Free paper session 2: Scaffold-based approaches I

KL2.1

Biomaterials for biofabrication

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Over the years the list of requirements for biomaterials has changed substantially. Desired specifications have evolved from (bio)inert non-degradable and non-immunogenic to biointeractive degradable and immunomodulatory. The application with biofabrication techniques imposes an extra set of requirements. These depend on the technique employed and may include rheological properties (e.g. for extrusion or dispensing) gelation/solidification kinetics (influencing shape fidelity) and mechanical properties (determining stability). Materials used with biofabrication techniques can generally be classified as either:

A) Scaffolding or substrate materials
B) Bio-ink or artificial extracellular matrix materials
C) Processing aids or supporting materials

The lack of suitable biomaterials has been identified as a major hurdle for a more rapid progress of the field. This keynote will give an overview of the biomaterials used in the area of biofabrication and provide insight in the opportunities limitations and challenges associated with specific biomaterials and biofabrication techniques.

F2.1

Biofabrication of anatomically shaped implants for regeneration of the rabbit humeral head

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Purpose: The eventual treatment for many degenerated articular joints is replacement of the entire joint with a non-degradable prosthesis. The field of regenerative medicine seeks for options to restore the diseased tissues on a biological level rather than to replace it. Regenerative treatments so far have addressed only small tissue defects. In this study we aim to take regenerative treatments to the next level by the fabrication and implantation of biodegradable anatomically shaped implants to recreate the humeral head in rabbits.
Materials and methods: The implants were based on a CT-scan of a rabbit humeral head identifying the bony and cartilaginous compartments. The bone component was printed from poly-ε-caprolactone (PCL) with fused deposition modelling (porosity 74%) and was coated with calcium phosphates. The cartilage component consisted of a gelatin-based hydrogel that was reinforced with organized PCL microfibers (porosity 92%) printed with melt electrospinning writing. Both components were thermally fused prior to hydrogel infusion. The biphasic scaffolds were implanted in the shoulder joints ex vivo in cadaveric rabbits (n=3) and in vivo (n=3) as a pilot study to assess construct strength and fixation and to monitor the rehabilitation of the animals. Three variations of the cartilage compartment were compared i.e. fiber reinforced GelMA containing 1) expanded rabbit chondrocytes 2) TGF-β or 3) an empty GelMA control group. Chondrogenesis of expanded rabbit chondrocytes in GelMA was confirmed in vitro.
Results: Rabbit chondrocytes produced an interconnected cartilage matrix within the reinforced GelMA hydrogel in vitro. Next biphasic scaffolds with the anatomical dimensions of the rabbit humerus were successfully implanted in cadaveric rabbits. These implants remained intact after abduction/adduction cycles of the rabbit shoulders. Preliminary evaluation of the in vivo study at the four-week end point showed all implants in their original location of which one was partially fractured under the head. Abundant ingrowth of bone tissue into the stem was observed with microCT. The formation of cartilage and bone tissue and the degeneration of the scaffold will be further analysed with histology and immunohistochemistry.
Conclusion: Anatomically shaped implants based on GelMA and PCL were biofabricated and implanted in the shoulder joint of rabbits. This approach for the regeneration of both cartilage and bone tissue in a customized implant potentially offers a biological solution for the replacement of (partially) degenerated joints.

F2.2

Bioprintable hydrogels for vascularized bone constructs

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Introduction: A major focus area in the field of bone tissue engineering is timely vascularization. Early onset of vascularization is needed to ensure survival of large clinically relevant-sized cell-based bone tissue engineered constructs.1
3D bioprinting is a technology that enables production of living bone replacement constructs reproducible in size shape and porosity. Furthermore heterogeneous constructs can be created by using different print heads filled with distinct matrix materials cell types and other biologicals.2

Aim: The general aim of this study is to investigate whether constructs with heterogeneous cell organization can lead to specific tissue formation in the defined locations.
In this work different available bio-printable hydrogels were compared with respect to their capacity to support endothelial- and bone-progenitor cell differentiation.
Materials & Methods: Various hydrogels were used at concentrations suitable for bioprinting. Hydrogels consisted of methacrylated gelatin (GelMA) methacrylated hyaluronic acid (HAMA) Matrigel alginate gellan gum chitosan and mixtures of these gels.
Osteoprogenitors (MSCs) and endothelial progenitors (EPCs) were seeded in different hydrogels as mentioned above and assessed for cell viability osteogenic differentiation and network formation at different timepoints in mono- and co-culture experiments.
Furthermore hydrogels were assessed for printability by measuring the accuracy of actual printed construct.
Results: Viability of the seeded cells after 7 days was >60% in all gels except those containing chitosan. Constructs composed of GelMA and Matrigel with MSCs resulted in the highest alkalic phosphatase activity per cell at day 7 and most prominent matrix deposition at day 20.
Cellular network formation was only seen in the constructs containing Matrigel and GelMA.
Overall printability of GelMA and alginate were found to be satisfactory based on stability of the structure.
Conclusion: From the different natural hydrogels selected to optimize the printing of heterogenic bone tissue engineered constructs GelMA alginate and Matrigel appeared to be best performers both with respect to technical and biological output parameters

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1. Unger RE et al. Adv Drug Deliv Rev. Mar 2015
2. Fedorovich NE et al. Tissue Eng Part A Feb 2011

F2.3

Tissue spheroids encaged into microscaffolds (lockyballs) as a promising bottom-up strategy in biofabrication and bioprinting.

