Poster session 10: Scaffold based biofabrication approaches

P10.1

Stereolithography of poly(ethylene glycol) hydrogels produces micro-containers for cell culture and micro-channels for vascular networks.

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Three-dimensional (3D) soft biomaterial scaffolds for long-term cell culture are critical components in tissue engineering and regenerative medicine. However it is still challenging to construct such scaffolds with desired structural stability and resolution using soft hydrogel. We've developed a method to fabricate 3D biocompatible hydrogel scaffolds at sub-200 µm resolution using projection-based stereolithography to address the biomedical challenges of stem cell culture and synthetic vasculature. Poly(ethylene glycol) (PEG) hydrogels were 3D printed by spatially controlled light-induced solidification of an aqueous pre-polymer solution (PEG-diacrylate 700 Da and 5000 Da lithium acylphosphinate photoinitiator Quinoline Yellow as absorber) using a modified commercial stereolithography printing system (envisionTec Micro 405 nm illumination). Optimization of the optical properties (proximity effects) and material properties (composition of pre-polymer solution) allowed for printing of pyramid-shaped micro-containers for long-term 3D stem cell culture and cuboids with internal channels approaching arteriole dimensions (100 µm cross-section). Human mesenchymal stem cell (hMSC) culture on the pyramidal micro-containers showed that hMSC spheroids formed spontaneously after 24 h incubation and high cell viability (> 80%) was sustained in the stable cultured spheroids for 7 days of incubation. Compared to the technically delicate state-of-the-art hanging drop methodology used for spheroid formation our time- and work-efficient approach in 3D printed low cell adhesion hydrogels provides improved control of hMSC spheroid size and shape. As synthetic arteriole and venule analogs our internal channel structures could be freely designed and constructed inside a hydrogel volume at sub-200 µm resolution in a single automated process (100 µm X 100 µm square channels and <Ø200 µm circular channels) a resolution few methods can achieve in soft hydrogels with full design freedom in all three dimensions. The aim of printing micro-channels within bulk hydrogels is to further fabricate 3D microvascular scaffolds for tissue engineering since vascularization is generally considered as the most important obstacle in the field. On-going cell culture experiments show high compatibility of the printed micro-channel structures to an endothelial cell line (CRL2922) to be employed for endothelialization of the printed vascular network analogs.
Founding Source: DTU Nanotech

P10.2

Continuous electrojet writing as a robust technique for bio-interface patterning

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With growing demands for tailored cellular microenvironments in bioengineering there is tremendous potential in combining nanotechnology and new biomaterial fabrication techniques to construct the defined biochemical and physical inputs of an extracellular matrix (ECM). However fabricating biointerface geometries in a scalable and adaptable manner remains a challenge that critically limits the translation of interface engineering for use in day-to-day cell culture study. Here we introduce a new direct writing technique continuous electrojet writing (cEJW) to directly "print out" designed biomimetic fibril structures. In particular the fibril resolution can be tuned crossing three orders of magnitude from 100nm to hundreds of microns. This technique can be applied to a wide range of materials including proteins such as gelatine biodegradable polyesters and various synthetic polymers. Especially it enables continuous deposition of volatile inks by utilizing a jet initiation mechanism. Furthermore cEJW can be performed on a variety of material substrates with conducting or insulating nature from pre-fabricated structures such as PDMS to hydrogels pre-casted with microchannels. Comparing to conventional electrospining methods with operation voltages exceeding 1kV cEJW enables low operation voltages of typically sub-100V which permits direct printing of charge-sensitive materials and bio-elements. The cEJW technique reported here illuminates new pathways for incorporating well-defined ECM-like structures within microfluidic devices further extending to applications in tissue scaffolds and organ-on-chips.

