCs were isolated enzymatically, culture expanded in a Quantum® bioreactor and grown in normal oxygen, low oxygen and pro-inflammatory conditions (IL-1β, TNF-α, IFN-γ). EVs were isolated from UCMSC conditioned media by differential ultracentrifugation using a 30% sucrose cushion and characterised by Transmission Electron Microscopy (TEM) and Nanoparticle Tracking Analysis. Common EV markers were analysed using a europium-based immunoassay, MACSPlex Exosome Detection kit and immunoblotting. A proximity-based extension assay was used to identify the expression of 92 inflammatory proteins in the EV cargo.

RESULTS:There was no difference in EVs cultured in normal oxygen, low oxygen and pro-inflammatory conditions when analysed for size, particle concentration and morphology. TEM images of EVs showed a characteristically round morphology with a phospholipid bilayer. All EVs displayed the common tetraspanin markers (CD9/63/81) and cytosolic proteins (ALIX, HSP70). There were no differences in EV protein cargo between normal and low oxygen conditions. However, EVs from primed cells showed an increased expression of 11 pro-inflammatory cytokines including a >2-fold increase of 6 CC chemokines and a 10-fold increase in CXCL5 and CSF-1.

DISCUSSION & CONCLUSIONS:Overall, the pro-inflammatory primed UCMSC-EVs displayed an upregulation of proteins associated with chemotaxis and angiogenesis. Next-generation sequencing will identify anti- and pro-inflammatory miRNAs in EV cargo.The next step is to determine if the differential protein expression from primed EVs correlates with a change in T-cell proliferation and polarisation.

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REFERENCES:

Keywords: Extracellular vesicles, Multipotent (mesenchymal) stem cells

Clinical grade MSC-secretome promotes human cartilage recovery in vitro

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INTRODUCTION:Osteoarthritis (OA) is a disabling joint disorder causing articular cartilage degeneration. [1] Currently, treatments are mainly pain- and symptom-modifying, rather than disease-modifying. Human bone marrow stromal cells (hBMSCs) have emerged as a promising paracrine mechanism-based approach for the treatment of OA. [2,3]

METHODS:We developed a "donor-to-patient" closed, scalable and automated system for aseptic therapeutic cell manufacturing using a a xeno-free culture system (XFS). We characterized cells, secretome and extracellular vesicles (EVs) of hBMSCs, grown in XFS compared to a conventional fetal bovine serum (FBS) culture system. We investigated also the therapeutic potential of conditioned media (CM) and EVs in an in vitro model of OA. We treated primary cultures of human articular chondrocytes (hACs) with IL-1α to induce an inflammatory process, and with hBMSC-CM or hBMSC-EVs for 16 and 48 h. A parallel experiment was performed involving pre-treatment of hACs with hBMSC-CM or hBMSC-EVs for 3h before the induction of inflammation.

RESULTS:First, we observed that XFS promoted enhanced growth and viability of hBMSCs compared to FBS-containing medium while preserving their typical phenotype. The secretome of these cells comprised biological factors involved in homeostasis, wound healing and angiogenesis, as well as a marked production of EVs. The biological effects of the CM and EVs derived from hBMSC cultivated in XFS- and FBS-based medium were tested on IL-1α treated hACs in an experiment designed to mimic the OA environment. We observed that under inflammatory conditions hACs are able to recruit and internalize more MSC-derived EVs, especially those derived from cells cultured in our XFS system. Treatment with CM derived from XFS-cultured hBMSC inhibited IL-1α-induced expression of IL-6, IL-8 and COX-2 by hACs compared to FBS-based condition. Furthermore, we observed that hBMSCs grown in XFS produced a higher amount of EVs compared to FBS culture. The hBMSC-EVs not only inhibited the adverse effects of IL-1α-induced inflammation but exerted a significant chondroprotective effect in vitro.

DISCUSSION & CONCLUSIONS:In conclusion, the XFS medium was found to be suitable for isolation and expansion of hBMSCs with increased production of EVs and active paracrine factors. The application of cells cultured exclusively in XFS overcomes issues of safety associated with serum-containing media and makes ready-to-use clinical therapies more accessible.

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Keywords: Cartilage / joint and arthritic conditions, Translation and commercialisation (inc. clinical trials and regulatory approval)

The application of osteoblast-derived extracellular vesicle enriched fractions for hard tissue regeneration

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INTRODUCTION:Extracellular vesicles (EVs) could provide an acellular means of inducing tissue regeneration. Within hard tissues, attention has historically focused on vesicles bound within the calcified extracellular matrix - so called 'matrix vesicles'. However, liberation of these vesicles is time consuming and applies potentially disruptive digestion protocols that are likely to restrict their biotherapeutic activity. Research into the function of unbound osteoblast EVs generated during mineralisation has been much less intensively studied. We propose that unbound extracellular vesicles can be efficiently collected in vitro from continuous cell cultures and presents a potentially viable alternative to cell-based approaches for inducing hard tissue regeneration.

METHODS:In this study EV concentrated fractions were isolated from the culture medium of mineralising osteoblasts (16/SS/0172) over a period of 3 weeks using differential ultracentrifugation. The presence of EVs was validated using nanoparticle tracking analysis and transmission electron microscopy. The presence of exosome-associated proteins (ALix, TSG101, CD63) was verified by Western blotting. Temporal variations in the EV proteome were analysed weekly using liquid chromatography tandem-mass spectrometry (LC-MS/MS). The capacity of 5 µg/mL of the EV concentrated fraction to induce mineralisation in stem cell cultures was assessed against a clinical gold-standard, BMP-2. Cellular alkaline phosphatase activity was quantified as a measure of osteogenic induction. Calcium accumulation was visualised using the alizarin red assay. Mineral phase was analysed using X-ray fluorescence (XRF) and infrared spectroscopy (IR).

RESULTS:EV concentrated fractions significantly enhanced alkaline phosphatase levels, mineralisation rate and mineral volume beyond BMP-2. XRF elemental mapping showed enriched areas of calcium and phosphorus co-localisation in EV supplemented cultures. Infrared spectroscopy analysis of the mineral phase confirmed the presence of octacalcium phosphate, an intermediate phase in the formation of hydroxyapatite. Principal component analysis and accompanying TEM-coupled energy dispersive X-ray spectroscopy (EDX) localised mineralisation to the EV phospholipid membrane, implicating EVs as sites of mineral nucleation. Proteomic analysis of EVs revealed the presence of bridging collagens, calcium chelating proteins and extracellular binding proteins. Temporal proteomic comparison of EV fractions over the course of 3 weeks revealed that the relative intensity of these proteins was significantly ($P<0.05$) upregulated as mineralisation advanced.

DISCUSSION & CONCLUSIONS:Our data suggests that EV concentrated fractions are able to enhance mineralisation in MSC culture and have potential value in regeneration medicine. However, it will be important to define whether the these effects are purely EV mediated and how these therapies can be standardised for the production of clinical products.

Keywords: Extracellular vesicles, Bone and bone disorders (osteoporosis etc)

Toward Retinal Tissue Engineering by Using a Novel Electrospun Nanofibrous Scaffold

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INTRODUCTION:Age-related macular degeneration (AMD) is the principal cause of blindness in the elderly worldwide, affecting about 30-50 million individuals[1]. AMD consists in a degeneration of the central retina due to age-related changes in Bruch's membrane (BM) and in the retinal pigment epithelium (RPE). Currently, periodic intravitreal injections of anti-vascular endothelial growth factor drugs are the gold standard therapy in the management of AMD. However, these drugs are unable to restore tissue functionality. Retinal tissue engineering may be helpful in providing better solutions. This work aims at designing a functional BM.

METHODS:We fabricated electrospun nanofibrous membranes composed of *Bombyx mori* silk fibroin (BMSF) and polycaprolactone (PCL) and we investigated their properties: surface morphology, thickness, permeability, mechanical properties, and *in vitro* cytocompatibility. We employed ARPE-19 cells for *in vitro* studies.

RESULTS:The BMSF/PCL membranes were successfully produced by electrospinning. The membranes were constructed of randomly oriented fibres and displayed a thickness of 40 µm, an average fibre diameter of 938±223 nm, and a fibre packing density of 49.25±1.2%. The maximum tensile strength of the membrane was 3.15±0.33 MPa, with an ultimate strain of 35.4±6.22% and a Young's modulus of 13±3.6 MPa and its permeability was $2.7\times10^{-18}\pm9.8\times10^{-19}$ m². Cells were viable after 5 days of direct contact with the retinal membranous scaffold.

DISCUSSION & CONCLUSIONS:An ideal substrate for constructing a prosthetic BM with attached RPE cells has yet to be found. The obtained scaffolds showed structural and mechanical similarity to human BM, which has a random fibrillar network, a packing density of 48%, and a Young's modulus ranging from 6 to 14 MPa[2]. Furthermore, we found out that scaffolds are suitable for cell culture and we are carrying out additional investigation with ARPE-19 cells. However, to more closely imitate the BM, fibre diameters and membrane thickness need to be further reduced. The successful outcome of this study will inform the treatment of an optimal substrate as a basal support for RPE. Moreover, this work may lead to new studies where a second layer of photoreceptors can be bioprinted on the RPE.

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Keywords: Eye, Biomaterials

Extracellular matrix hydrogels derived from waste corneal and scleral donor tissue for delivery of corneal mesenchymal stem cells to the injured ocular surface

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INTRODUCTION:Inflammation to the ocular surface can induce tissue damage and cause blindness. Though corneal transplantation is a viable treatment option, surgery cannot be performed in the presence of active inflammation, leading to waiting times of up to 18 months. Therefore, there is an unmet clinical need for an early intervention therapy. Adult stem cells, including corneal mesenchymal stem cells (C-MSCs), have unique wound healing properties, secreting trophic factors in response to injury, communicating with the wounded environment, dampening inflammation, and implementing a dynamic healing response. In order to provide this healing response, C-MSC need to be delivered to the eye in a favourable environment; we propose doing so through ECM hydrogels. Although ECM hydrogels have manufactured using corneal tissue [1], scleral tissue has never been used, a much more abundantly available waste product from corneal transplant. This project is an initial step in comparing ECM hydrogels formed from corneal and scleral tissue, and their ability to support C-MSC populations.

METHODS:Corneal and scleral tissue were decellularised by 3 different methods to determine the optimal technique: freeze-thaw, SDS treatment and hypertonic NaCl. Decellularisation efficiency was determined by histology, immunohistochemistry and DNA analysis. Decellularised tissue was dried, pepsin digested and ECM hydrogels formed by neutralization. Hydrogels from both cornea and sclera were assessed for light transmittance, biochemical composition assessed by SDS-PAGE and structure assessed by histology, immunohistochemistry and environmental-SEM. C-MSC were cultured within/on the hydrogels and viability, proliferation, phenotype and secretome assessed compared to conventional culture.

RESULTS:ECM hydrogels from both scleral and corneal tissue formed after all three decellularisation techniques, although sodium chloride and freeze-thaw techniques were found to be more efficient in removal of DNA. Composition of the pepsin digest were similar between decellularisation techniques and similar to non-decellularised controls. There were no differences in transparency between ECM hydrogels formed from digested scleral and corneal tissue and structure appeared similar. Both corneal and scleral hydrogels supported the viability, proliferation and phenotype of C-MSC.

DISCUSSION & CONCLUSIONS:This study showed that ECM hydrogels can be formed from waste donor tissue, either using corneal tissue deemed unsuitable for transplant or scleral tissue that is often discarded as waste. These hydrogels can support the growth and proliferation of C-MSC, and are ideal candidates for a topical delivery system for stem cells to the front of the eye.

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Keywords: Hydrogels and injectable systems, Multipotent (mesenchymal) stem cells

Anterior cornea tissue equivalents based on decellularized stromal sheets and cell-laden collagen hydrogels

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INTRODUCTION:To overcome the important shortage of donor corneas for transplantation, alternatives based on tissue engineering need to be developed. Decellularized corneas are a great biomaterial, but their densely packed collagen architecture difficults their recellularization *in vitro*. Therefore, alternative fabrication methods that ensure high cellularity throughout the scaffold in a rapid manner are desirable. In this study we have developed constructs based on the use of decellularized corneas but using a bottom up approach by layering sheets of decellularized tissue and cell-laden hydrogels.

METHODS:Corneal lenticules were obtained from porcine corneas by cryosectioning, decellularized as previously described [1] and air-dried for storage. Degradation was analysed by digestion with collagenase I. Human stromal cells were encapsulated in collagen I hydrogels and casted in between dried sheets. Constructs were cultured in serum-free medium supplemented with ascorbic acid and insulin for 3 weeks. Epithelial cells were then seeded on the surface. Transparency, cell viability and phenotype were analysed by qPCR, histology and immunofluorescence. Constructs were sutured onto an *ex vivo* porcine cornea and cultured for one week.

RESULTS:Lenticules were successfully decellularized, achieving dsDNA values of 13 ± 1.2 ng/mg dry tissue, and were more resistant to degradation than collagen I hydrogels. Constructs presented high cell viability and keratocyte-like phenotype, by up-regulation of keratocan, decorin, lumican, collagen I, ALDH3A1 and CD34. Corneal epithelial cells stratified and presented cobblestone morphology. Sutures were well tolerated and no tearing of the construct occurred. After 7 days, constructs were covered by a neo-epithelium from the host porcine cells and integration into the host stroma was observed.

DISCUSSION & CONCLUSIONS:This study describes a method of fabricating anterior corneal substitutes in a simple and rapid manner, obtaining mature and suturable constructs using only tissue derived materials.

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Keywords: Decellularised matrices, Biomaterials

Human Induced Pluripotent Stem Cells Derived Lacrimal-Gland-On-Chip

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INTRODUCTION:Lacrimal gland (LG) is an exocrine gland that secretes lacrimal fluid, which moistens and protects the eye surface. LG related dry eye disease leads to discomfort, inflammation and even vision loss. Human induced pluripotent stem cells (hiPSCs) harbor the potential to differentiate into cell types that can form functional LG *in vitro*. Combining tissue constructs with microfluidics offers recapitulation of cell microenvironment by allowing precise control of mechanical and biochemical parameters. This study aims to construct a 3D LG from hiPSCs in an organ-on-a-chip system.

METHODS:Developmentally, LG is formed from the interaction of periocular mesenchyme (POM) with conjunctival epithelium (CE) cells. To obtain CE cells, hiPSCs are cultured in 2.5D Matrigel® and multi-zone ocular cells (MZOCs), are obtained[1]. MZOCs have been shown to contain ocular surface ectodermal cells (OSE), which are the ancestors of CE cells. To sort out CE cells, FACS is employed at different stages of differentiation. POM differentiation is carried out by inhibiting TGFβ and WNT pathways[2]. The obtained cells are cultured in 3D in a custom made microfluidic chip to mature them into LG cells. The LG formation is induced with FGF10 and BMP7. Tear production is assessed by monitoring lactoferrin secretion with ELISA.

RESULTS:The differentiation processes were monitored with immunofluorescence and gene expression analysis of cell specific marker proteins. POM cells were obtained after 2 weeks of inhibitor treatment. These cells have the ability to be subcultured and remain viable in 3D Matrigel® culture. We have shown that 3-week culture of MZOCs give rise to OSE and extended culture can allow direct differentiation of OSE to CE. We later showed that 8 weeks old MZOCs offer the highest proportion of culturable CE cells after FACS.

DISCUSSION & CONCLUSIONS:To bioengineer a functional lacrimal gland, here we adapted a developmental approach where iPSCs are separately differentiated to epithelial and mesenchymal cells. During LG development, POM secretes FGF10, which induces CE to thicken and invade POM. Further BMP7 secretion results in branch formation. This interaction gives rise to the LG tissue with LG epithelium and LG mesenchyme. For this purpose, hiPSCs have been firstly differentiated into CE and POM. Further these cells are merged in 3D hydrogel and cultured within a microfluidic system.

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Keywords: Induced pluripotent stem cells, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Testing the functionality of a biosynthetic poly-ε-lysine based corneal endothelial graft in a rabbit model of corneal endothelial damage

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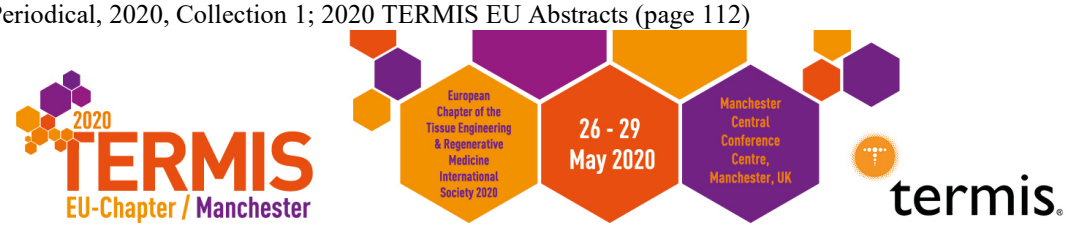
INTRODUCTION:Corneal endothelial (CE) dysfunction resulting from progressive cell loss leads to visual impairment and a requirement for a corneal endothelial transplant. There is a worldwide donor cornea shortage so biosynthetic graft alternatives are being developed using *in vitro* expanded CE cells. We have developed a synthetic peptide hydrogel using poly-ε-lysine (pεK) that is thin, transparent, porous and robust. Here we investigate its functionality as a tissue engineered corneal endothelial graft in a rabbit model of endothelial damage.

METHODS:PεK hydrogels were synthesised from pεK crosslinked to 60% with nonanedioic acid to a polymer density of 0.111g/ml using N N-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI). The polymer solution was cast in the condensation rings on the lid of a 48 well plate or punched into 8mm circles for *in vivo* grafts. Gels were seeded with primary porcine corneal endothelial cells (CECs) at a density of $1x10^5$ cells/gel and cultured for 3 weeks. An *ex vivo* anterior chamber (AC) model (Barron) using porcine corneas was used to optimise delivery and attachment of the graft to the posterior cornea. OCT imaging was used to assess graft attachment and thickness of the cornea. PεK hydrogels (+ and – cells) were transplanted into the right eyes of New Zealand white rabbits with Descemet's membrane removed. A group with no gel acted as control. OCT imaging and pachymetry was used to measure corneal thickness for the experimental period (3 weeks). Photographs of rabbit corneas were taken to assess corneal clarity and eyes were dissected for histological analysis.

RESULTS:Porcine CECs adhered to the pεK hydrogels and formed monolayers. Gels delivered to the *ex vivo* AC model using an Endoserter adhered to the posterior porcine cornea. They remained attached and corneas with gels + cells were shown to be thinner than control corneas using pachymetry and histology. Gels+cells transplanted into rabbit eyes *in vivo* remained attached and the thinness of the corneas shown using pachymetry was maintained in the group with gel+cells (379.11µm SD 23.5), to a lesser extent with the gel alone (676.06µm SD 374.11) but not in eyes with no gels (all above detection limit of pachymeter >1000µm).

DISCUSSION & CONCLUSIONS:The *ex vivo* and *in vivo* data suggest that the bio-synthetic grafts are able to function to maintain the hydration levels of the corneal stroma and retain clarity of the cornea. The partial success of the acellular graft is an interesting finding that requires further investigation.

Keywords: Biomaterials, In vivo and animal models



Local non-viral gene delivery to immunomodulate and enhance fracture healing

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INTRODUCTION:Although 80% of fractures typically heal without complications, there is a small proportion (≤ 20%) that experience delayed healing or non-union. In patients with complications, there is a typical involvement of excessive pro-inflammatory cytokines and/or failure to resolve inflammation due to defective regulatory mechanisms. Thus, immunomodulation of the local fracture microenvironment, such as by enhancing anti-inflammatory cytokine production, could be an effective way to enhance fracture healing. Accordingly, the overall objective of this study is to develop an innovative gene-based therapy that mitigates the negative effects of inflammation while providing a structural template for new bone formation.

METHODS:In this study, a collagen-hydroxyapatite scaffold is used as a platform for the delivery of pDNA, encoding for interleukin-1 receptor antagonist (IL-1Ra), complexed to the robust non-viral gene delivery vector, polyethyleneimine (PEI). We utilize pDNA encoding for GFP and Gaussia luciferase as reporter genes to determine the transfection efficiency and gene expression profile in rat bone marrow-derived mesenchymal stem cells (MSCs). The effect of PEI-pDNA nanoparticles on cell viability was evaluated using cell titer blue assay. The conditioned medium of PEI-pIL-1Ra transfected cells was checked for IL-1Ra bioactivity using HEK-Blue-IL1b reporter cell line. The capacity of IL-1Ra gene activated scaffolds to mitigate IL-1b-induced inhibition of osteogenesis was determined by micro-CT analysis.

RESULTS:We have determined that PEI-pDNA nanoparticles can achieve a transient gene expression profile in MSCs, with a transfection efficiency of 14.8±1.8%. The PEI-pDNA nanoparticles had a limited effect on cell viability after 10 days in culture, in terms of their metabolic activity. Cells transfected with PEI-pIL-1Ra were found to produce functional IL-1Ra, with the capacity to antagonize IL-1b-mediated alkaline phosphatase activity. Nanoparticles carrying pDNA encoding for IL-1Ra have been successfully incorporated into collagen-hydroxyapatite scaffolds, as verified by reporter assays. Most encouragingly however, was the finding that mineralization within scaffolds can be inhibited by exposure to IL-1b, and that this negative effect could then be mitigated in scaffolds incorporating PEI-pIL-1Ra nanoparticles.

DISCUSSION & CONCLUSIONS:The transient nature of therapeutic gene expression in our approach offers a key advantage that potentially enables the preservation of the initial pro-inflammatory response to fracture, which is crucial to the healing cascade. Utilizing our therapy is also advantageous over other approaches including recombinant protein and viral gene therapies. Studies are currently on-going to evaluate the therapeutic efficacy of our approach in a femoral osteotomy model in rats.

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Keywords: Bone and bone disorders (osteoporosis etc), Immunity / immunomodulation / macrophage

Characterisation of collagen-based scaffolds as three-dimensional models for breast cancer tumours and bone metastasis

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INTRODUCTION:Breast cancer (BC) is the most common form of cancer in women worldwide. Cancers are ideally placed for targeted gene therapy as they are characterised by various genetic mutations. However, 2D models fail to accurately model the tumour microenvironment of breast cancer. Collagen-based scaffolds can be utilised to bridge the gap between 2D and animal models [1,2]. The technology proposed here seeks to use collagen-based scaffolds to firstly develop a more accurate representation of the tumour microenvironment and secondly use it to assess the potential of nucleic acid delivery to abrogate primary and metastatic BC cell growth. METHODS:Triple-negative BC cells (MDA-MB-231 and MDA-MB-436) and epithelial cells (MCF-10A) were grown on collagen-glycosaminoglycan (GAG) scaffolds to model BC primary tumours and on collagen-nanohydroxyapatite (nHA) scaffolds to model bone metastasis. BC cells grown on scaffolds were visualized using scanning electron microscopy. Cell growth was assessed over 21 days and gene expression comparison was performed by qPCR comparing BC cells and epithelial cells cultured on 3D scaffolds and in 2D to validate the model. microRNA (miRNA-146a-5p) and siRNA (siVEGF) delivery was performed using Lipofectamine RNAiMAX to inhibit breast cancer cell growth in 2D and 3D. Uptake was confirmed by confocal imaging and functionality was confirmed by quantitative real-time PCR. DNA and metabolic assays were performed to evaluate effects on proliferation and metabolic activity after nucleic acid delivery.

RESULTS:Significant changes were identified in gene expression comparing BC cells cultured on the two different scaffolds and in 2D. Nucleic acids were successfully delivered and significant inhibition of cell growth was measured. Furthermore, differences in the effects of miR-146a and VEGF manipulation in 2D compared to the two different scaffolds were demonstrated.

DISCUSSION & CONCLUSIONS:This study characterises collagen-based scaffolds to adequately model BC primary tumours and bone metastasis. It demonstrates the uptake and functionality of nucleic acid delivery to BC cells cultured on the scaffolds. The therapeutic effect of nucleic acid delivery correlates with in-vivo effects as evident in the literature [3,4]. The results show the suitability of the scaffolds to model different BC tumour microenvironments and evaluate the effectiveness of gene therapy.

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Keywords: Microenvironment and niche engineering, Cancer

Design of injectable hydrogels based on hyaluronic acid-peptides derivatives for tissue engineering

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INTRODUCTION:Shear-thinning injectable hydrogels have emerged as promising biomaterials as they eliminate the need for surgical implantation and can be obtained ex-vivo and easily injected.[1] Among them, small peptide hydrogelators have attracted great attention due to their ease of synthesis, biodegradability and their ability to self-assemble under physiological conditions, mainly in response to stimuli.[2] However, one of the main drawbacks of these systems is their low resistance to strain. Indeed, this can be a problem for tissue engineering applications due to the exogenous strains and the forces exerted by cells.[2] With this in mind, we hypothesized that grafting short gelling peptides on hyaluronic acid (HA), a polysaccharide widely distributed in body tissues,[3] would allow formation of injectable hydrogels with properties particularly suitable for engineering artificial soft tissues.

METHODS:Different beta-sheets forming peptides were thus selected to physically crosslink HA. In order to optimize the properties of these new hybrid hydrogels, we carefully investigated the relationships between the structural parameters of the HA-peptide conjugates (peptide structure, degree of substitution and molar mass of HA) and the morphological and mechanical characteristics of the hydrogels. Then, in-vitro effect on cell viability of the hydrogels was explored.

RESULTS:In this sense, two different peptides have successfully been grafted. These HA-peptide conjugates are capable of forming hydrogels at physiological pH, due to crosslinks made by the formation of beta-sheets between the grafted peptides as confirmed by circular dichroism, thioflavin T binding studies and transmission electron microscopy. Furthermore, dynamic rheological measurement demonstrated that the formation of these beta-sheets is a time-dependent and temperature-dependent process, thus allowing possibility to tune the gel strength. In addition, these hydrogels present self-healing and injectability properties and are capable of encapsulating cells without damaging them.

DISCUSSION & CONCLUSIONS:All together, these results let us believe that these new HA-peptide hydrogels are promising biomaterials for tissue engineering as well as other biomedical applications.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

A biocompatible and injectable hydrogel for the sustained treatment of neurodegenerative diseases

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INTRODUCTION:Neurodegenerative diseases (NDs) are one of the most challenging medical problems, being associated with tremendous human suffering and socio-economic impact. Advanced therapy medicinal products (ATMPs) emerged as treatment alternatives due to their potential to cure severe chronic conditions[1]. However, despite high expectations, the clinical use of ATMPs is still very limited. To treat neurological diseases the therapeutic agents generally need to cross a major obstacle, the blood–brain barrier, which can severely limit the treatment efficacy[2]. Consequently, the direct injection into the central nervous system (CNS) can boost and accelerate the therapeutic effects. The ATMPs efficacy can be further improved by their engineering with natural-based biomaterials and/or through their combination with adjuvant therapies. Therefore, we designed a hydrogel for the direct injection into the CNS and consisting of compounds naturally present in it. Liposomes were used to physically crosslink the hyaluronic acid-based hydrogel to present the ability to carry cells and other therapeutic agents (e.g. growth factors).

METHODS:Unilamellar liposomes characterization comprised the determination of their size, polydispersity index (PDI), surface charge, temperature transition and distribution in the hyaluronic acid matrix. The hydrogel thermal and rheological behaviours were also assessed. Cytocompatibility was investigated using bone marrow mesenchymal stem cells, since they have several features relevant to NDs treatment. In vivo compatibility assessment was performed by direct injection of the hydrogel into the rat ventricular space, since the intracerebroventricular route is safe and well-tolerated, even within the pediatric population. Finally, a fluorescently-labelled hydrogel was used to determine its brain distribution.

RESULTS:LUVs presented a size of 115.7+3.5 nm with a PDI of 0.088+0.022 and a zeta-potential of -33.4+3.7 mV. The uniform distribution of liposomes within the gel matrix was also demonstrated, being this mixture thermally stable. The cells encapsulated were able to adhere to, survive and proliferate within hydrogels. In vivo assays demonstrated the hydrogel compatibility and diffusion into corpus callosum.

DISCUSSION & CONCLUSIONS:The physicochemical characterization and in vitro and in vivo assays demonstrated that the gel displays appropriate properties to be directly injected into the CNS. Moreover, being corpus callosum damage responsible for important neuronal deficits, the predisposition of the hydrogel for this bundle of nerve tissue can be ideal to treat NDs patients. Therefore, the engineering of ATMPs using this natural carrier can be a new hope in the treatment of those highly debilitatin conditions.
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Keywords: Nervous system (brain-central-peripheral / disorders), Advanced therapy medicinal products

Laponite hydrogel scaffolds containing graphene oxide and phosphonate moieties for bone tissue engineering

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INTRODUCTION:Interest in materials for bone tissue engineering (BTE) has increased with the increasing need for treatments for bone disorders, especially in ageing populations. This work aims to develop a composite hydrogel containing Laponite nanoclay, graphene oxide (GO) and phosphonate-containing polymer for BTE. Laponite is an osteoinductive synthetic clay that forms injectable hydrogels and degrades into nontoxic products [1]. Phosphonates are organic phosphorous compounds that aim to mimic the function of bone-protecting bisphosphonate drugs [2]. Graphene is a stiff and readily modifiable form of carbon that can both tune the mechanical properties of composites and serve as a delivery platform for therapeutic agents [3]. Therefore, this composite would promote accelerated bone repair and regeneration due to the synergistic effect of all three components.

METHODS:Laponite XLG, GO and poly(vinylphosphonic acid-co-acrylic acid) (PVPA-co-AA) were dispersed in deionised water at desired concentration using magnetic stirrer. Osteoblast-like cells were encapsulated in Laponite-based hydrogels and added drop-wise into culture medium. After 24 hours, one group of samples was transferred to osteogenic conditions. At 1 and 3 weeks post-culture, samples were fixed and harvested for analysis. Also, cell spreading was investigated by phalloidin staining at 1, 3 and 7 days post-seeding.

RESULTS:Laponite-based hydrogels were injectable and exhibited a shear-thinning behaviour. Phalloidin staining revealed cell morphology and degree of cell spreading within hydrogels. After 1 week post-seeding, positive stain of calcium deposition was observed in Laponite-GO-(PVPA-co-AA) hydrogels in both media with and without osteogenic inducers, whereas Laponite hydrogels showed positive stain only in osteogenic condition. Mineralised matrix was observed in all Laponite-based hydrogels after 3 weeks of culture.

DISCUSSION & CONCLUSIONS:Phalloidin staining suggested the restricted cytoskeletal organization of osteoblast-like cells within hydrogels due to a small porous structure. GO and PVPA-co-AA could accelerate cell mineralisation in the absence of osteogenic inducers. It is hypothesised that the P–C bond in PVPA can mimic the P–C–P backbone in bisphosphonates and can induce osteoblast maturation subsequently improving mineralisation.2 However, Laponite-based hydrogels tended to degrade in culture medium after 1 week, resulting in difficulty of analysis for in vitro model.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

Self Assembling Peptide Scaffolds to deliver Schwann-like Stem cells for Nerve Regeneration

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INTRODUCTION:Transection of a peripheral nerve (typically following trauma) leads to loss of function and significant physical and psychological morbidity. To repair divided nerves, the severed ends are brought together surgically; this isn't possible if there is a defect/gap between the two. Current management to transplant a less essential nerve (i.e. Autologous Nerve Graft; ANG) to the injured defect can cause significant donor site morbidity. Engineered Nerve Guidance Conduits (NGC, typically hollow, biocompatible tubes) are available instead of ANG's to bridge a nerve gap, but these have poorer outcomes, especially if the gap is large (>2cm). Unlike ANG's, NGCs lack Schwann cells (SC) which have an essential role in supporting regenerating axons after injury in the peripheral nervous system. Therapeutic delivery of SC within an engineered nerve guidance conduit (NGC) could improve outcomes. Human adipose derived stem cells (hASC) are easily sourced and can be differentiated to a Schwann-like phenotype (hdASC), although stability of the hdASC phenotype has been questioned. We present our initial findings investigating the use of novel self-assembling peptide (SAP) hydrogels produced by Manchester Biogel® as a scaffold to stabilise/improve hdASC differentiation and a plan of further research to combine this with a novel NGC (POLYNERVE®) developed by our group.

