Free paper session 6: Scaffold based II

KL6.1

Novel approaches to bioprinting by means of multi-channel 3D plotting

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Additive Manufacturing (AM) technologies offer fascinating new possibilities for biomedical applications. Most suitable for bioprinting of real three-dimensional objects are extrusion-based methods like 3D plotting. In the last couple of years we have developed and characterised a number of pasty biomaterials suitable for 3D scaffold manufacturing and biofabrication purposes. Amongst others we have successfully utilised a pasty calcium phosphate bone cement bioglass-biopolymer composites biopolymer hydrogels like alginate gelatine gellan gum and collagen and blends thereof. We could demonstrate the fabrication of hollow strands and 3D scaffolds made thereof from high concentrated alginate/polyvinyl alcohol (PVA) blends as well as strands with core/shell morphology made of several biomaterial combinations. Due to the fact that all pastes can be processed at room or physiological temperature and no harsh conditions are applied sensitive biological components like growth factors can be included in the plotting process.
Most suitable for the biofabrication of three-dimensional open porous cell-laden constructs is an alginate/methylcellulose blend hydrogel which provides sufficient strength (viscosity) during extrusion but also suitable conditions for cell cultivation after alginate crosslinking. We could demonstrate the applicability of this new hydrogel for cell-plotting with human mesenchymal stroma cells as well as with microalgae (“green bioprinting”). An overview about our work in the field of AM and biofabrication can be found on our website www.biofabrikation.de.

F6.1

Kinetics of tissue spheroids spreading on synthetic fluorescent electrospun matrices.

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Aim: Tissue spheroids (TS) have been proposed as building blocks for biofabrication. Chondrospheres are already successfully used in clinical practice for treatment of cartilage defects. However potential washing out of non-attached tissue spheroids suggests that attachment and spreading of tissue spheroids on electrospun matrices could potentially eliminate this undesirable effect and optimize clinical outcome of implantation of tissue engineered constructs biofabricated from TS. In order to estimate potential of using electrospun matrices as a carrier for tissue spheroids we studied kinetics of TS spreading on electrospun matrices.
Methods: The battery of electrospun matrices from different biomaterials including fluorescent electrospun matrices with the luminescent Tris (8-Aluminium hydroxiquinolate III) has been fabricated using home-made and commercial electrospinning devices. The TS (chondrospheres osteospheres and desmospheres) have been biofabricated using micromolded non-adhesive hydrogel. The kinetics of TS spreading have been evaluated using light microscopy fluorescent microscopy and scanning electron microscopy. Surface Evolver (SE) has been used in order to calculate the thickness of tissue engineered constructs as a function of tissue spheroids pattern and surface density of robotic seeding. The thickness of tissue engineered construct after tissue spheroids spreading and fusion on electrospun matrices has been estimated on histological sections. The was hability test was used for estimating the level of attachment to electrospun matrices of spreaded tissue spheroids Results: TS strongly attached and spread on tested electrospun matrices. It has been shown that kinetics of tissue spheroids spreading is depended on physico-chemical and geometrical properties of tested electrospun matrices as well as the material properties of tissue spheroids. Initial pattern and density of tissue spheroids robotic placing determines the resulted thickness of tissue engineered constructs. Experimental data were in agreement with mathematical modeling and theoretical predictions using SE.
Conclusions: It has been demonstrated that tissue spheroids strongly attached and spreaded on different electrospun matrices. Theoretical and experimental data demonstrated that it is possible to calculate the desirable thickness of tissue engineered constructs resulted after spreading of tissue spheroids on electrospun matrices. Thus electrospun matrices could be used as carriers for implantation spreaded tissue spheroids without loss of tissue spheroids as a result of undesirable washing out effect. Moreover tissue spheroid spreading on fluorescent electrospun matrices could enable the real time quantitative recording of TS spreading kinetics and be employed for high throughput screening of tissue biocompatibility of synthetic electrospun matrices.

F6.2

Bioprinted soft tissue models for compound testing

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Three-dimensional (3D) cell cultures are well established. Standard tissue engineering approaches generate 3D models with random distribution of cells and matrix which is not representing the in vivo situation. There's an urgent demand for standardized and reliable in vitro 3D models for substance testing in the cosmetic and pharma industry. Bioprinting allowing the precise deposition of cells matrix and biological factors in 3D is expected to generate advanced in vitro tissue models in a reliable manner better reflecting the in vivo situation.
We establish robust protocols to print soft tissue models in a reproducible way. The bioprinter is equipped with micro valve-based inkjet printheads for cell jetting and contact printing. A chemically-defined ECM-like BioInk that is print- and cyto-compatible was developed. For the light-induced polymerization a UV-LED (365 nm) was integrated into the instrument. Tissues are printed as follows: First one layer of BioInk is printed and polymerized with UV and second cells suspended in cell culture media are jetted in the same pattern onto the BioInk followed by the next BioInk layer. In this way the 3D tissue is printed.
In a proof-of-concept study we were printing full-thickness skin equivalents. Dermal equivalents were printed with eight layers of human primary dermal fibroblasts. Viability stainings (MTT) showed proliferating cells throughout the whole culture period of 7 weeks and cells were populating the entire constructs. At different time points human primary keratinocytes were seeded on top of the dermis leading to an epidermal-like layer as shown by immunostaining. Bioprinting could provide customized skin models for the cosmetic industry.
In an ongoing project we develop in vitro muscle/tendon tissues in a customized labware allowing read-out in the same well plate. The specialized 24 well plate contains two posts in each well to produce muscle fibres between the posts. With the previously developed BioInk we printed primary human myoblasts in a dumbbell-shape that differentiated into striated myotubes as shown with myosin heavy chain- (MHC-) staining. Printed primary rat tenocytes showed characteristic collagen I distribution around the cell nuclei after differentiation. In the future bioprinted muscle/tendon tissues could be used in compound screening for muscle-related diseases.
With the developed bioprinter we are able to produce in vitro skin and muscle/tendon tissue models with primary cells combined with customized labware for read-outs.
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F6.3