P10.3

Polymer processing for 3D printing of tissue engineered heart valve scaffolds

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Aim: Polylactid-co-glycolides (PLGA) in different percentual compositions were selected for 3D-printing relating to their promising suitability for heart valve tissue engineering. Using an appropriate additive manufacturing process i.e. 3D printing for selective laser sintering custom-made scaffolds are to be fabricated for further seeding with vascular cells from human umbilical cords. PLGA compositions were processed by two different methods to evaluate the most suitable and were analyzed for their suitability as potential scaffold materials.
Methods: The optimal particle size and shape of PLGA for 3D-printing techniques were evaluated as about 50µm and spherical. Unfortunately all primary materials are available only as granules which are not suitable for 3D printing due to the large particle size. Special grinding procedures in a cryomill are one method to generate the powder-like structure. Fabrication of polymeric microspheres is an alternative method but needs large amounts of toxic solvents. Molecular weight was analyzed before and after processing to elucidate the influence of the processing methods on the materials. Scaffold samples were produced using a custom-made contactless stripe-light 3D scanner and were analyzed for their cell-seeding capabilities. Vascular cells from human umbilical cords were expanded in a rotating bed bioreactor and were subsequently seeded onto the surface of the 3D-printed PLGA.
Results: Both processing methods were very extensive procedures resulting in a mostly flaked rough shape after grinding in a cryomill and in very small amounts of round microspheres using high amounts of solvents. Cytotoxicity assays showed no toxic influence of potential solvent residues on the cells. Molecular weight after the processing is similar to the molecular weight before processing. Polymeric flakes as well as microspheres were used to fabricate scaffolds by selective laser sintering. Human vascular cells attached to the scaffold surface with migration into the inner structure of the polymeric samples.
Conclusion: Since appropriate powder-like resorbable polymeric materials for 3D printing of heart valve scaffolds are not available on the market special processes such as grinding and fabrication of microspheres had to be evaluated. This study demonstrates the general suitability of PLGA for 3D-printing and tissue engineering after extensive processing. For future experiments much effort needs to be devoted to establishing the optimal material processing procedure with the aim of fabricating an adequate human heart valve scaffold.

P10.4

Degradation of TMC-based network structures prepared by stereolithography

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Aim: Additive manufacturing is of great interest for application in the biomedical field. Stereolithography is recognized as one the most versatile and accurate of the additive manufacturing techniques. A major advantage of this technique is the ability to prepare designed structures with desired mechanical properties. For this we have developed resins based on poly(trimethylene carbonate) (PTMC) for the preparation of medical implants and tissue engineering scaffolds. These resins can be photo-crosslinked into networks with excellent mechanical properties [1].
In this study the degradation characteristics of TMC networks and of TMC and D L-lactide (DLLA) copolymer networks are investigated.
Methods: Three-armed PTMC oligomers and three armed P(TMC-co-DLLA) oligomers were synthesized by ring opening polymerization of trimethylene carbonate (TMC) and DL-Lactide (DLLA) using trimethylol propane as initiator. Subsequently the oligomers were functionalized with methacrylic anhydride. Networks were prepared by stereolithography using PTMC-based resins containing 30 wt% propylene carbonate 5 wt% (relative to the macromer) TPO-L photo-initiator and 0.15 wt% (relative to the macromer) Orasol Orange G.
Enzymatic degradation of the networks was assessed using a PBS solution containing 20 µg/ml cholesterol esterase at 37 ˚C.
Results: After ring opening polymerization and functionalization macromers with Mn of approximately 5 kg/mol were obtained. With these macromers resins were formulated and used to prepare network films with thicknesses of 200-300 µm by stereolithography. It was shown that photo-crosslinked networks based on PTMC homo-macromers degraded slowly by the action of the enzyme. The degradation rate of the TMC and DLLA copolymer networks was significantly higher.
With these resins designed structures for application in intervertebral disk regeneration were prepared as well.
Conclusions: TMC-based photo-crosslinked networks prepared by stereolithography are biodegradable in vitro. The degradation characteristics of the networks can be tuned by synthesizing copolymer macromers of DLLA and TMC. These macromers are well suited to be used in the preparation of biodegradable structures by stereolithography.