METHODS:Viability of hdASC cultured on SAP supplied by Manchester Biogel® in 2D and hASC in 3D were assessed with LIVE/DEAD staining. Effect of cell seeding and culture conditions on gel degradation and stiffness were assessed rheologically. Gene expression for maintenance of a neurogenic phenotype was monitored in hdASC cultured on the gels. Rat dorsal root ganglia (DRG) viability and functionality on gels were investigated through LIVE/DEAD assays and by measuring intracellular calcium increase following KCl induced depolarisation.

RESULTS:SAP gels supplied by Manchester Biogel® enable functional DRG adherence and neurite outgrowth without the need for laminin coating. Culture of hdASC in SAP hydrogels favour a neurotrophic phenotype compared to collagen controls. Gel degradation rates are greatly increased in the presence of cell culture conditions.

DISCUSSION & CONCLUSIONS:SAP gels provide an ideal medium of culture for ASC1, dASC and DRG. Given these initial findings, we aim to investigate a SAP hydrogel containing hdASCs that could be delivered within our novel nerve conduit (POLYNERVE®) for further in vivo trials.

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Keywords: Nervous system (brain-central-peripheral / disorders), Cell therapy

Spatiotemporal biomaterial modification via cytocompatible supramolecular complexation

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INTRODUCTION:Native tissues are characterized by a dynamic nature. Recapitulating such dynamicity in engineered tissues requires the temporal control over their biochemical composition. Typically, spatiotemporal modification of biomaterials relies on photoresponsive strategies, which pose the inherent risk of cytotoxic UV-light and radical-based reactions. Here, we pioneered supramolecular desthiobiotin/avidin complexation to enable the dynamic modification of biomaterials. Desthiobiotin is a non-sulfur containing analog of biotin that also interacts with avidin, but with substantially lower binding affinity than biotin (Kd,biotin~10-15 M vs Kd,desthiobiotin~10-13 M). We hypothesized that a supramolecular desthiobiotin/biotin displacement strategy would grant spatiotemporal control over the biochemical composition of biomaterials in a novel, facile, and cytocompatible manner.

METHODS:Dextran-tyramine-biotin (Dex-TA-biotin) was synthesized as previously described. Hydrogels were prepared by mixing 5% Dex-TA-biotin, 3 U/ml horseradish peroxidase, and 0.05% H2O2. Hydrogels were further functionalized with 1 µM tetravalent neutravidin (i.e., avidin analog) and 1 µM desthiobiotin-FITC, biotin-atto565, and/or biotin-FITC, and subsequently analyzed using fluorescence recovery after photobleaching (FRAP) and fluorescence confocal microscopy.

RESULTS:Fluorescence confocal microscopy and FRAP confirmed that biotin-FITC was coupled to Dex-TA-biotin hydrogels via neutravidin, but not to non-functionalized (i.e. Dex-TA) hydrogels, which validated the successful generation and functionality of Dex-TA-biotin hydrogels. As shown in Figure 1, the reversible and sequential modification of hydrogels was demonstrated by displacing desthiobiotin-FITC (i.e. green) with biotin-atto565 (i.e. red). By tuning the concentration and incubation time of biotin-atto565, we could reproducibly control its penetration depth into the hydrogels. This strategy granted spatial control over the hydrogels' biochemical composition by determining the thickness of the biotin-displaced shell. Performing the supramolecular displacement strategy in the presence of cells did not reveal a cytotoxic effect, as assessed by live/dead cell staining. Moreover, the method enabled the spatiotemporal capturing and presentation of, for example, bioactive peptides (e.g., RGD) and endogenous growth factor, which was validated using surface plasmon resonance.

DISCUSSION & CONCLUSIONS:In situ tuning of the biochemical composition of engineered tissues is key to mimic the dynamic nature of native tissues. We have successfully demonstrated a novel method for the spatiotemporal modification of biomaterials based on reversible and cytocompatible desthiobiotin/avidin complexation.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive

Comprehensive analysis of the structural and functional state of liver during regeneration

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INTRODUCTION:A healthy liver has a high regenerative potential, but this potential is significantly reduced in the presence of background diseases or after extensive resection. Standard methods for assessing the structural and functional state of the liver do not allow studying the processes occurring in liver cells in dynamics during regeneration. Modern label-free methods of multiphoton microscopy with the FLIM (fluorescence lifetime) and SHG (second harmonic generation) modes will expand the possibilities of studying the metabolic state at the cellular level due to its non-invasiveness and high sensitivity.

METHODS:The experiments were performed on Wistar rats weighing 400-500 g. Removal of the left lobe of the liver is a model of 30% hepatectomy (30% HP), removal of the left and middle lobes of the liver is a model of 70% hepatectomy (70% HP). Metabolic imaging was performed on 3th and 7th days after surgery. A separate analysis of NADH and NADPH was presented to evaluate the overall synthetic activity in hepatocytes. The study of the lipid composition of liver tissue is carried out by the method of Time-of-Flight secondary ion mass spectrometry - ToF-SIMS 5 (ION-TOF, Germany). Resected part of liver were examined as a control.

RESULTS:The analysis results showed an increase in the overall metabolic activity of hepatocytes with an increase in the contribution of phosphorylation oxidation processes to hepatocytes, which may indicate an increase in the energy requirements of proliferating cells. Analysis of lipid composition showed that non -specific signal of amino acids decrease, phosphatidylcholine decrease, sphingomyelin increase, triacylglycerides increase, cholesterol greatly increase.

DISCUSSION & CONCLUSIONS:The obtained parameters may be useful in determining the criteria for the evaluation of the liver regenerative potential after surgery.

Acknowledgements:This work was supported by the Russian Science Foundation grant № 19-15-00263.

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Keywords: Imaging - advanced, In vivo and animal models

Morphological phenotype mapping for optimization of bio-molecule coating for cell culture

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INTRODUCTION:In cell culture, surface coating with functional bio-molecules, such as antibodies, ECM proteins, or peptides, is one of the promising methods to succeed in difficult cultures. However, most of such coating functional bio-molecules are technologically difficult to produce in large volume with high purity. As a result, most of functional bio-molecules for the culture surface coating are expensive and limited in available volume. Furthermore, the effect of such coating can only be checked after long period of culture time with multiple staining evaluations. Therefore, for researchers who try to develop new culture methods, it is extremely difficult to optimize the coating condition. Such limitations in the surface coating optimization is a critical issue for developing new cell cultures to advance regenerative medicine.

Our group has been reporting morphology-based cell evaluation methods using image processing and AI (artificial intelligence) technologies. In this work, we have applied this morphology evaluation technique to profile the effect of coating conditions.

METHODS:Five coating bio-molecules (recombinant peptide, fibronectin, gelatin, laminin, and vitronectin) were coated to culture well-plates with different conditions. Human bone marrow derived stem cells (BMSCs) were seeded and cultured for 5 days. During the cell culture, phase-contrast microscopic images were acquired automatically with automatic cell imaging system, BioStation CT (Nikon). The morphological profiles were quantified as described previously [1,2].

RESULTS:By the developed morphological profile mapping method, a modified principal component analysis, the un-functional and functional coating conditions could be discriminated and visualized only from the cellular morphological information. Our transcriptomic analysis also showed that the differences of morphological profiles highly correlated with the differences of expression profiles.

DISCUSSION & CONCLUSIONS:Our investigation results indicate that the cellular morphological pattern reflects the functional coating status, and therefore can be used as an indicator to feasibly and rapidly investigate the optimum bio-molecule coating condition only from the early cultured cell images.

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Keywords: Imaging - advanced, Interfaces – engineered

Multimodal Label-free Microscopy to Study Stem Cell Reprogramming In-vitro in 3D Nichoid Scaffolds

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INTRODUCTION:We developed a 3D in-vitro model for mesenchymal stem cells (MSCs) expansion and differentiation based on the Nichoid scaffold[1]. This microstructure, thanks to its precise geometry at the microscale which emulates the tensional states of the native niche, promotes stem cells proliferation maintaining cell stemness. The hypothesis is that the Nichoid controls the oxygen tension and it shapes the cell morphology thus varying the nuclear pore permeability to transcription factors. In this work, fluorescence microscopy and multimodal label-free microscopy[2] were applied to investigate MSCs response to differentiation media inside 3D Nichoids.

METHODS:Nichoid scaffolds were obtained via two-photon laser polymerization of SZ2080 photoresist deposited on different glass surfaces. MSCs at a density of 20,000 cells/cm2 were seeded on the Nichoids and 5,000 cells/cm2 on flat control substrates and cultured with complete, adipogenic and chondrogenic culture media for 21 days. To evaluate cell response to the 3D environment, cells were investigated with standard fluorescence microscopy and label-free multi-photon microscopy at different time points. Image processing was performed using MATLAB and Fiji-ImageJ.

RESULTS:Preliminary results showed that MSCs expanded in the 3D environment and conditioned towards the chondrogenic lineage produced smaller collagen type I deposits with respect to flat substrates. Fibrils were more numerous in scaffolds corridors than inside the pores. MSCs induced towards the adipogenic phenotype behaved in the opposite way, showing a two-fold numerosity in adipocytes inside the 3D microstructure compared to flat substrates. The use of Multimodal label-free microscopy allowed to chemically identify and spatially localize collagen and lipids in 3D, by filtering out any other signal, without the use of fluorescent probes.

DISCUSSION & CONCLUSIONS:Our results suggest that the Nichoid design and stiffness combine to significantly promote stem cell differentiation towards the adipogenic lineage. On the contrary, the Nichoid reduced the efficacy of the chondrogenic differentiation protocols with respect to flat controls. Multimodal microscopy, thanks to its chemical specificity and low invasiveness, demonstrated to be a promising technique for observation of the evolution of cell culture in-vitro in a 3D environment.

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Keywords: Microenvironment and niche engineering, Multipotent (mesenchymal) stem cells

Label-free detection of macrophage activation triggered by degradable biomaterial particles

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INTRODUCTION:Macrophages are the key player for phagocytosis. Many biomaterial devices formed by hydrolytic polymers are known as promoting the wound healing during degradation, as an example, the poly glycolic acid (PGA). Macrophage activated and further polarized by local acidification in phagosomes due to PGA particle internalization and sequentially degradation. In this project, alongside the conventional ELISA assay, a label-free detection method of macrophage activation has been developed with Raman spectrometry.

METHODS:The RAW 264 cell line was used as the macrophages' model. The PGA pellets were obtained from Corbion Purac and PGA porous scaffolds were produced by super critical CO_2 foaming technique. Some PGA scaffolds were hydrolyzed for 8 weeks and denoted as 8-week-degraded PGA. The non-degraded and 8-weeks-degraded PGA scaffolds were then grounded into particles with ≤ 5 um size for macrophage internalization at density of 30 particles/cell, The cells were imaged at 2, 6, and 24 hours by a Raman microscope at 532 nm excitation. The cell culture media were collected at day 2 and day 4 for ELISA measurement.

RESULTS:For the Raman spectra of the macrophages. we paid extra attention on lipid regions since the strong combined peaks around 2850 cm^{-1} to 2940 cm^{-1} represent various compounds of CH_2 and CH_3 groups which massively exist in lipids. The aggressive phagosomal membrane showed intensity increasing of lipid peaks after co-culturing with PGA particles for 6 hours, and the non-degraded particles contributed a higher activation of macrophage after 6 hours incubation comparing to control and 8-weeks-degraded particle stimulation. The IL-1β (M1 macrophage marker) secretion showed an increasing trend in the groups of non-degraded particles stimulation while decreased in the groups of 8-weeks-degraded particles along with incubation time. The IL-10 (M2 macrophage marker) barely secreted in non-degraded particles groups, but highly expressed by 8-weeks-degraded particles stimulation.

DISCUSSION & CONCLUSIONS:Non-degraded PGA contained certain amorphous region which could degrade fast and reduce the pH value rapidly; whilst the 8-weeks-degraded particles remained most of the crystalline structure with slow degradation rate and pH value reduction in the culture. Both Raman and ELISA outcome demonstrated that the activation and polarization of macrophage was pH-dependent. Fast pH value reduction in macrophage could induce IL-1β production; whilst slow degradation of PGA triggered higher IL-10 production. Hence, the macrophage activation can be measured with Raman spectrometry that dynamically monitors the pathogen internalization and macrophage phagosome maturation continuously and label-freely.

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Keywords: Imaging - advanced, Biomaterials

Modulation of inflamed synovium and its residing macrophages improves in vitro migration of mesenchymal stromal cells

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INTRODUCTION:Therapeutic solutions aiming to stimulate endogenous repair of osteochondral defects by using scaffolds or hydrogels are emerging. To achieve good integrative repair, mesenchymal stromal cells from the underlying bone marrow (BMSCs) should migrate into the hydrogel/scaffold and deposit extracellular matrix. Osteochondral defects are often accompanied by synovial inflammation. We investigated how synovial inflammation influences BMSC migration, and whether modulation of inflammation improves migration.

METHODS:Osteoarthritic synovial tissue explants were cultured with/without 1 µM triamcinolone acetonide (TAA) for 24 hours to obtain synovium conditioned medium (SCM). The effect of 6 SCM donors on migration of passage 3 BMSCs was examined in a Boyden chamber assay. Inflammation of the synovial explants was assessed with gene expression analysis and flow cytometry of synovial macrophages. Human peripheral blood monocytes were stimulated with TNF-α/IFN-γ towards pro-inflammatory macrophages, with IL-4 towards repair macrophages, and with IL-10 towards anti-inflammatory macrophages. After polarization they were cultured with/without 1 µM TAA for 24 hours, the conditioned medium was used to assess BMSC migration. Synovial fibroblasts were isolated and cultured with/without 1 µM TAA for 24 hours, the conditioned medium was used to assess BMSC migration.

RESULTS:SCM resulted in a donor-dependent increase in MSC migration. Modulation of synovial inflammation with TAA significantly decreased expression of TNFA, IL1B, and IL6, genes associated with inflammation, and increased gene expression of CD163, associated with anti-inflammatory macrophages, in synovial tissue explants. The percentage of CD14+/CD80+($p<0.001$) or CD14+/CD86+($p<0.001$) pro-inflammatory macrophages was lower in TAA-treated samples, whereas the percentage of CD14+/CD163+ anti-inflammatory macrophages was higher($p<0.001$) than without TAA. Modulation of synovial inflammation with TAA resulted in a 1.5-fold increase($p<0.01$) in migration. Moreover, BMSC migration increased 3.1-fold($p<0.001$) in response to medium conditioned by repair macrophages modulated with TAA, and 2.3-fold($p=0.02$) by modulated anti-inflammatory macrophages. Migration was unaffected by TAA modulated pro-inflammatory macrophages and synovial fibroblasts.

DISCUSSION & CONCLUSIONS:Decreased synovial inflammation increased BMSC migration. The effect of TAA is possibly through repair- and anti-inflammatory macrophages. Modulation of inflammation and macrophage phenotype in synovium using TAA seems promising to enhance BMSC migration. This knowledge could be used in approaches stimulating endogenous repair of osteochondral defects.

Keywords: Immunity / immunomodulation / macrophage, Cartilage / joint and arthritic conditions

Neutrophil Activation Affects Healing of Diabetic Wounds

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INTRODUCTION:A common secondary complication in individuals with Type-2 diabetes is the development of foot ulcers that show signs of compromised wound healing. Current clinical strategies to improve healing include frequent cleaning and dressing, offloading, and topical application of antibiotics. While these strategies may be sufficient in some individuals, in many, healing does not occur naturally, and eventually the limb needs to be amputated. Hence, new strategies to promote healing of diabetic wounds are essential. Additionally, recent reports suggest that diabetic wounds are likely to be chronically inflamed [1,2]. Given these recent findings, we hypothesized that wound healing is compromised in individuals with diabetes due to alternate activation of innate immune cells, and that treating the local inflammatory microenvironment may promote better healing.

METHODS:To determine immune responses that may be different in diabetic individuals, peripheral venous blood and biopsy of the foot ulcer site were collected from over 50 diabetic individuals, following informed consent. Phenotype of immune cells in blood was characterized by flow cytometry. In addition, the functional activity of neutrophils in terms of their phagocytic capacity as well as ability to produce ROS was determined. To develop hydrogels that may act as bandages to promote healing, chitosan scaffolds were prepared by crosslinking chitosan with tripolyphosphate. Immuno-modulatory drugs as well as growth-factors were loaded and kinetics of release studied. These scaffolds were then tested on skin wounds in diabetic rats.

RESULTS:Phenotypic and functional characterization of neutrophils obtained from blood do not show any significant differences at the basal level in diabetic individuals with healing versus non-healing ulcers. However, following ex vivo activation, neutrophils from individuals whose ulcers do not heal show much higher capacity for phagocytosis and ROS production, suggesting a propensity for higher activity. We are currently testing if this activity may be reduced using immuno-modulatory agents. Simultaneously, chitosan scaffolds containing immuno-modulatory drugs and growth-factors were fabricated. In vitro release studies suggest that drugs release over a week and growth-factors over two days. Preliminary studies in a diabetic rat model for wound healing suggest that the drug and growth-factor containing scaffolds show accelerated healing.

DISCUSSION & CONCLUSIONS:Our data suggests neutrophils are differentially activated in diabetic individuals, and that hydrogels releasing a combination of immuno-modulatory agents and growth-factors may serve as a new therapeutic strategy to treat diabetic wounds.
ACKNOWLEDGEMENTS:Work funded by RGUHS-IISc initiative and DBT, Govt. of India.
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Keywords: Wound healing, Drug delivery

Towards the Biohybrid Lung: Computational Fluid Dynamics Assessment of Hollow-Fiber Membrane Oxygenators

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INTRODUCTION:Extracorporeal membrane oxygenation (ECMO) represents the gold standard for treating respiratory failure, as a bridge to lung transplantation. However, ECMO devices demonstrate several issues, which limit their usage to 3-4 weeks. Thrombus formation is a common cause of failure, which limits gas-transport and has been associated with the haemodynamics of these devices. Endothelialisation of the hollow-fibres (HF) gas-exchange membranes offers a potential solution to thrombogenicity. However, inhomogeneous and extra-physiological haemodynamics would subject the seeded endothelial cells (ECs) to variable and potentially damaging blood wall shear stress (WSS). This study developed computational fluid dynamics (CFD) models for investigating the haemodynamics of HF membrane oxygenators, with a view to assessing the haemodynamic effect on seeded ECs.

METHODS:3D-heterogeneous CFD models of a clinically-available paediatric HF membrane oxygenator (MiniLung) and an experimental rat HF membrane oxygenator were developed to mimic fluid flows up to 0.8 l/min using blood, DMEM (for simulating the flow dynamics during EC seeding), or water (for CFD model validation purposes). Coupled O2-transport and blood-flow CFD models were developed to predict pO2, O2 saturation, blood velocity, and WSS, under different HF configurations (in-line, staggered) on the outer HF walls.

RESULTS:The presence of inhomogeneous WSS patterns on the HF oxygenators, as well as significant differences in the WSS and pressure were observed between the three simulated fluids in both oxygenator models. The blood-based models predicted significantly higher WSS and pressure compared to the DMEM- and water-based models at the corresponding locations investigated. Lower blood-flow velocity was associated with very low WSS (<0.1 Pa) and areas of recirculating flow. A higher incidence of thrombus formation and clotting is expected to occur in such regions. The results suggested that O2 diffusion resistance across the HF wall, void fraction and fibers configuration played a relevant role in O2 transport efficiency, as well as the magnitude and distribution of the WSS.

DISCUSSION & CONCLUSIONS:This study developed CFD models used to assess the performance of ECMO devices. Such models predicted regions of variable WSS, including stagnant, as well as recirculating flow regions, suggesting increased flow-induced blood damage and clotting formation. Moreover, the computational predictions highlighted the need for design optimisation of the oxygenators in order to minimising thrombus formation, while allowing efficient endothelialisation of the devices.
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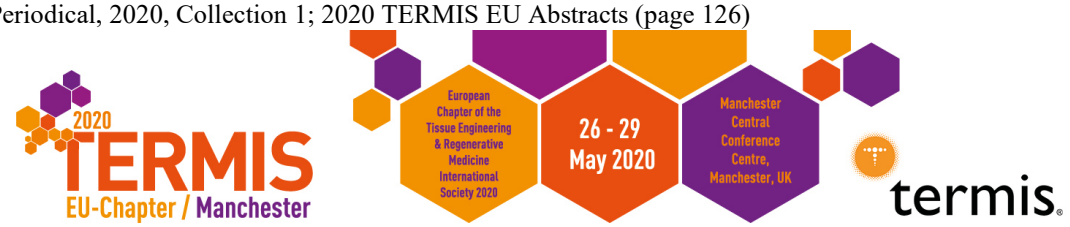
Keywords: Cardiovascular, Biofabrication



Towards a mathematical model of vascular network formation in a hydrogel to aid the design of engineered tissues

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INTRODUCTION:Accelerated vascularisation of clinically-viable engineered tissues is essential to ensure sufficient oxygen and nutrient levels for the survival and function of therapeutic cells once implanted in vivo [1]. Pre-vascularisation of the engineered tissue by the inclusion of endothelial cells (ECs) that form capillary-like structures in vitro is one such promising technique [1]. Here, we use mathematical and computational modelling to investigate the impact of ambient and intercellular oxygen concentration on the formation of EC networks in vitro. We focus on the development of vascular endothelial growth factor (VEGF) gradients, a factor produced by ECs under low oxygen that guides EC migration and promotes vascular network formation [2, 3].

METHODS:Coupled partial differential equations (PDEs) were used to describe the time and spatial development of the endothelial cell density, oxygen concentration, and VEGF concentration. Interaction terms include uptake and production of VEGF, uptake of oxygen, and chemotaxis, whereby ECs migrate towards a positive VEGF gradient. The model was parameterised using existing values from literature including cellular, VEGF, and oxygen diffusion, and VEGF degradation [4], with less known parameters refined by sensitivity analysis.
The equations were discretised and solved using second order finite differences in Python, considering a typical timescale of 48h. The geometry chosen represents an in vitro setup of a hydrogel in a well, with appropriate no flux or fixed boundary conditions implemented, and initially uniform distributions of cells and chemical factors.

RESULTS:We demonstrate parameter sets under which cell clusters and capillary-like structures form. Resulting cell distributions are analysed using metrics typical of in vitro network formation analysis, such as total vessel length, and complexity, a ratio between perimeter and area of cell clusters, to allow comparison with available in vitro data from within the Centre for Nerve Engineering at UCL.

DISCUSSION & CONCLUSIONS:We demonstrate a flexible computational model of vascular network formation with a mathematical framework able to adapt to changes in geometry, material properties, and cell type (including co-culture). Further development of the PDE model will allow us to consider optimum culture conditions and initial endothelial cell distributions for effective pre-vascularisation of engineered tissue constructs.

Acknowledgements:This work was supported by an EPSRC studentship EP/R512400/1 to GFAB.
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Keywords: Vascular systems / vascularisation and heart, In vitro microenvironments

Understanding the mechanisms behind scaffold-supported bone regeneration through in silico modeling

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INTRODUCTION:Large bone defects remain a clinical challenge, with a gold standard treatment – autologous bone graft transplantation – that presents many drawbacks. Design optimized scaffolds appear as a promising alternative [1]; however, the current scaffold design process remains trial and error mainly due to a lack of understanding of the scaffold-supported bone regeneration process. This results in high costs, ethical issues and the inability to find the optimum design due to the large number of variables involved. Thus, the aim of this study was to investigate the mechanisms behind scaffold-supported bone regeneration and identify underlying rules using an in silico modelling approach.

METHODS:A multiscale computer model previously validated for uneventful bone healing [2] was further developed to investigate scaffold-supported bone regeneration within a large bone defect model in sheep [1]: a 4 cm tibial osteotomy stabilized with a locking compression plate and augmented with a mechanically optimized titanium scaffold. The multiscale computer model couples an agent-based model describing biological activity and a finite element model evaluating the mechanical signals within the regenerating region. The model was further developed to include an often reported cell-scaffold interaction, namely surface guidance [1].
Predicted tissue formation were compared to histological and C-ray data over the healing progression.

RESULTS:Using a previously validated bone regeneration computer model resulted in overestimated bone formation within the defect and a slower bone bridging compared to in vivo data. Implementing surface-guided cell migration matched the bone formation along the scaffold struts found experimentally; however, the simulated healing process was slower and lateral bridging was not reached after 24 weeks. In addition, more bone growth was predicted along the fixation plate, what was not seen so extensively in vivo.

DISCUSSION & CONCLUSIONS:Currently proposed and generally accepted bone regeneration mechano-biological rules do not accurately explain timeline and patterning of the bone regeneration process in a large defect filled with a scaffold. Surface guidance was also not enough to explain experimental observations. Future work will further investigate the relative role of potential mechanisms behind scaffold-supported bone regeneration and use the knowledge for the optimization of scaffolds to promote bone regeneration.

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Keywords: In silico models, Biomechanics / biophysical stimuli and mechanotransduction

In silico modelling to optimize the design of Engineered Neural Tissue conduits to promote growth of neurites after a peripheral nerve injury

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INTRODUCTION:Peripheral nerve injury (PNI) affects 1M people in Europe and the USA p.a.[1] Patients experience debilitating symptoms associated with paralysis and loss of sensation.[1] Consequently, patients live with long-term disability, requiring care with large costs of the order of 100 billion $/year in the USA.[2]
The gold-standard treatment for large gaps aims at connecting the proximal and distal nerve stumps through autografts.[3] Although this approach is common, it requires the extraction of healthy nerve from the patient resulting in donor-site morbidity, and functional outcomes are poor in many cases.[4] A compounding challenge lies in matching grafts to host tissues with different neuron populations (motor, sensory).[5] Neurites from motor and sensory neurons respond in different ways to their local microenvironment, e.g. to material-based, durotactic and chemotactic cues.[6] This provides an opportunity to design engineered replacement tissues that exploit these differences to promote the growth of distinct neurite populations.
Here we focus on Engineered Neural Tissue (EngNT), comprised of anisotropic cellular hydrogels that mimic features of the autograft. EngNT has achieved comparable efficacy to autografts, and enables careful positioning of material and cellular components.[3] We aim to propose new EngNT designs that control the growth of both populations via the distribution of biomaterial and chemical cues. Given the cost and time constraints of exploring this using experiments, we propose the use of computational modelling to inform the design process.

METHODS:A mathematical model is developed to consider the two nerve populations following a random-walk model. The two populations (sensory, motor) are distinguished through their respective growth rates and response to cues. The base model is parameterised using in vivo neurite counts at the proximal and distal stumps.

RESULTS:Preliminary results show good agreement with data. This parameterised framework can now be used to explore the optimal arrangement of materials and cells to promote neuronal regeneration and propose new conduit designs.

DISCUSSION & CONCLUSIONS:An in silico model of PNI was developed within a multidisciplinary framework. The predictions made will enter a cycle where they get tested using an in vivo model, whose results will refine the model. Iteration for this process will be repeated until an optimal design is achieved to be tested clinically.

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Keywords: Nervous system (brain-central-peripheral / disorders), In silico models

On the evaluation of the circadian clock in a polymer based porous scaffold

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INTRODUCTION:Angiogenesis, the formation of new blood vessels, is driven by the sprouting of endothelial cells (EC) and recruitment of pericytes (PC), ensheathing the new capillary and plays a crucial role in regenerative medicine and tissue engineering (1). Understanding the molecular mechanisms regulating this process is key to harnessing its reparative potential. Circadian dysregulation (CD), the disruption of the natural 24-h cycle of an organism produced by environmental cues or pathology, affects many aspects of human health, including angiogenesis and in particular EC behaviour. In this study, we propose the development of a 3D system based on a tuneable polymer porous scaffold and primary human vascular cells to study circadian regulation of the vasculature. This will provide an essential tool for bridging the gap between simplified 2D *in vitro* experiments and complex animal models. Furthermore, we aim to produce a co-culture within these scaffolds including both EC and PC to recapitulate the complex interactions occurring in the capillaries and study the effect of CD in vascular cells.

METHODS:Highly porous polyurethane (PU) scaffolds, fabricated by Thermal Induced Phase Separation (TIPS) (2), were coated with fibronectin/gelatin to mimic the extracellular matrix. Primary human EC and PC were cultured alone or co-cultured into PU scaffolds, or in a Matrigel 3D gel as a control. The circadian genes were measured by PCR or luminescence, after synchronisation (serum shock).

RESULTS:Confocal imaging showed abundant EC and PC growth and proliferation on the PU scaffold in close proximity to the pores, for a period of two weeks. Co-cultures indicated close interaction between the two cell types, forming physical connections and organising in capillary-like structures in 3D. Luminescence indicated that PC, but not EC, expressed circadian clock genes and could be efficiently synchronised both in 2D culture and 3D. Furthermore, results on Matrigel showed that both the circadian clock synchronisation and its genetic ablation influence angiogenesis. Importantly, EC and PC were able to influence each other's circadian rhythm in co-culture and PC synchronisation increased EC pro-angiogenic potential.

DISCUSSION & CONCLUSIONS:This preliminary data provides the basis for the future study of how vascular cells influence each other's circadian clock in a 3D tissue engineered scaffold, and how the circadian clock can directly influence angiogenesis.

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Keywords: Vascular systems / vascularisation and heart, Microenvironment and niche engineering

3D Screening platform of cell-adhesion peptides: going beyond RGD

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INTRODUCTION:In tissue engineering, cell-adhesion peptides (CAPs) such as the ubiquitous arginine-glycine-aspartic acid (RGD) sequence have allowed the functionalization of synthetic materials to mimic macromolecules of the extracellular matrix (ECM). However, the variety of ECM macromolecules makes it challenging to reproduce all of the native tissue functions with only a limited variety of CAPs. Screening of libraries of CAPs, analogous to high-throughput drug discovery assays, can help to identify new sequences directing cell organisation. However, challenges to this approach include automation of cell seeding in three dimensions and characterization methods. Here, we report a method for robotically generating a library of 16 CAPs to identify microenvironments capable of directing a chain-like morphology in olfactory ensheathing cells (OECs), a cell type of particular interest for spinal cord injury repair to guide axon growth.

METHODS:Hydrogels loaded with mouse OECs genetically modified to express a green fluorescent protein were made with a liquid handling robot. Stock solution of 4-arms poly(ethylene glycol) (PEG) terminated with vinyl sulfone (PEG-4VS) or thiol (PEG-4SH) were prepared manually. CAPs terminated with a cysteine that can react with the PEG-4VS were prepared. The robot was program to dispense and mix the crosslinker PEG-4SH, PEG-4VS, the peptide and the cell suspension in a well plate. Confocal imaging of immunostained OECs was conducted and the metabolic activity of cells was assessed.

