Free paper session 9:Bioinspired biofabrication

KL9.1

Technology and biomedical applications of advanced microwell arrays – from 3D cell culture to bottom-up tissue engineering

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There is a growing demand for organotypic cellular aggregates be it as advanced in vitro model systems means to research on such aggregates or as functional (units of) regenerative/therapeutic tissue engineering constructs means to implant them. Potential application fields of complex functional three-dimensional (3D) microtissues in academia industry and the clinics are manifold ranging from stem cell research cancer research and developmental biology over tissue replacement and bioartificial organs to toxicity and pharmaceutical drug testing. The requirements for the corresponding culture substrates include control over shape and size of the cell-supporting biomaterial areas or lumen large numbers of applicable substrate materials and possibilities for their spatially resolved surface modification defined supply of cells with nutrients gases and biomolecules accessibility for controlled cell seeding and potential harvesting of aggregates microscopic observability and addressability for high-throughput screening of factors of the solid or liquid cell microenvironment. Conventional substrates or scaffolds do not meet these requirements. This talk will discuss the micro- and nanoscale 3D polymer film (thermo)forming and patterned functionalization of and 3D cell culture in advanced microwell arrays with each well representing an artificial cellular microenvironment. Besides soluble factor screening this technology also allows the integration and screening of libraries of substrate-bound factors such as geometry or surface chemistry. Furthermore the talk will discuss how arrays of geometric microwells replica-moulded in hydrogels can be applied in multistep bottom-up engineering of tissues by successive assembling of corresponding cellular building blocks on increasing scales the final tissues having a clinically relevant size in the millimetre range and complex outer geometries and internal architectures. Thereby tissues can be engineered as (self-scaffolding) cells-only constructs or as constructs of cells aggregated together with micro- or nanoengineered objects as instructive microscaffolds. The talk will also give tools at hand to by simple means without expensive equipment engineer microwell arrays be it as hierarchical and multiscale 3D microenvironments for advanced in vitro studies or for microfabrication of heterogeneous 3D tissues.

F9.1

Fabrication of scaffold mimicking the architecture of articular cartilage using direct-write electrospinning

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Aim: Direct-write (DW) near-field electrospinning and melt electrospinning have been emerged to overcome the limitations of traditional electrospinning technique in terms of design of scaffold's architecture and a densely packed structure. However the above mentioned methods either showed limitation in fabricating large structures or a requirement of a high temperature melting system as well as the difficulty in obtaining submicron fibres. Here we developed a new technique for fabrication of fibrous structures in a precise and controllable way using a DW electrospinning process. Furthermore a scaffold mimicking the zonal organization of articular cartilage was produced by this method and its performance on cartilage tissue formation was evaluated through chondrogenic differentiation of seeded Human Mesenchymal Stromal Cells in vitro.
Methods: 300PEOT55PBT45 was dissolved in a mixture of chloroform and 1 1 1 3 3 3-hexafluoro-2-propanol (v/v=75:25) at a concentration of 28% (w/v) for electrospinning. The applied voltage working distance and flow-rate were varied from 4 to 8 kV 2 to 7 cm and 0.08 to 0.5 ml/hr respectively. Fibers were collected using a moving collector controlled by a CAM software. The scan-speed was varied from 1 to 13 mm/s. To mimic the architecture of articular cartilage four patterned meshes were stacked and bonded together to achieve a final construct.
Results: The DW scaffold was able to direct tissue organization and fibril matrix orientation compared to a typical electrospun mesh. RT-PCR results revealed that expression of chondrogenic markers Sox9 and ACAN in DW scaffolds in chondrogenic medium was significant enhanced compared to conventional electrospun scaffolds at day 14 (3.5 and 17 fold respectively) and at day 21 (4.6 and 67 fold respectively). The formation of cartilage-like matrix was further confirmed by Alcian Blue staining at day 21.
Conclusions: In the present work to the best of our knowledge this is the first time we showed a technique which allows engineering fiber deposition from single fiber to core-like bundles of fibers by controlling the processing parameters. A scaffold mimicking the architecture of articular cartilage was produced using this method. Biological results revealed that the DW scaffold was able to direct tissue organization and fibril matrix orientation compared to a typical electrospun mesh. Moreover a higher expression of chondrogenic markers was also observed in the DW structure thus highlighting the potential of this new electrospinng technique for cartilage regeneration.