References:
[1] Schüller-Ravoo S et al. Acta biomaterialia 8 3576 (2012).

Acknowledgements:
We would like to acknowledge the AO Foundation for financial support through their Annulus Fibrosus Rupture Program (SCAFFLEX project). DLLA and TMC were respectively supplied by Corbion Purac (The Netherlands) and Huizhou Foryou Medical (China).

P10.6

A single-step method to obtain additive manufactured fiber-based scaffolds with different surface topographies

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Aim: Surface topography from micro- to nanoscale influences cell behavior. However most of the studies have been carried out on 2D polymeric substrates with just a few reports in 3D[1] mainly due to the challenge to transfer in a controlled manner the tailored surface topographies onto 3D scaffolds. In this work we combined wet-spinning a non-solvent induced phase separation technique with additive manufacturing (AM) which allows the layer-by-layer fabrication of the scaffolds from a 3D model data[2]. Scaffolds with different surface topographies were obtained to study the effect on human mesenchymal stem cells (hMSCs) behavior.
Methods: Poly(ethylene oxideterephtalate)/poly(butylene-terephtalate) was used to produce scaffolds using different solvent/non-solvent (S/NS) combinations (Table 1). A syringe pump was used to control the extrusion of the polymer solution. To collect the polymer filaments a container with different polymer non-solvents was fixed to the fabrication platform and used as precipitation bath. Scaffolds with a defined structure were fabricated using a bioplotter[3] and were characterized by scanning electron microscopy (SEM) and laser scanning microscopy. HMSCs were seeded and cellular activity and morphology were assessed by SEM fluorescence microscopy and biochemical assays.
Table 1. Parameters used for the processing of the scaffolds

SOLVENT/NON-SOLVENT
A) CHL/80v% EtOH in water
B) CHL/90v% EtOH in water
C) CHL/ISOP
D) CHL:DCE/90v% ISOP in water

CHL: chloroform; DCE: dichloroethane; EtOH: ethanol; ISOP: isopropanol
Results: From all the different S/NS combinations studied the ones reported in Table 1 allowed the most reproducible plotting of 3D scaffolds with defined geometry and different fiber-surface topographies. HMSCs seeded on the different scaffolds attached proliferated and differentiated towards osteo- or chondrogenic lineages.
Conclusions : The combination of AM with wet-spinning allowed the manufacturing of PEOT/PBT scaffolds with a defined structure and more interestingly with different fiber-surface topographies by varying the S/NS combinations. In vitro studies indicated that these scaffolds support hMSCs culture and differentiation.

References:
[1]Kumar Biomaterials 33:4022-30;2012
[2]Mota J Bioact Compat Polym 28:320-40;2013
[3]Moroni Biomaterials 27:974-85;2006

Acknowledgements:
This work was funded by the European Regional Development Fund (Program COMPETE) Fundação para a Ciência e a Tecnologia research grant PEst-C/SAU/LA0002/2013 and North Portugal Regional Operational Programme(ON.2).

P10.7

Fabrication of anatomically shaped biodegradable scaffold using 3D printing technology for treatment of orbital wall fracture