RESULTS:In blank PEG hydrogels, OECs maintained a rounded shape which was also observed in several CAPs of our library. In contrast, the OECs spread into a spindle shape - typical of cells cultured on planar tissue culture poly(styrene) - in the PEG hydrogels functionalized with CRGDSGK, CIKVAV, CYIGSR, CAELDVP, CDGEA, and CTWYKIAFQRNRK. Conversely, for CLDV and CLALERKDHSG, the OECs organised into spheroids. Interestingly, the sequences CPRARI and CKRSR directed the OECs to form a chain. OECs cultured in the PEG hydrogels functionalized with CRGDSGK, CPRARI and CKRSR were positive for marker characteristic of this lineage. In addition to preserve OECs phenotype, these three selected CAPs supported cell expansion.

DISCUSSION & CONCLUSIONS:This approach resulted in the identification of two CAPs not previously reported to interact with OECs to direct their morphology into structures potentially suitable for axon guidance. The same screening approach should be applicable to any cell types to discover new CAPs to identify relevant peptide sequences to direct the organization of cells into structures pertinent for tissue regeneration.

Keywords: In vitro microenvironments, Biologics and growth factors

Recapitulation of the Human Bone Marrow Niche in a 3D Microphysiological System

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INTRODUCTION:The maintenance and differentiation of hematopoietic cells are tightly controlled within the bone marrow (BM) stroma. Replication of the complex architecture and cell composition using 3D multicellular cultures are necessary to predict in vitro therapeutic effects of compounds, particularly in the context of oncology. We demonstrate the use of a BM microphysiological system for long term culture and generation of differentiated hematopoietic cells.

METHODS:To recapitulate the BM niche in vitro, we have cultured human mesenchymal cells (MSCs) on a porous ceramic scaffold for 7 days. Then, hematopoietic stem cells (HSCs) are seeded on this scaffold with serum-free media containing SCF, FLT3, G-CSF, TPO, and EPO for 7 days within a recirculating microfluidic chip. During and after treatment with oncology compounds, the hematopoietic cell population is assessed with a 9-color flow cytometry panel. To monitor the effect of oncology compounds on the microenvironment, MSCs are treated with carboplatin, cisplatin, etoposide, and paclitaxel and DNA damage response and cell cycle were evaluated.

RESULTS:This co-culture platform supports differentiation of erythroid, myeloid, and megakaryocytic lineages over a 5-week culture (1.7x10^3, 4.9x10^3, and 3.6x10^3, respectively). Stem and progenitor cells are also maintained in culture (4.7x10^3 and 6x10^2, respectively). Following treatment for 5 days with a lineage-specific compound, we observed that the percentage of erythroid lineage cells drops from 10.9% of the overall population to 7.6% (1uM), 2.9% (5uM), and 1.4% (10uM) in the treated samples. We also observe a dose-dependent decrease in erythroid cells with active mitochondria, as indicated by TMRE and MitoTracker fluorescence. Post compound withdrawal, the erythroid population recovers, mirroring the known clinical profile of this drug. MSCs treated with oncology compounds show dose-dependent increases in yH2AX foci formation and decreases in Ki67+ nuclei.

DISCUSSION & CONCLUSIONS:To validate the use of this BM microphysiological system, we have studied the effects of oncology compounds on the BM microenvironment and hematopoietic differentiation. We show that the hematopoietic population dynamics mirror the clinical profile of an erythroid-specific compound. Assessment into whether standard-of-care oncology drugs affect the microenvironment, which could alter the response of hematopoietic cells to drugs, show that MSCs display DNA damage response and cell cycle effects. Further characterization, including more detailed mitochondrial and metabolomic profiling, will provide mechanistic information about the lineage specificity of compounds and the role of the microenvironment in supporting recovery. This platform represents a key advancement for the study of human in vitro hematotoxicity and marrow biology.

Keywords: Microenvironment and niche engineering, Stem cell niche

Mimicking Ameloblastoma in 3D

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INTRODUCTION:Ameloblastoma is a locally aggressive tumour of the jawbones that arises from the tooth bud or the odontogenic epithelium. Ameloblastoma is associated with disruption of bone homeostasis, which is the balance between the removal of mineral and organic constituents of the bone matrix by osteoclasts and the new bone formation by osteoblasts. Previous studies have focused on matrix-metalloproteases (MMPs), in the bone resorption of ameloblastoma. The precise molecular mechanisms remain unknown. We have been working on the development of a biomimetic 3D model of ameloblastoma and its relevant tumour microenvironment, in order to investigate the cellular mechanisms and molecular pathways behind the bone resorption of ameloblastoma.

METHODS:Here, we have managed to establish 3D tumoroid models for two different histological subtypes of ameloblastoma; plexiform and follicular. The models are composed of an artificial cancer mass-composed of either AM-1 cells (plexiform ameloblastoma cell line) or AM-3 (follicular ameloblastoma cell line) and a surrounding bone-like stroma with active bone-forming osteoblasts. The surrounding stroma was developed by incorporating primary osteoblasts from the calvaria of neonatal rats. All cell types were grown in 2D to be used as controls.

RESULTS:Our findings show that the tumoroid models present histological features similar to those of classical ameloblastoma from patient samples. AM-3 cells in 3D released ~3 times higher MMP-2 (***$p<0.001$) compared to 2D by day 3 and ~8 times higher MMP-9 (***$p<0.001$) compared to 2D by day 5 to the cell culture media. These findings were supported by immunofluorescence staining of AM-3 for MMP-2 and MMP-9 (n=3). The bone nodules form at earlier day 2D control (*$p<0.05$). The number of bone nodules were ~7 times higher in our 3D active bone forming models compared to 2D control (*$p<0.05$). When ameloblastoma cancer-mass was introduced to active-bone forming model, bone formation is inhibited (n=9).

DISCUSSION & CONCLUSIONS:Histology H&E analysis and invasion patterns of 3D ameloblastoma tumoroid model proves biomimicry of our model. 3D ameloblastoma tumoroid models give more advanced understanding of the molecular characteristics of the disease than 2D monolayers of AM cell lines.
Our data first to indicate that, ameloblastoma cells inhibit osteoblast-driven bone nodule formation. 3D ameloblastoma tumoroid model can be used to further investigate inhibition of bone formation.

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Keywords: Cancer, Microenvironment and niche engineering

Breast cancer spheroid models using elastin-like recombinamer hydrogel microenviroments

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INTRODUCTION:The tumor microenviroment (TM) is a three-dimensional (3D) system formed by cells, non-cellular components and extracellular matrix (ECM), being all important in tumor progression and metastasis. Current two-dimensional (2D) *in vitro* models used in drug screening cannot fully mimic TM complexity, and more predictive 3D *in vitro* models are needed. Among them, cancer spheroids (cS) can reproduce many tumor features, like the 3D environment, drug resistance and ECM deposition. Hydrogels (HGs) can be used as platforms for cS production. Elastin-like recombinamers (ELR) are elastin-inspired polymers. ELR can self-assembles forming gels above certain temperatures, and can be cross-linked by click chemistry.[1] Their tunable properties and biocompatibility make them promising materials for cancer research.
The aim of this work is to evaluate if ELR HGs can be used for developing breast cancer cS models, that can be used for drug screening.

METHODS:HGs were fabricated by mixing i) MMP-sensitive ELR with cyclooctine groups (C-ELR) and ii) RGD-carrying ELR with azide groups (N-ELR), both dissolved in cell media. Breast cancer (MCF-7, MDA-MB-231) or non-tumor breast (MCF10A) cells were dispersed in C-ELR, mixed with N-ELR, and incubated at 4ºC for 8 min and 37ºC for 15 min. Then, cell media was added on top. Cell density in the HGs was 1million cells/mL. Cell proliferation and viability were determined by alamarBlue and calcein AM/propidium iodide. Cell distribution in HGs was studied with Phalloidin-Alexa488/DAPI staining. ECM deposition was evaluated by immunomfluorescence. For drug resistance experiments, doxorubicin (Dox) was incubated 48h with MCF-7 in hydrogels/well-plates. IC50s were measured with alamarBlue.

RESULTS:MCF-7, MCF10A and MDA-MB-231 were successfully encapsulated in the HGs, being homogeneously distributed. HGs were biocompatible, and MCF10A and MCF-7 proliferated forming cS. MDA-MB-231 did not form cS. All cell types deposited ECM proteins (collagen IV and fibronectin) after 7d. MCF-7 cells had a higher Dox IC50 when cultured in HGs when compared with 2D culture.

DISCUSSION & CONCLUSIONS:MCF-7 and MCF10A were able to form spheroids in ELR HGs, meanwhile MDA-MB-231 formed cell networks in the HG; depositing all of them ECM proteins in the HGs. Also, it was observed resistance to Dox. These findings suggest that ELR HGs are a promising material for developing in vitro breast cancer models for drug screening.

Acknowledgements:1. González de Torre I. et al. Acta Biomater.10, 2014, 2495

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Keywords: In vitro microenvironments, Cancer

Miniaturized bioreactors to create off-the-shelf bone pockets

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INTRODUCTION:We aim to explore the concept of liquefied and multilayered capsules (LMC) as alternative bioencapsulation systems aiming bone regeneration. The concept relies on the use of hydrogels as temporary hydrophilic 3D structures to produce liquefied systems encapsulating cells and microparticles [1,2]. Herein, we propose the use of nanogrooved microdiscs (topodiscs) to provide topographical cues for co-encapsulated adipose-derived stem (ASCs) and endothelial cells (HUVECs), to create pre-vascularised bone-like microtissues. Additionally, the immunomodulatory ability of LMC will be evaluated by producing an indirect co-culture system with macrophages and ASCs. For that, LMC ending with different types of polyelectrolytes are placed on top of 2D cultured macrophages. The crosstalk-effect between both cell phenotypes is evaluated.

METHODS:High-production of alginate hydrogels containing cells and polycaprolactone microparticles by electrospraying. Layer-by-layer is then performed with poly(L-lysine) (PLL), alginate (ALG), and chitosan (CHT). The liquefied core is obtained by EDTA immersion. Topodiscs production by nanoimprinting of microparticles between nanogrooved polyvinyl alcohol counter-moulds. LMC encapsulating HUVECs and ASCs-topodiscs microaggregates are produced. LMC encapsulating microparticles and ASCs, and with variable last layers (PLL, ALG, or CHT) are added on top of THP-1.

RESULTS:Topodisc presented a homogeneously nanogrooved surface, allowing cell adhesion and alignment. The core liquefaction treatment allowed direct contact of HUVECs on top of co-encapsulated microaggregates. Upon 21 days in basal culture, osteopontin was detected. LMC without cells were cultured on top of macrophages for 7 days. IL-6/IL-10 ratio significantly decreased over time, particularly for CHT. Expression of pro-healing markers CD163 and CCL13 was increased for PLL and CHT, regardless the significant enhancement of inflammatory genes CCL20 and CXCL10 for PLL. ASCs increased the macrophages release of IL-10.

DISCUSSION & CONCLUSIONS:Mineralised topodisc-ASC within LMC were obtained for static mono and co-cultures, even without osteoinductive factors. This was not observed for monoculture spherical particle conditions, highlighting topodiscs nanogrooves as an impacting factor to induce osteogenesis. We believe that LMC once injected would easily adapt to lesions with complex shapes, and recruit new vessels while also stimulating macrophages that aid in the healing process. On the inside, the new microaggregates of cells-microparticles would also contribute to accelerate the bone regenerative process by providing healthy microtissues.

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Keywords: Differentiation, Immunity / immunomodulation / macrophage

Development of Novel Human Epithelial Mucosal Tissue Models and The Role of Tissue Specific Fibroblasts

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INTRODUCTION:Epithelial tissues are found throughout the body and are a component of larger structures such as the skin, intestine and oral mucosa. They share a common, highly conserved structure: a surface epithelium supported by a basement membrane and an underlying fibroblast-populated submucosal compartment. However, amongst different tissue types the structure of the surface epithelium differs and is highly specialised according to its function.
We hypothesise that signalling from the fibroblasts in the submucosal compartment is critical to the differentiation of the overlying epithelial layer. We propose to investigate whether these signals are tissue specific and assess the paracrine and juxtacrine influences between these cell populations.
The advent of advanced 3D tissue culture and tissue engineering technologies has enabled the production of complex, physiologically relevant in vitro tissue equivalents. We propose to test our hypothesis by developing models using tissue matched epithelial cells and fibroblasts in comparison to fibroblasts from different tissue types. We aim to determine whether tissue matched fibroblasts are required for the specialised differentiation of the epithelial layer or if generic fibroblasts from a common source can enable appropriate differentiation.

METHODS:Reconstructed human full thickness skin equivalent models have been developed utilising primary commercially available epidermal and dermal cells. Oral mucosal and intestinal models have also been developed using primary cells and existing cell lines. In each model, fibroblasts are seeded in Alvetex® Scaffold well inserts followed by epithelial cells seeded on top of this construct, which subsequently differentiate.

RESULTS:Our respective tissue models demonstrate structural morphology resembling the structure of native skin, intestine and buccal mucosa. Fibroblasts produce endogenous extracellular matrix proteins which provides support for the overlying epithelia. Extensive characterisation of the mucosal models shows evidence of basement membranes and multiple other structural and functional features shared by the corresponding real tissue. When epithelial cells were cultured with non-tissue matched fibroblast sources this had a significant influence on the differentiation and morphology of the overlying epithelia. Intestinal epithelial cells showed greater polarisation. Oral epithelial cells underwent a more epidermal-like differentiation pathway to produce a terminally differentiated layer. Presence of a stratum corneum-like structure suggests plasticity and the capacity of the buccal epithelial cells to transdifferentiate resulting in a more skin-like morphology when cultured with dermal fibroblasts.

DISCUSSION & CONCLUSIONS:Our data provide preliminary evidence that tissue specific fibroblasts are required for the formation of fully differentiated mucosal tissue constructs and alternative fibroblasts sources can alter epithelial cell differentiation.

Keywords: Microenvironment and niche engineering, Differentiation

Peptide-protein co-assembling hydrogel as an effective 3D model of ovarian cancer

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INTRODUCTION:There is a need for more realistic 3D models that enhance our capacity to study cancer progression and metastasis in a biologically relevant, yet controlled, manner. Supramolecular materials offer an inventive avenue to engineer hydrogels that recreate molecular and physical features of the native extracellular matrix (ECM). Here, we report the design, synthesis, and validation of a novel 3D model of the tumour microenvironment (TME) based on the co-assembly of peptide amphiphiles (PAs) with the ECM proteins keratin and fibronectin.

METHODS:The material platform takes advantage of multicomponent self-assembly to create a tuneable nanofibrous architecture that mimicks the TME of ovarian cancer. The main design element is the use of self-assembling peptide amphiphiles (PAs) to serve as "molecular chaperones" to guide the assembly of ECM proteins into multifunctional supramolecular ensembles to serve as ECM-like gels. Towards this goal, PAs were co-assembled with keratin and fibronectin and used to conduct 3D triple-cultures.

RESULTS:3D mono-cultures of human ovarian cancer cells led to tumour spheroid formation, with comparative results obtained in Matrigel controls. 3D tri-cultures of ovarian cancer cells with human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (hMSCs) revealed an extensive F-actin network surrounding the tumour spheroids and integrated cell-cell interactions. A proof-of-concept study with clinically-used chemotherapeutics was conducted to validate the functionality of the 3D model.

DISCUSSION & CONCLUSIONS:The resulting materials can be used to precisely recreate the TME and enable more effective testing of anti-cancer therapeutics. Overall, the work demonstrates the potential of the peptide-protein co-assembling platform to serve as a controllable and tuneable multicompoment matrix for modelling of TMEs in vitro.

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Keywords: Biomaterials, Other

Neurovascular 3D Cell Model to Investigate the Role of Pericytes in Dementia

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INTRODUCTION:Pericytes, together with brain microvascular endothelial cells (BMECs), astrocytes, and neurons, form the neurovascular unit (NVU). All these cell types influence the barrier function in the brain capillaries and breakdown of the NVU is a common early symptom of vascular dementia and Alzheimer's disease (Kisler et al., 2017). Pericytes have previously been shown to contract capillaries and thus reduce cerebral blood flow in a downstream response to the addition of amyloid-β, a hallmark protein of Alzheimer's disease (Nortley et al., 2019). However, the full role of pericytes in health and disease remain poorly understood, partially due to a lack of adequate models that can recapitulate the complexity of the multi-cellular NVU. The aim of this study is to incorporate pericytes into a 3D *in vitro* model to study the function and dysfunction of the NVU and the pericytes within it.

METHODS:Brain pericytes-like cells and BMECs were differentiated from human induced pluripotent stem cells (iPSCs; Stebbins et al., 2016; Faal et al., 2019) and co-cultured in a transwell-type NVU model. Integrity of the endothelial barrier function was evaluated by transendothelial electrical resistance measurements. Fluorescent Fluo4-AM was used as a calcium efflux indicator.

RESULTS:Brain pericyte-like cells stained positively for pericyte markers NG2, PDGFR-β, α-SMA, and Desmin. Co-culture with BMECs resulted in a significantly increased transmembrane resistance compared with mono-cultured BMECs. Furthermore, our iPSC-derived brain pericytes-like cells release calcium after carbachol stimulation showing their potential ability to contract. DISCUSSION & CONCLUSIONS:These results suggest that functional brain pericytes-like cells can be derived from iPSCs and incorporated into a NVU model. We will build on these findings to develop a robust model to study pericytes within the NVU to investigate their function and dysfunction in health and disease.

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Keywords: Nervous system (brain-central-peripheral / disorders), Disease models

Towards high quality cartilage from induced pluripotent stem cells; comparing neo-cartilage deposited by primary chondrocytes and by differentiated iPSCs

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INTRODUCTION:Use of induced pluripotent stem cells (iPSC) is proposed an excellent approach for tissue regeneration, disease modelling, and drug screening. Regeneration of cartilage, however, still shows inadmissible variability. Moreover, cells are prone to become hypertrophic, and expansion of cells following differentiation rapidly decreases quality of deposited cartilage extracellular matrix (ECM). Aiming at an efficient and consistent protocol for autologous-like cartilage we compared chondrocytes derived from iPSCs taking different approaches with primary chondrocytes and bone marrow derived mesenchymal stromal cells (BMSCs).

METHODS:Two control iPSC lines were differentiated (N=5-8) either using a step-wise protocol towards chondroprogenitor cells (CPCs) or towards iMSCs. Characteristics of cells were determined by flow cytometry. Subsequently, chondrogenesis was performed and characteristics of cells and ECM were determined with RT-qPCR and histology.

RESULTS:Flow cytometry analyses showed over 90% of CD73+/CD90+/CD31-/CD45- cells, confirming resemblance of generated iMSCs with BMSCs. In contrast, less than 10% of CPCs were CD73+/CD90+/CD31-/CD45-. These cells express CD146 and CD166 as described before[1]. Following chondrogenesis, analyses demonstrated significant differences between chondrocytes generated by the different protocols. Specifically, differences in Alcian Blue intensity indicated higher levels of glycosaminoglycans in neo-cartilage deposited by CPCs as compared to iMSCs. This is in line with gene expression levels of *COL2A1* in CPC-derived chondrocytes within 3 weeks following chondrogenesis (500-fold higher compared to chondrocytes generated from iMSCs; $P<0.001$). Conversely, *COL1A1* expression was significantly lower in CPC-derived chondrocytes (45-fold lower; $P<0.001$). When comparing CPC-derived chondrocytes with primary chondrocytes, *COL2A1* and *COL1A1* expression was at least similar following 3 weeks of chondrogenesis (4-fold higher and 9-fold lower, respectively, with $P=0.09$ and $P=0.13$). Chondrogenesis with iMSCs as compared to CPCs appeared less reproducible and gene expression suggested similarity between chondrogenesis of iMSCs and BMSCs. *COL2A1* expression was comparable ($P=0.27$) while *COL1A1* expression tended to be higher in iMSC-derived chondrocytes ($P=0.08$). We did not observe differences in *COL10* expression ($P=0.15$). Overall, we found that similarity of primary chondrocytes was over 70% for CPCs while it was not even 50% for iMSCs.

DISCUSSION & CONCLUSIONS:We demonstrate that neo-cartilage deposited by iPSC-derived chondrocytes via generation of CPCs is highly comparable to neo-cartilage of primary chondrocytes. The suggested resemblance make them highly suitable to further explore applications in regenerative medicine and disease model systems.

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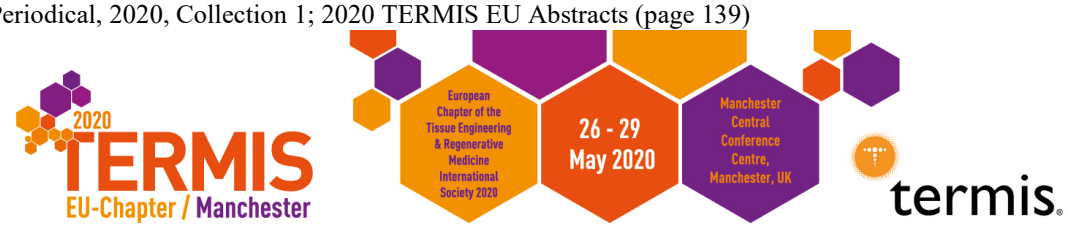

Cyto- and biocompatibility of an antibacterial hybrid surface

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INTRODUCTION:Implant associated infection is a fear complication in orthopedics. Surface properties and local antibiotic delivery can affect the race for the surface and support material integration while inhibiting bacterial growth. The implant modification must be easy and ideally allow the perioperative loading with specific antibiotics. This study aimed at the development of a porous titanium surface for loading with antimicrobial substances.

METHODS:The surface of titanium k-wires were modified by laser structuring (Q-switched Nd:YAG laser, λ = 1064 nm). Metal silver particles were embedded in a titanium dioxide layer by physical vapor deposition (PVD). Surface topography and cell interaction were characterized using scanning electron microscopy (SEM). Gentamicin and silver content as well as release were determined by direct quantification and microbiological analysis. Cytocompatibility was investigated using primary human osteoblast like cells (n = 6/group) and the biocompatibility was assessed in a rat osteointegration model (approval No: G0072/18, in compliance with the Animal Welfare Act). Briefly, k-wires (1. CTRL - titanium implant, 2. modified surface - pores and silver, 3. hybrid surface - modified surface with gentamicin, n = 8 rats/group) were implanted in the rat femur and the integration analyzed by mechanical push-out testing and histology after 8 weeks. Statistics: Kruskal-Wallis, Dunn´s (Prism, GraphPad, USA).

RESULTS:Laser structuring resulted in open pores with dimensions of 70 - 160 µm in lengths and 40 - 70 µm in widths. Gentamicin load was 430 µg/cm², and 4 µg/cm² silver particles were embedded in the dioxide layer. An almost complete gentamicin released occurred after 15 minutes, whereas silver was released very slowly. Growth of S. aureus was inhibited by samples from the elution experiment. Neither the surface modification nor the gentamicin impacted primary human osteoblast like cells. The in vivo study showed a good biocompatibility of the surface over 8 weeks. A significantly better osteointegration of the k-wires with a modified surface and the hybrid surface (with gentamicin) compared to the pure titanium k-wires (CTRL) was determined in the push-out test. This was confirmed by a better osteointegration seen in the histology.

DISCUSSION & CONCLUSIONS:The surface modification can be easily applied to titanium surfaces and allows the loading with antibiotics as needed. Fast gentamicin release protects the surface from bacterial colonization without compromising cytocompatibility and osteointegration. This approach allows for the production of “off-the-shelf” orthopedic implants with this surface modification and the loading with the antibiotic just prior to implantation.

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Keywords: Drug delivery, In vivo and animal models

Non-invasive sterility testing and quality control of cell-based therapeutics using Raman Trapping Microscopy

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INTRODUCTION:The combination of Raman spectroscopy with optical trapping provides a non-invasive, fast and easy approach for identifying bacteria and infected cells ensuring quality of cell-based therapeutics. Increasing occurrence of antibiotic resistant bacteria is a heavy threat in modern hospitals. In order to ensure safety of patients it is essential to check for and characterize remaining microorganisms on a routine basis. Intracellular pathogens have devised various mechanisms to subvert the host immune response in order to survive and replicate in host cells.
Raman spectroscopy is an analytical method solely based on the interaction of molecules with focused laser light. The generated spectroscopic patterns are unique for every cell type and cell state and could thus be called a metabolomic fingerprint.

METHODS:Raman Trapping microscopy (RTM) arrests motile cells in solution during Raman spectral analysis. In this approach, RTM was used to distinguish strains and subtypes of patient derived Pseudomonas and Staphylococcus aureus. Monocytes purified from peripheral blood mononuclear cells by negative depletion were infected with C. pneumoniae. RTM measurements was conducted on blood cells isolated from human donors.

RESULTS:Raman spectroscopy analyses the entire metabolome of individual cells providing spectral information that is as characteristic as a fingerprint. RTM was able to discriminate different strains and subtypes of patient derived bacteria. It was furthermore possible to identify the percentage of infected monocytes. In addition, change of Raman spectra of erythrocytes as well as of thrombocytes with time was consistent with routine quality control studies of blood products. Bacterial contamination revealed clear changes in Raman spectra within less than 1 hour of incubation.

DISCUSSION & CONCLUSIONS:Current methods like mass spectrometry analyses entire bacterial colonies. Thus, only an overall picture of the bacterial culture can be shown, whereas subpopulations may be underrepresented or even masked in the results. Results from Raman spectroscopy are consistent with standard oligonucleotide DNA microarray, quantitative real-time PCR and immunofluorescence, but save time and costs. Guaranteeing the quality of all blood products is difficult to achieve. Only about 1% of the blood bags are actually tested for quality and purity and so far there is no control of blood products immediately prior to transfusion.
RTM can also monitor quality and sterility of blood products, by monitoring the effect of storage conditions on the functionality of blood cells products over time. Furthermore, RTM is showing a great potential to identify bacterial infection and effect on blood cells, offering a promising approach to detect sepsis. RTM is a fast and non-invasive method requiring less than 500 cells for analysis. Thus, RTM can be used as a rapid diagnostic tool for the detection of infected cells and has the potential to become a standard for quality control of cell products.

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Keywords: Cell therapy, Enabling technologies

Development of Porous Polyurethane based Tissue Engineering Scaffolds with Controlled Pore Interconnectivity and Cell Adhesion inducing Nanoscale Interfacial Features

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INTRODUCTION:Interconnectivity and the surface properties are the defining parameters for foam based tissue engineering scaffolds. Polyurethane has advantages as a polymer for scaffold fabrication; however its surface properties are not suitable for cellular colonisation. In this study, in order to improve the capacity of Polyurethane scaffolds we have used sacrificial sphere templating for enabling control over pore interconnectivity together with the optimisation of hard segment: soft segment ratio for better cell attachment and nanoscale surface modifications (plasma treatment and polydopamine coatings) for better cellular in-growth (1).

METHODS:First the sintering process was optimised and the experimental results were compared to determine the optimal model for the simulation of the pore formation. Following the optimisation, foams with the same pore size but different interconnectivities were manufactured and tested for their ability to induce colonisation by fibroblasts and mesenchymal stem cells over 21 days. Following the determination of the optimal interconnectivity, the effect of hard:soft segment ratio on the attachment of cells were analysed in 2D and 3D.

RESULTS:The experimental results fit better with Milner's visco-elastic theory of sintering, which can be used to predict the eventual pore sizes. Pores with bigger interconnections induced better cell in-growth; when applied concamitantly with oxygen plasma treatment large interconnections resulted in even faster migration of stem cells, higher proliferation and ECM secretion. We have also determined an optimal hard:soft segment ratio (an NCO index of 300 as a threshold) for improved cell adhesion; an effect which was observed also in 3D foams.

DISCUSSION & CONCLUSIONS:Sacrificial sphere templating as a well controlled process which can create porous structures with precise pore sizes and pore interconnectivity. Together with the control of the polyurethane chemistry and additional nanoscale surface modifications; application specific, optimised porous scaffolds can be produced. By the step-by-step adjustement of the sintering parameters (interconnectivity), polyurethane chemistry (initial cellular response) and inner surface properties of pores (for colonisation); porous scaffolds with better microenvironments for functional tissue regeneration was achieved.

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Keywords: Interfaces - engineered, Polymers - natural / synthetic / responsive

Investigating the Physiological Relevance of *Ex Vivo* Disc Organ Culture Systems for the Development of Cell Therapies based on Nutritional Demands

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INTRODUCTION:There has been significant interest in developing cell-based therapies aiming to repopulate the nucleus pulposus (NP) and augment tissue repair. *Ex vivo* disc culture systems have become a valuable tool during development and preclinical testing, providing a platform between cell culture and *in vivo* studies. It is critical to assess these therapies under the hostile biochemical niche typically experienced *in vivo*, ensuring normal cellular function and regeneration[1]. However, it remains to be elucidated whether the *ex vivo* culture microenvironments are comparable to human degeneration. This work aims to create a validated computational model which can be used to predict the metabolite gradients generated in *ex vivo* culture systems.

METHODS:Finite element models of cultured disc were created using COMSOL Multiphysics®. These models were governed by coupled reaction-diffusion equations, taking into account geometrical differences, cell viability, cellular metabolism and nutrient diffusion through the different tissue domains. Metabolic rates were dependent on local oxygen and pH levels by employing equations derived previously[2] [3]. Experimental verification of these models was performed by measuring the metabolite concentrations in discs cultured for 7 days, in a custom-made bioreactor.

RESULTS:The diffusion distance across the NP was significantly different between bovine caudal disc locations (n=6, $P<0.05$). The viable cell density employed computationally was 5,578±801 cells/mm^3 and 14,465±3,937 cells/mm^3 for the NP and AF (n=3), respectively. Cell density remained constant as there was no significant difference in NP viability at day 7. The predicted central glucose concentration of a disc cultured in 25mM media was 6.22mM, which was within the standard deviation (SD) of the experimental value 5.35±1.47 mM (n=3). The lactate concentration predicted under the same conditions, was 10.44mM, which also lay within the SD of the experimental measurement, 9.64±1.49 mM (n=3). The experimental pH level decreased from 7.3±0.1 in the media to 6.4±0.1 in the disc centre (n=8). While the predicted pH in the disc centre ranged from 6.3 to 6.6 depending on disc size. DISCUSSION & CONCLUSIONS:This work presents the first experimentally verified predictive model, advancing the knowledge of the microenvironment within *ex vivo* disc cultures. Ultimately, it is imperative that the critical metabolite values (minimum glucose, oxygen & pH values) are matched to those at a stage of human IVD degeneration, where regenerative cell-therapy is an appropriate strategy, to realise successful clinical translation based on nutritional demands.

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Keywords: In silico models, In vitro microenvironments

BMP Antagonists – A Possible Cause for Spinal Non-Fusion?

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INTRODUCTION:Spinal fusion is a procedure where the intervertebral disc (IVD) is removed and two adjacent vertebrae are forced to fuse by compression. This procedure is the most commonly applied procedure to achieve spinal stability and relief of back pain. However, non-successful fusion leads to pseudo-athrosis and ongoing pain. There is increasing evidence that supraphysiological doses of BMP2 and burst-release of this cytokine did not generate satisfying results in clinical studies. Current hypothesis was raised that IVD cells and/or tissue seem to inhibit the action of BMP2. In this overview we summarize the current evidence that BMPs might be inhibited by the secretome of human IVD cells, i.e., nucleus pulposus cells (NPC), annulus fibrosus cells (AFC) and cartilaginous endplate (CEPC) cells.