Layer-by-layer microfabrication of cellularized poly (lactic acid) constructs for bone tissue engineering

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Introduction: Bone Tissue Engineering (BTE) requires tissue specific cells biochemical growth factors and a porous biocompatible scaffold to support cell proliferation and differentiation. There are different methods to fabricate BTE scaffolds and rapid prototyping (RP) is of growing interest allowing the fabrication of custom made tridimensional (3D) scaffolds with high resolution. Conventional BTE is based on the seeding of cells onto macroporous scaffold ("top-down"). The major limitation of this approach is the poor cell viability frequently observed inside the scaffold. Layer-by-Layer (LBL) microfabrication relies on another approach ("bottom-up") based on the assembly of small seeded blocks.
The aim of this work was to evaluate proliferation and differentiation of human bone marrow stromal cells (HBMSCs) and endothelial progenitor cells (EPCs) in two dimensions (2D) and 3D using a LBL assembly of polylactic acid (PLA) membranes seeded with human stem cells.
Materials and Methods: PLA membranes were fabricated by direct 3D printing a RP method based on extrusion of PLA dissolved in chloroform through a nozzle deposition process (3Dn-300 Sciperio/nScrypt Inc. Orlando Florida). HBMSCs and EPCs were seeded onto PLA membranes as mono- and co-cultures. Cell morphology (Scanning Electron Microscopy – SEM) cell survival (Live-Dead) proliferation (DNA synthesis-CyQuant) and differentiation (alkaline phosphatase for HBMSCs and Von Willebrand factor for EPCs) were evaluated in 2D. Then 3D LBL assemblies (4 layers) of the seeded membranes were prepared. Two photons microscopy (2PM) was performed after specific cell labeling for 3D constructs characterization. Phenotype characterization of LBL constructs was performed using Quantitative Polymerase Chain Reaction (qPCR).
Results: 2D mono- and co-cultures have shown cell survival on PLA during 21 days. Proliferation assay displayed an increase of DNA synthesis in all cultures after 7 days. Cell differentiation markers were expressed in all cultures. SEM showed different cell morphology of the mono- and co-culture as well as sample topography. 2PM have shown viability of the cells implanted in the 3D LBL constructs. qPCR have shown specific gene expression for endothelial and osteoblastic phenotype.
Conclusions: LBL biofabrication enables a controlled 3D organization of cells inside the scaffold as well as osteogenic and endothelial differentiation. Results obtained by now indicate that LBL approach could be suitable for bone tissue engineering in order to promote homogenous cell distribution into the scaffold and gene expression specific to the cells implanted.

F6.4

Scaffolds surface modification via irradiation laser treatment

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The aim of the study was to investigate the influence of laser irradiation treatment of polymeric scaffolds on their biological properties. Scaffold matrices 5x5x3mm from Poly (ε-caprolactone) (average molecular weight (~Mw) 45 000) have been fabricateed via Fused Deposition Modeling (FDM). The obtained scaffolds had structures with 40% porosity and interconnected pores with average size 150 µm.
Laser treatment of the scaffolds surface was done to make physical and photochemical modification in order to enhance cell's adhesion and proliferation. In order to improve the porosity femtosecond laser modification is performed. A detailed study of the influence of the pulse energy pulse length and number applied laser pulses on the morphology of FDM fiber meshes was done. The 3D structure before and after laser treatment were investigated by scanning electron microscopy (HITACHI SU8000). The irradiated areas have been examined by means of spectroscopic techniques microtomographical analysis (SkyScan 1172) and contact angle measurements. Cellurar studies were made in order to proove the capablility of the method to control cell distribution and to increase cell infiltration into fibrous scaffolds. The hFOB cells were seeded in the scaffolds (0 1 x 106/scaffold) and cultured for 1 3 and 7 days in osteogenic medium. Cell's adhesion and proliferation were monitored to tested by WST8 test.
The results show that using the optimal laser parameters micropores are formed. FS laser modification method is effective for microscale structuring of FDM fiber tissue
Moreover the btained results have shown an influence of the laser treatment on scaffolds surface morphology and cell's adhesion and proliferation on the the construct.
Summarizing irradiation laser treatment of the patterned biopolymer influence on cells adhesion of modify surface. Structuring of FDM meshes surfaces with topological features having sizes on the order of 100 µm can provide directed tissue development. Using laser treatment it is possible to increase the biological properties of the porous 3D constructs with controllable parameters.
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F6.5

Computational design of biotransportation network for biofabrication

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Vascularization plays a critical role to maintain viability of cells and functionalization of tissue during biofabrication. It nowadays becomes an important topic drawing great attention in tissue engineering and regenerative medicine. The formation of a well-organized 3D sophisticated network with tubules which mimics native blood vessels and capillaries and allows gradients of oxygen and nutrients in the construct system remains a major challenge to date. Moreover a proper integration between the tissue constructs and the host vasculature is imperative. This paper aimed to develop an integrated procedure by combining computational topology optimization of vascular network in silico photolithography bioprinting and experimental test in-vitro. In this study we propose a novel strategy for biofabrication to improve nutrient's transportation in the hydrogel-cell construct. In the design we consider the nutrient diffusion in the optimized vascular system and uptake of nutrient by cells through validation. The results demonstrate that the new fabricated tissue construct has much better nutrient delivery and more favorable microenvironment for cells to survive and proliferate thereby exhibiting the importance of addressing transport problem in biofabrication.