Free paper session 5: Engineerd tissues and organs II

KL5.1

Bioprinted engineered tissues for translational applications

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Advances in tissue engineering and regenerative medicine have led to the development of many clinical therapies. However challenges still exist in developing complex tissue systems. One challenge that has delayed rapid clinical translation is the lack of effective delivery methods for cells and biomaterials to build complex tissue constructs. Living tissues maintain inherent multi-cellular heterogeneous structures and rebuilding of such complex tissue structures requires subtle arrangements of different cell types and extracellular matrices at their specific anatomical target sites.
3D bioprinting has emerged as an innovative tool that enables rapid construction of complex 3D tissue structures with precision. This developing field promises to revolutionize the field of medicine addressing the dire need for tissues and organs suitable for surgical reconstruction. In this session novel and versatile approaches to building tissue structures using 3D printing technology will be discussed. Clinical perspectives unique to 3D printed structures will also be discussed.

F5.1

Prevention of apoptosis in epithelial-cell-spheroids.

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Three dimensional (3D) culture systems like forming multicellular spheroids are thought recapitulating in vivo-like environments. However inside of multicellular spheroids often shows apoptotic cell death when we use epithelial cell. This is not a result of restriction of gas and nutrients because apoptosis is detected in spheroids having diameters less than 200 µm. Previously we reported a method to form network-like microchannels in multicellular spheroids using micro hydrogel beads [1]. The inner architectures of such spheroids with microchannels seem to those in the liver lobule. The objective of this study is to prevent apoptotic cell death in multicellular spheroids by forming adequate microenvironments for epithelial cells. We used mouse E14.5 fetal hepatocytes as an epithelial cell. To form microstructures we injected 1 ul fresh medium containing 1000 fetal hepatocytes and same number of alginate hydrogel beads (20 um in diameter) into the 3% methylcellulose (MC) medium. Spheroids with and without structures were cultured with 10-7 M dexamethasone and 10 ng/ml oncostatin M to induce hepatic differentiation. After 3 and 7 days spheroids were removed from the MC medium fixed and embedded to paraffin blocks to make sections. Staining with active form of Caspase 3 (17 kDa protein) antibody revealed that apoptotic cell death in conventional spheroids was started at day 3. According to hematoxylin-eosin staining massive cell death was detected in conventional spheroids at day 3 and day 7. On the other hand there was lesser amount of apoptotic cells in spheroids with microstructures. CK8/18 and E-cadherin expression showed that it was possible to culture the spheroids even after 7 days. We also confirmed a cellular function of spheroids with channels by albumin secretion activity. The prevention of apoptosis thought to be depending on the formation of mono to bi-layered cell structures in the spheroids. This 3D culture method would be adaptable for various epithelial cells.

[1] Kojima N. et al. Fabrication of microchannel networks in multicellular spheroids. Sensor. Actuat. B-Chem. 198 249-254 (2014)

F5.2

Bioprinting of human pluripotent stem cells and their directed differentiation for the generation of 3D liver-like micro-tissues