F9.2

3D Bioprinting of branched artery model with live cells

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Aim: In this research work novel methodologies for biomodeling and 3D biomimetic bioprinting of scaffold-free complex branched vascular constructs are presented.
Method: The principal aim of this study is to develop novel computer aided algorithms and strategies to model and 3D bioprint a three-branched vascular construct biomimetically. Computer algorithms were developed to model branched vascular model from medical images. Novel biomimetic path planning and optimization methods were developed to support bioprinted cell aggregates. Agarose based hydrogel was prepared as a support material. NIH 3T3 fibroblast cells were cultured and then optimized as a bioink for direct cell-printing.
Results: The developed algorithms were implemented for a sample coronary artery with three-branches. NIH 3T3 fibroblast cell aggregates and support structures are 3D printed layer-by-layer according to the developed horizontal bioprinting path plan. The printed tissue construct was checked against the computer model. The results show that the accuracy of the printed constructs are around 96% to 93% for the support and cellular constructs respectively. The cell viability of the printed cellular aggregates after printing was analyzed using a Live/Dead assay Kit. The cell viability after printing and after 7 days incubation was 95% and 90% respectively. The imaging analysis showed the fusion of the cellular aggregates occurred within 2-4 days after printing.
Conclusions: Novel biomimetic modeling and scaffold-free 3D bioprinting strategies were developed for printing complex branched vascular structures. A bioink made out of cell aggregates and bioprinting topology were optimized for automated direct cell printing. The modeled three branched artery model was bioprinted with cell aggregates successfully. The results show high printing accuracy and cell-viability after printing and incubation.

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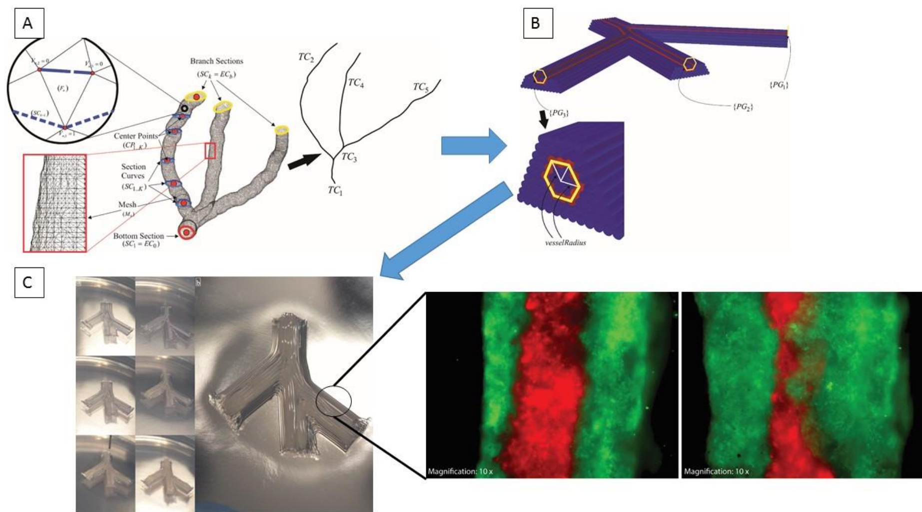

Figure 1: (A) Modeling of branched vascular structure (B) Self-supported path planning (C) 3D Bioprinted constructs with fibroblast cells and their fusion.

F9.3

Controlled assembly of engineered micro-objects and bone marrow stromal cells as a bottom-up tissue engineering approach