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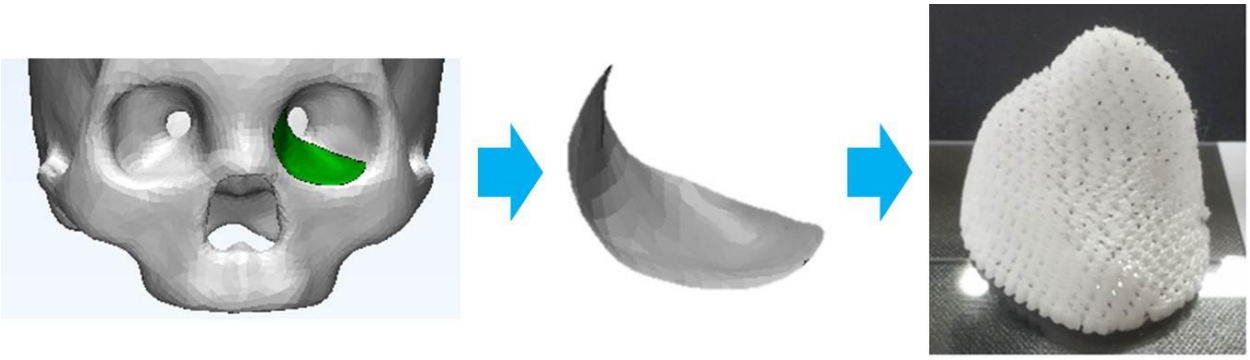
Orbital bone around eyeball consisting of diverse and complex shaped bones is able to be easily broken by trauma including traffic accidents sports activities. Especially facture of medial orbital wall associated with complex fracture is very difficult to treat. The purpose of present study was to fabricate anatomically curved mesh using polycaprolactone (PCL) by three-dimensional (3D) printing technology for treatment of medial blow-out fractures. In general flat non-biodegradable mesh has been manually formed into curved shape to fit the contour of orbital wall in the surgical room. However it is very difficult to make anatomically equivalent shape. Moreover the non-biodegradable mesh should remain in patient body for the rest of life after the implantation. Therefore anatomically shaped biodegradable curved mesh was fabricated using 3D printing technology. To achieve anatomically relevant modeling a large number of CT data was analyzed and contour of orbital region was standardized. Then the standardized model was compared with that extracted from Korean standardized CT model of Korea Institute of Science and Technology Information (KISTI). It was concluded that persons having same sex possess similar geometry in orbital wall region. In addition the anatomically curved PCL mesh was successfully fabricated using 3D printing technology.



Schematic of 3D printing of anatomically shaped mesh

P10.8

Preparation of porous tubular scaffolds for vascular tissue engineering by photo-crosslinking of PTMC in a glass mold

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Vascular grafts with an inner diameter less than 6 mm are urgently needed due to increasing coronary artery interventions. Current methods for manufacturing porous tubular scaffolds include solvent casting electrospinning and 3D printing. However these methods are not feasible for all polymers and may yield scaffolds with unsuitable properties. Poly(trimethylene carbonate) (PTMC) is a biocompatible and biodegradable polymer that can be crosslinked by gamma or ultraviolet (UV) light irradiation. The aim of this study was to develop a simple way to manufacture porous tubular PTMC scaffolds for vascular tissue engineering. Methacrylate-functionalized 3-armed PTMC of 13 000 g/mol and Irgacure 2959 as photo-initiator were dissolved in propylene carbonate. The porous tubular scaffold was obtained by four steps: mixing polymer/Irgacure solution with salt UV irradiation in a glass mold demolding and salt leaching. The space between the two glass tubes of the mold (Figure 1a) containing the polymer/salt suspension had an inner and outer diameter of 5 mm and 8 mm respectively. An elastic PTMC scaffold was made by photo-crosslinking of PTMC in a glass mold. The scaffold had an inner diameter of 3.37 mm and a wall thickness of 0.95 mm. The porosity as determined by gravimetry was 82 %. Pore sizes as observed by SEM varied from 150-250 µm. The E-modulus maximum strength and elongation at break in the radial direction were 0.465 MPa 0.234 MPa and 1018 % respectively. We optimized a molding method for the preparation of porous tubular PTMC scaffolds for small-diameter vascular tissue engineering and also obtained elastic scaffolds with suitable pore sizes and porosity for subsequent cell culturing.