METHODS:We stimulated low-passage (2-3) human bonemarrow-derived mesenchymal stromal cells (MSCs) and femoral hip-derived osteoblasts (OBs) and co-cultured these with allogeneic IVD cells obtained from spinal surgery. We then stimulated MSCs and the OBs in monolayer and osteogenic medium, whereas IVD cells were kept in 3D alginate bead culture and separated by high density pore culture inserts (0.4 µm pore size). We quantified relative gene expression at bone-relevant genes, alkaline phosphatase (ALP) activity and Alizarin red (ALZR) staining after 21 days. Furthermore, to test the effect of a previously investigated BMP2 analog to block the inhibitors, cells were further stimulated with 100 ng/mL BMP2 and/or L51P.

RESULTS:We found significant inhibitory effects of IVD cells onto MSCs undergoing differentiation in presence of NPC, AFC and CEPC as shown in reduced osteogenic gene expression, ALZR staining and ALP activity (N = 11 donors paired on each side). In the case of allogeneic human OBs only a trend towards inhibition could be demonstrated (N = 7 donors on each side). The addition of L51P to the co-culture recovered ossification. On the side of the IVD cells BMP2 and/or L51P had a strong chondrogenic effect.

DISCUSSION & CONCLUSIONS:Our data suggested evidence for inhibition for MSCs. However, OBs did not show the same inhibitory effects but showed a trend in presence of IVD's secretome. This warrants for animal models where the donor variance can be better controlled.

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Keywords: Intervertebral disc / spine and their disorders, Biologics and growth factors

Non-Viral Transfection of Nucleus Pulposus Cells with FOXF1 induces reprogramming to a Healthy Phenotype in-vitro and in-vivo

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INTRODUCTION:Intervertebral disc (IVD) degeneration is characterized by matrix degradation, inflammation and neurovascular invasion. Viral gene therapy approaches raises concerns of mutagenesis and unwarranted immune response. To avoid these risks, engineered-extracellular-vesicles (eEV) carrying developmental transcription factors can be introduced into endogenous diseased cells without host or permanent integration of native DNA to stimulate tissue repair. FOXF1 is highly expressed in the healthy nucleus pulposus (NP) and promotes regeneration in tissues such as lung. This study aims to 1) Examine effects of FOXF1 on human NP cell phenotype and function and 2) assess FOXF1 delivery to NP cells via eEVs in-vitro (human) and in-vivo (mouse).

METHODS:Aim1: Cells were isolated from patient NP tissue (IRB:2015H0385), electroporated with FOXF1 plasmids and cultured over 4-weeks in 2%-agarose. Cell viability (Calcein/Ethidium), gene expression (qPCR), and proteoglycan (DMMB/Hoescht) were assessed at 0, 2 and 4-weeks with statistical Mann-Whitney tests.
Aim2: eEVs were generated via electroporation of FOXF1 or empty- plasmids into human/mouse fibroblasts respectively, isolated, and quantified via Nanosight. For in-vitro feasibility, diseased human NP cells were treated with eEVs for 7-days and uptake assessed (fluorescent imaging and qPCR). In-vivo, FOXF1 or empty-plasmid eEVs were injected into lumbar IVDs of B6-mice (IACUC:2016A00000074-R1) with viability and gene assessed at 7-days. Ongoing 12/24-week studies are in progress with pain/behavioral assessment data.

RESULTS:Aim1: Cell viability remained high for all groups and FOXF1 expression maintained over 4-weeks. Phenotypic markers (T,KRT19) were significantly upregulated, with down-regulation of inflammatory cytokines (IL-6,IL1-B), NGF and matrix degrading enzymes (MMP12/MMP13). Proteoglycan content was significantly increased compared to controls. Aim2: Human eEV treatment in-vitro showed cell specific uptake of eEVs along with >1000-fold increase in FOXF1. Significant upregulation of FOXF1 was seen at 2 and 7-days. Mice studies showed comparable cell viability to controls and upregulation of FOXF1 in treated IVDs

DISCUSSION & CONCLUSIONS:We have previously identified Brachyury as a potential therapeutic transcription factor and FOXF1 further validates this concept. Aim1 demonstrates our ability to successfully transfect diseased human NP cells with high cell viability, increases in healthy phenotypic markers including proteoglycan, critical for IVD function. Aim2 demonstrates successful uptake of transcription factor-loaded eEVs both in-vitro (human) and in-vivo (mice) with high efficiency and no cytotoxic effects. Ongoing behavioral assays have demonstrated differences in grip strength and will further assess this regenerative therapeutic method in-vivo as a minimally-invasive therapy for Low back pain.
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Keywords: Gene therapy, Extracellular vesicles

Can interleukin-1beta and cathepsin-D modulate the terminal complement complex formation in human disc tissue cultures?

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INTRODUCTION:The formation of the terminal complement complex (TCC), a complement system activation product that acts as inflammatory trigger and induces cell lysis, was previously identified in degenerated discs, correlating positively with the degree of degeneration [1]. However, it is unclear which molecular factors play a role in complement activation during disc degeneration (DD). Therefore, here we have investigated possible triggers of TCC formation in the context of DD.

METHODS:Disc tissue biopsies were collected from adolescent idiopathic scoliosis (AIS, n=8, age 16±3) and DD (n=11, age 56±15) patients with ethical approval and informed consent. Standardized tissue punches from nucleus pulposus (NP), annulus fibrosus (AF) and endplate (EP) were separately cultured. Isolated cells were analyzed by flow cytometry and gene expression. Cells and tissues were stimulated with medium containing 5% human serum alone or supplemented with interleukin-1β (IL-1β, 10 ng/mL), cathepsin-D (0.5 µg/mL) or zymosan (100 µg/mL). Serum-free medium cultures were used as control. In cell cultures, TCC formation and lytic activity were determined by ELISA and CH50 assay. In tissues cultures, TCC and CD59 formation were analyzed by immunohistochemistry. Statistics: Kruskal-Wallis in cell cultures, one-way ANOVA in explant cultures.

RESULTS:In isolated cells, the deposition of TCC and expression of CD46, CD55 and CD59 significantly increased with culture ($p<0.05$). However, no differences were found in response to proinflammatory/degenerative stimuli, neither for scoliosis nor DD patients. In tissue cultures, compared to non-treated tissues, IL-1β stimulation led to lower percentage of TCC+ cells in AF and EP ($p<0.05$), whereas the presence of cathepsin-D significantly increased TCC formation in NP ($p<0.01$). The percentage of CD59+ cells significantly increased in AF and NP after stimulation with cathepsin-D and zymosan ($p<0.05$).

DISCUSSION & CONCLUSIONS:These data suggest that complement activation and TCC formation can be induced in vitro if disc cells are kept in their native tissue environment. Interestingly, the presence of IL-1β, a pro-inflammatory molecule, led to less TCC formation in AF and EP. Although strong TCC deposition may be a degeneration-associated event, proinflammatory conditions may influence it via a negative feedback mechanism. Nevertheless, TCC formation was shown to be triggered by cathepsin-D, an important player in osteoarthritis, but poorly described in DD. Although further mechanistic studies are ongoing, overall, these results suggest a functional relevance of IL-1β and cathepsin-D in modulating TCC formation, being TCC proposed as a possible new target for DD therapy.

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Keywords: In vitro microenvironments, Immunity / immunomodulation / macrophage

Mimicking the Intervertebral Disc Microenvironment for Expansion of Nucleus Pulposus Progenitor Cells in a Context of Cell Therapy

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INTRODUCTION:Low back pain (LBP) is a global health concern that affects as many as 75–80% of people during their lifetime. Although the causes of LBP are multifactorial, increasing evidence implicates intervertebral disc (IVD) degeneration as a major contributor. In this respect, tissue-specific progenitors may play a crucial role for tissue regeneration, as these cells are perfectly adapted to their niche. Recently, progenitor cell population was described in the nucleus pulposus (NP) of the IVD. These cells, positive for Tie2 marker, have self-renewal capacity and in vitro multipotency potential. However, extremely low numbers of the NP progenitors limit the feasibility of cell therapy strategies. Here, we study the influence of the culture method and of the microenvironment on the human NP progenitors and their differentiation potential in vitro.

METHODS:Cells were obtained from human NP tissue from trauma patients undergoing spinal surgery. Briefly, after mild overnight digestion, the NP tissue cells were cultured in 2D (monolayer) or 3D (alginate beads) conditions with medium supplemented in ascorbic acid. After 2 weeks, cells from 2D or 3D culture were expanded on fibronectin-coating flasks with medium supplemented in FGF-2 to mimic the native microenvironment of NP cells. Subsequently, expanded NP cells were then characterized by cytometry (CD105, CD90, CD73, CD45, CD34 and Tie2) and tri-lineage differentiation, which was analyzed by qPCR and histology.

RESULTS:Cytometry analysis, after 2D- or 3D-expansion showed the presence of 0.1 % and 78.2 % of Tie2+ NP progenitors, respectively. Concerning the chondrogenic differentiation assay, the detection of glycosaminoglycans in the culture medium was drastically increased for 3D-expanded cells (11-fold) vs 2D-expanded cells. Moreover, the relative gene expression of collagen type 2 and aggrecan was also increased (600-fold and 2-fold, respectively). Regarding osteogenic differentiation assay, relative gene expression for osteopontin increased for 3D- (150-fold) vs 2D-expanded cells. However, no difference was observed between 2D and 3D expansion for the adipogenic differentiation assay.

DISCUSSION & CONCLUSIONS:The present study shows that 3D expansion of NP cells better preserves the progenitors cells population and increases the chondrogenic and osteogenic differentiation potential compared to 2D expansion. This project not only has a scientific impact by evaluating the role of native physiological niches on the functionality of NP progenitors but could also lead to an innovative clinical approach with cell therapy for IVD regeneration and repair.

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Keywords: Intervertebral disc / spine and their disorders, Microenvironment and niche engineering

Investigating the Transcriptome-Wide Expression Changes of Differentiating Stem Cells Targeted For Intervertebral Disc Regeneration

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INTRODUCTION:Novel cell-based regenerative therapies have the potential to restore function and relieve pain in patients with low back pain (LBP) caused by intervertebral disc (IVD) degeneration. We have previously shown that stimulation of adipose-derived stem cells (ASCs) with growth differentiation factor-6 (GDF6) promotes differentiation into nucleus pulposus (NP) cells of the IVD, which then have potential for stimulating IVD regeneration. Mechanistically, GDF6 stimulation activates the Smad1/5/8 and ERK1/2 signaling cascades leading to downstream gene expression. The aim of this study was to progress our understanding of the immediate/early response mechanisms in ASCs which may regulate GDF6-induced differentiation.

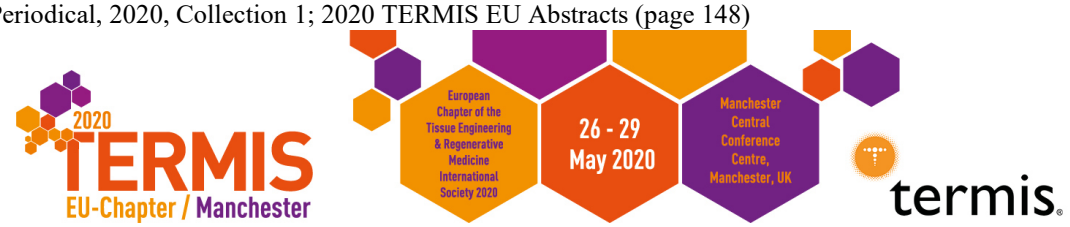
METHODS:Transcriptome analysis was undertaken on ASCs 2hr, 6hr and 12hr after GDF6 stimulation (N=3) using RNAseq analysis (Illumina HiSeq) and assessed using gene ontology (GO) (Gprofiler, padj<0.05, fold change ≥1.5) and ingenuity pathway analysis (IPA) ($p<0.05$, fold change ≥1.5). The genes identified were validated using RT-qPCR and western blot analysis at 2hr, 6hr and 12hr as well as at 1hr and 24hr in the original cohort (N=3) and in an additional cohort (N=6).

RESULTS:Bioinformatic analysis of the RNAseq dataset revealed a number of genes that play important roles in stem cell lineage commitment. Specifically, the chondrogenic-related transcription factors (TFs) SOX8, SOX9, SCX, EGR1 were initially shown to be significantly upregulated and subsequently downregulated across the time course. Additionally, the TFs CEBPA, CEBPD, PPARγ and NR4A1 that are involved in the regulation of adipogenesis were differentially expressed over the time course, suggesting inhibition of adipogenesis (N=3, paired t-test, padj $p<0.05$, fold change ≥1.5). RT-qPCR and western blot analysis confirmed RNAseq analysis as well as demonstrating equivalent TF expression in an additional cohort (N=6, One-Way ANOVA and nonparametric, $p<0.05$).

DISCUSSION & CONCLUSIONS:Bioinformatic analysis identified a number of genes that are known from literature to play roles in lineage commitment. SCX, for example, is more commonly associated with the annulus fibrosus of the IVD however, is known to potentiate SOX9 and therefore may be critical in controlling early NP differentiation. EGR1 regulates many different genes and was found to be consistently upregulated at 1h, maybe indicating that this TF precedes expression of the other TFs that regulate differentiation. Finally, TFs involved in promoting adipogenesis (CEBPA, CEBPD, PPARγ) were attenuated over the time course, whilst NR4A1 (an inhibitor of PPARγ) showed significant upregulation at 1h and 2h, potentially highlighting an important factor in lineage inhibition.

ACKNOWLEDGEMENTS:Funding sources: NIHR Manchester Biomedical Research Centre and The RoseTrees Trust

Keywords: Multipotent (mesenchymal) stem cells, Cell therapy



Chemical free antimicrobial surfaces based on engineered 3D micro/nano-environments

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INTRODUCTION:The excessive use of antimicrobial agents can lead to the increase of antimicrobial resistance (AMR) in bacterial populations. This may occurs when microbes express pumps that increase the efflux of antimicrobial agents, which include disinfectants, antibiotics and antiseptics. Developing strategies that minimize the need to use chemicals is therefore critical to reduce the rise of AMR.
Micro and nano-engineered surfaces have shown resistance against microbes colonization and biofouling without the need to use any chemicals [1, 2]. However, the mechanisms for which this is achieved are still poorly understood. In this work we present a library of 20 micro/nano-engineered surfaces and analyse their antimicrobial properties against 3 different bacteria.

METHODS:The antimicrobial surfaces were developed using two technologies. Standard SU-8 lithography rendering sharp square like cross-sections, while a novel technology, developed by the authors and known as diffusion induced lithography (DIL), enabled the manufacturing of engineered 3D micro/nano-environments.
The common bacterial pathogens Escherichia coli (Ec), Klebsiella pneumoniae (Kp) and Pseudomonas aeruginosa (Pa) where incubated over the surfaces for 24 hr. Then the surfaces were washed to remove non-adhered bacteria and the remaining ones were stained for fluorescent imaging.

RESULTS:Bacteria adhesion and micro-colonies (clusters of over 10 bacteria) formation was measured over the micro/nano-engineered surfaces and compared against the control surfaces. Different adhesion behaviours were observed for each of the three bacteria, with a maximum reduction in the formation of micro-colonies of 98% for Ec, 96% for Kp, and of 80% for Pa. When using surfaces developed by standard lithography the number of attached bacteria was often much higher than in the control samples. Conversely, for the DIL-fabricated samples a maximum reduction of adhesion of 84% for Ec, 74% for Kp, and of 65% for Pa was recorded.

DISCUSSION & CONCLUSIONS:It was possible to find an engineered surface that, despite not being the most optimal for any of the individual bacteria, it had significant increased antimicrobial properties for the three of them. The use of 3D micro/nano-environments exhibited enhanced antimicrobial properties, signalling this technology as candidate for the development of chemical free antimicrobial surfaces.

Acknowledgements:Financial support was received from Monash Infrastructure. This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF).

References:[1] E.P. Ivanova, et al., Nature communications, 2013. 4: p. 2838.
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Keywords: Infection, Interfaces – engineered

3D bilayer spheroid modelling of the mammalian cornea to identify novel limbal stem cell markers

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INTRODUCTION:Corneal disease accounted for approximately 1,500,000 cases of blindness worldwide in 2015[1]. Yet whilst effective treatments are available, a key clinical limitation is the availability of good quality corneal epithelial cells that *in vivo* derive from the stem cell compartment, the limbus. Characterising the limbal stem cell phenotype is therefore crucial for enabling the clinical translation of corneal therapies. Traditionally, this research uses primary mammalian corneal tissue due to a lack of an alternative *in vitro* high-throughput, multi-cell type, 3D limbal model.

METHODS:We have therefore developed a novel hanging drop spheroid model of the rabbit cornea, by surrounding stromal keratocyte (SK) cores of ~3000 cells with an envelope of ~6000 limbal epithelial cells (LECs), thus creating bilayered spheroids. At multiple time points over 10 days, models were incubated with EdU for 24h, snap frozen, cryosectioned and immunolabelled for key corneal markers or examined by transmission electron microscopy.

RESULTS:Batches of up to 100 models per donor were created with consistent cellular architecture. LECs within the model showed discrete cellular organisation and differentiation in as little as four days of co-culture. This was characterised by the formation of a proliferative p63+, CK14+, vimentin+ basal epithelial population, with suprabasal and superficial LECs subsequently differentiating and upregulating differentiation markers CK12/3 and PAX6. The SK core adopted an *in vivo*, non-wound healing phenotype, which we determined by the positive expression of mesenchymal markers vimentin and fibronectin, and the negative expression of α-SMA. Furthermore, TEM analysis of the model revealed potential basement membrane deposition at the epithelial-stromal interface, and a distinct gradient of keratin expression throughout the epithelial layer.

DISCUSSION & CONCLUSIONS:This data therefore shows that our novel 3D models recapitulate key corneal limbal markers in both epithelium and stroma. Furthermore, polarisation of the epithelium reveals the active epithelial-stromal intercommunication taking place within our model in a short time frame. Work is ongoing to examine model responses to WNT pathway modulators and to create high-quality human equivalents. Further work will aim to develop our model as an alternative *in vitro* high-throughput drug-testing platform for use by the drug/cosmetics industry, and as a novel, functional means of selecting high-grade limbal epithelial cells suitable for transplantation.

ACKNOWLEDGEMENTS:We thank the BBSRC for funding, and members of the Durham LSSU and Electron Microscopy Unit for help and materials.

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Keywords: Eye, Stem cell niche

Bone marrow-inspired 3D ex-vivo mesenchymal stem cell niche models for stemness acquisition of clinically relevant hematopoietic stem and progenitor cells

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INTRODUCTION:It is wieldy accepted that hematopoietic stem and progenitor cell (HSPC) behavior within the bone marrow (BM), in the specialized 3D microenvironments called “niches”, is triggered biophysically and biochemically by the molecular crosstalk among the niche cells, their extracellular matrix (ECM) components and ECM-bound or diffusible biomolecules. Characterizing the composition of different BM niches is still a matter of ongoing research and, in spite of the progress made so far, engineering a fully functional artificial BM niche is considered to be one of the major challenges in human hematopoietic research [1]. Bone marrow mesenchymal stem cells (MSCs) are assessed here for their role within a biochemically and biophysically well-defined xeno-free niche model to elucidate mechanisms of BM niche action as well as their potential role as a rheostat to regulate HSPC fate for further clinical translation applications.

METHODS:Using bio-chemically and mechanically tailorable hydrogels as scaffolds, several ex-vivo, xeno-free, BM-mimicked 3D HSPC-MSC niche models are developed by systematic optimization approach with high clinical relevance by considering the combination of ease of use, reasonable throughput and multiple readouts. Primary human CD34+ HSPCs were cultured for one and seven days within several different 3D niche models to decipher early and late regulation effects of MSCs itself and their soluble/unsoluble niche components on HSPCs phenotype and function. The ability of the cellular and extracellular niche components to promote HSPC’s self-renewal or differentiation towards myeloid/lymphoid lineages was assessed by quantitative flow-cytometry immunophenotyping, based on CD34, CD38, CD45RA, CD49f, CD90, CD135, and CD10 surface marker panels. Their functionality was evaluated in vitro by Long-Term Culture Initiating Cell assay, Colony Formation Unit assay and RT-PCR. Confocal and electron microscopy imaging was used to determine the functional, structural and cellular composition of the niches.

RESULTS:Characterization studies, imaging analysis of the 3D niches and viability/functionality assays of harvested cells from the niches confirmed successful development of the biophysically and chemically active BM-mimicked 3D model niches. Functionality and immunophenotyping assays have revealed different roles for the secretion factors and ECM components of human BM-MSCs on HSPCs self-renewal and lineage selection within the 3D model niches.

DISCUSSION & CONCLUSIONS:Newly generated knowledge on MSC-related cellular and extracellular niche factors, in future, might hold a key to facilitate early and late fate decisions of HSPCs, and therefore, pay way for their successful ex-vivo regulation that is currently missing and limiting their future therapeutic potential.

REFERENCES:[1] P.E.Bourgine, I.Martin, and T.Schroder. Cell Stem Cell 22, 298 (2018).

Keywords: Stem cell niche, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Perivascular Niche-on-a-Chip Platform for Label-Free Investigation of h-MSCs Metabolism

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INTRODUCTION:Mesenchymal stromal cells (MSCs) experience many intercellular and extracellular forces and specific oxygen tension (pO_2) inside their native in vivo stem cell perivascular niche. Many questions still remain regarding the complexity of this micro-environment and how oxygen concentration gradients and elasticity at the niche-tissue interface can affect MSC metabolism; which could be a physiologically significant feature to enhance differentiation. Here by the use of a miniaturized optically accessible bioreactor (MOAB)[1] coupled with fluorescence lifetime imaging microscopy (FLIM)[2] we profile cellular metabolism of human-MSCs (h-MSCs) in a simulated stem cell niche environment by controlling substrate stiffness and pO_2 gradients via interstitial flow velocity levels. The combination of these advanced modalities can profile metabolic activity of cells in very specific micro-anatomic environments to analyse physiology and diseases.

METHODS:h-MSCs were cultured within MOAB with continuous flow at 0.5 - 5 - 10 - 30 µL/min up to 7 days, with and without stiffness tuned polyacrylamide gels. Computational Fluid Dynamic (CFD) analysis was used to predict shear-stress levels and the pO_2 gradients within the culture chamber. Real-time FLIM was performed to quantify free and protein bound nicotinamide adenine dinucleotide (NADH) at successive time points. The effect of oxygen depletion and substrate stiffness in combination or independently, on MSC metabolism was investigated.

RESULTS:h-MSCs cultured on stiffness tuned substrates exhibited distinct metabolic transitions to oxidative phosphorylation on stiffer substrates. Flow rates up to 5 µL/min, still in the perivascular interstitial level, did not yield significant differences in the average NADH level indicating a stable metabolic activity. Computational analysis predicted a linear drop in pO_2 along the perfusion chamber, after imposing cell consumption, which agrees with FLIM analyses finding a bound NADH fraction linear decay towards the outlet hypoxic region, as early as day 1 for 0.5 µL/min and 5 µL/min, indicating a glycolytic shift in metabolism.

DISCUSSION & CONCLUSIONS:The pO_2 spatial depletion along the micro-chamber coupled with interstitial flowrates and tuned stiffness resemble the stem cell niche interface in the perivascular region. The hypoxic outlet models the inner niche environment where we were able to measure, by FLIM, significant spatial metabolic transition towards glycolytic activity. Our platform represents a powerful tool for MSC studies in metabolic controlled conditions.

ACKNOWLEDGEMENTS:This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 825159-MOAB).

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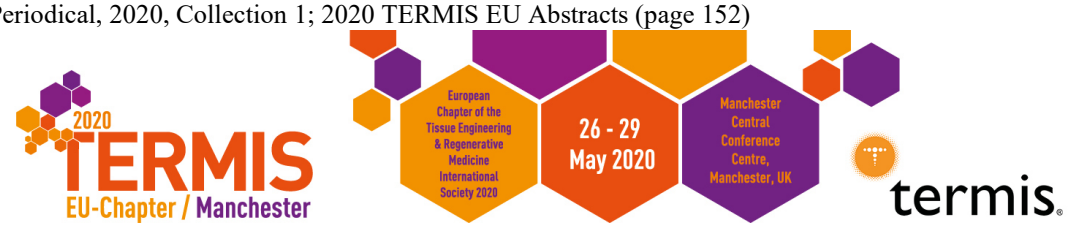
Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, In vitro microenvironments



Microfluidically generated single cell microgels as pericellular niches with temporally controlled biochemical and biophysical properties

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INTRODUCTION:The modular design of tissues is of indispensable importance for proper organ function. A key example of this phenomenon is extracellular matrix, which is naturally modular. Specifically, cells are entrapped in a niche composed of a thin layer of pericellular matrix, which in turn is located in a bulk of territorial matrix. These two matrix types are highly distinct in their biochemical and biophysical properties: while the pericellular matrix provides a stimulating cellular microniche, the territorial matrix gives rise to organ level characteristics. Incorporating such a modular design into biomaterials is expected to allow engineered tissues to more accurately emulate native tissues. However, it has remained a grand challenge to engineer the functional counterpart of life's smallest living building block: a cell within its pericellular matrix.

METHODS:A microfluidic droplet generation platform with delayed gelation was designed to produce enzymatically crosslinked single cell microgels that were mere micrometers larger than the single cell they encapsulated. The single cell microgels were engineered with on-demand tunable biophysical (temporal stiffening) and biochemical (dynamic macromolecular displacement) properties. The effects of temporal stiffening on the microgels were investigated using AFM and fluorescence quantitation and its effect on stem cell lineage commitment using histology, immunohistochemistry, label-free imaging (i.e., Hyperspectral Raman and CARS), qPCR, and RNAseq.

RESULTS:Single cell laden microgels with a diameter of 35 micrometer were produced in a monodisperse manner via on-chip enzymatic crosslinking of discrete prepolymer droplets. The single cell microgels remained metabolically active for at least month without any notable cell egression. The microgels' Young's modulus could be dynamically tuned from 2 to 50 kPa. Single cell analysis revealed that softer microgels stimulated adipogenesis, while stiffer microgels induced osteogenesis. Importantly, temporal stiffening of microgels revealed that the first three days of differentiation determined the stiffness-induced stem cell fate decision. Subsequently, we combined our single cell microgels with distinct biomaterials to create advanced bioinks. This modular approach effectively uncoupled the engineered tissues pericellular and territorial environments, which allowed for an unprecedented control over the design and behavior of living implants.

DISCUSSION & CONCLUSIONS:We here present a novel microfluidic single cell microgel-based concept that advances the engineering of hierarchical tissues by incorporating pericellular microniches within biomaterials in a facile yet highly controllable manner.

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Keywords: In vitro microenvironments, Enabling technologies

Senescence modulate angiogenic activity of mesenchymal stromal cells

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INTRODUCTION:Mesenchymal stem/stromal cells (MSCs) are an important member of the stem cell family and can be found in most postnatal organs and tissues. The MSC secretome influences the microenvironment upon injury, promoting cytoprotection, and tissue repair of the damaged area. These effects are of particular interest for the treatment of ischemic damaged tissues in which promoting vascularization and reinstalling perfusion via angiogenesis. Modulation of secretory activity revealed as an acquisition of senescence associated secretory phenotype (SASP) of MSC by aging cells is affected the neighboring cell functions and, inter alia, may have a tumorogenic effect as well. In this study, we examined the angiogenic activity of senescent MSCs.

METHODS:MSCs were isolated from human adipose tissue. Subculture was done at 80-90% confluence of the cell layer. Long-term cultivation was continued until replicative senescence was observed in the culture (19-28 passages). Senescence of MSCs was identified by assessing the activity of β-galactosidase, population doubling (PD) per passage, cell morphology etc. Conditioned medium (CM) from young and senescent cells was collected for further analysis, including chorioallantoic membrane assay in ovo, capillary-like tube formation on Matrigel, non-targeted cell migration assay, analysis of MSC secreted proteins. Forty one cytokines were measured by multiplexed fluorescent bead-based immunoassay detection (MILLIPLEX® MAP system) using a Human Cytokine / Chemokine panel. Total RNA was extracted from MSCs for qPCR analysis.

RESULTS:Attenuation of proliferative activity is one of the main indicators of the cell senescence in vitro. The data demonstrated that PD was decreased significantly after 18 passages (126 days). Cell senescence was confirmed by β-galactosidase staining and increased MSC size and vacuolization. Angiogenic potential of senescent MSC CM was attenuated which was characterized by decreased number of blood vessels in chorioallantoic membrane assay in ovo. In the same time long-term cultivation did not affect endothelium cell capillary-like network of tubule complexes on Matrigel and non-directed endothelium cell migration in vitro. Analysis of CM had shown increased concentration a number of cytokines (MCP-3, IL-4, IFN-a2, MDC, IP-10, FGF-2, RANTES, GRO, IL-8, IL-6, MCP-1) with pro- and antiangiogenic activity. PCR analysis revealed both down- and upregulation of some angiogenesis-associated genes.

DISCUSSION & CONCLUSIONS:Thus, modulations of MSC secretory phenotype under senescence result in decreased angiogenic activity in complex model in ovo, but not on Matrigel with endothelium cells only.

Acknowledgements:This study was supported by the RFBR (project no. 19-015-00150).

Keywords: Stem cell niche, Vascular systems / vascularisation and heart

Single cell RNA sequencing of Human Bone Marrow reveals new targets for enrichment of Skeletal Stem Cells

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INTRODUCTION:There remains an unmet need to develop robust regenerative strategies to reduce bone loss and improve quality of life. Cell-based strategies aim to harness the regenerative potential of skeletal stem cells (SSCs), a population of specialised stem cells residing in bone marrow that possess the capacity for self-renewal and differentiate along osteogenic, adipogenic and chondrogenic lineages. However, the successful application of SSCs in reparative medicine is limited significantly by the lack of specific target markers for isolation of SSCs. Single cell RNA sequencing platform, DropSeq, is a high-throughput methodology that utilises microfluidic systems to encapsulate single cells in droplets for parallel sequencing of thousands of single cell transcriptomes. Drop-Seq technology enables the identification of rare cell types within heterogeneous bone marrow populations and suggests cell type-specific molecular signatures for delivery into isolation protocols.

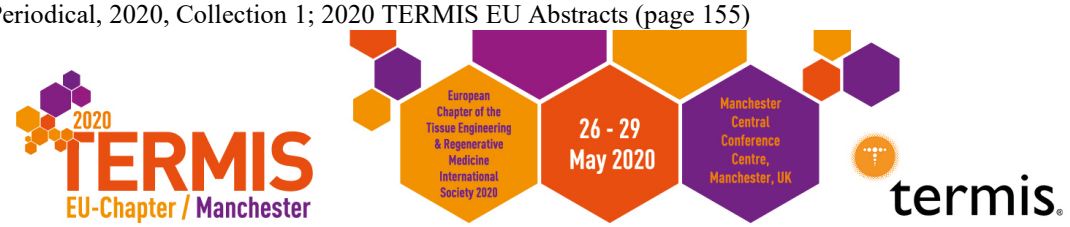
METHODS:We have previously developed a protocol to isolate potential SSC/progenitor populations from human bone marrow using oligonucleotide-coated gold nanoparticles that isolate cells from heterogeneous populations based on the expression of target mRNA. We have performed DropSeq on the enriched SSC populations, profiling >8,000 cells, to dissect the cellular heterogeneity within these populations and identify new targets for nanoparticle-based sorting. We have subsequently, assessed the differentiation and proliferation potential of the novel SSC-enriched populations.

RESULTS:Delivery of the enriched SSC populations into the DropSeq methodology revealed cellular heterogeneity in the bone marrow populations. Differential gene analysis between cell types presented new distinct targets for SSC-isolation, which, when implemented into the initial nanoparticle-based cell sorting, showed significantly enhanced enrichment of SSCs, compared to previous targets and other cell-sorting technologies, including magnetic cell-sorting using Stro-1 antibody.