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Introduction: In the drug development process only around 16% of the drugs that begin preclinical testing are approved for human use. Some of this low success rate can be attributed to the different responses between animals and humans to the drugs being tested. A possible solution to this might be the creation of human micro-tissues derived from pluripotent stem cell sources for drug testing instead of current animal models. This would result in scalable faster and potentially more reliable drug testing platform and hopefully an end to animal testing. Here we report the first investigation into the bioprinting of human induced pluripotent stem cells (hiPSCs) their response to a valve-based printing process as well as their post-printing differentiation into hepatocyte-like cells (HLCs) within a bioprinted 3D hydrogel structure.
Methods: Post-printing cellular viability and pluripotency was evaluated using fluorescence-activated cell sorting (FACS). Multiple human pluripotent stem cell lines and pluripotency markers were tested. HLCs differentiated from both hiPSCs and human embryonic stem cells (hESCs) sources were bioprinted and examined for the presence of hepatic markers using immunofluorescence labelling. HLCs were then printed into a 3D hydrogel matrix and the resulting cell-laden hydrogel structures were used to verify that the differentiation proceeded normally in 3D.
Results and Discussion: Examined cells were positive for nuclear factor 4 alpha and were demonstrated to secrete Albumin and have morphology that was similar to that of hepatocytes. Both hESC and hiPSC lines were tested for post-printing viability and pluripotency and were found to have negligible difference between the printed and non-printed cells. 3D printed alginate hydrogel structures containing hESC-derived HLCs were tested for viability and Albumin secretion during the differentiation protocol and were found to be hepatic in nature. 3D alginate structures with 40 printed layers containing HLCs reached peak Albumin secretion at Day 21 of the differentiation protocol.
Conclusions: This work demonstrates that the valve-based printing process is gentle enough to print human pluripotent stem cells (both hESCs and hiPSCs) while either maintaining their pluripotency or directing their differentiation into specific lineages. The ability to bioprint human pluripotent stem cells will pave the way for producing organs or tissues on-demand from patient specific cells which could be used for animal-free drug development and personalised medicine.

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F5.3

Design and fabrication of a biomimetic oriented scaffold with multi-branch network for myocardial tissue engineering

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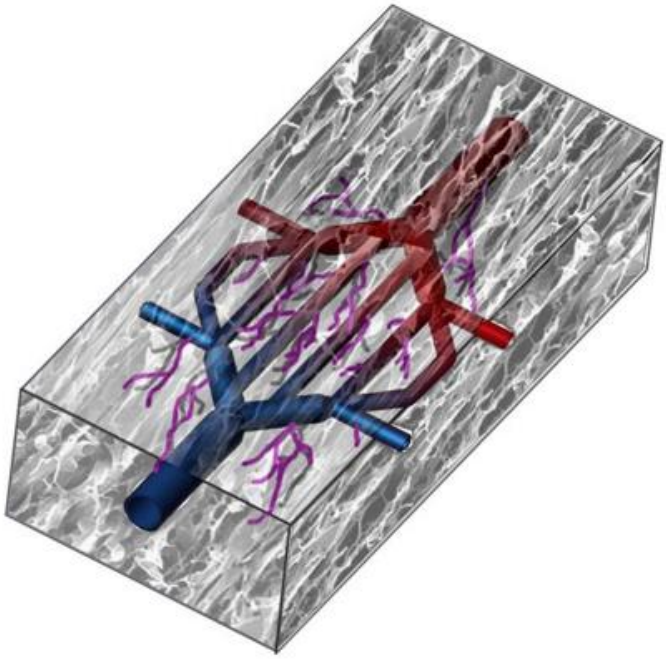
Microenvironmental suitablibility is essential for engineering a functional cardiac tissues. In an attempt to biomimic native cardiac extracellular matrices we developed am oriented porous myocardial scaffold with multi-branch network for cardiac tissue engineering and regenerative medicine. A novel multihydrogel materials and a multi-hierarchy porous structure were designed for scaffold in order to biomimic the morphological and structural characteristics of the cardiac extracellular matrix (ECM). The oriented porous scaffold was fabricated by unidirectional thermal induced phase separation technology. The branched vascular network structure was fabricated by 3D printing as a sacrificial element using carbohydrate due to its biocompatibility and good mechanical property. Then the network structure in engineered myocardium constructs was dissolved to generate vascular networks. To achieve an optimal scaffold design the mathematical model of oriented scaffold with vascular network was established and computational fluid dynamic analysis was performed. The experimental evaluation of the designed scaffolds was performed with seeded cardiomyocytes compared to simulation results. These results indicated that the biomimetic scaffold with multi-branch network could promote viability and function of cardiomyocytes and may serve as a potential matrix for the regeneration of cardiac tissue.