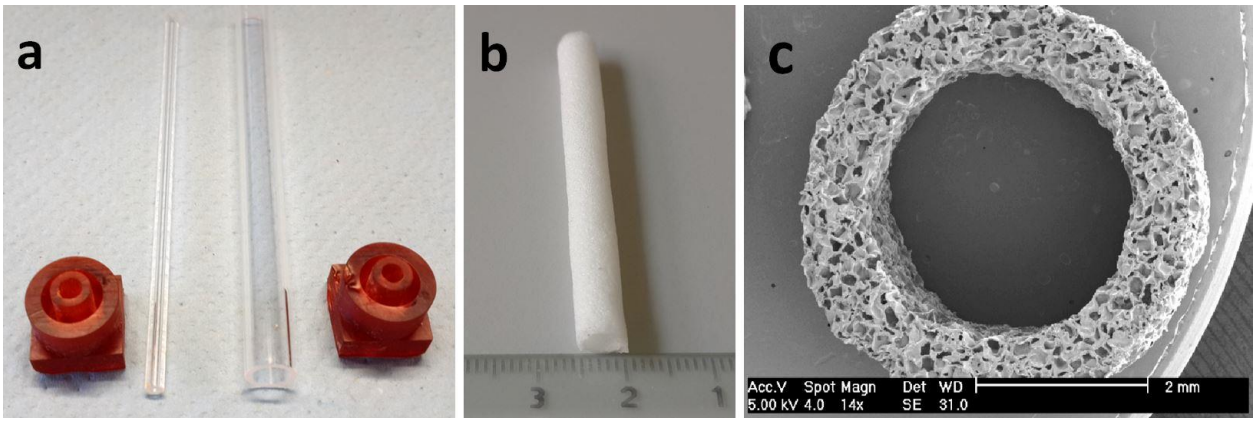

Figure 1. (a) glass mold for photo-crosslinking, (b) porous PTMC scaffold, (c) SEM picture of scaffold cross-section.

P10.9

Tailoring Bone Morphogenetic Protein-2 pharmacokinetics using different Poly (Lactic-co-Glycolic Acid) (PLGA) copolymer microspheres in Poly(Propylene Fumerate) (PPF).

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To determine the effect of Bone Morphogenetic Protein-2 (BMP-2) pharmacokinetics on bone formation a composite biomaterial consisting of a Poly(Propylene Fumarate) (PPF) scaffold and Poly(Lactic-co-Glycolic Acid) (PLGA) delivery vehicles was investigated in this study. Whereas the 75% porous PPF scaffold structure was not altered the degradation rate of the PLGA microspheres was changed in an attempt to tailor BMP-2 release by varying the lactic-to-glycolic acid ratio. Four microsphere formulations with a 50/50 65/35 75/25 and 85/15 L:G ratio and an increasing in vivo degradation rate were fabricated. The in vitro and in vivo release kinetics were determined by analyzing radiolabeled 125I-BMP-2. Biological activity was tested using a W20-17 cell culture model and a subcutaneous rat model for 14 weeks. Outcome parameters were defined as capacity to increase the in vitro AP activity in weekly consecutive cell cultures and the in vivo bone formation after 7 and 14 weeks. The composites showed similar release profiles in vitro with a minimal burst release between 2.7% (±1.8) and 8.2% (±2.8). In vitro bioactivity was shown until day 31.5 and at 73 76.5 and 108 days for all composites. In vivo the BMP-2 release rate from all composites was significantly increased. PPF/PLGA 85:15 released significant less BMP-2 per time point in the first weeks. Micro-CT and histological analysis after 7 and 14 weeks of implantation showed bone formation which significantly increased over time for all composites. No significant differences were seen between the composites. Overall the results of this study show that release of bioactive BMP-2 could be modified to a limited extend using various lactic molar ratios of PLGA microspheres as delivery vehicles embedded in a PPF scaffold.