DISCUSSION & CONCLUSIONS:Nanoparticle-based cell sorting, combined with DropSeq, delivers enriched human SSC populations for enhanced sequencing depth and reveals novel targets for SSC-isolation protocols. These molecular targets, when implemented in isolation protocols, allow enhanced SSC enrichment and offer an improved source of SSCs for research and ultimately therapeutic evaluation.

ACKNOWLEDGEMENTS:This work is funded the Rosetrees Trust, Wessex Medical Research and the Biotechnology and Biological Sciences Research Council.

Keywords: Omics / bioinformatics and systems biology



Heparan glycosaminoglycans enhance the expansion and therapeutic potency of human mesenchymal stem cells

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INTRODUCTION:Mesenchymal stem cells (MSCs) offer great hope to the treatment of age-related degenerative diseases. However, their ex vivo expansion capabilities and therapeutic potency decline with age. MSC aging is associated with reduced 6-O-sulfation of extracellular heparan sulfate (HS) glycosaminoglycan sugar chains that in turn leads to defective fibroblast growth factor-2 (FGF2):HS:FGF receptor complexes and subsequent signalling.

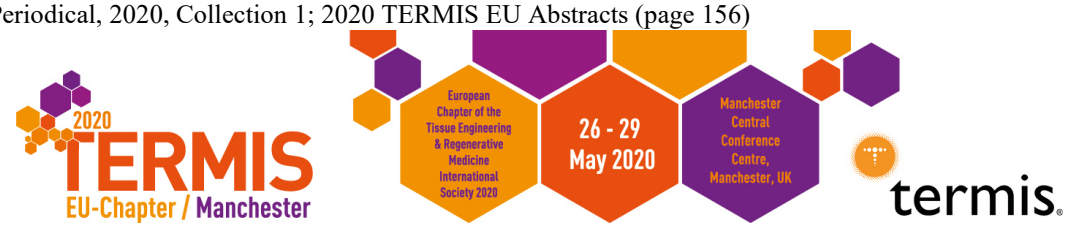
METHODS:To restore FGF2-mediated MSC growth and therapeutic potency, using affinity chromatography we developed a heparan sulfate variant (HS8) with enhanced FGF2 binding.

RESULTS:Disaccharide analysis revealed that HS8 is enriched for 6-O sulfation, which is 13% more than the crude HS. Its ability to enhance FGF2 signaling, and thus the ex vivo expansion of MSCs, were confirmed by both Western blotting and viable cell number analysis. Despite an increase in the number of population doublings, HS8-expanded MSCs (MSCHS8) were not compromised with respect to telomere length, colony-forming efficiency, biomarker expression or tri-lineage potential. HS8 supplementation reduced the accumulation of senescence-associated β-galactosidase by ~3-fold during long-term ex vivo expansion. When MSCs were extensively expanded, the levels of the senescence marker p21Cip1 significantly elevated in the control MSCs, but minimally changed in HS8-expanded MSCs. A qPCR array of 84 genes associated with human aging revealed that genes involved in inflammation were significantly altered during hMSC senescence, and that HS8 supplementation delayed such changes. Therapeutically, HS8-expanded MSCs administered into osteochondral defects within the femoral trochlear groove of NIH nude rats resulted in 20% more defects achieving high ICRS II and O'Driscoll scores over those administered with control MSCs. Magnetic Resonance Imaging confirmed reductions in the severity of osteochondral lesions following MSCHS8 treatment in yet larger animals (micropigs), and there was marked improvement in joint biomechanical properties shown by Instron Biomechanical testing.

DISCUSSION & CONCLUSIONS:Our study thus suggests that media supplementation with HS is a useful approach to help delay the process of MSC aging that mars their essential ex vivo therapeutic expansion. Furthermore, this study also advocates for the further development of affinity-isolated HS species capable of mimicking cellular microenvironments as a strategy for clinically relevant regeneration.

Acknowledgements:The work was funded by the National Medical Research Council, Singapore under its Bedside and Bench Grant Call (NMRC Project No. NMRC/BnB/0003b/2013) and the Biomedical Research Council (BMRC) of the Agency for Science, Technology and Research (A*STAR), Singapore.

Keywords: Cell therapy, Translation and commercialisation (inc. clinical trials and regulatory approval)



MiR-218 Promotes Chondrogenesis by Targeting Hypertrophic Markers During Differentiation of Human Mesenchymal Stromal Cells

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INTRODUCTION:Mesenchymal stromal cells (MSC) derived from human bone marrow are a source of adult stem cell population that could be used for cartilage regenerative therapy due to their chondrogenic differentiation potential. However, induction of hypertrophic phenotype markers in MSC during chondrogenesis limits clinical application of these cells. It is therefore important to identify specific targets to repress hypertrophic degeneration of chondrocytes. We addressed the question whether microRNAs (miRs) play a role in development of hypertrophy. Our aim was to identify new miRs that are directly involved in post-transcriptional regulation of the hypertrophy-related genes.

METHODS:MiR expression profiles in human articular chondrocytes (AC) were compared to hypertrophic chondrocytes derived from human MSC by microarrays, and, after in silico array analysis, miR-218 was selected for further validation. The expression profile kinetics for miR-218 was evaluated during in vitro chondrogenic differentiation of MSC versus AC. Next, its putative target mRNAs were validated by miR reporter assay in HEK293T, as well as by functional assays (western blotting and ALP activity) in transiently transfected by miR-218 mimic Saos-2 cells. Finally, to confirm a putative role of miR-218 in chondrogenesis, MSC were transiently transfected with miR-218 mimic and subjected to 28 days of chondrogenesis time-course, to evaluate effects caused by gain of miR-218 on chondrogenic versus hypertrophic differentiations.

RESULTS:MiR-218 was selected as a top candidate miR predicted to target hypertrophy-related genes. It was found that downregulation of miR-218 expression over a time-course of chondrogenesis in MSC correlates with induction of hypertrophic markers. It was confirmed that miR-218 directly targets hypertrophy-inducing COL10A1, MEF2C and RUNX2 mRNAs, and gain of miR-218 mimic in Saos-2 cells leads to reduction of MEF2C and RUNX2 proteins accumulation, with significant attenuation of ALP activity. In human MSC, miR-218 mimic transfection boosted chondrogenesis (GAG deposition, COL2A1 and KLF15 expression) and reduced expression of hypertrophic markers (COL10A1/COL2A1 and MMP3).

DISCUSSION & CONCLUSIONS:This study demonstrated for the first time an anti-hypertrophic role of miR-218 in regulation chondrogenic differentiation in human MSC, providing a better understanding of post-transcriptional regulation mechanisms of hypertrophic phenotype development in chondrocytes.
Acknowledgements:Financial support was received from the German Research Foundation (DFG2/707/12-1).

Keywords: Multipotent (mesenchymal) stem cells

The secretome of mesenchymal stromal cells drives functional heterogeneity

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INTRODUCTION:Heterogeneity within mesenchymal stem/stromal cell (MSC) populations is a significant barrier to their translation as regenerative therapies. We identified a biomarker, CD317, which discriminates elongated, migratory, regenerative MSCs (CD317-) from flattened/spread, non-migratory, differentiation-incompetent MSCs (CD317+)[1]. Here we demonstrate how the secreted signals from MSC subtypes, including soluble proteins, extracellular matrix (ECM) and extracellular vesicle (EV) cargo, can alter the phenotypes of others.

METHODS:Whole secretomes were concentrated from conditioned media of immortalised clonal lines of CD317- and CD317+ human MSCs and EVs were isolated by differential ultracentrifugation. We used LC-MS/MS to quantify secreted and EV proteins and Nanostring to determine EV-miRNA expression. MSC-derived ECM was decellularised with ammonium hydroxide and Triton-X100 after 2 weeks' cell growth. ECM was imaged by scanning electron microscopy or reseeded with cells for morphometric analysis using Phase Focus ptychography.

RESULTS:We identified 44 significantly increased secreted proteins and 162 significantly increased EV proteins in CD317- MSCs versus CD317+ MSCs ($p<0.05$), with significant enrichment of upregulated proteins in "Focal Adhesion" and "ECM-receptor interaction" KEGG pathways ($q=1.83e^{-13}$ and $3.75e^{-14}$ for secreted proteins and $q=2.73e^{-24}$ and $9.27e^{-2°}$ for EV proteins respectively). The matrisome of regenerative CD317- MSCs was particularly enriched in fibronectin, type VI collagen, periostin, aggrecan, biglycan, decorin and thrombospondin 1. We identified 12 differentially expressed miRNAs between the EVs of CD317- and CD317+ MSCs ($p=0.0066$-0.0438); 10 EV-derived miRNAs (mir376a-3p, mir29a 3p, mir221-3p, mir145-5p, mir630, mir21-5p, mir100-5p, mir320e, mir125b-5p and mir29b-3p) were upregulated in CD317- MSCs versus CD317+ MSCs and gene set enrichment analysis of their cumulative predicted targets from the TargetScan prediction algorithm highlighted significant involvement of pathways related to "ECM-receptor interaction" and "Focal Adhesions" ($q=1.48e^{-8}$ and $3.92e^{-9}$ respectively). ECM substrates secreted by CD317- MSCs had a more varied, globular topography compared to matrices derived from CD317- MSCs. Exposure of CD317+ MSCs to conditioned medium or ECM from CD317- cells induced a morphological conversion to a CD317- phenotype, with significantly reduced cell volume and area, increased length:width ratio and increased average migration speed and distance.

DISCUSSION & CONCLUSIONS:We have demonstrated considerable plasticity in MSC phenotypes, and that these phenotypes can be dictated by the dominant secretomes and/or matrisomes derived from specific MSC subtypes within a mixed cell population. These findings will have implications for the selection and treatment of heterogeneous MSCs prior to clinical application as well as improving our understanding of the role of secreted signals in cell competition.

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Keywords: Stem cells - general, Extracellular vesicles

There's more to fat than stem cells, characterising fat graft materials to understand the regenerative properties of adipose tissues

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INTRODUCTION:The discovery of mesenchymal stem cells isolated from adipose tissue in 2001 led to an explosion of studies investigating the potential of adipose derived stromal cells (ADSCs) for a range of clinical applications. In parallel, plastic surgeons have been developing novel fat grafting techniques following observations that fat grafting can not only restore volume but can also improve tissue repair and regeneration (Rigotti et al., 2007). At present we have a limited understanding of how adipose tissue processing affects the biological function of injected tissues, which is urgently needed to optimise novel adipose therapies for tissue regeneration. This study aims to characterise the cellular, structural and secretory phenotype of lipoaspirate and formulations of adipose tissues (FAT); bridging the gap between *in vitro* ADSC studies and clinical fat grafting results.

METHODS:Adipose tissue was collected from consenting patients undergoing routine surgery (NHS ethics 15/YH/0177). Four different FAT were produced to enable comparison of clinically relevant materials. These included: lipoaspirate, emulsified fat (also called "nanofat"), stromal vascular fraction (SVF) gel (where adipocytes are ruptured and removed) and cultured ADSCs. Structural characterisation was performed using confocal laser scanning microscopy following staining for key components of the adipose tissues. Secreted cytokines were measured using a cytokine array and the functional effects of FAT on skin and scars was examined *in vitro*.

RESULTS:Confocal imaging of the FAT showed lipoaspirate and emulsified fat both retained the microstructure of adipose tissues with intact adipocytes and evidence of blood vessels. The SVF-gel had a more homogeneous and irregular structure with very few intact adipocytes visible. The cytokine array data showed several differences in the relative secretion of cytokines; adipose tissues secreted more pro-inflammatory signals (including IL6 and IL8) while cultured ADSCs secreted more signals involved in tissue remodelling (including TGFβ, EGF and TIMP2). Functional assays demonstrated conditioned medium from adipose tissue but not cultured ADSCs were able to inhibit myofibroblast differentiation.

DISCUSSION & CONCLUSIONS:This study provides important data on the composition and secretory phenotype of adipose tissue and highlights important differences between cultured ADSCs and whole tissue. It is evident the regenerative effects observed from fat grafting arise from heterogeneous tissues rather than ADSCs alone, with work ongoing to understand the mechanism of action.

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REFERENCES:Rigotti et al. Plast Reconstr Surg. 2007;119(5):1409-24

Keywords: Wound healing, Trauma / surgery and rehabilitation

Dexamethasone regulates circular RNA expression during human bone marrow mesenchymal stromal cells differentiation

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INTRODUCTION:Understanding the molecular mechanisms underlying *in vitro* differentiation of human bone marrow mesenchymal stromal cells (MSCs) is of utmost importance for regenerative medicine. The role of circular RNAs (circRNA) in gene expression regulation was recently acknowledged, with mechanisms including miRNA sponging, impacting on mRNA biogenesis or competing for RNA-binding proteins [1]. The expression and role of circRNA in stem cell differentiation is currently unclear. Therefore, the goal of the present study was to identify the differential expression of circRNA in early osteo- and chondrogenesis of human MSCs.

METHODS:MSCs were isolated from human bone marrow obtained with full ethical approval. Osteogenic and chondrogenic differentiation and the undifferentiated controls were induced in monolayer and pellet cultures, respectively (n=9 donors each). Cultures were maintained for 7 days, then samples were collected for RNA isolation. Samples from 3 donors were used for RNA hybridization arrays in order to identify differential circRNA expression during differentiation. After this initial screening, samples from all 9 donors were used for target validation by qPCR. In an additional experiment (n=4 donors), the effect of dexamethasone or the glucocorticoid receptor agonist (+)-ZK216348 on gene expression in monolayer or pellet cultures of undifferentiated cells was assessed.

RESULTS:Microarray data have been deposited in NCBI's Gene Expression Omnibus [2]. Bioinformatic analysis identified several differentially expressed circRNA during differentiation. Among others, four different circRNAs derived from *FKBP5* gene were upregulated in both osteo- and chondrogenesis. In validation experiments, the upregulation of total and circular isoforms of *FKBP5* was confirmed in both osteo- and chondrogenesis. Dexamethasone alone was shown to strongly influence the expression of total *FKBP5* expression. This effect was mediated by a transactivation mechanism, since (+)-ZK216348, which preferentially induces transrepression, did not have the same influence on *FKBP5* gene expression.

DISCUSSION & CONCLUSIONS:The differential expression of several circRNAs was identified. Validation experiments showed that total and circular *FKBP5* (of which the linear transcripts encode for a co-chaperone of glucocorticoid receptor) were consistently upregulated in both osteogenic and chondrogenic differentiation and this effect was likely associated to dexamethasone treatment through a transactivation mechanism. The functional role of *FKBP5*-derived transcripts in differentiation should be explored in follow on studies, allowing to better understand the role of dexamethasone during *in vitro* differentiation.

ACKNOWLEDGEMENTS:This Investigation was financially supported with the assistance of the AO Foundation via an AOTRAUMA Network grant.

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Keywords: Differentiation,

Predicting and promoting human bone marrow MSC chondrogenesis by way of TGFβ receptor profiles

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INTRODUCTION:The use of monolayer expanded mesenchymal stromal cells from bone marrow (MSCs) as source material for cartilage regeneration has been hampered by the inherent and unpredictable donor variation of primary cells. Although CD markers are typically used to characterize cell populations, there is little correlation between CD marker profile and functional outcomes. Therefore, we aimed to discover novel predictive markers of MSC chondrogenic potential.

METHODS:Human Bone marrow was obtained after informed consent (Freiburg:EK-326/08). hMSCs were cultured up to passage-10 and mRNA analysis performed. Chondrogenesis in pellet culture (1% ITS, 1% non-essential amino acids, 1% pen/strep, 10ng TGFβ, 50µg/ml AA, 100nM Dexamethasone, DMEM/HG) was additionally assessed at multiple passages. Gene expression for a panel of TGF-β superfamily receptors was performed on day 0 prior to chondrogenic stimulation. After 28 days of chondrogenesis, gene expression and histological evaluation (Safranin-O and Col2A1 immunostaining) was performed. Spearman's rank correlation was used to calculate a Ratio for chondrogenic prediction. R=2^(-(ΔCt_1-ΔCt_2)), where ΔCt1=Ct hTGFβ-RI–Ct hRPLP0 and ΔCt2=Ct hTGFβ-RII–Ct hRPLP0 was used to predict the chondrogenic potential. To demonstrate the functional role of TGFβ-Rs during chondrogenesis, TGFβ-RI, TGFβ-RII, and ACVRL-I were transiently inhibited using siRNA.

RESULTS:The TGFβ-RI/TGFβ-RII ratio at the time of passaging reliably correlated with chondrogenic differentiation on D28. The ROC analysis established a ratio above 0.136 to be predictive of chondrogenic responders with an accuracy of 95.8%, while a lower ratio resulted in poor chondrogenic potential. In order to confirm the role of the receptor profile in hMSC fate, TGFβ-RI, TGFβ-RII and ACVRL1 were transiently knocked-down using a single dose of siRNA on day 0, prior to chondrogenic stimulation. Donors with a low ratio responded positively to the silencing of TGFβ-RII, with a marked enhancement of chondrogenic matrix deposition.

DISCUSSION & CONCLUSIONS:The ratio of TGFβ-RI / TGFβ-RII at the time of cell passaging reliably predicts chondrogenic outcome regardless of passage or donor, and a low ratio is associated with poor chondrogenic potential. By transiently increasing the ratio with a single siRNA knockdown of TGFβ-RII the chondrogenic differentiation ability of non-responsive MSCs is recovered. The ability to prospectively stratify cell populations into chondrogenic responders and non-responders can alleviate one of the major challenges for MSC therapy, the functional prediction of a donor on any given day, offering a more reproducible autologous cell therapy approach for cartilage regeneration. Additionally, modulation of the receptor profile offers an opportunity to correct non-responding populations.

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Keywords: Multipotent (mesenchymal) stem cells, Cartilage / joint and arthritic conditions

Tissue engineered hydrogel blocks as a model to study bone-tendon interface development

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INTRODUCTION:Tendon tears lead to patient discomfort and never fully heal due to scar tissue formation, which weakens the tendon to bone attachment site, or enthesis. This leads to high re-tear rates, contributing to post-surgical failure rates of up to 95%, depending on tear size. Regenerating the enthesis has shown to be challenging due to its highly complex nature, but tissue engineering provides a promising way to increase understanding of enthesis formation to aid the future development of strategies for enthesis regeneration. In this study, we aim to establish a tissue engineered model to study enthesis development by encapsulating human bone marrow-derived mesenchymal stromal cells (hBMSCs) in a biocompatible gelatin and poly(ethylene glycol) (PEG)/poly(D,L)-lactic acid(PLA)-based hydrogel. Methods to form bone and tendon 'blocks' were optimised forming a platform to study cell-cell interactions in enthesis formation.

METHODS:Methacrylated gelatin (GelMA) and PLA-PEG-PLA were synthesized and characterised by NMR spectroscopy. Hydrogels were formed under visible light (λ=400-500nm) in the presence of 0.3% (w/v) photoinitiator and characterised using rheometry, degradation, swelling and mechanical robustness tests. hBMSCs were encapsulated at 2.5x10^6 cells/ml. Biocompatibility was assessed by cell viability (Live/Dead staining), spreading (actin staining), and proliferation (Ki67 expression). Tenogenesis was optimised under mechanical and biochemical (TGF-ß3) stimulation. Osteogenic differentiation was performed by culture in osteogenic medium. Tissue blocks were analysed by qPCR, histology and immunofluorescence.

RESULTS:NMR analysis of hydrogel precursors showed successful synthesis. The resulting hydrogels had a storage modulus of 1.93±0.23kPa, a swelling ratio of 16.5±0.4 with an equilibrium water content of 93.9±0.2% and 6.8±0.6% gel fraction. Hydrogel degradation was assessed until day 57, and samples demonstrated the long-term stability required for extended cell culture. Importantly, no adverse effects on hydrogel structure appeared after 24h mechanical stimulation. High cell viability (>75%), spreading and Ki67 expression confirmed biocompatibility. Under osteogenic conditions, osteogenic marker expression increased and mineralisation was observed throughout the hydrogel confirming successful differentiation. Optimum tenogenic differentiation was achieved by combining both biophysical and biochemical stimulation, as determined by an increased expression of SCX, COL1A1 and COL3A1 and tendon-like tissue formation.

DISCUSSION & CONCLUSIONS:Overall, this work has optimised the conditions for both bone and tendon block formation in a biocompatible 3D culture system. This provides a platform for studies investigating enthesis formation and development using a tissue engineering approach. With this system, we enable further studies into the role of paracrine signalling at the enthesis and influence on development, thereby expanding our fundamental understanding of artificial enthesis development.

Keywords: Multipotent (mesenchymal) stem cells, Hydrogels and injectable systems

Remote triggering of TGF-β/Smad2/3 signaling promotes tenogenic commitment via magnetically actuated MNPs-ActRIIA labeled hASCs laden on magnetic scaffolds

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INTRODUCTION:Load bearing tendons are transmitters of forces generated by muscle to bone resulting in joint movement. Injuries affecting these tissues are a significant clinical burden and efficient treatments are still unmet. Tackling tendon regeneration, magnetically-assisted tissue engineering strategies aim to develop functional substitutes that recreate native tendon milieu, and capable of remote magnetic responsiveness [1]. Moreover, functionalised magnetic nanoparticles (MNPs) targeting cellular mechanosensitive receptors are potential instructive tools to mediate mechanotransduction in guiding tenogenic responses [2].

METHODS:In this work, we combine magnetically responsive scaffolds and targeted Activin A type II receptor (ActRIIA) in human adipose stem cells (hASCs), under alternating magnetic field (AMF), to synergistically facilitate external control over signal transduction.
Aligned magnetic fibrous scaffolds were fabricated by 3D-printing made of a blend of starch and polycaprolactone (SPCL) and incorporating iron oxide MNPs (magSPCL) [3]. After hASCs labeling with carboxyl functionalised MNPs previously coated with anti-ActRIIA by carbodiimide activation, hASCs were seeded onto magSPCL scaffolds and AMF exposed for 10min twice a week (f=50Hz, B=1mT) for up to 21 days of culture.

RESULTS:Firstly, the phosphorylation assessment of Smad2/3 proteins, by ELISA assay and pSmad2/3 immunocytochemistry, revealed stronger pSmad2/3 detection and signals in the nuclei of hASCs labelled with MNPs-ActRIIA cultured in magSPCL under AMF stimulation when compared to non-stimulated conditions, suggesting a more efficient activation of the ActRIIA receptor. Additionally, hASCs treatment with SB431542 was able to inhibit the receptor and subsequent pSmad2/3 cascade.
Moreover, MTS assay, dsDNA quantification and reactive oxygen species quantification were performed to assess hASCs viability, proliferation, and oxidative stress, respectively. The tenogenic commitment was assessed by real time RT-PCR, immunocytochemistry and Sirius Red/Fast Green Collagen assay.

Results showed an increase in metabolic activity and cell proliferation after 21 days, and the effect of AMF on hASCs response was not deleterious.

DISCUSSION & CONCLUSIONS:The combination of remote triggering TGF-β/Smad2/3 using MNPs tagged hASCs, through magnetically actuated scaffolds stimulates overall expression of tendon related genes, downregulation of non-tendon related genes, and the deposition of Tenomodulin and Scleraxis by hASCs, in comparison to non-stimulated conditions.
In sum, engineered magnetically-responsive constructs may act as cell signaling instructive platforms to enable downstream activation of key phospho-Smad2/3 cascade and inducing tenogenic differentiation.

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Keywords: In vitro microenvironments, Differentiation

Remote magnetic field instructs the crosstalk between macrophages and tendon cells in IL-1B enriched environments

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INTRODUCTION:Tendon injuries are relevant debilitating conditions in athletes, occupational workers and active ageing. Untreated lesions often progress into pathological conditions and chronic degenerative diseases compromising tendon function and the quality of life of patients. Macrophages can modulate the differentiation capacity of mesenchymal stem cells (MSCs) while MSCs can regulate macrophage plasticity [1]. Moreover, human tendon cells (hTDCs) can induce tenogenic cues in MSCs suggesting that the cellular interactions within a niche are extremely important for resolving inflammatory cues, promote healing and restore normal tissue functions.
In this work, we propose exploring the crosstalk between macrophages and hTDCs establishing co-culture systems and assessing their inflammatory cytokine profile. hTDCs were previously stimulated with IL-1β, used to recreate the inflammatory cues associated to tendinopathy conditions.
Furthermore, in a previous study by our group [2], we showed that remote actuation of a magnetic field modulated the inflammatory response of hTDCs treated with IL-1β. Thus, we also aim to investigate the influence of magnetic stimulation in the response of macrophage/IL-1β-treated hTDCs systems.

METHODS:THP-1 macrophages obtained after PMA differentiation were either i) added to IL-1β-treated-hTDCs or ii) seeded in transwell (1.0 um pore size) for direct and indirect cell-cell contact cultures, respectively. Cells were exposed to magnetic forces of 5Hz, 4mT and 50% duty cycle [2] for 1hour. The outcomes of macrophage-hTDCs co-cultures were compared to individual cultures for cytokine profile at gene and protein levels. Macrophage phenotypic markers were also assessed.
RESULTS:Our results show the gene expression of ARG-1 and MRC-1 (M2 markers) increases in magnetic actuated conditions especially in direct co-cultures while NOS-2 (M1) decreases. Also, under magnetic actuation both intracellular and extracellular IL-4 (pro-inflammatory cytokine) levels increase in direct co-cultures as well as the release of IL-10 but the release and gene expression of TNFα and IL-6 decreases.

DISCUSSION & CONCLUSIONS:Interestingly, magnetic actuation leads to a decrease in the IL-1β production in all cultures suggesting a powerful effect of magnetic forces over IL-1β response in accordance to our previous results. Overall, magnetic actuated IL-1β-treated-hTDCs influence the polarization of macrophages into M2 phenotype in co-culture systems suggesting the potential benefit of magnetic actuated approaches in orchestrating repair mechanisms for tendon oriented strategies.
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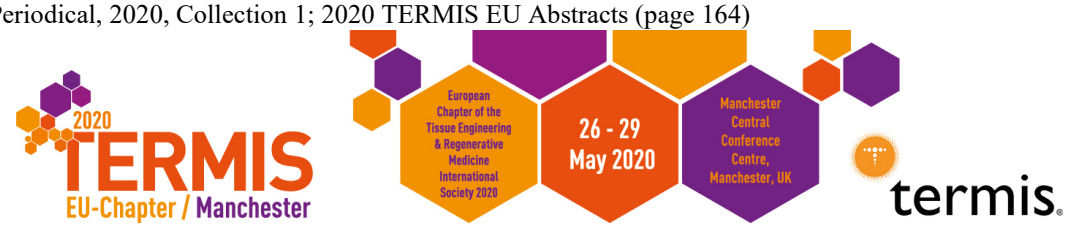
Keywords: Immunity / immunomodulation / macrophage, Biomechanics / biophysical stimuli and mechanotransduction



New generation modified graphene oxide reinforced HDPE/UHMWPE biomaterial for load bearing orthopedic applications

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INTRODUCTION:The generation of wear debris at articulating surface induces osteolysis resulting in aseptic loosening, bone degradation and suppression of bone formation. In an effort to combat such clinically relevant issues, this study explores the development of new generation UHMWPE based nanocomposites.

METHODS:In present work, a 'dual-hybrid' approach has been used for the fabrication of polymer based biomaterial which consists of a blend of high density polyethylene (HDPE) and Ultra high molecular weight polyethylene (UHMWPE) reinforced with modified graphene oxide (mGO). The nanocomposite (NC) is processed using melt-mixing which resulted in better dispersion of both polymeric phases and mGO. The samples were then γ-sterilized with a dosage of 25 kGy in order to understand its clinically relevant performance limiting properties. These properties mainly include wear properties using a pin-on-disc tester and cytocompatibility of the NC using assays like WST and live/dead assay.

RESULTS:It was observed that γ-ray sterilized nanocomposite exhibited an improvement in the oxidative index (16%), free energy of immersion (-12.1 mN/m), surface polarity (5.0 %) and hardness (42%) which further resulted in better wear properties predominantly coefficient of friction (CoF). In addition, the generated wear debris were observed under transmission electron microscope (TEM) which demonstrated that most of the particles were phagocytosable ranging between 0.5 µm to 4.5 µm. Despite of such size range these wear particles did not induce any cytotoxic effects to the proliferation and morphology of both human mesenchymal stem cells (hMSCs) and MC3T3 murine osteoblast cells when seeded in 5 mg/ml of concentration for 72 h.

DISCUSSION & CONCLUSIONS:The enhancement observed in the properties of the NC is critically dependent on the uniform dispersion and scavenging effect of mGO in the polymer matrix even after gamma-sterilization. In the above backdrop, combining our observations it can be concluded that these newly developed mGO reinforced HDPE/UHMWPE based nanocomposite can be utilized as a promising candidate for load bearing orthopedic applications.

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Keywords: Bone and bone disorders (osteoporosis etc), Polymers - natural / synthetic / responsive

Spontaneous 3D Micropatterning of BMP-2 in Self-assembling Nanoclay gels for Bone Tissue Regeneration

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INTRODUCTION:Limitations of current clinical treatments for critical size bone defects necessitate the development of new strategies for bone tissue engineering (TE). In this regard, emulating the three-dimensional (3D) organization of biochemical cues present in the native cellular microenvironment is likely to be key to develop biomaterials with distinct levels of functionality. However, despite advances in TE, achieving stable structures incorporating 3D-micropatterning of biochemical cues, particularly that preserve resolution with an increase in size has proven challenging1. Nanoclay-gels have established potential in TE due to their capacity to sequester proteins for sustained, localised bioactivity2. The current study reports a simple biomimetic method to applying self-assembling nanoclay-gels for spontaneous 3D micropatterning of proteins, allowing the delivery of localized stable niches for enhanced bone regeneration.

METHODS:Hydrous suspensions of Laponite®, a synthetic smectite clay, were added to a solution containing biomolecules and ions present in blood plasma to initiate a diffusion-reaction mediated self-assembly process. The assembled structures were tested for their ability to pattern fluorescently-labelled model proteins (albumin, avidin, streptavidin, immunoglobulin, casein) and to localize the activity of bone morphogenetic protein (BMP-2) The structures were analysed using a range of imaging techniques, including fluorescent, polarized light and electron microscopy, and a 28-day murine subcutaneous implantation assay.

RESULTS:Nanoclay/protein scaffolds possessed an internal degree of order able to template punctuated or gradual 3D gradients of all proteins tested. By changing the assembly parameters, such as concentration, ionic strength, incubation time and temperature, it was possible to demonstrate control over the spatial localization of the proteins. Furthermore, the assembly of structures at scale with a range of dimensions (0.2-1mm) and shapes (droplets, cylinders, strings) while preserving the resolution of protein patterning. The assembled structures displayed a radial birefringence under polarized light, indicating the presence of periodical arrangements of nanoparticles. Finally, the in vivo study revealed that punctuated localisation of BMP-2 inside the scaffold provided the potential to control the spatio-temporal formation of mature bone.

DISCUSSION & CONCLUSIONS:This study reveals, for the first time, the potential to harness interactions between clay-nanoparticles and biomolecules present in physiological fluids to design customized scaffolds with complex biochemical gradients, dimensions and shapes for bone with clinical relevance.