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In the past few decades a number of different biomaterial strategies have been developed for the regeneration of skeletal tissues. These typically comprise the use of porous scaffolds or injectable formulations like hydrogels or pastes. For both approaches several hurdles could be identified. These include non-uniform cell or tissue distribution because of a limited accessibility of the scaffold's inner part and in case of natural or synthetic hydrogels inadequate mechanical properties insufficient diffusive transport of gases nutrients and metabolites and unfavourable chemically reactive and toxic microenvironments for the cells when cross-linking cell-containing hydrogel precursors.
In our study we have shown a novel bottom-up approach able of overcoming these technical limitations [1]. By introducing polymeric micro-objects with defined size and shape in combination with bone marrow stromal cells (hMSCs) in vitro mechanically stable aggregates were obtained with a shown homogenous distribution of the cells throughout the construct. These aggregates were fused into shaped tissue constructs of clinically relevant size without showing cell death in the inner parts. By modulating the size and shape of the objects and the object-to-cell ratio more open or more dense and compact aggregates were obtained.
To show the potential of the bottom-up approach in bone tissue engineering the differentiation of hMSCs towards the osteogenic lineage cultured on radical-free medical-grade PLDLA micro-objects was studied in vitro. In gene and protein expression levels no difference was observed between soluble-factor-induced differentiation of hMSCs cultured with or without micro-objects. From these results one can conclude that these non-functionalized bare micro-objects do not inhibit osteogenic differentiation and do not induce a loss in hMSCs' phenotype.
To not only allow yet also promote osteogenic differentiation osteoconductive or even osteoinductive properties can be added to the micro-objects by functionalization. Several approaches such as the application of osteoinductive coatings and controlled osteoinductive nanotopographies are currently being explored. Octacalciumphosphate coatings were successfully applied to the micro-objects and hMSCs have shown to remain viable on these functionalized structures. We expect that the presence of this ceramic coating positively influences the osteogenic potential of our micro-objects without compromising the favourable mechanical properties of the formed aggregates.

1. Leferink A. Adv Mater 2014. 26(16): p. 2592-9.

F9.4

Investigation on the influence of laser wavelength and pulse duration on the laser-induced forward transfer of cells

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Aim: Different groups use the laser-induced forward transfer for printing biomaterials including living cells. Therefore they apply lasers with different wavelength (193 – 355 nm and 1064 nm) and different pulse durations (1 – 30 ns and 500 fs) and different laser absorption materials (metals polymers bi-layered systems or the biomaterial to be printed). Dinca et al. [1] compared printing of alginate and cell medium with 500 fs and 15 ns pulse duration at 248 nm wavelength. However so far there is no systematic investigation of the influence of these laser parameters on the printing of biomaterials and cells.
We applied lasers with different parameters like wavelength (355 532 1064 and 2940 nm) pulse durations (1 ns – 1 µs) and pulse energy for laser-induced forward transfer with different absorption materials to investigate the influence of these parameters on printed hydrogel droplets and cell viability.
Methods: The principle setup for the laser-induced forward transfer consists of two coplanar glass slides. The upper slide is coated underneath with a laser absorbing layer and a layer of the biomaterial (usually cells embedded in a hydrogel) to be transferred. Laser pulses are focused through the upper glass slide into the absorbing layer. By evaporating this layer in the laser focus a vapor bubble is generated that expands and propels the biomaterial underneath towards the lower glass slide [2]. After a few micro-seconds the bubble re-collapses. Due to inertia and surface tension the gel forms a jet flowing to the lower glass slide where it deposits as a droplet.
Results and Conclusion: The laser-induced forward transfer of hydrogels and the printing of vital cells can be initiated with laser pulses with wavelength from ultraviolet to mid-infrared and a wide range of pulse durations from femto-seconds to micro-seconds. However the successive temporal parts of the laser pulse have varying impact on the printed droplet volume. Additionally there are differences in the jet formation process achievable droplet size and applicable laser absorption material.

1. Dinca V. et al. Thin Solid Films 516 (2008) 6504–6511
2. Gruene M. et al. BioMedical Engineering OnLine (2011) 10:19

F9.5

Muscle-neuron co-culture on poly (lactic acid) ultra-thin films for biohybrid actuation

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Aim: The scope of this work was the fabrication of poly lactic acid (PLLA) freestanding ultra thin films characterized by an anisotropic pattern of C2C12 muscle cells co-cultured with PC12 neural cells in order to obtain bio-hybryd constructs with enhanced muscle contraction performances. The micromolding in capillaries (MIMIC) technique a simple method based on capillary filling of liquid in enclosed channels between a substrate and a PDMS microfluidic layer was exploited to selectively adhere C2C12 muscle cells within a micrometer-sized pattern and at the same time to print laminin in the other regions thus assuring the subsequent adhesion of PC12 cells. PLLA nanofilms represent a suitable matrix for C2C12 skeletal muscle cell adhesion and differentiation [1]. Our hypothesis is that the synergistic effect of anisotropic patterning and co-culture with PC12 cells will significantly improve the formation of mature muscle tissue [2] and the overall device contractility thus achieving effective biohybrid actuator components .