P10.10

Surface modification of high definition 3D printed scaffolds via self-initiated photografting and photopolymerization

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The capacity to write well-defined micron and/or nanoscale fibrous substrates suitable for tissue engineering applications is a key to advance the field of biofabrication. One such emerging additive manufacturing method melt electrospinning writing (MEW) accurately deposits polymer filaments as small as 820 nm in a solvent free approach. So far only poly(ε-caprolactone) (PCL) and poly(2-oxazoline) have been successfully used for MEW [1 2]. Through the combination of MEW and self-initiated photografting and photopolymerization (SIPGP) of 2-hydroxyethyl methacrylate (HEMA) a hydrogel can be attached to the surface of the printed fiber allowing independent control of scaffold shape and chemistry. Since PCL is readily processed via MEW a hydrogel coating is an important development in the technology.
Unlike many solution electrospun materials melt electrospun PCL scaffolds are fully penetrated by cells which produce extracellular matrix and have been used in vivo with excellent integration [3]. However the performance of the device may be inhibited by interactions occurring between the PCL scaffolds and plasma proteins or blood cells. Consequently proper design of the surface chemistry is essential to control and predict the behavior of this complex system. Here we report the surface modification of PCL scaffolds obtained by MEW so that they are hydrophilic and readily wettable. We used scanning electron microscopy micro-Raman spectroscopy and fluorescence spectroscopy to examine shape homogeneity and properties of the hydrogel coating. Furthermore we studied protein adsorption on non-modified as well as on modified scaffolds.
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[2] G. Hochleitner J. F. Hümmer R. Luxenhofer J. Groll Polymer 2014 55 5017-5023
[3] L. Thibaudeau A. V. Taubenberger B. M. Holzapfel V. M. Quent T. Führmann P. Hesami T. D. Brown P. D. Dalton C. A. Power B. Hollier D. W. Hutmacher Dis. Mod. Mech. 2014 7 299-309

P10.11

Comparative study between 3D printed bioabsorbable membrane and titanium membrane for alveolar bone regeneration

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Titanium membrane has been widely used in dentistry for reconstruction of alveolar bone defect. However an implanted titanium membrane must be removed therefore the secondary surgery for removal has to be performed and this is a major disadvantage of titanium membrane. In order to overcome this disadvantage we fabricated thin microporous membrane composed with blended bioabsobable polymer of polycaprolactone poly(lactide-co-glycolide) and β-tricalcium phosphate using a 3D printing system. Then fabricated membrane was transformed into 3D shape with a pressing apparatus. The performance of fabricated membrane was demonstrated with in-vitro experiment and in-vivo alveolar bone defect of canine model. The result of experiment showed that defected alveolar bone was well regenerated and the implanted membrane was entirely degraded within 8 weeks. This results means that fabricated bioabsorbable membrane has promising potential as a substitution for titanium membrane.

Acknowledgement:
This work was supported by the R&D program of MSIP/COMPA [2014K000161]

P10.12

Strategies for biofunctionalizing photocurable gelatin methacrylamide hydrogels for cartilage tissue engineering

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Covalently incorporating biomolecules in gelatin-methacrylate (Gel-MA) hydrogels has been highlighted for its biofabrication scope of use. However this strategy requires chemical modification and may result in structural damage to the bioactives. Thus this study aims to compare the effect of thiolation and methacrylation of heparin on its native bioactivity retention in Gel-MA hydrogels and ability to promote chondrogenic differentiation.
Gel-MA thiolated heparin (Hep-SH) and methacrylated heparin (Hep-MA) were synthesized using proven protocols [1 2 3]. Modified heparins' native capability to administrate bioactive factors was evaluated via coagulation and BaF3 proliferation assays. Human articular chondrocytes were encapsulated in Gel-MA hydrogels incorporated with Hep-SH or Hep-MA using photopolymerisation techniques to assess differentiation. Constructs were cultured for 5 weeks under chondrogenic conditions. Cell viability (Live/dead®) metabolic activity (alamarBlue®) glycosaminoglycan (DMMB) and DNA content (CyQUANT®) were quantified. Matrix deposition and cytoskeletal architecture were visualised and macromer retention (DMMB) was monitored.
This study showed that thiol-modification of heparin is a superior protocol for preserving growth factor signalling and anticoagulation activity compared to methacrylation (Fig.1). Both Hep-MA and Hep-SH were covalently incorporated in Gel-MA hydrogels successfully although the latter revealed lower retention. Covalently incorporating small amounts of heparin in Gel-MA hydrogels supported cell viability and chondrocytic morphology while only Hep-SH yielded significantly greater differentiation and matrix deposition (Fig.2).
Thus combining Hep-SH and Gel-MA represents a potential platform for cartilage tissue engineering with enhanced cell differentiation and minimum influence on native bioactivity. Future work will focus on tailoring degradation rates of Gel-MA using thiol chemistry.
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2.Smeds et al.J Biomed Mater Res 2001 54(1) 115-21
3.Tae et al.Biomac 2007 8(6) 1979-86