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Keywords: Biomaterials, Hydrogels and injectable systems

Evaluation of a scaffold-free nerve tube for peripheral nerve regeneration of a rat sciatic nerve defect

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INTRODUCTION:Even with recent advancements, the treatment for peripheral nerve transsections still remain a challenge. Nerve damage can lead to severe loss of sensibility and motor function, therefore greatly affect the patient's quality of life. Our goal is to produce a scaffold-free nerve tube (NT) produced solely from patient own cells. A viable conduit pre-seeded with endothelial (ECs) and Schwann cells (SCs) could allow a faster recovery for patients with major peripheral nerve transsections. METHODS:NT's are made of human fibroblasts sheets seeded with both EC and SC's and then rolled to form a filled tubular structure, a technique based on the self-assembly method.[1] NT's were implanted in immunodeficient RNU rats for 22 weeks to repair a 15 mm sciatic nerve defect. Graft innervation was observed by immunofluorescence and toluidine blue staining. The gastrocnemius muscle weight was used as a reliable measurement of reinnervation status. The horizontal ladder rung test was used to determine functional outcome assessment.[2] In addition, the sciatic functional index (SFI) was determined from DigiGate treadmill walking following nerve injury.

RESULTS:The nerve tube filled with a cellularized sheet structure enhanced nerve fibers migration. Moreover, toluidine blue staining revealed mature myelinated fibers after 22 weeks. Equivalent muscle mass was observed in grafted NT's vs gold standard control autograft and resulted in comparable nerve reconnection. Furthermore, the rats analyzed gait showed partial motor function recovery.

DISCUSSION & CONCLUSIONS:A pre-vascularized NT with autologous cells increases the efficiency of axonal migration by increasing the supply of nutriments and oxygen through the rapid establishment of a capillary network. Moreover, long term primary SC's survival may release nerve regeneration factors and increase axons migration. Finally, in the rat long-gap model, it has been shown that pre-vascularized and pre-seeded NT's allows for motor function recovery.

Acknowledgements:This research was supported by grants from the CIHR (Canadian Institutes for Health Research)

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Keywords: Trauma / surgery and rehabilitation, In vivo and animal models

Developing a 3D iPSC-Derived Blood Brain Barrier

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INTRODUCTION:The blood brain barrier (BBB) is an interface where brain microvascular endothelial cells (BMEC's) form tight junctions around the lumen of neural blood vessels to selectively control influx and efflux of compounds to and from the brain1. In neurodegenerative disorders such as Alzheimer's disease, damage to the BBB and influx of neurotoxic components is often an early cause of cognitive decline. The BBB is of significant interest in the study of dementia but studies that replicate the BBB in vitro often fail to recapitulate the complex multicellular nature of the in vivo environment. This study presents an approach for better recapitulating the in vivo environment with the combination of induced pluripotent stem cell (iPSC) derived neurovascular cells with a 3D culture system that mimics aspects of the brain extracellular matrix.

METHODS:Cortical neurons and BMEC's were differentiated from iPSC's using previously reported protocols2,3. To form a 'neural' component of the BBB model, iPSC neural precursor cells were embedded in a type I collagen hydrogel and matured into cortical neurons for 3 weeks. The hydrogel was then coated with a basement membrane of Matrigel and iPSC BMECs were seeded onto the surface. The resulting structure was cultured for 10 days and various analyses were performed including RT-PCR, immunofluorescence microscopy (IFM) and trans-endothelial electrical resistance (TEER) to determine the phenotypic and functional properties of the neurons, BMECs and the BBB model as a whole.

RESULTS:Analysis of the co-culture revealed phenotype retention and physiological function in both the neurons and BMECs. RT-PCR and IFM demonstrated that neurons embedded in the hydrogel expressed key markers of neuronal maturation. TEER measurements and IFM also showed that BMECs seeded on the hydrogel surface expressed tight junction proteins and formed a functional barrier that was further improved by the presence of neurons in the co-culture. Moreover, a 3D confocal map of the co-culture highlighted a homogenous distribution of neurons throughout the hydrogel while the BMECs remained on the surface and formed a functional barrier and did not migrate into the gel.

DISCUSSION & CONCLUSIONS:These data highlight the successful generation of a 3D co-culture of iPSC derived BMECs and neurons into a structure that replicates functional aspects of the BBB. This could provide a platform probing the role each cell type plays in BBB maintenance and loss of function in neurodegenerative diseases.

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Keywords: Nervous system (brain-central-peripheral / disorders), Disease models

Determination of the appropriate dosage of Adipose tissue-derived Stem Cell Secretome for Spinal Cord Injury Regenerative Medicine Approaches

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INTRODUCTION:Traumatic spinal cord injury (SCI) is a neurological condition which leads to impairments of several body functions. As existing treatments for this condition have been inefficient for tissue repair and functional recovery, current research has been focusing on cell free-based therapies employing mesenchymal stem cell (MSC) secreted products. MSCs, and among them Adipose tissue-derived Stem Cells (ASCs), have been shown to exert beneficial effects on the CNS through soluble factors and small extracellular vesicles that they secrete, commonly referred as secretome. However, the therapeutic dosage of ASC secretome still has to be determined. In order to bridge this gap, this work aimed to analyze the therapeutic efficacy of different concentrations of ASC secretome for SCI repair.

METHODS:Adult female mice were subjected to a complete transection at T8 level and then took, intravenously, secretome or vehicle on day 0, 1 and 2 after injury and once a week for 7 weeks. Based on distinct treatments, mice were divided into five groups: 1 – Vehicle (Neurobasal A Medium); 2 to 4 – three different secretome concentrations: 1x, 25x and 50x. For the evaluation of locomotor recovery, Basso Mouse Scale (BMS) Test was performed weekly. Motor function, coordination and mechanical allodynia, were also assessed through Open Field (OF), Beam Balance (BB) and Von Frey (VF) tests, respectively.

RESULTS:Animals treated with 50x secretome presented a consistent increase in the mean score of BMS test throughout the experiment, in comparison to the 1x secretome or the vehicle groups. In OF and BB tests, as the concentration of secretome increases, the means value of velocity, distance traveled, and coordination score are also increased when compared to control groups. No major differences were found among groups on sensory function.

DISCUSSION & CONCLUSIONS:The impact of ASCs secretome on the recovery of a mice model of SCI is concentration dependent, with the 50x concentration group disclosing increased levels of motor recovery.

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Keywords: Multipotent (mesenchymal) stem cells, In vivo and animal models

The Bioengineered Nichoid Scaffold Stimulates Pluripotency of Neural Precursors Cells and Potentiates Their Therapeutic Efficacy In Vivo

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INTRODUCTION:In regenerative medicine, biomaterials have been used to create 3D micro-scaffolds, allowing the generation of active biophysical signals for directing stem cell fate. The Nichoid, has been reported to maintain the stemness capacity in cells of different origins [1]. The aim of this study was to investigate: i) the proliferation, differentiation and stemness properties of neural precursor cells (NPCs) expanded inside the Nichoid (NIC-NPCs); ii) the therapeutic effect, safety and mechanism of action of NIC-NPCs in preclinical experimental model of Parkinson's Disease (PD) [2,3].

METHODS:Nichoids were fabricated by two photon laser polymerization using a home-made SZ2080 photoresist. NPCs expanded inside the Nichoid were counted and characterized by immunofluorescence, western blot, and Real Time PCR analysis. Moreover, a RNA-seq analysis of NPCs grown inside the scaffold was performed to evaluate the gene expression profiles. Parkinsonism was induced by the administration of MPTP in C57/black mice by using an acute protocol [3]. The recovery of function was investigated with vertical and horizontal grid behavioral tests and, after the animals' sacrifice, observations were corroborated by immunofluorescences studies performed on brain sections of transplanted animals versus controls.

RESULTS:NIC-NPCs presented a specific 3D organization and morphology, by forming a carpet-like structure. 7 days after plating, the NIC-NPCs show a significantly higher proliferation and viability than in standard floating conditions (controls). Moreover, NIC-NPCs show an increase in stemness and pluripotency potential, as demonstrated by immunofluorescence, Real Time-PCR, Western blot and methylation assays. The whole transcriptome analysis identified 1935 deregulated RNAs between NIC-NPCs and the controls: 81,5% (1577 out of 1935) were coding genes and most of them were up-regulated (927). The most deregulated pathways confirmed the upregulation of pluripotency, cellular proliferation and mechano-transduction genes. To evaluate the in vivo efficacy, after intra-striatal infusion in PD mice, NIC-NPCs showed an increase in their therapeutic features by counteracting neurodegeneration and promoting recovery of function. Specifically, NIC-NPCs favored the expression of tyrosine hydroxylase and dopamine transporter. Moreover, NIC-NPCs downregulated gliosis.

DISCUSSION & CONCLUSIONS:Here we provide evidences that the expansion of NPCs in the Nichoid both favors their pluripotency and potentiates their efficacy showing great promise in the field of regenerative medicine.

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Keywords: Stem cell niche, In vivo and animal models

A 3D Organotypic "*"ex vivo"*" Bone Marrow Slice Culture Model to Investigate Cell Responses to Bacterial Infection

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INTRODUCTION:Infections of the bone marrow by *Staphylococcus aureus* (*S. aureus*) species, one of the main colonisers in prosthetic joint infections and osteomyelitis, are often associated with increases in inflammation and influx of pro-inflammatory cytokines leading to osteonecrosis and bone loss. It is at these damaged necrotising areas that bacterial species colonising an implant invade into the bone marrow cavity. The pathologic mechanisms leading to bone loss are poorly understood with limited reproducible model systems avaiable that can be carefully manipulated to improve understanding. The aim of this study was to develop an *ex vivo* osseous model that provides a 3D organotypic culture environment promoting viability of cell populations and to validate the responsiveness of this system to bacterial virulence following infection with *S. aureus* species.

METHODS:Femoral slices of 1.5mm thickness were prepared from 28 day old male Wistar rats, embedded in a semi-solid agar based medium and cultured at the liquid-air interface for 48hr in αMEM, supplemented with 30ng/mL RANKL and 20ng/mL M-CSF, along with a microinjection of mixed bone marrow cells. Maintenance of cell populations was confirmed by digital histomorphometry and immunocytochemistry identifying CD105, CD14, CD68 and neutrophil elastase as markers for mesenchymal stromal cells (MSCs), monocytes, macrophages and neutrophils respectively. Femoral slices were also cultured for 24h in the presence of agar innoculated with two different *S. aureus* strains (Oxford reference and osteomyelitis isolate 7791). Following infection, tissue slices were examined by digital histmorphometry and immunocytochemistry for apoptosis markers and necrosis LDH activity. Secretion of pro-inflammatory cytokines, IL-1β, IL-6, TNFα was assessed by ELISA. Heat inactivated bacteria provided negative controls.

RESULTS:Maintenance of cell types following culture were observed with immunocytochemistry. Histological examination of tissue slices following infection with either strain demonstrated attachment of bacteria to the extracellular matrix of cultured tissue. Cell viability analysis following immunocytochemistry suggested apoptosis of cells following culture with bacteria and LDH analysis suggested increased evidence of necrosis by 19hrs in culture with viable bacteria compared to controls. Cells within cultured tissue slices secreted pro-inflammatory cytokines TNFα and IL-1β with secretion of cytokines significantly higher in tissue infected with strain 7791 ($p<0.005$). IL-6 secretion by cells in both infected tissue slices and controls was low.

DISCUSSION & CONCLUSIONS:This data suggests potential successful development of an *ex vivo* osseous slice culture model capable of supporting heterogenous cell populations. Infection of the culture model demonstrates an immune response and secretion of pro-inflammatory cytokines representative of the clinical environment.

Keywords: Bone and bone disorders (osteoporosis etc), Disease models

Microfluidic bone organ model

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INTRODUCTION:Engineered bone tissue often lacks the complexity of the native microenvironment in terms of structural hierarchy, vascularisation, and cellular composition. Bone regeneration and cellular dynamics are highly depended on the non-immunological regulatory roles of macrophages/monocytes, which are often neglected. To harness the secretory influence of immunogenic cells for bone tissue engineering, this study aims to construct an osteogenic niche in a microfluidic bioreactor utilizing primary mesenchymal stem cells and monocytes later to be differentiated into osteoblasts and osteoclasts respectively.

METHODS:Microporous beta-tricalcium phosphate (βTCP) based ceramic scaffolds were manufactured in-house using polymer foam replica technique. The custom microfluidic bioreactor was designed and fabricated from polymethylmethacrylate sheets and assembled with double side adhesive. Primary mouse bone marrow cells were isolated and sorted for mesenchymal stem cells (MSC) and monocytes. MSCs and monocytes were seeded in scaffold and co-cultured for 4 weeks followed by subcutaneous implantations to nude mice. The implanted constructs were assessed by histology and gene expression levels with cell-free control constructs.

RESULTS:In vitro material and structural characterization of ceramic scaffolds demonstrate preservation of the beta phase of TCP with interconnected porous architecture. Microfluidic bioreactor successfully assists the cell seeding and maintenance of the cellular physiology. In vitro osteogenic differentiation towards osteoblasts and osteoclasts has been achieved.

DISCUSSION & CONCLUSIONS:Tissue-engineered bone grafts that lack osteoclastic cells cannot form a physiologically relevant bone microenvironment. Cross-talks between the osteoblast-osteoclast highly affect the overall success of bone turnover. In addition, utilization of osteoclasts along with biomaterials often face inhibition due to material characteristics such as crystallinity, grain size, and surface chemistry. Tailoring the tissue scaffold for both osteoblast and osteoclasts is the optimum solution for a healthy and native-like environment.
A microfluidic bioreactor that can allow in vitro modelling of osteogenic niche and can alter the bone remodelling process is of high importance. Such a system not only will bring a tremendous amount of therapeutic tools for many bone diseases but also will allow a close investigation and experimentation for osteogenic niche cross-talks and processes. Using immune cells to alter bone regulation and repair mechanisms while improving the nutrient supply to the cell-biomaterial construct, our system holds promise for a high-performance tissue platform.

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Keywords: Biomaterials, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Modelling the Microbiota-Gut-Brain Axis in vitro: focus on a novel Blood-Brain-Barrier-on-a-chip

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INTRODUCTION:In last decades, scientists have been accumulating several evidences that intestinal microflora, collectively named gut-microbiota, could alter the brain pathophysiology. There exists an outstanding hypothesis suggesting that the microbial-released secretoma could pass the blood-brain-barrier (BBB) and potentially affect brain functionality. In a context where 3D cultures and millifluidics become essential, the ERC MINERVA project (G.A: 724734) [1] aims at developing the first engineered microbiota-gut-brain platform to investigate the intestinal microflora impact on brain functionality. For this purpose, we have developed a novel millifluidic organ-on-a-chip device, modelling in vitro the BBB, the organ that prevents neurotoxins and pathogens from entering the brain. Here we describe hydraulic and mass transport validation of the device coupled with multi-physics computational analysis.

METHODS:We have designed a device with three independent optically accessible culture chambers made up of two perfused micro-compartments separated by permeable microporous membranes for cell culturing and secretome diffusion. Through piezoelectric sensors we have tested the hydraulic sealing at different flow rates. We have developed CFD models with COMSOL Multi-physics to predict velocity profiles, shear stress, oxygen distribution and molecules diffusion across the membrane. To evaluate the effective diffusion rate through the membrane, we have performed a mass transport studies with a fluorescent lipopolysaccharides (LPS) during perfusion with cells.

RESULTS:CFD analysis showed the presence of laminar flow, which both guarantees shear stress experienced by endothelial cells and astrocytes, within physiological levels, replicating in this way the capillaries-brain interstice interface conditions. Mass transport analysis allowed us to set proper input flow rates which guarantee molecule diffusion across the micro-porous membrane with adequate diffusive fluxes. The model showed a linear decrease in oxygen concentration along the chamber with pO_2 levels that guarantee continuous oxygen supply to the cells. Experimental mass transport studies with LPS simulating the microbial secretoma confirmed a correct solutes diffusion across the membrane during perfusion with cells.

DISCUSSION & CONCLUSIONS:Computational analysis allowed us to set optimal input parameters to guarantee physiological conditions inside the BBB-on-a-chip device while hydraulic tests demonstrate an excellent hydraulic sealing with consequent good maintenance of sterility. Experimental analysis with LPS confirm the suitability of the device for mass transport studies.
Acknowledgements:MINERVA project received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (Grant Agreement no. 724734).
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Keywords: Bioreactors, In vitro microenvironments

Towards the Development of a Fracture Healing on a Chip Platform

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INTRODUCTION:Endochondral ossification (EO) is the process by which the longs bones are formed during development, and is reactivated during fracture healing. During fracture healing, a cartilaginous template at the fracture site is invaded by vasculature; initiating a cartilage to bone transition. Most of what is known about EO in fracture healing has been derived from animal models. Though essential, animal models are time consuming, expensive, and their utility as analogues for humans has been questioned (1). Microphysiological systems (MPS), or organs-on-chip, are complex 3D in vitro models aimed to recreate biological processes at the microscale. Herein, we describe the development of a fracture healing-on-chip platform, detailing the interplay between human cartilage micro-tissues (µtissues) and vasculature.

METHODS:Devices: PDMS moulds were fabricated by casting PDMS using a laser cut master. PDMS and glass coverslips were then plasma treated and bonded.
Cartilage µtissues: human bone marrow stromal cells (hBMSCs) were seeded in agarose µwells, centrifuged to form µtissues, and cultured for 14 days in chondrogenic medium at 5% O_2.
Cartilage-vasculature co-culture: At day 14, µtissues were harvested and encapsulated (60, 300, or 600 µtissues / 100µL) with GFP-tagged human umbilical vein endothelial cells (HUVECS) in a fibrin hydrogel and injected into mesofluidic devices. Cartilage µtissues and HUVECs were cultured for 7 days.

RESULTS:The concentration of cartilaginous µtissues in the sample effected vascular morphology. At low concentrations of cartilage tissue (60 µtissues / device), HUVECs do not form vascular structures. However, as concentration of cartilage increases, HUVECs form open vascular networks. Interestingly, the vessels that formed grew away from and around the µtissues, avoiding the cartilage spheroids rather than towards them as has been observed with cancer spheroids (2).

DISCUSSION & CONCLUSIONS:We have observed that vascular structures are formed in the presence of cartilage µtissues, and that network morphology is effected by µtissue concentration. Future work will seek to probe the specific interaction between endothelial cells and µtissues, and specifically how this is influenced by the phenotype of the cartilage phase as it progresses towards hypertrophy. Ultimately, realisation of a fracture healing on-chip model could be used as a tool to augment fracture healing research. In addition, such a system could be used to screen conditions to prevent vascularisation and hypertrophy of chondrocytes; a key challenge in cartilage tissue engineering.

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Keywords: Cartilage / joint and arthritic conditions, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Mature and functional liver organoids self-assembled from hiPSC-derived hepatoblasts suitable for regenerative medicine and toxicology applications

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INTRODUCTION:The self-organizing nature of cells enables the production of organoids resembling native tissues. Indeed, the idea of reproducing a whole organ is challenging, however, mimicking organs as complex as the liver have proved to be very difficult. In particular, when derived from human induced Pluripotent Stem Cells (hiPSCs), adult and not fetal characteristics are required from hepatic cells to be used in toxicology and drug screening studies. In this work we present the generation of liver organoids by self-assembling of hiPSC-derived hepatoblasts (iHBs) possessing features beyond the fetal or neonatal stage.

METHODS:iHBs were self-assembled in inert hydrogel microwells then further differentiated into hepatocytes (iHeps) and maintained in culture for 30 days.

RESULTS:Aggregation of the cells generated smooth and well defined spheroids of 250 µm in diameter with no sign of significant cell death. They were then characterized in terms of functionality. Gene expression studied by RT-PCR and immunofluorescence analysis showed that a variety of important markers of the hepatic lineage maturation were acquired such as HNF4α, HNF1α, UGT1A1, clotting FIX, BSEP. Moreover the complete disappearance of the intracellular and secreted AFP was observed within the first 6 days of culture with a 4-fold increase of albumin secretion, reaching the level of that secreted by primary human hepatocytes in 2D cultures. Urea production and response to hormones (insulin/glucagon) have been also recorded alongside bile salt excretion. Organoids showed the complete disappearance of the fetal isoform of Cytochrome P450 3A (CYP3A7) while CYP3A4 (adult isoform) and CYP1A1/2 exhibited a statistically significant increase in expression, inducibility and activity (EROD/BROD metabolized up to 81%) along the time. Finally, apical marker expression highlighted the complex polarization of the cells and the existence of a well-defined bile canaliculi network which extends inside the core of the organoids, as assessed through imaging, computational analysis and 3D reconstruction. Being able to go through fusion when placed in close proximity, organoids generated larger tissues (0.6-1 mm Ø) with no sign of necrosis suitable as building blocks for the assembly of complex bioartificial liver constructs.

DISCUSSION & CONCLUSIONS:Data recorded suggest that we were able to generate with high reproducibility organoids exhibiting all the liver functions tested at a level comparable to that of PHHs as required for real-life applications such as screening in drug discovery and personalized medicine. We postulate that these liver organoids could be used as biological components for tissue engineering applications such as liver-on-chips, bioartificial liver and liver bio-fabrication.

Keywords: Cell therapy, Induced pluripotent stem cells

Development of a 3D bioprinted human-based bone organoid using patient-derived cells

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INTRODUCTION:The current gold standard for understanding bone biology and pathology involves the use of animal models. While extremely valuable, the generation of transgenic animals remains a costly and inefficient process, which is ultimately not representative of human bone pathophysiology. *In vitro* strategies using human cells may help to closely resemble the human bone biology and thus gain a deeper understanding of the human bone pathophysiology. We aim to establish personalized and clinically relevant bone models to study bone pathophysiology by developing novel three-dimensional (3D) bioprinted bone organoids using patient-derived cells.

METHODS:Human long bone specimens (n = 2) were obtained from pediatric patients undergoing subtractive osteotomies for deformity correction. Cells were isolated based on tissue culture plastic adherence. After expansion, cells were combined with alginate-gelatine (0.8-4.1% w/v) hydrogels and bioprinted to 3D cell-laden scaffolds (n = 3) to mimic the trabecular bone structure [1]. 3D bioprinted scaffolds were cultured in static bioreactors for 6 or 7 weeks under osteogenic conditions. Live/dead assays were performed to determine cell viability after bioprinting. Weekly micro-computed tomography (micro-CT) scans were taken non-invasively to assess mineral volume. Osteoblast and osteocyte cell phenotypes of the 3D bioprinted organoids were assessed by histology, immunohistochemistry and gene expression analysis after 6 or 7 weeks of culture. Organoids were compared to human bone tissues to demonstrate resemblance to *in vivo* bone architecture and phenotype.

RESULTS:More than 90% of patient-derived cells survived the bioprinting process. Alizarin Red S staining shows a high degree of mineralization after 6 weeks of culture and micro-CT data revealed an average mineral volume of 53.6 ± 4.3 mm³ in our 3D bioprinted organoids. The cells resided in cavities in the mineralized bone-like tissue, resembling the morphology of osteocytes embedded in lacunae. Osteocalcin expression in 3D bioprinted organoids was similar to this of bone explants. Furthermore, upregulation of the phosphate regulating endopeptidase homolog X-linked gene (PHEX) showed that our 3D bioprinted bone organoids are able to support cell differentiation towards the osteocyte lineage.

DISCUSSION & CONCLUSIONS:These results are a proof of concept for 3D bioprinted human bone organoids using patient-derived cells supporting co-existence and bioactivity of both osteoblastic and osteocytic cells. The established 3D bioprinted bone organoid will be an especially powerful tool to elucidate pathomechanisms in rare human bone diseases such as osteogenesis imperfecta and facilitate the development and testing of advanced therapies on a patient-specific level.

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Keywords: Biofabrication, 3D printing and bioprinting

An *Ex Vivo* Human Osteochondral Explant Culture System for Osteoarthritis Treatment Evaluation

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INTRODUCTION:Currently, *in vivo* animal models are used to evaluate and optimize regenerative or tissue engineered cartilage implants. However, this is time consuming, expensive, and not well controlled. Furthermore, translation of their results to humans is questionable. The overall objective therefore is to develop an *ex vivo* human-tissue based osteochondral explant culture model that could reduce and refine animal studies. The present study aims to demonstrate that human osteochondral explants at various stages of osteoarthritis (OA) can be kept in culture while preserving its viability and composition.

METHODS:Osteochondral explants (Ø10 mm, bone length ±4 mm) with either a smooth or fibrillated cartilage surface, representing different OA stages, were harvested from fresh human tibia plateaus obtained from total knee replacement surgeries (Máxima Medical Centre, Eindhoven) under IRB approval. Explants were cultured for two or four weeks in a double-chamber culture platform in which bone and cartilage compartments are separated and can be supplemented with tissue-specific medium components[1]. Fresh and cultured explants were evaluated for their cartilage and bone metabolic activity as well as cartilage cell viability, biochemical content and proteoglycan/collagen distribution. Data were statistically analyzed using a Kruskal-Wallis test (statistical significance at $p < 0.05$) and a Dunn's multiple comparison post-hoc test.

RESULTS:Biochemical content and proteoglycan/collagen distribution in the cartilage of explants did not significantly change in culture over four weeks. Chondrocyte viability was preserved in both the core and periphery of smooth cartilage explants. In fibrillated cartilage explants however, viability decreased in the periphery of the sample in cultured compared to fresh explants (fresh: 94±6%, cultured: two weeks 64±17%, four weeks 69±17%, $p < 0.05$). Metabolically active bone and marrow was observed in the periphery of all explants, while activity was decreased in the bone core in cultured compared to fresh explants.

DISCUSSION & CONCLUSIONS:This study shows an *ex vivo* human osteochondral culture system with the potential to be developed into a treatment evaluation system. Although biochemical and histological results did not show changes within the cartilage tissue, viability of the explants needs to be carefully controlled for each specific use. In fibrillated cartilage osteochondral explants, the chondrocytes in the periphery are more sensitive, and these explants might not be ideal to culture as a defect model.

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Keywords: Cartilage / joint and arthritic conditions, Disease models

Human induced pluripotent stem cell (hiPSC)-derived endothelial cells for vascularization of liver organoids

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INTRODUCTION:Organoids are miniaturized and simplified versions of an organ produced in-vitro by multicellular 3D self-organization showing realistic micro-anatomy and part of functions. The techniques for growing organoids have rapidly improved since the early 2010s, yet in-vitro) mimicking the complexity of a real organ is far from trivial. Vascularization of organoids may contribute to the acquisition of a more in-vivo-like phenotype and functionality thanks to the role endothelial cells (ECs) play in diverse physiological processes, particularly during organogenesis. Due to the great heterogeneity of the endothelium, the source of ECs used to this purpose remains debatable. METHODS:Our work focuses on the generation of EC differentiated from hiPSCs (iECs) to vascularize a liver bud in-vitro. A homogeneous population of CD144+CD31+CD34+vWF+ iECs was obtained after 10 days of differentiation. These cells upregulated ICAM upon addition TNF-α, endocytosed acetylated-LDL, expressed eNOS and formed tubular networks when cultured on a matrigel layer. To generate vascularized liver buds, iECs were further cocultured with hepatoblasts (iHBs) and mesenchymal cells (iMSCs) derived all from the same hiPSC line. Obtained organoids were maintained up to 30 days in coculture.

RESULTS:Cells showed the ability to self-assemble and rearrange after 24h as demonstrated by formation of spheroids exhibiting an initial average diameter of 150 µm that increased to 300-500 µm at the end of the culture. Moreover, 20% of the cells still expressed the Ki67 after 30 days, confirming maintenance of viability and proliferation capacity while no sign of apoptosis or necrosis was detected. Interestingly, each spheroid showed a reproducible distribution of the three cell populations and an EC network was observed within the organoids as highlighted by CD144 and CD31 expression. RTqPCR revealed the expression of liver sinusoidal (LSEC) markers such as FVIII, Stab2 and Lyve1 independently of the presence or absence of exogeneous VEGF. Immunofluorescence and immuno-histochemistry analyses confirmed the iHB differentiation into hepatocytes (iHeps) and cholangiocytes: the expression of specific markers such as BSEP, ALB and CK7 was detected during the organoid culture time, while still proliferating iHBs expressed CK19 and AFP. iHep maturation was confirmed by detection of albumin secretion.

DISCUSSION & CONCLUSIONS:Our preliminary results suggest that our iECs have the potential to express LSEC markers even in the absence of exogeneous VEGF. Further in-vivo experiments will be carried out to study their ability to vascularize liver organoids through anastomosis to the host vasculature network.

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Keywords: Vascular systems / vascularisation and heart, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Recapitulating the monocyte extravasation process to the osteoarthritic synovial membrane in a joint-on-a-chip model

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INTRODUCTION:Osteoarthritis (OA) is a whole joint disease in which inflammation plays a pivotal role [1]. OA synovium is characterized by an accumulation of macrophages originating from extravasated monocytes and contributing to joint damage. Advanced 3D organotypic models represent powerful tools to study complex biological mechanisms in in vivo-like microenvironments. Here, we developed and validated a microfluidic system that recapitulates monocyte extravasation in an OA-like microenvironment by modelling joint tissues in inflammatory conditions.

METHODS:The microfluidic model included synovium and cartilage compartments modeled by human OA synovial fibroblasts and chondrocytes embedded in fibrin hydrogels. A synovial fluid channel separated these compartments. Two synovial compartments lined an endothelialized channel mimicking the post-capillary venule. To optimize the model, the effect of fluid flow and pro-inflammatory stimulation (Tumor Necrosis Factor-α, TNF-α) on the expression of adhesion molecules (Intercellular Adhesion Molecule-1, ICAM-1; Vascular Cell Adhesion Molecule-1, VCAM-1) regulating the interactions between endothelial cells and monocytes was tested. The model was validated monitoring monocyte extravasation in response to a mix of chemokines. Finally, the model was used to quantify monocyte extravasation in response to synovial fluid from OA patients in comparison to a control group. Data were analyzed by t-Test or Two-Way Anova depending on the experimental set-up.

RESULTS:We obtained an organotypic model of OA joint by co-culturing human OA synovial fibroblasts and chondrocytes, which included a perfusable endothelialized channel and OA synovial fluid. We showed that ICAM-1 expression was enhanced by the flow applied on endothelial cells, and even more by the combination of flow and TNF-α. Differently, VCAM-1 was downregulated in response to fluid flow, but the stimulation with TNF-α rescued VCAM-1 expression. The validation experiments proved that monocytes extravasated only in the presence of chemokines and that this process was enhanced when the endothelium was pre-activated by TNF-α and flow at 30 µL/h ($p<0.001$). Additionally, OA synovial fluid induced monocyte extravasation ($p<0.01$) and, accordingly with the validation experiments, endothelial pre-activation was needed for the process to occur.

DISCUSSION & CONCLUSIONS:We developed a microfluidic organotypic model including patient-derived cells and synovial fluid to investigate monocyte extravasation in an OA-like microenvironment. We provided the first direct evidence that OA synovial fluid induces monocyte extravasation acting as a chemoattractant. This platform will be used to test therapeutic agents to counteract monocyte extravasation.
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Keywords: In vitro microenvironments, Immunity / immunomodulation / macrophage

A novel ex-vivo model for studies of tissue repair

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INTRODUCTION:Current strategies used for treating lung diseases are merely symptom-relieving and do not actively repair injured tissue. Common degenerative lung diseases such as chronic obstructive pulmonary disease (COPD) arise from inadequate tissue repair processes. COPD alone accounts for 3 million deaths per year and is projected to be the third highest cause of deaths worldwide by 2030[1]. A promising biological approach to repair lung damage is by manipulating the signals (proteins) controlling lung generation during development[2]. Wnt5a is a known factor that stimulates the non-canonical Wnt-Planar Cell Polarity pathway, critical for both lung development and repair of adult lung tissue[3].