Methods: Si wafer (Fig. 1A) were spin coated with poly vinyl alcohol (PVA) (Fig. 1B) and then with PLLA (Fig. 1C). Then the substrates were incubated with fibronectin (10 μg/ml at 37°C for 1 h Fig. 1D). A PDMS stamp characterized by 50 μm-wide and 100 μm-high grooves spaced by 50 μm was previously immersed in laminin (5 μg/ml 37°C for 1 h) and then printed on PLLA (Fig. 1E). Myoblasts (C2C12 cells) were seeded and confined in the open spaces corresponding to the mold grooves (Fig. 1F). Once C2C12 cells reached confluence (Fig. 1G) neurons (PC12 cells) were seeded over the PLLA substrate and preferentially adhered on the laminin-printed areas (Fig. 1H). At the end the PLLA nanofilm was detached in water (Fig. 1I).

Conclusions: The MIMIC technique applied to nanofilms allows the fabrication of microstructures in which C2C12 muscle cells are anisotropically aligned and neurons are confined in lateral regions with the possibility to efficiently develop neuro-muscular junctions thus giving rise to a new type of biohybrid actuators [3].

Reference s:

[1] L. Ricotti et al. Biomed. Microdev. 12(5) 2010: 809-819.
[2] S. Ostrovidov et al. J. Tissue Eng. Regen. Med. 2014.
[3] L. Ricotti et al. Biomed. Microdev. 14(6) 2012: 987-998.

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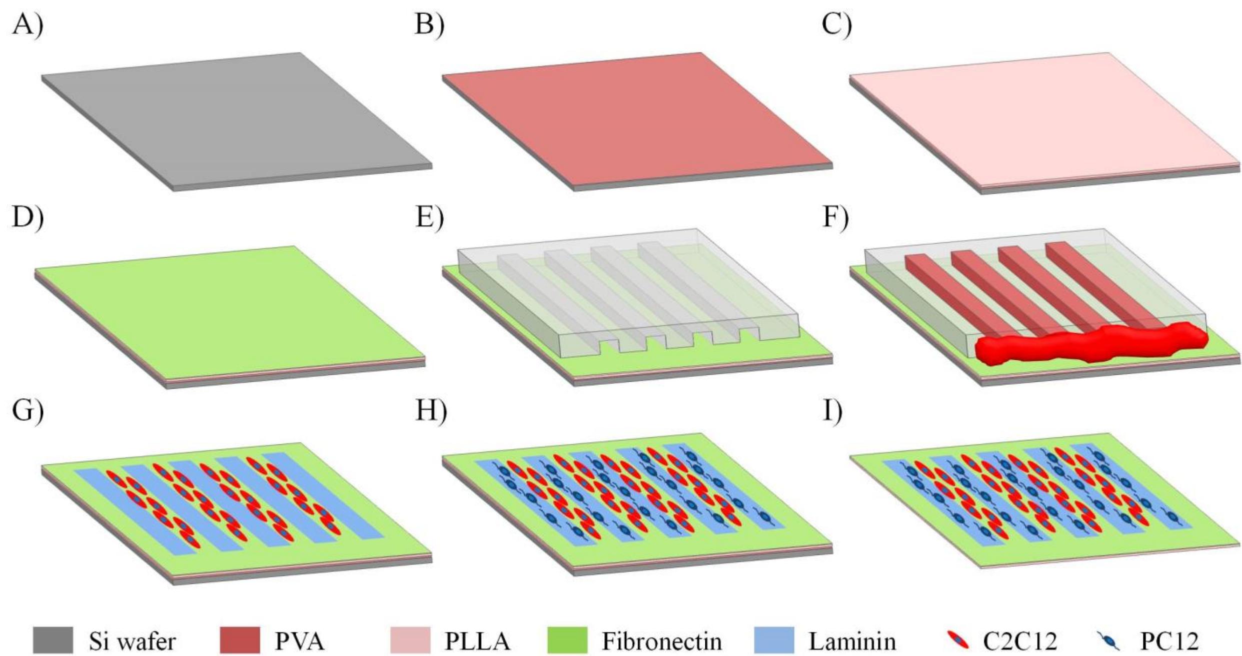

Depiction of the fabrication steps.