Figure 1

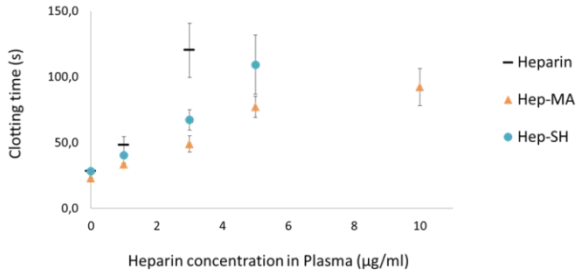

Figure 2

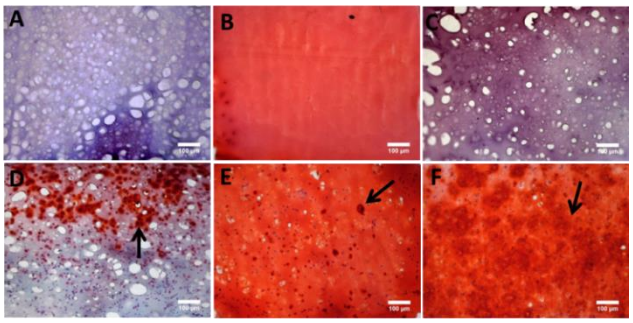

Figure 1. Activated Partial Thromboplastin Time of heparin and modified heparins. Figure 2. Safranin-O staining of cell free (A-C) and cell laden (D-F) hydrogel constructs containing Gel-MA (A,D), Hep-MA (B,E) and Hep-SH (C,F) at 5 weeks.

P10.13

3D printed polyisocyanopeptide – gelatin methacrylate (GelMA) hydrogels for bone tissue engineering