F5.4

Challenges in biofabrication of alginate based matrices for bone tissue regeneration: aspects of degradation and application of co-culture

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Aim: Biofabrication has been suggested as a promising method for hydrogel-cell printing in the field of regenerative medicine. The challenges of the fabrication of bone-like tissue are controllable degradation of the matrix and cell development in a suitable constructs that facilitate bone remodelling. As biofabrication has the potential to mimic native tissues the use of different cell types in a construct is essential. In this regard our study focuses on the degradation behaviour of the hydrogel which is strongly connected to the tissue development in general and thus with the cultivation of co-cultures for bone tissue engineering approaches.
Methods: As biofabrication implies the generation of viable cells all fabrication processes should be "cell-friendly" and performed under sterile and biocompatible conditions. The bioplotting technique was used to fabricate 3D hydrogel-based constructs layer by layer. The employed hydrogel for biofabrication approaches is based on an ADA-GEL system alginate-dialdehyde covalently crosslinked with gelatin 1. Bulk degradation behaviour of the hydrogel was studied without cells by monitoring Ca-ion and protein release as well as mechanical characteristics up to 21 days of cultivation. Beside material properties and process optimization single cells and co-culture system of RAW 264.7 and ST-2 cells were analysed. Cell proliferation was analysed by mitochondrial activity and DNA-synthesis. Furthermore cell differentiation was detected by specific markers TRAP ALP osteocalcin and VEGF. The cell morphologies were evaluated by fluorescence and scanning electron microscopy.
Conclusion: The applied hydrogel a gelatin modified alginate based ADA-GEL system promoted cell adhesion and migration in 3D by strong cell-material interactions. It was shown that different cell types2 grew through the hydrogel matrix and also in bioplotted constructs3. Furthermore promising results indicate that the degradation behaviour of the hydrogel can be controlled by the material composition and gelation process. Additionally the results of this study show that alginate based hydrogels used in biofabrication can support and promote the growth and repair of natural tissues including adjusted bulk degradation.

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F5.5

A comparison of different hydrogels for 3D bioprinting of hybrid mechanically reinforced constructs for cartilage tissue engineering

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Tissue engineering can potentially be used to develop grafts for total joint resurfacing. Challenges to be overcome include the scaling-up of engineered cartilaginous grafts and recapitulating the mechanical properties of the native tissue. Hydrogels have been used extensively for cartilage tissue engineering with success in supporting matrix formation; however they lack the mechanical stability of native cartilage. 3D bioprinting technology allows for the mechanical reinforcement of cell-laden hydrogels with polycaprolactone (PCL) fibres. The aim of this study was to firstly compare various hydrogels (agarose alginate Gelatin methacrylamide(GelMa) and BioINK™) for 3D bioprinting applications. We then sought to print these hydrogels alongside PCL to engineer mechanically reinforced constructs with properties appropriate for load bearing applications.
Porcine bone marrow derived mesenchymal stem cells (BMSC) were encapsulated in each gel and were cultured for 4 weeks in chondrogenically defined media. They were assessed biochemically for DNA and sGAG content and histologically for collagen type I II and X deposition. Each gel was printed alone or beside PCL with 4 different line spacing: 1.5 2.5 3.5mm and a graded scaffold of 1.5 2 and 2.5mm (to mimic the three zones of cartilage).Printability of the hydrogels and PCL reinforced hydrogels were determined by analysing percentage line spreading. Mechanical testing was undertaken to determine the equilibrium and dynamic modulus.
The results of this study demonstrated that agarose and alginate best supported chondrogenesis of encapsulated BMSCs as evidenced by positive staining for sGAG and collagen type II deposition and weak staining for collagen type I and X. GelMa supported high proliferation but positive staining for collagen type I indicated the development of a more fibrocartilage tissue. Most control over line width was achieved with alginate and all gels printed well alongside PCL. Reinforcing the hydrogels with printed PCL fibres in a graded scaffold resulted in the development of a construct with an equilibrium modulus (1.4MPa) similar to that of articular cartilage. These findings suggest that a hybrid scaffold of PCL and a BMSC laden alginate hydrogel can be used to tissue engineering mechanically robust articular cartilage.