METHODS:A spatially restricted area of mouse precision-cut lung slices (PCLS) was injured using hydrochloric acid (HCl). Optimal concentrations of HCl required to provide either mild or severe injury were determined using viability assays. PCLS were treated with pro-repair molecules for 24 and 48 hr post-injury. Tissue repair was assessed using validated markers, Ki67 and pro-surfactant protein C (proSP-C), that respectively identify proliferating cells and alveolar type II/progenitor cells. Cells were semi-automatically quantified in injured and uninjured regions of PCLS.

RESULTS:High cell viability was maintained in uninjured regions of acid treated PCLS for 48 hr, compared to control (uninjured) PCLS. Greater changes in the percentage of Ki67 and proSP-C positive cells were present at 48 hr post-injury compared to the 24 hr time point. The proportion of proSP-C positive cells in the acid-injured regions increased from 6.3 % to 15.0 % at 24 hr and from 8.0 % to 22.8 % at 48 hr and proSP-C percentage increased further upon treatment with Wnt5a. Interestingly, the percentage of Ki67 positive cells significantly increased in the uninjured region from 8.6 % to 12.8 % at 48 hr but there was no such increase at 24 hr.

DISCUSSION & CONCLUSIONS:Ki67 and proSP-C provide robust readouts of repair in mouse PCLS at 48 hr post-injury. ProSP-C, a marker of endogenous progenitor cells, increased significantly upon injury. The increase in Ki67 in uninjured regions distant from the injured area of PCLS suggests cell proliferation in tissue surrounding the injury stimulated by paracrine factors may be a mechanism of tissue repair. We have characterised a novel ex-vivo model to study lung injury and repair, which may become a new tool for respiratory research and regenerative medicine.

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Keywords: Disease models, Wound healing

Biological assessment of gut environment in vitro models for an innovative multi-organ-on-a chip platform to model the microbiota-gut-brain axis communication pathway

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INTRODUCTION:The gut-brain axis is based on a complex multi-organ interaction that involves: (1) the microbiota population; (2) the gut-associated lymphoid tissue (GALT) mainly characterized by the gut epithelium-immune system interaction; (3) the brain. The comprehension of the microbiota-gut-brain biochemical mechanisms represents a challenging aspect in the neuropathological research [1]. It is known that gut microbiota influences neurological function and cell population viability by releasing neurotoxin molecules, such as LPS [2]. In this work, we developed and preliminary assessed two in vitro cell-based models aimed at representing the GALT components to be used in MINERVA, a project funded by the European Research Council (ERC), aimed at developing an innovative multi organ-on-a-chip-based platform to investigate microbiota-gut-brain connection in neurodegenerative disorders [3].

METHODS:We modeled (1) the intestinal epithelium using the Caco2 cell line and (2) the immune system by co-culturing U937 and MV4-11 cell lines. To reach the optimal epithelium integrity, we cultured cells in transwell plates for 21 days, measured cell viability and analyzed tight-junctions formation by immunocytochemistry and western blotting. Furthermore, we assessed the epithelium permeability to FITC-dextran. For the immune system cell based model, we assessed cell viability and cytokines production. To validate both the in vitro models and to assess cell response once exposed to the detrimental effect, we incubated E.coli LPS at increasing concentration (from 0.5 to 10 µg/ml) and repeated the previous analyses measuring cell viability, epithelial barrier integrity and cytokines production.

RESULTS:The intestinal epithelium model showed a strong tight-junctions network that is compromised under LPS treatment in a dose-dependent manner. The FITC-dextran permeability assay confirmed the lower barrier integrity under toxic conditions. Similarly, the immune system model showed LPS toxicity in a dose-dependent manner, evident by reduced cell viability and increasing cytokines production.

DISCUSSION & CONCLUSIONS:In conclusion, both the in vitro models resulted responsive to detrimental treatments and suitable for mimicking the gut permeability and immune response to microbiota-derived bioactive molecules. Therefore, they appeared appropriate to be exploited in the MINERVA engineered multi-organ platform. Within the MINERVA, they could be cultured in single organ-on-chips or integrated in a unique multi-organ-on-a-chip in which the intestinal barrier and the immune system directly interact to better mimic the GALT environment.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Immunity / immunomodulation / macrophage

A Vascular Microphysiological System Model of Early Atherosclerosis

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INTRODUCTION:The initiation and progression of atherosclerosis is often studied using animal models, which suffer from a number of limitations including (a) important differences in disease progression compared to humans, (b) expense and long follow-up times for disease progression, and (c) difficulty of isolating factors involved in such a complex disease. Human microphysiological systems (MPS) can address many of these pitfalls and examine the effect of genetic variants in disease development. In this study, we developed and validated a human vascular MPS model of early stages of atherosclerosis and show that disease progression can be inhibited with statins or by blocking P2Y↓11.
METHODS:All primary human cell isolations were performed using a protocol approved by the Duke University Institutional Review Board. The vascular MPS consisted of vessels containing dermal fibroblasts and/or vascular smooth muscle cells embedded in a dense collagen gel and primary human endothelial colony forming cells. Plastic compression was used to increase the mechanical strength. Vessels were perfused for one week before treatment with modified low-density lipoprotein (LDL) and monocytes

RESULTS:To model early atherosclerosis, Vessels were exposed to a shear stress of 0.27 Pa for one week and then perfused with media containing 50 μg/mL enzyme-modified LDL (eLDL) with or without TNF-α. Perfusion with either eLDL for 96 h or TNF-α for 8 h reduced vasoactivity and promoted expression of leukocyte adhesion molecules on ECs. Adding TNF-α and eLDL together further elevated leukocyte adhesion molecule levels but did not have an additive effect upon vasoactivity. Perfusion of vessels with eLDL or TNF-α increased monocyte adhesion, transmigration, and foam cell formation and the combination of both had a synergistic effect. Pro-inflammatory responses and inhibition of vasoactivity were blocked by lovastatin or NF157, a P2Y11 inhibitor that blocks pro-inflammatory effects of oxidized LDL (1).

DISCUSSION & CONCLUSIONS:These results are consistent with clinical findings of early atherosclerosis development and showed that inflammation can be removed after removal of pro-inflammatory stimuli. Monocyte accumulation and foam cell formation can occur at shear stresses between 0.1-0.3 Pa, values consistent with those obtained in animal models (2). A novel finding is that by blocking P2Y11, vasoactivity is maintained, which may reflect the relative metabolism of nucleotides at the endothelial surface.

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Keywords: Vascular systems / vascularisation and heart, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Biohybrid lung development – Gas exchange membrane endothelialization by endothelial cells from induced pluripotent stem cells

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INTRODUCTION:The development of a biohybrid lung (BHL) may provide an alternative therapy option for patients with end-stage lung disease, replacing curative lung transplantation. The BHL working principle is based on extracorporeal membrane oxygenator (ECMO) technology, which enables respiratory support by gas exchange through artificial membranes. The major disadvantage of contemporary ECMO systems, thrombus formation with insufficient gas exchange capacity, will be solved by establishment of an endothelial cell (EC) monolayer on all blood contacting surfaces. Since the generation of sufficient EC numbers from autologous sources is problematic, alternative EC sources must be considered. In addition to immune-tolerable ECs, ECs from induced pluripotent stem cells (iPSC) may be used. Here, we assessed and compared the eligibility of ECs from different iPSC-clones to endothelialize the gas exchange membrane (GEM).

METHODS:ECs were generated from three different human iPSC lines (Clone1-3), following a scalable differentiation protocol and seeded onto fibronectin coated polymethylpentene GEM. Following the phenotypical characterization via immunofluorescence staining, ECs were analyzed for the expression of pro-thombogenic and pro-inflammatory markers with or without the presence of TNFα, using FACS and qRT-PCR. Leukocyte- and platelet adhesion assays were carried out to assess the haemocompatibility of the endothelialized GEM. A scratch assay was conducted in a parallel plate flow chamber to assess monolayer regeneration under dynamic conditions (30dyne/cm², 24h). ECs sourced from umbilical cord blood (ECFCs) were tested as control.

RESULTS:iPSC-derived ECs formed confluent monolayers on the membrane. Stimulation with TNFα strongly induced the EC-adhesiveness for thrombocytes and leukocytes for ECs derived from two iPSC-clones, while ECs derived from the third clone did not respond. This result was confirmed by qRT-PCR and FACS analyses, which showed strong upregulation of E-Selectin and Tissue Factor expression upon stimulation with TNFα for two of the three iPSC-EC lines. Also, faster proliferation was noted for the non-responsive clone. The tested EC monolayers were capable to withstand flow exposure for 24h and showed regenerative behavior in scratched areas.

DISCUSSION & CONCLUSIONS:iPSCs offer the possibility to provide patient-specific ECs in abundant numbers needed to cover blood contacting surfaces of the biohybrid lung. It must be taken into account that different clones can differ in terms of their proliferation, proinflammatory response and function. However, this can be tested in vitro prior to clinical application. Nevertheless, reduced or delayed activation of the iPSC-ECs may be advantageous, as the monolayer may be invisible for pro-thrombogenic and pro-inflammatory activations and reactions.

Keywords: Interfaces - biological, Advanced therapy medicinal products

Towards a Biohybrid Lung: Cell Aerosolization for Efficient Endothelialization of Gas Exchange Membranes

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INTRODUCTION:Extracorporeal membrane oxygenation is limited to several days or a few weeks due to multiple complications. To improve hemocompatibility, efforts are taken to seed endothelial cells on gas exchange membranes. To achieve a confluent cell layer, current seeding methods require large volumes of highly concentrated cell solutions.
Cell aerosolization is an innovative application process used in regenerative medicine. Recently, research interest in this technology has grown, because cells can be applied to substrates with different topographies with a high efficiency and little or no damage. Here we evaluated the influence of aerosolization on endothelial cells for membrane seeding.

METHODS:Human umbilical cord vein endothelial cells (HUVECs) up to passage 4 were used. For aerosolization, a Vivostat® system was used. Pipetted cells served as control. The general suitability was evaluated with varying flow velocities, substrate distances and cell concentrations of 0.5-5x10^6 cells/mL. Cells were analyzed for survival, proliferation, apoptosis and necrosis levels. In addition, aerosolized and pipetted cells were cultured either static or under flow conditions in a microfluidic bioreactor for three days. Evaluation included immunocytochemistry and gene expression via RT-qPCR.

RESULTS:Cell survival of aerosolized cells was higher than 90 % for all tested parameters. A slight decrease in survival was only seen for low nozzle-substrate distances. Cell concentration did not influence survival. HUVECs show typical cobblestone morphology after aerosolization and no changes in proliferation were seen for up to 5 days. In addition, no increase of apoptosis and necrosis levels was seen 24 hours after aerosolization. For dynamic culture, cells quickly formed a confluent layer and withstood wall shear stresses of 5 dyn/cm² already after 2 hours. Immunocytochemistry revealed typical expression of CD31 and von-Willebrand-factor as well as partial cell alignment in flow direction. Via RT-qPCR, cell behavior of aerosolized and control cells was evaluated with a focus on mRNA expression levels of shear dependent and inflammatory marker genes.

DISCUSSION & CONCLUSIONS:High shear and elongation stresses can affect cells during aerosolization, and could thereby influence their survival and behavior after processing. With this study, we have proven the feasibility of endothelial cell aerosolization with no significant changes in cell behavior. The amount of cells needed for a confluent coating can be reduced compared to conventional seeding techniques. Thus, this technique can be used for efficient endothelialization of gas exchange membranes for biohybrid lung applications.
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Keywords: 3D printing and bioprinting, Interfaces – biological

Electrosprayed chitin nanofibrils/electrospun poly-hydroxyalkanoate fibers as bio-based functional mesh

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INTRODUCTION:Chitin is a polysaccharide derived from natural resources with renewability and antimicrobial, anti-cicatrizing and anti-inflammatory properties that have great potential for biomedical and coating applications [1]. Polyhydroxyalkanoates (PHAs) are a group of bio-based polyesters produced in nature and have found biomedical and packaging applications. We aim to develop chitin nanofibril (CN) coated poly(hydroxybutyrate-co-hydroxyvalerate)/olive leaf extract (PHBHV/OLE) fibers and evaluate their metabolic activity and antibacterial properties for different applications. Sprayed CN coatings, PHBHV/OLE fibers and CN-coated PHBHV/OLE fibers with desirable size and morphology were successfully obtained using the electrospinning technique. The materials were tested with different bacteria to assess antimicrobial effects.

METHODS:Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) (Sigma-Aldrich) was dissolved in a dichloromethane/methanol (10:1 w/w) mixture at 15 w/w%. OLE (16.8 w %) was added to the solution to produce electrospun fibers at optimal process conditions (Linari s.r.l, Pisa, Italy). CNs (Texol, Pescara, Italy) were dissolved in aqueous acetic acid at 50% (v/v) at 0.52 w/w% and the solution was electrosprayed. Size and morphology of particles and fibers were investigated using scanning electron microscopy. Human Caucasian Foreskin Fetal Fibroblasts (HFFF2) was used to test fibrous mesh compatibility (alamarBlue assay) and microbial growth inhibition test and overlay diffusion assay were used to test antimicrobial activity of fibrous mesh against E. coli, S. aureus and L. innocua bacteria using.

RESULTS:CNs with an average size of 180 nm±0.047 were obtained. The TP content in Tuscan OLE varied in 14.99-27.83 mg Gallic Acid Equivalent (GAE)/g. Oleuropein (14.69 ± 0.92 mg/g of OLE) was the main component. Homogeneous fibers composition with improved hydrophilicity due to the presence of -OH groups attributable to OLE were obtained. The presence of OLE inside the fiber did not significantly change the fiber diameter while led to the formation of a few beads in the PHBHV/OLE mesh. PHBHV/OLE fibrous mesh showed desirable metabolic activity (56.81% ±0.80%). CN-coated PHBHV/OLE presented an inhibition effect towards E. coli and S. aureus. Differently, L. innocua was less affected by the fibrous mesh.
DISCUSSION & CONCLUSIONS:PHBHV/OLE fibrous meshes possessed surface, and texture properties suitable for HFFF2 growth. CN-coated PHBHV/OLE showed antimicrobial activity. The presence of CNs improved antibacterial activity and is also involved inmunomodulation [1]. This natural and eco-sustainable mesh is promising in wound healing and coating applications.

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Keywords: Polymers - natural / synthetic / responsive, Wound healing

Antibacterial and antifouling silicone modified polyurethanes for urinary tract reconstruction

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INTRODUCTION:Pathological disorders of urinary tract are fairly prevalent and estimated to affect 400 million people worldwide. Such conditions necessitate medical intervention, wherein the diseased tissue is conventionally replaced by an intestinal autograft to correct the functionally deficient physiology. However, the usage of intestinal mucosa is invariably fraught with many short and long term complications related to absorption of the waste products in urine.
This creates a constant demand for alternate structural and functional substitutes of native tissues including ureters, bladder and urethra. Realizing the challenge, we have designed a unique set of silicone based antibacterial polyurethane blends and assessed their potential for the application of permanent urological implants.

METHODS:We have harnessed the highly amenable yet easily scalable methodology of reactive co-extrusion for material fabrication. Briefly, silicone (PDMS) is allowed to crosslink within the molten polyurethane (TPU) matrix. To our knowledge, this methodology of dynamic vulcanization has not been attempted for TPU/PDMS in past. The final binary blends can withstand large deformations and multiaxial stresses and are also endowed with antifouling behavior. Further, they are covalently modified with quaternized ammonium, pyridinium and phosphonium compounds to inhibit of urinary tract infections.

RESULTS:The successful incorporation of silicone phase is confirmed through the torque profile, electron microscopy and contact angle goniometry. Nearly 18° increase in contact angle, from 88.6° in neat TPU to 105.1° in TPU/PDMS: 80/20, is observed. A wide of range of structural parameters, including an ultimate tensile strength of 3.4 - 24.1 MPa, failure strain of 637% - 1020% and elastic modulus of 4.8 - 9.1 MPa could be encompassed for the designed library of blends. As high as three-fold log reduction in bacterial growth is achieved in artificial urine infected with three recurrent uropathogens (P. mirabilis, E.coli and S. aureus). The preliminary evaluation of cytocompatibility has been also conducted using mouse fibroblast cells and reveals no toxic response with respect to medical-grade control.

DISCUSSION & CONCLUSIONS:The present work is aimed at designing a polymeric biomaterial with long-term sustainability in urinary environment. Therefore, the bio-stable, polycation functionalized silicone/polyurethane blends have been accordingly tailored to possess superior mechanical and surface properties. The collective performance from uniaxial, biaxial, dynamic tensile testing; infected artificial urine study and "in vitro" cytocompatibility assessment establishes their prospect for reconstructive urology.

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Keywords: Biomaterials, Infection

Bio Fabrication to Clinical Translation – Precise Bio Cornea

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INTRODUCTION:Bioengineering has made impressive strides in recent years, yet the commercialization of bio-fabricated tissues and organs is still a great challenge. Precise Bio has taken up the challenge and established an industry-oriented workflow for taking academic products into the market, composed of experienced biology, physics and chemistry teams working under regulatory compliant labs, with strong engineering support.

METHODS:To achieve commercial biofabrication, we developed a high-end laser-based bioprinting method, allowing the deposition of pico-liter sized drops composed of single cells within highly viscous bioinks, achieving high cell printing densities. This continuous laser-induced-forward-transfer printing (co-LIFT) is capable of printing several bioinks simultaneously at high rates, controlled drop-size and while maintaining high (over 95%) cells viability. The co-LIFT bioprinter is incorporated into a designated 3D platform, which can be customized to combine additional bio-fabrication technologies. We present here the Precise Bio development of a first bio-fabricating technology for tissue transplanted, currently in preclinical study. We show results for bio- fabricated corneal grafts, but other ophthalmologic tissues are in development. This bio-fabrication platform can be readily adapted to other tissues and organs.
Corneal endothelial cells dysfunction leads to swelling of the collagen fibrils in the corneal stroma, leading to edema and blindness. This can be reversed using corneal transplantation. Nevertheless, there is a worldwide shortage of high-quality corneal tissue, currently obtained exclusively from organ donors.

RESULTS:To meet this shortage, Precise-Bio developed an engineered corneal tissue substitute to replace damaged endothelial layers. Human donor corneal endothelial cells are expanded under GMP compliant conditions and later processed onto a bio-fabricated ultra-thin collagen scaffold, which has similar mechanical and chemical properties as the natural membrane. These corneal implants contain high cell density and display efficient endothelial biological activity. Importantly, one donor may produce hundreds of corneal tissue constructs for keratoplasty transplantation, thus potentially alleviating the corneal shortage. Preclinical animal trials demonstrate that Precise-Bio bioengineered corneal implants can be delivered into NZ rabbits' corneas, where they adhere and reduce corneal swelling over the period of the experiment. Towards commercialization, each corneal batch is tested for cell density, identity, purity, potency and sterility.

DISCUSSION & CONCLUSIONS:Precise-Bio demonstrates here the ability to produce functional corneal endothelial implants for the clinical procedure of human keratoplasty in a quality-controlled process. Our platform can be readily extended to bio-fabricate other potential tissues and organs with the potential for upscaling towards industry-oriented commercialization.

Keywords: 3D printing and bioprinting, Eye

Treatment of a 95% total body surface burns patient with a Novel Polyurethane-based Composite Cultured Skin

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INTRODUCTION:The biodegradable temporising matrix (BTM) is our standard of care for patients with >30% TBSA deep burns. A 95% TBSA burn presented in December 2018. After total eschar excision on arrival, BTM was applied to 85% of the body two days post injury. With no meaningful donor site, TGA Special Access Scheme B was obtained with approval to use Composite Cultured Skin (CCS). We have been developing this technology, alongside the BTM, since 2004 and now report on its first use in an adult human burn patient.

METHODS:Twenty six CCSs were grown in an ISO 7 cleanroom environment in a semi-automated bioreactor. Two small split thickness skin grafts were obtained to provide the fibroblasts and keratinocytes, which were isolated and mass cultured in Cell Factories to obtain relevant cell numbers. The matrix (1mm thick polyurethane foam) was pre-soaked in plasma. In the bespoke bioreactor cassette (25cm x 25cm), fibroblasts were seeded onto the matrix with thrombin until the keratinocytes were seeded. The total co-culture period was 14 days. The CCSs were applied after BTM delamination and dermabrasion, and dressed with Mepitel and Acticoat. Punch biopsies, transepidermal water loss readings and Vivascope confocal imaging were performed to indicate wound healing and skin barrier function.

RESULTS:Clinically, robust epithelium was visible with CCS 'take' by at least day 18 post-application. Punch biopsies taken at day 12/14 confirmed integrating CCS with epidermal formation and the presence of superficial CCS polyurethane and BTM in the deeper subcutaneous section. Evaporative water loss readings also indicate closure and the formation of a barrier like normal skin with readings at Day 33 post application of 14.9g/m²h compared with normal skin of 10.8g/m²h. Early CCS batch take was irregular and additional CCSs produced. Some widely meshed split skin, and Meek, grafting were used to cover difficult areas, yielding poorer cosmetic results.

DISCUSSION & CONCLUSIONS:At 1 year post burn, this 95% TBSA deep burn patient has survived, is healed and has now left rehabilitation. Although further CCS refinement and optimisation of the clinical care regime is required, this approach has the potential to provide an alternative treatment for care of life-threatening burns.

Keywords: Wound healing, Biomaterials

Hacking the skin's tolerance to mechanical load

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INTRODUCTION:Skin is vulnerable to injury when exposed to pathological mechanical loads, leading to blisters, skin tears and pressure ulcers[1]. Load tolerance varies by anatomical location, with plantar skin (on the foot sole) particularly load tolerant [2]. Here, we ask what makes plantar skin load tolerant, and can we use this knowledge to re-engineer non-plantar skin for enhanced load-tolerance?

METHODS:We quantified differences in the composition of plantar and non-plantar human skin (NHSREC 17/W/0161) using immunofluorescence of structural proteins [3]. We measured how each skin type deforms under load and measured material properties at the sub-micron scale using Atomic Force Spectroscopy. We separated out the effects of plantar morphology and composition on load tolerance using computational models of load-bearing skin. Finally, we re-programmed keratinocytes from non-plantar skin to become more plantar-like using in vitro co-culture with plantar fibroblasts.

RESULTS:We found that morphology and composition of plantar skin play distinct and complementary roles in protecting it from injury. More specifically, plantar morphology protects against skin tears and blisters, while plantar composition protects against pressure ulcers. We were able to induce keratin-9 expression (usually only present in plantar skin) in non-plantar keratinocytes and found that these re-programmed cells could tolerate higher mechanical loads than non-plantar keratinocytes.

DISCUSSION & CONCLUSIONS:The load tolerance of skin depends on both its morphology and composition. In particular, plantar skin morphology protects against tears and blisters, while composition protects against pressure ulcers. Skin load tolerance is malleable, demonstrating the potential for preventative therapies that augment native skin's load tolerance. For regenerative therapies, targeting both morphology and composition will lead to more robust engineered skin.

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Keywords: Skin, Biomechanics / biophysical stimuli and mechanotransduction

Bioprinting Skin Models Using Reactive Jet Impingement

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INTRODUCTION:In Vitro skin models have become a popular alternative to the use of animal models for the evaluation of industrial products. However, their implementation at industrial level has been hindered by their low reproducibility and long manufacturing time. In the last years, bioprinting has emerged as a new approach to the automated production of skin equivalents, but the complexity and manufacturing times of these models have barely improved.
To overcome these limitations, we present a novel bioprinting system named Reactive Jet Impingement(ReJI). This system is based on the jetting of two liquid bio-ink precursors from opposing cartridges, which meet and react in mid-air forming a stable gel. In comparison with other technologies, ReJI allows the printing of high cell densities without exposing the cells to shear stresses.
Here, we will report how the printing of high fibroblast densities directly affects the formation of the dermal layer, which could help to reduce their manufacturing time.

METHODS:Two inks based on collagen-alginate-fibrinogen and thrombin-CaCl2 with suspended neonatal Normal-Human Dermal Fibroblasts (neo-NHDF) at 500,000cells/mL and 5,000,000cells/mL were printed using the ReJI system. Proliferation of the printed neo-NHDF was assessed by quantifying double stranded-DNA content of each printed after 1, 3 and 7 days of printing, whereas their morphology and distribution was determined by Immunostaining. Dermal protein expression and their reorganization over time was evaluated by WesternBlot and Scanning Electron Microscopy.

RESULTS:Fully cross-linked dermal models were manufactured at a rate of 124seconds/gel, improving throughput when compared to other bioprinting processes. The possibility of printing high cell densities was also confirmed by the comparable number of live cells and proliferation rates between both printed densities.
Fibroblasts in gels printed with higher cell density presented a faster adaptation to the gels, showing a spindle-shaped morphology after only 1 day. In addition, degradation of these gels and production of new extracellular matrix could be observed on day 3 (5,000,000cells/mL) instead of 7 days (500,000cells/mL), which could potentially speed up the skin model production.

DISCUSSION & CONCLUSIONS:These results highlight the role of the initial cell density on the tissue maturation and the production of extracellular matrix. ReJI system has proven to be a suitable bioprinting technique for the fast manufacturing of highly dense dermal models, which could reduce the time taken to produce human skin models.

Acknowledgements:This project is funded by the EPSRC through the Centre of Doctoral Training in Additive Manufacturing and 3D Printing(EP/L01534X/1), CRODA International plc. and Newcastle University.

Keywords: 3D printing and bioprinting, In vitro microenvironments

Tunable substrate functionalities guide electric-field mediated neural differentiation of mesenchymal stem cell (hMSCs) on PVDF nanocomposites

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INTRODUCTION:Among the various biophysical and biochemical cues to direct stem cell differentiation, the critical role of external electric field (EF) has been established in the recent past. In this context, the role of the electroactive property of the substrate becomes important in evaluating EF mediated stem cell differentiation. In order to address this, the present study demonstrates the effect of electroactive properties in PVDF-nanocomposites to direct stem cell differentiation using external EF-stimulation.

METHODS:PVDF nanocomposites were prepared by melt mixing of 2wt% MWNT and 20wt% Barium-titanate(BT) using twin-screw extruder at 220°C and 60rpm, which are subsequently rolled at 80% strain. The fraction of electroactive phase (β-phase) was quantified using FT-IR and XRD. The dielectric measurements were performed using Impedance Analyzer. The morphology of nanofillers in PVDF was analysed using electron-microscopy (SEM and TEM). The EF-stimulation to hMSCs on functional substrates were performed using a DC source (1 V/cm and 2 V/cm) for 10min in every 24hr, after 3rd day of culture. The effect of EF on cell viability was performed using WST-1 assay. The expression of neural markers was studied using immunocytochemistry and gene expression analysis by RT-PCR. The electrophysiology of differentiated hMScs were studied using patch-clamp recordings and Ca^{2+} oscillations.

RESULTS:The electroactive β-phase in PVDF was tuned from ~27% to ~94% and the conductivity from ~10^{-11} S/cm to ~10^{-3} S/cm. Also, dielectric constant of PVDF-nanocomposites was varied from ~10 to ~400. hMSCs cultured on PVDF-nanocomposites having MWNTs exhibited filopodial extensions like neurite outgrowths. The neurite length on rolled-PVDF/MWNT was higher compared to unrolled-PVDF/MWNT. A greater number of neurite outgrowths with more branch points on non-elongated hMSCs were observed on rolled-PVDF/BT/MWNT. The genotypical analysis using immunocytochemistry and RT-PCR of neural markers at day 7 and day 14 confirmed the early differentiation of hMSCs towards neuronal pathway on rolled-PVDF/MWNT and late differentiation towards glial pathway on rolled-PVDF/BT/MWNT. The stem cell committed to neuronal lineage has shown Ca^{2+} oscillation with KCl depolarisation. The glial like cells have shown negligible response to KCl depolarisation but prolonged Ca^{2+} oscillation with mechanical stimulation.

DISCUSSION & CONCLUSIONS:The present study reports, the strategy to tune EF mediated differentiation of human-mesenchymal-stem-cells (hMSCs) towards neuronal and glial pathways, using tailored functional properties of the substrate. The EF stimulation of hMSCs on substrate with higher conductivity directed neuronal differentiation and low conductivity with high dielectric property of the substrate supported glial differentiation of hMSCs without any biochemical growth factors in vitro.

Keywords: Multipotent (mesenchymal) stem cells, Differentiation

Successful isolation and culture of multipotential distal airway stem cells from COPD patients

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INTRODUCTION:Chronic Obstructive Pulmonary Disease (COPD) presents a significant, incurable, worldwide health burden, identifying it as a prospective regenerative medicine target. Sourcing epithelial cells for lung therapies has proved challenging due to issues of cell senescence and dedifferentiation, although progress has been made in culturing airway basal cells, information on the culture of more distal lung cells remains scarce.

METHODS:Distal lung tissue was obtained from human donors with and without COPD was minced and digested overnight at 4°C in 1mg/mL protease XIV, 0.005% trypsin, and 10ng/mL DNAse I in F12:DMEM (1 : 1). The resulting suspension was passed through sterile gauze and a 70 µm cell strainer before pelleting by centrifugation. Cells were plated on type I collagen-coated tissue culture flasks in either cFAD medium with rock inhibitor supplementation (cFAD+)[1] for epithelial cell isolation, or DMEM (10% FBS) for fibroblast isolation. Cells were characterised by immunocytochemistry for vimentin, smooth muscle actin (SMA), pan-cytokeratin, TP63, cytokeratin-5, E-cadherin, Club cell secretory protein (CCSP), and β IV tubulin. Cells were expanded then differentiated using air-liquid interface culture with measurement of trans-epithelial electrical resistance (TEER), in a matrigel organoid culture system and seeded to porous collagen scaffolds.

RESULTS:Culture in DMEM resulted in fibroblastic colonies strongly positive for vimentin and weakly positive for SMA, cFAD+ resulted in predominantly epithelial cells expressing pan-cytokeratin and E-cadherin. Further, the epithelial cells expressed TP63 and cytokeratin-5 suggesting a distal airway stem cell (DASC) identity. At air-liquid interface cells differentiated, demonstrating the capacity to develop tight junctions with increased TEER (≥350 Ω.cm2), had increased levels of the club cell marker CCSP, stained positively for β IV tubulin and had visible, motile cilia. Culture in matrigel produced self-organising, mucus producing organoids, with visible motile cilia on the surface in addition to thin-walled, cavitated, alveolar-like organoids. Both fibroblasts and DASCs attached readily to collagen scaffolds, had excellent viability and exhibited proliferation on the scaffolds over a 14 day period.

DISCUSSION & CONCLUSIONS:We have successfully isolated and culture-expanded fibroblasts and epithelial progenitors from the distal lung tissue of COPD and healthy human donors. Preliminary work demonstrates the multipotential differentiation capacity of DASCs in a variety of formats making them a valuable source for a range of regenerative medicine therapeutic approaches.

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Keywords: Disease models, Differentiation

Medical Device Principal Investigator Perspectives on Impact: Focus, Barriers and Approaches

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INTRODUCTION:There is a burgeoning empirical focus on the role of principal investigators (PIs) in public science. PIs are central actors in the creation, translation and eventual impact of medical device research from bench to beside. However, little is known about how these important actors understand and conceptualise impact from their perspective. The focus of this paper is to address this gap in knowledge through the exploration of PI impact orientation, the awareness, attitudes and approaches of PIs in relation to the generation and prioritisation of research impacts.