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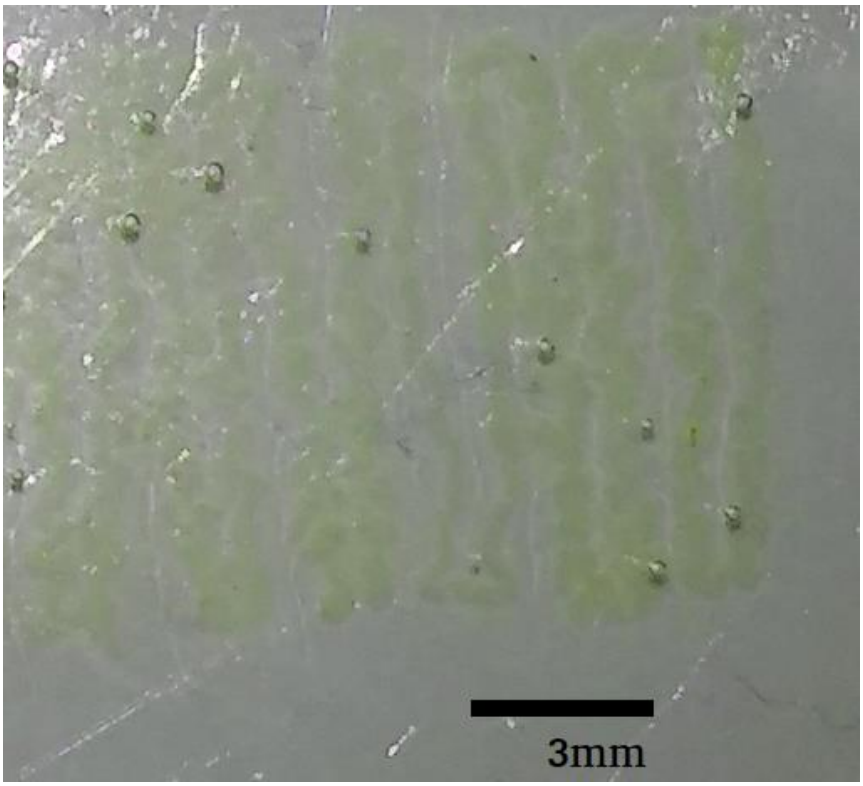
Bioprinting has gained much attention in the scientific community and is currently the state of the art technology in biology engineering and medicine. This technique needs to be advanced in order to be able to create complex tissues as in its current state it falls short in creating tissues with multiple interconnected cell structures. Furthermore it is crucial to design engineered tissues where different cell types could communicate during the tissue regeneration.
In this research a method has been explored which allows the fabrication of Polyisocyanopeptide – GelMA hydrogels by using the 3D printing technique to biomimic the early stage of bone regeneration. (Figure 1) The macrophage encapsulated polyisocyanopeptide hydrogel has been printed in osteoblast carrying GelMA hydrogels. Migration and cell survival of both cell groups has been analyzed via confocal microscopy. The printing conditions were optimized for low pressure and mild temperatures to allow the cells to survive during the printing process.



Polyisocyanopeptide hydrogel printed inside GelMA

P10.14

Determining the influence of scaffold size on cell seeding efficiency and behaviour in culture

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Aim: It is well known that the internal dimensions of a tissue scaffold strongly influence the behaviour of cells in culture and their generation of extra cellular matrix (ECM). Here we present a method of separately quantifying the contribution to the scaffold architecture to the seeding of the scaffold and subsequent behaviour of cells in culture.
Methods: Cubic lattice scaffolds of pore size 300 - 400 □m were fabricated from bioactive glass powder (70% SiO2-30% CaO2) using moulds fabricated by stereolithography. Two scaffold concepts were used 1) an approximately cubic one piece "solid" scaffold of a given pore size and 2) a scaffold of identical pore size but fabricated in slices that are stacked vertically to after sintering to produce a "sliced" scaffold of equivalent dimensions to the solid scaffold. Both scaffolds were seeded with human osteoblasts (HOBs) C-12720 (Promocell Heidelberg Germany). The solid scaffolds were statically seeded from a cell suspension whereas the sliced scaffolds were stacked using individual slices which were seeded prior to assembly. Thus the sliced scaffolds contained a uniform distribution of cells prior to culture. Both the solid and sliced scaffolds were cultured under identical conditions. After periods of up to 21 days in culture the two scaffold types were tested for total cell count cell viability alkaline phosphatase activity alazarin red staining and collagen deposition. The sliced scaffolds were disassembled to determine whether cell behaviour within the scaffolds was different from those on the exterior.
Results: In all cases the sliced scaffolds show a higher level of initial cell density (determined after 24 hours in culture) than was found using the solid scaffolds. Using the Alamar Blue metabolic assay coupled with total cell number determined from a DNA assay we found that metabolic activity per cell was also lower in the solid scaffolds when compared with the sliced scaffolds and this was also found to be the case with the ALP assay. However for both measures the activity was greater with the larger scaffold dimensions. Disassembled sliced scaffolds showed similar response to Alazarin red and collagen staining on each slice.
Conclusions: The use of slice scaffolds confirms that the seeding of porous scaffold structures is not uniform in a static cell suspension. The activity of cells within the scaffold is also a function of scaffold dimensions. This confirms that the dimensions of a scaffold influence both seeding efficiency and cell behaviour in culture.