METHODS:Taking a micro level focus, this study employed a qualitative approach to explore the perspectives of publicly funded medical device PIs. Set in an Irish context of the Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), a total of 38 PIs were interviewed in-depth, and the resulting data was analysed using a systematic thematic analysis approach.

RESULTS:We found that PIs in this study presented a wide variety of perspectives in relation to the intended impacts of their research, with the main emphasis placed on the more established scientific and economic metrics associated with impact (e.g. citations, patenting), and a surprisingly strong human capital impact focus. With respect to broader research impacts, PIs struggled or where unable to identify specific impact and beneficiaries, particularly PIs at the more basic end of the translation continuum. PIs proffered barriers to impact, including complexities, misunderstandings and negative opinions of impact and the impact 'agenda'. To enhance the impact potential of their research projects, PIs used a variety of approaches including collaboration strategies, project formation strategies, and career planning and long-term strategising.

DISCUSSION & CONCLUSIONS:Findings of this study have highlighted the divergent understandings of impact as perceived by PIs, as well as some factors and barriers to enhancing PI impact orientation in the medical device research field, particularly for more basic-oriented medical device PIs. Based on the findings of this study, an ecological framework for PI impact orientation has been developed, utilising Urie Bronfenbrenner's bioecological theory of development, which can support PIs in addressing, mapping and analysing the translational potential and intended impacts of their medical device research across environmental systems. A number of recommendations and future research directions were also identified.

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Keywords: Translation and commercialisation (inc. clinical trials and regulatory approval),

Characterisation of mesenchymal stem/stromal cells in clinical trials: critical analysis and implications for their use in regenerative medicine

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INTRODUCTION:Advanced therapy medicinal products (ATMPs), unlike other biological medicines, may be produced outside of the standard medicines manufacturing paradigm. This possibility, and ubiquity of mesenchymal stem/stromal cells (MSCs), may encourage unlicensed clinics offering unapproved treatments. Literature reporting MSC clinical trials is thus critical in conveying the extent of reliable research; therefore data quality should withstand scrutiny.
This study evaluates cell characterisation in clinical trial publications and discusses implications for use of MSCs in regenerative medicine, with the aim of defining appropriate reporting standards.

METHODS:A literature search identified 1986 potentially relevant primary research articles based on title and abstract content. Application of stringent pre-defined inclusion/exclusion criteria yielded a relevant dataset.

RESULTS:84 papers published between 2010–2019 met the criteria. Trials spanned 28 countries, involving autologous (51%) and allogeneic (49%) MSCs from five tissue sources and 44 indications. 63% were Phase 1/IIa, 35% Phase II and 2% Phase III trials. 47.6% of studies reported bulk values for expression of typical MSC surface markers (CD73, CD90, CD105) and absence of haematopoietic markers (CD34, CD45). An additional 16 markers were referenced in <20% of studies. 11 (13.1%) studies included individual values per cell lot. 33 studies (39.3%) omitted characterisation data and 39 (46%) omitted cell viability. Functional or potency assessment of the cells was limited to osteogenesis and adipogenesis assays (26% of papers) and chondrogenesis (19%). Five papers (6%) mentioned specific functional assays, of which only two appeared biologically relevant. 23% omitted discussion of mechanism of action. Compliance with International Stem and Gene Therapy Society minimal identification criteria for MSC was claimed, but not demonstrated, in 13 (15%) papers.

DISCUSSION & CONCLUSIONS:This analysis revealed a surprising lack of characterisation. Most papers omitted basic identity of the study drug. This is problematic: key attributes of MSCs cannot be conveyed by a single term such as “mesenchymal stem cell” due to well-documented difficulties in defining cell type. Less than half of the studies included some assessment of cellular identity, purity and viability. Characterisation may have been performed and not published; likely given such data are necessary to obtain a clinical trial authorisation. However, without accepted objective requirements for clinical MSCs, trial publications should include at least basic information on the cell population being administered to provide evidence that cells are “MSCs”. Poor definition of drug substance (phenotypic identity) also raises questions of impurity profile (safety) and relevant biological activity (efficacy).

Keywords: Advanced therapy medicinal products, Multipotent (mesenchymal) stem cells

A new microfluidic model that allows monitoring of complex vascular structures and cell interactions in a 3D biological matrix

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INTRODUCTION:Microfluidic devices offer an ideal solution to improve traditional cell culture models and to bridge the gap between in vitro and in vivo research. This technique aims to resemble the biological microenvironment and is used to mimic complex human tissues, including the vasculature. However, the focus of most vascular microfluidic devices is limited to interaction between endothelial cells (ECs) and the surrounding cell and environment. Here we present a novel microfluidic model that allows EC interaction with supporting pericytes and extracellular matrix (ECM), in a 3D vessel structure subjected to hemodynamic flow in a polydimethylsiloxane (PDMS) chip, enhancing the complexity to mimic natural conditions.

METHODS:We designed a PDMS device with a reservoir for a 3D fibrinogen gel containing supporting pericytes. Open channels were created to enable ECs to form a functional monolayer in the gel. Flow was introduced via a pump system while the dimensions of the design facilitated clear 3D confocal imaging.

RESULTS:In our system, ECs interact with pericytes and create a human cell derived blood vessel that maintains an open and perfusable lumen. Dextran diffusion verified the successful establishment of endothelial barrier function while demonstrating the beneficial role of supporting pericytes. Furthermore, the artificial blood vessel reacts on biological stimuli such as thrombin validating the natural barrier response. The blood vessel in this microfluidic device allowed circulation of monocyte. Immune cells and the vasculature shows a natural interaction with immune cells in response to TNFα induced inflammation.

DISCUSSION & CONCLUSIONS:In this study we validated a novel vasculature microfluidic device that can be easily produced and live-imaged. This design enables the co-culture of multiple cell types in a 3D ECM environment while being perfused. Our current model provides a unique tool to conduct in vitro analysis of the human microvasculature during inflammation. The flexibility of this model allows researchers to study specific cell-cell and cell-ECM interactions using different stimuli immune cells to mimic (disease) environments.

Keywords: In vitro microenvironments, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Drivers for angiogenesis in the arteriovenous in vivo bioreactor

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INTRODUCTION:Reproducing vascularised and viable tissue following injury or disease remains an important goal in the field of tissue engineering. The surgical creation of an arteriovenous (AV) loop within an engineered scaffold has been shown to spontaneously generate vascularized tissue. This vascular conduit directs highly pressured arterial blood into a thinner walled vein resulting in hydrostatic gradients that increase angiogenesis, vascular tissue formation with an intricate microcirculation. Understanding the mechanisms that underlie this phenomenon may result in directing growth to specific tissue or organ end-points and may be applicable to the field of reconstructive surgery and tissue transplantation.

METHODS:The study involved creation of AV loops in mouse and rat limbs. These AVLs were placed within a number of custom-designed 3D printed in fibrin gel filled porous chambers and harvested at numerous time points over 28 days. Comparisons were made to no AVL controls and arteriovenous flow through models. Chambers were weighed and analysed for proliferation (BrdU), collagen synthesis (Hsp 47), Laminin, and vascular smooth muscle (a-SMA) with immunohistochemistry in mice. Patency was assessed by FITC Dextran perfusion. Inflammatory cell recruitment was also assessed using flow cytometry. Proteomic assessment of the generated matrix was performed on the rat tissue.

RESULTS:We show that the AVL increases angiogenesis and the volume of tissue generated in the mouse and rat. The AVL encourages a wave of proliferating tissue to develop in the provisional matrix along with sprouting angiogenesis and persistent macrophage recruitment. Proteomic analysis suggests the presence of the AV loop up-regulates the RXR pathways, a key enhancer of angiogenesis through specific macrophage phenotypes.

DISCUSSION & CONCLUSIONS:The mouse AV shunt model provides us with a genetic tractability system to interrogate the mechanisms by which new tissue forms from the shunting phenomenon. Validation studies can be performed in larger models that generate more tissue for analysis using the rat. A specific proangiogenic phenotype of macrophage is highly prevalent in the formation of tissue in the AVL. Identifying these novel mechanisms will allow us to encourage angiogenesis through surgical means in engineered tissues.

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Keywords: Vascular systems / vascularisation and heart, In vivo and animal models

Combination of neural crest derived stem cells (NCSCs) and microvascular fragments (MVF) from adipose tissue: Are pre-vascularized neurospheres a better chance for the treatment of Hirschprung´s disease?

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INTRODUCTION:Transplantation of neural crest derived stem cells (NCSCs) from the enteric nervous system (ENS) as a cell therapy of dysganglionosis is one of the major goals for the treatment of Hirschsprung´s disease patients. Such ENS spheroids could be transplanted into the affected area of the gut and lead to a re-establishment of the missing neuronal network through differentiation. However, due to the diffusion limitation of oxygen, particularly large spheroids usually undergo apoptosis and would therefor fail the application. To address this problem, we used adipose tissue-derived microvascular fragments (MVF) to generate pre-vascularized neurospheres for the first time.

METHODS:Enteric single cells were isolated from the plexus myentericus of postnatal C57BL/6 mice and combined with MVF isolated from epididymal adipose tissue of C57BL/6 donor mice. In a 96-well plate, both these components were mixed in various ratios to generate ENS neurospheres with or without MVF as biologically intact blood vessel fragments. The formation of the spheres was first characterized in vitro by means of scanning electron microscopy (SEM), histology and immunohistochemistry. Furthermore, the dorsal skinfolds chamber model was used to analyze the in vivo vascularization of implanted spheroids by means of intravital fluorescence microscopy over an observation period of 2 weeks.

RESULTS:We could demonstrate that a successful incorporation of MVF did not affect proliferation, apoptosis or cellular composition of spheroids. However, SEM revealed a differing topography between spheroid of ENS cells only and those combined with MVF. Lastly we showed that the included MVF led to an extremely rapid vascularization of implanted neurospheres in vivo.

DISCUSSION & CONCLUSIONS:By incorporating MVF into ENS neurospheres we were able to generate pre-vascularized spheroids that were able to rapidly established a fully formed vascular system within them. This could overcome the issue of the diffusion limit of transplanted neurospheres and offer an attractive solution for further clinical applications.

Keywords: Nervous system (brain-central-peripheral / disorders), Disease models

Excitation-contraction coupling in vascularised myocardium-on-a-chip

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INTRODUCTION:Within the heart, beating cardiomyocytes (CM) share their local microenvironment with endothelial cells (EC), fibroblasts (FB), and other non-myocytes. A critical determinant of healthy myocardial function is efficient bi-directional communication between CM and neighbouring cells, and disruption of these interactions leads to pathogenesis. However, detailed study of the relationship between CM and cardiac non-myoctes has been hampered by a lack of in vitro models which recapitulate the cellular and architectural composition of adult tissue. For example, EC in vivo are polarised and rely on luminal exposure to circulatory shear stress to dynamically regulate their phenotype, including interaction with extravascular cells such as CM. Therefore, in order to gain a therapeutically useful understanding of cardiac cellular communication, there is a need to develop in vitro models which replicate physiology.

METHODS:To recapitulate myocardial-endothelial association, we have developed microfluidic chips which subject 3D hydrogel-based preparations to precisely controlled fluid flow. Within these chips, we have co-cultured human induced pluripotent stem cell-derived cardiomyocytes, human cardiac microvascular endothelial cells, and human left ventricular fibroblasts for 5 days under a pro-vasculogenic protocol (0.5 ul/min flow rate, supplementation with VEGF and Ang-1). To evaluate the role of vasculogenesis on CM function, we have incorporated CM differentiated from a genetically modified stem cell line which expresses the calcium (Ca2+) biosensor GCAMP6F. As the mediator of cardiac excitation-contraction coupling, the cellular phenomenon whereby an electrical impulse is converted into mechanical motion, Ca2+ mediates acts as a useful surrogate for myocardial function.

RESULTS:Via live and fixed fluorescence microscopy, we have observed spontaneous endothelial vasculogenesis in microfluidic chips, resulting in a perfusable microvascular network with a continuously open lumen embedded within beating iPSC-derived myocardium. We have observed the presence of a tight endothelium via retention of perfused rat erythrocytes and 40kDa FITC-Dextran. We then assessed calcium transients of iPSC-CM in co-culture with EC in both control and vasculogenic conditions, and found that mycoytes in microfluidic chips display significantly abbreviated Ca2+ handling kinetics (time to peak reduced by 27.5%, p=0.004, time to 50% decay reduced by 18%, p=0.001).

DISCUSSION & CONCLUSIONS:This work describes a novel, physiologically relevant in vitro model of the human myocardium via generation of perfusable microvasculature within a beating preparation. Ca2+ handling, a fundamental determinent of cardiac output, was significantly altered in vasculogenic conditions, indicating the importance of microenvironmental factors. This work therefore emphasises the need for biomimetic in vitro platforms to provide theraputically relevant insights into cardiac

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Vascular systems / vascularisation and heart

Prevascularization of pseudo islets as a novel strategy to improve islet transplantation

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INTRODUCTION:The transplantation of pancreatic islets is a promising therapeutic approach for the increasing number of diabetic patients worldwide. However, insufficient engraftment of transplanted islets is still a major problem. In this study, we introduce a novel strategy to improve the critical islet revascularization process by incorporating microvascular fragments (MVF) into pseudo-islets (PI).

METHODS:Primary pancreatic islet cells and MVF were harvested from C57BL/6 mice for the experiments. Islets were dissociated into single cells and cocultured with MVF by means of the liquid overlay technique for 5 days to generate prevascularized PI (PI+MVF). In vitro, we analyzed the morphology, viability, cellular composition, endocrine and angiogenic function of PI+MVF by scanning electron microscopy, immunohistochemistry, flow cytometry, enzyme linked immunosorbent assay as well as sprouting assay. Freshly isolated islets (FI), cultivated islets (CI) and PI served as controls. In vivo, we assessed the revascularization and endocrine function of the grafts by means of the mouse dorsal skinfold chamber model in combination with intravital fluorescence microscopy and the kidney capsule transplantation model in diabetic mice.

RESULTS:Stable and compact PI+MVF were generated within 5 days. These exhibited a roundly shaped, smooth surface pattern and contained a significantly higher number of endothelial cells when compared to controls. The incorporation of MVF did not influence the cellular viability and insulin secretion of PI+MVF while mitochondrial reactive oxygen species levels were found to be decreased. In addition, PI+MVF contained a significantly higher number of proliferative endothelial cells. Moreover, only PI+MVF exhibited a sprouting capacity when compared to controls. In vivo, we detected a massively accelerated vascularization of PI+MVF as indicated by an elevated functional capillary density when compared to FI, CI and PI. Furthermore, in diabetic mice the critical mass of 250 transplanted FI did not decrease hyperglycemia during the entire observation period of 28 days, whereas 250 PI+MVF restored normoglycemia within 4 days.

DISCUSSION & CONCLUSIONS:In the present study, we successfully incorporated MVF into PI, which ameliorated the revascularization process and significantly accelerated the restoration of normoglycemia. The herein described prevascularization concept markedly reduced the required number of transplanted islets in diabetic mice and, thus, has great potential to improve the success rates of clinical islet transplantation.

Keywords: Biofabrication, Vascular systems / vascularisation and heart

Characterising equine embryonic stem cell derived tenocytes to determine their use as a cell therapy

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INTRODUCTION:Tendon injuries occur commonly in human and equine athletes. Adult tendons undergo poor natural regeneration, resulting in scar-tissue which is prone to re-injury. Fetal tendons however are capable of completely scar-less regeneration, a property which is intrinsic to the fetal cells themselves. Novel cell therapies should therefore try to recapitulate this scar-less fetal tendon regeneration. This project builds on our previous research into the use of horse embryonic stem cells (ESCs) to aid tendon regeneration. Here we determine if tendon cells derived from ESCs are more similar to fetal or adult tendon cells and define the different target genes of scleraxis (SCX), an essential gene in tendon formation, at the different stages of tendon development.

METHODS:Using genome wide transcriptional analysis, we compared fetal, adult and ESC-derived tenocytes cultured and differentiated in a 3D culture system. We then determine the effects of knocking down the expression of SCX on gene expression in adult, fetal and ESC-derived tenocytes using RNA-sequencing and qPCR. ChIP-qPCR was then used to determine the differentially expressed genes that are directly regulated by SCX.

RESULTS:Of the 21,689 mapped genes, 542 genes were differentially expressed (DE) between the adult and fetal tenocytes, 2,940 genes were DE between the adult and ESC-derived tenocytes and 2,708 genes were DE between the fetal and the ESC-derived tenocytes (log fold change ±2; q-value <0.01). Genes which were significantly upregulated in adult tenocytes were expressed at similar levels in fetal and ESC-tenocytes and were mainly associated with immune system processes. Genes which were significantly upregulated in fetal tenocytes tended to be even further upregulated in ESC-derived tenocytes and were primarily associated with cell migration. SCX knock-down (KD) in adult and fetal cells had differential effects on gene expression. Over 200 genes were DE in fetal SCX KD tenocytes compared to the control, whereas less than 100 genes were DE in the adult case. ChIP-qPCR also revealed that SCX has differential binding partners in these two cell types. Preliminary results suggest that SCX KD in ESCs resulted in upregulation of cartilage markers.

DISCUSSION & CONCLUSIONS:In summary, our work towards has indicated that ESC-derived tenocytes are more similar to fetal than adult tenocytes, however all three cell types present as unique populations in which SCX has different regulatory roles.

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Keywords: Embryonic stem cells, Cell therapy

Fetal secretome – a novel therapeutic strategy for cartilage regeneration

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INTRODUCTION:Repair of injured adult cartilage tissue leads to formation of fibrocartilage with inferior biomechanical properties resulting in continuous cartilage degeneration and chronic disease. Fetal tissue, in contrast, has the inherent capacity to regenerate without fibrocartilage formation. Recapitulation of developmental processes is therefore discussed as a potential key to tissue regeneration.
Cell therapies based on mesenchymal stem cells (MSCs) have lead to promising results in enhancing cartilage regeneration. The efficacy of MSCs therapies has been shown to be largely due to their paracrine activity. As the paracrine activity and efficacy are dependent on the cells' origin, differentiation status and particularly donor age, the secretome of fetal cells constitutes a promising treatment avenue.
The aim of the presented study was therefore to test the effect of fetal secretomes on inflamed chondrocytes in vitro.

METHODS:A comprehensive in vitro experiment was carried out employing gene expression analysis (sheep specific whole genome microarrays), protein secretion analysis (label free bottom-up shot gun proteomics by high resolution orbitrap mass spectrometry), proliferation assays, wound healing assays (scratch assay) and beta-Galactosidase assays for cell senescence to characterize the effect of fetal secretomes (obtained from fetal chondrocytes and fetal MSCs) on adult injured chondrocytes (inflamed with 10ng IL1beta and TNFalpha per ml of medium). Secretomes of three fetal donors (for chondrocytes and MSCs) where pooled and tested on inflamed adult chondrocytes obtained from three different individuals.
We further compared the secretome composition and the response of the treated chondrocytes to key factors of fetal regeneration identified in previous studies. In these previous studies standardized cartilage lesions were surgically induced in adult (aged 2-4 years) and fetal sheep (80 days gestation). Time points for sampling were chosen according to the three stages of healing (inflammatory phase, reparative phase, remodelling phase). Samples were compared for protein secretion and on the histologic level with special emphasis on extracellular matrix composition, collagen organization and collagen types, qualitative and quantitative composition of growth factors and cytokines, inflammatory response, chemotaxis, cell proliferation, MMPs and migration as well as senescence.

RESULTS:Tremendous differences between adult and fetal regeneration were observed with regard to inflammation-related processes and extracellular matrix remodelling.
DISCUSSION & CONCLUSIONS:Comprehensive understanding of the underlying processes of fetal cartilage regeneration and the exploitation of the fetal regenerative potantial by applying a fetal secretome based therapy may lead to the development of novel therapeutic strategies inducing regeneration rather than fibrocartilage formation.

Keywords: Cartilage / joint and arthritic conditions, Biologics and growth factors

The culture and characterization of canine pancreatic organoids for sustainable treatment of Type I Diabetes Mellitus in dogs

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INTRODUCTION:Type 2 diabetes mellitus (T2D) is a frequently observed diseases in cats. Type 1 Diabetes (T1D) occurs in around 1-2% of pet dogs in the Western world. There seems not to be a specific breed preference, in contract to so many other diseases in dogs. The burden for the dogs and for their owners often leads to euthanasia of, apart from T1D, healthy dogs. Since long term daily blood glucose level measurements and insulin injections are not practically feasible, and les labor-intensive, less stressful and more sustainable solution to regulate blood glucose levels is of utmost importance.

METHODS:Based on the knowledge acquired during the culture of canine, feline, porcine and bovine liver and intestinal organoids, we developed a medium that allowed rapid proliferation of 3D pancreas organoids in Matrigel (1-6). Frozen pancreatic ductal tissues of surplus cadaver material (2 F and 1 M beagle dog, aged 1 year) was thaw after 7-8 moth liquid nitrogen storage and cultured in advanced DMEM expansion medium supplemented with various growth factors including amongst others R-Spondin, Wnt3a, NAC, Noggin. Gene-expression measurements are currently used to indicate the stem cell characteristics and differentiation potential of these organoids under expansion and differentiation culture conditions. Most of the primer pairs to measure gene expression of markers for various differentiation states of the cells are up-and-running.

RESULTS:Doubling times were around 2 days as measured with the Alamar Blue assay. Size of the hollow organoids varied from 200-400 µm. The balloon morphology and rapid proliferation suggest that the organoids consist of pancreas duct derived stem cells. As of early January results from the gene expression measurements were not available.

DISCUSSION & CONCLUSIONS:In order to replace damaged beta-cells to restore glucose-induced insulin release several steps need to be taken: First, create sufficient pancreatic stem cell mass, second, differentiate pancreatic stem cells into glucose responsive insulin releasing beta-cells. Third, create a method to have these cells sustainably connected to the dogs blood stream. The first step is taken. Almost a century of bantings discovery of canine insulin, the sustainable treatment of T1D in dogs seems one very small step closer to reality.

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Keywords: Stem cells - general, Diabetic healing

An allogenic 3D scaffold-free tissue engineered product for deep thickness skin regeneration: in vitro development to in vivo proof of concept

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INTRODUCTION:Deep thickness skin wound remains a major challenge for reconstructive surgery. A novel approach of tissue engineering, based on an allogeneic adipose-derived 3D scaffold-free technology, was proposed.

METHODS:Adipose-derived stromal cells (ASCs) were isolated from human adipose tissue to constitute the 3D-scaffold free graft by the production of the extracellular matrix (ECM, n=9). The ultrastructure of the graft was assessed by microtomography/SEM. The protein and growth factors contents were determined by proteomic analysis (LC-MS/MS) and ELISA, respectively. The in vivo biocompatibility (inflammatory reaction, biodegradation) was assessed in nude and Wistar rats up to 4 weeks (n=20) as well as the safety in terms of tumorigenicity/toxicity/biodistribution. The efficacy was then evaluated in a xenogenic (human to rat) model of ischemic (vs. non-ischemic) wound in hyperglycemic Wistar rats (n=42, 3D grafts vs. sham/Ctrl+).

RESULTS:The 3D-graft is a translucid and malleable membrane with a mean of 175±86 cells/mm² found to be embed in the ECM with a low level of mineralization (0.30±0.31%v/v). The proteomic and genes analysis revealed the stimulation of the biological pathways involved in early wound healing and the over-expression of pro-angiogenic genes (ANG, ANGPT1, EPHB4, VEGFA, VEGFB, VEGFC, EDN1, THBS1, PTGS1, LEP) in the graft (in comparison to ASCs alone), respectively. The VEGF and SDF1a contents (181±12 and 663±27 ng/g, respectively) were also improved in the scaffold-free implant. The biocompatibility and the safety of the 3D-graft were confirmed at 4 and up to 24 weeks post-implantation, respectively. The 3D-graft was easily handled and applied by a simple bandage on the ischemic/hyperglycemic wounds (on the leg) and promoted an earlier irreversible wound closure (27 vs. 34 days for sham, respectively) associated with angiogenesis, dermis/epidermis reconstruction, transient and reversible increase of aSMA, lymphocytes/macrophages recruitment at 10-15 days.

DISCUSSION & CONCLUSIONS:The scaffold-free approach with allogenic 3D-graft (derived from ASCs) demonstrated the safety and efficacy in stringent xenogenic model of hyperglycemic and ischemic deep-thickness wound.

Keywords: Cell therapy, Wound healing

Sustained Release of Oxygen to Accelerate Diabetic Wound Healing

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INTRODUCTION:Diabetes affects more than 9% of the population in the US alone. One in four people with diabetes develop severe chronic wounds that are slow to heal. Limited supply of oxygen and nutrients resulted from vasculitis and microcirculation dysfunction represents one of the major causes of compromised wound healing. Therefore, oxygenation of the wounds may accelerate wound regeneration. Hyperbaric oxygen therapy (HBOT) is a clinically available treatment. It delivers pure oxygen at a pressure of 2-3 atmospheres to the wounds [1]. However, 20-30 repeated treatments are required. In addition, it has potential to generate reactive oxygen species (ROS) in healthy tissues. Herein, we have developed an oxygen release system capable of long-term releasing oxygen to diabetic wounds.

METHODS:Oxygen release microspheres (ORMs) were fabricated by double emulsion using polyvinylpyrrolidone (PVP)/H2O2 complex as core and a biodegradable polymer as shell. The ORMs were conjugated with catalase for converting released H2O2 into molecular oxygen. A thermosensitive and ROS-capturing poly(N-Isopropylacrylamide)-based hydrogel was developed to deliver ORMs in the wound site.

RESULTS:The ORMs were capable of releasing oxygen for 4 weeks. Keratinocytes, fibroblasts and arterial endothelial cells were used for in vitro studies. dsDNA assay demonstrated that ORMs improved survival of skin cells under ischemic conditions. The released oxygen also stimulated endothelial lumen formation. In addition, it promoted the secretion of pro-angiogenic growth factors (IGF-1 and HGF), and suppressed the expression of inflammatory cytokines (TNFα and IL-1β) from keratinocytes and fibroblasts. The oxygen release system was delivered into db/db mice with 5-mm diameter of incisional wounds. Complete wound closure was observed at day 16, significantly faster than the non-treatment group. Immunohistochemical staining demonstrated that the oxygen release group exhibited greater density of blood vessels.

DISCUSSION & CONCLUSIONS:A novel oxygen release system to accelerate diabetic wound healing was developed. Both in vitro and in vivo studies showed that the released oxygen enhanced skin cell survival, promoted angiogenesis, inhibited inflammation, and stimulated wound closure.

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Keywords: Drug delivery, Hydrogels and injectable systems

Dense Collagen / PLGA Composite Hydrogels as Medicated Wound Dressings for the Treatment of Cutaneous Chronic Wounds: In Vitro and In Vivo Evaluation

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INTRODUCTION:Cutaneous chronic wounds are characterized by the absence of healing after six weeks. The classic treatment is the debridement of the wound bed followed by a compression method. When the treatment is not efficient enough, the application of wound dressings is required. To date, no dressings are appropriated to treat the different kinds and stages of wounds (1). Nowadays, research orientation is towards medicated wound dressings incorporating therapeutic molecules within biomaterials in order to favor skin repair or to prevent infection (2). In this study, dense collagen/PLGA composite hydrogels have been developed to deliver dexamethasone or spironolactone in a controlled manner to modulate inflammation in the wound bed.

METHODS:To evaluate composite hydrogels as a novel medicated wound dressing, hydrogel stability, mechanical properties, drug loading and release kinetic have been analyzed. Then, the in vivo performance of composites was evaluated in a pig model over 10 days. Dense fibrillar collagen hydrogels concentrated at 40 mg/mL were incubated in a PLGA solution (7 KDa) containing dexamethasone or spironolactone for 24 hours. Then, the mixtures were rinsed in PBS to closely associate the hydrophobic polymer with the collagen network.

RESULTS:This procedure permitted to obtain composites with high mechanical properties and an improved resistance against in vitro digestion by collagenase. The elastic modulus measured in composites was two times higher than that measured in pure collagen hydrogels. The composite hydrogels swelled up to 10 times their dried weight and recovered their original shape. The ultrastructural analysis by transmission electronic microscopy revealed the presence of PLGA domains scattered within the network of collagen fibrils. Compared to pure collagen hydrogels, the drug loading in composites was 5 times higher and the release rate was quasi constant over the first two weeks. Unlike pure collagen hydrogels, no burst release was detected. Cell viability experiments showed the absence of cytotoxic effect of composites hydrogels on fibroblasts and keratinocytes. Subsequently, the effect of composite hydrogels was evaluated in vivo in a model of impaired wound healing in pig. Spironolactone loaded composite hydrogels improved wound closure by 50% and permitted a complete re-epithelialization after 6 days.

DISCUSSION & CONCLUSIONS:Taken together, these results show that dense collagen/PLGA composite hydrogels are promising medicated wound dressings for the treatment of chronic wounds as they deliver constant doses of drugs favoring skin repair.
Acknowledgements:We thank ANR to have found this project.
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Keywords: Drug delivery, Composite materials

Amniotic Fluid Induces Regenerative Gene Expression Networks in Keratinocytes and Fibroblasts

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INTRODUCTION:Human cutaneous healing is dominated by an inflammatory response and subsequent tissue scarring. In early fetal wounds, healing is instead characterized by regeneration. The extracellular environment guides cellular behavior by causing measurable perturbations of gene expression networks. The aim of the present study was to evaluate the effect of amniotic fluid (AF) on gene expression in keratinocytes and fibroblasts in vitro.

METHODS:Keratinocytes and fibroblasts were isolated from skin obtained from healthy patients undergoing routine plastic surgery (Ethical Review no. 2018/97-31). Cells were subjected to twelve hours serum-free starvation and thereafter exposed to in Dulbecco's modified Eagle's medium with either 50% AF (pooled from approximately 100 women, Ethical Review no. 03-342) or fetal calf serum (FCS). Cells were analysed for global gene expression changes after 24h, using the Affymetrix HG-U133 Plus 2.0 array. Expression data was analyzed using WebGestalt for Gene Set Enrichment Analysis (GSEA), known pathways and transcription factor targets. Results were also cross-referenced using EnrichNet.

RESULTS:AF treatment resulted in 2,234 and 280 differentially regulated genes in keratinocytes and fibroblasts, respectively – and 1,327 and 1,965 genes after FCS treatment. The keratinocyte profile showed a shift towards differentiation and an increase in gene groups related to ectodermal-epithelial regulatory networks. For keratinocytes, both AF (671) and FCS (402) treatment caused up-regulated genes (266 in common), many of which relevant for keratinocyte differentiation and epidermal development. AF treatment up-regulated additional genes relevant for epidermal development, but also other transcription factors (TF) and TF protein-protein interactive networks (FOXA2, FOXM1, SOX2, EGR1, and SIX5, FLI1, PPARG, GATA4, GRHL3 respectively) compared to the expected epidermal gene regulation observed in FCS (canonical Notch, AP-1 and Wnt-signaling, SMAD2,3, and NFkB-p65). For the fibroblasts cultured in AF, many of the gene expression changes were related to metabolic state and changes of extracellular matrix components as well as up-regulation of pregnancy-related genes. For both cell types, genes active during stressor stimuli, such as immunological activation, were downregulated following exposure to AF.

DISCUSSION & CONCLUSIONS:The obtained results indicate that treatment with AF may trigger a differentiation program that includes higher degree of regeneration compared to proliferative FCS stimulus. These findings can serve as a source for further exploration of potential therapeutics with wound healing applications.

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Keywords: Skin, Biologics and growth factors