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Aim: The combination of conventional top-down solid scaffolds-based approach with novel bottom-up solid scaffold-free modular approach based on principles of directed tissue self-assembly opens a new exciting perspectives in the advancing of tissue engineering and biofabrication. In this combined or hybrid approach solid microscaffold will provide desirable material properties for supporting biofabricated tissue construct without compromising tissue spheroids (TS) fusion and or directed tissue self-assembly whereas enabling initially high cell density. The diversified family of microscaffolds suitable for rapid in situ tissue biofabrication has been designed fabricated and tested. We report here the recent progress in this direction.
Methods: The microscafolds have been designed using 3D Studio Max and then exported in stereolithography file format suitable for two photon polymerization (2PP). Finite element analyses have been used for designing concentric microscaffolds with enhanced material properties. Microscaffolds have been fabricated using 2PP of photo-sensitive organically modified biomaterials. Material properties of microscaffolds have been estimated using parallel plate compression test with Microsquisher. TS encaged into microscaffold using recessions in non-adhesive agarose hydrogel fabricated using commercially available silicone micromolds. The cell viability has been estimated using standard Life and Dead assay. The interlockability of lockyballs as well as tissue fusion capacity of tissue spheroids encaged into interlockable microscafold has been estimated.
Results: The diversed family of microscaffolds including interlockable microscaffolds or lockyballs mechanically enhanced concentric lockyballs arrow-headed lockyballs or velospheres; and finally harpoon-like microscaffold or capilinsers have been designed and fabricated using 2PP of photo-sensitive biomaterials. Theoretical and experimental estimation confirmed the desirable increasing of material properties of concentric microscaffolds. The lockyballs arrow-headed design (velospheres) and harpoon-like design (capilinsers) enable better interlocking with each other and enhanced fixation of microscaffold in surrounding tissues.TS encaged into microscaffolds did not compromise their capacities to undergo tissue fusion process.
Conclusions: The TS encaged into microscaffolds is a novel and original concept or so-called third strategy in tissue engineering based on combination of conventional solid-scaffold based approach with rapidly emerging of bottom-up modular tissue engineering approach based on directed tissue self-assembly of tissue spheroids. Functionalization of microscaffolds will enable their rapid translocation using of magnetic levitation and development of novel type in situ bioprinter for rapid in vivo treatment of cartilage bone and skin defects.

F2.4

Development of polymer/hydrogel scaffold using hybrid bioprinting system

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Hydrogels such alginate collagen and gelatin are widely used for cell encapsulation cell transplantation and scaffold for tissue engineering. However it is very difficult to fabricate long term stable 3D hydrogel scaffold due to their low mechanical properties.
In this study we have developed two-head hybrid bioprinting system which can extrude bio-compatible polymers and hydrogels simultaneously to fabricate 3D polymer/hydrogel hybrid scaffold. The 3D polymer/hydrgel hybrid scaffold composed of load-bearing bio-compatible polymer matrix and cell-laden hydrogel matrix in a single 3D scaffold. We used polycaprolactone (PCL Sigma-Aldrich) to increase the mechanical strength of the scaffold and sodium alginate (Sigma-Aldrich) to encapsulate a fetal cartilage derived stem cell (FCSC) in the scaffold. We also developed a PDMS chamber which can separate the scaffold into two parts. The hybrid scaffolds are co-cultured in the PDMS chamber and the FCSCs are differentiated into chondrocytes and bone cells separately. To measure the cell proliferation of the FCSCs WST-1 cell proliferation assay kit (Takara) was used. The FCSCs in the hydrid scaffold are stained with alizarin red and safranin O and analyzed using ImageJ to compare the cell differentiation. In order to compare the mechanical strength of the polymer/hydrogel hybrid scaffold with the conventional hydrogel scaffold we used universal testing machine(UTM).
The results shows that the compressive modulus of the polymer/hydrogel hybrid scaffold is 10 times higher than the conventional hydrogel scaffold. Moreover the FSCSs are well differentiated into chondrocytes and bone cells separately in a single scaffold.
In this work we can increase the mechanical property of the hydrogel scaffold by fabricating polymer/hydrogel hybrid scaffold using the hybrid bioprinting system. And we can differentiate the FSCSs into chondrocytes and bone cells separately in a single hybrid scaffold. Therefore hybrid bioprinting technology will be simple and powerful tool for fabricating customized scaffold in tissue engineering.

F2.5

Biofabrication of scaffolds for middle ear repair.

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Aim: Ear physiology at the middle ear (ME) level relies on minute highly performing tissues such as the tympanic membrane (TM) and the ossicular chain (OC). Conductive hearing loss is a major problem that impairs the normal auditory function as a result of infections or trauma leading to permanent damages of these tissues. The surgical repair usually involves autografting or synthetic prostheses showing suboptimal performance. The aim of this study was the fabrication of biomimetic scaffolds to be cultured with human mesenchymal stromal cells (hMSCs) for optimal restoration of the ME functions.
Materials & Methods: An additive manufacturing technique known as 3D fiber deposition (3DF) was used to fabricate OC replacements with poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT) copolymer. Furthermore the combination of 3DF with electrospinning enabled the fabrication of TM scaffolds with radially and circumferentially micro-patterns [1]. The scaffolds were cultured in vitro with hMSCs for 7 and 28 days for TM and OC respectively the latter under osteogenic differentiation.
Results & Discussion: TM scaffolds were produced with anatomic size showing anisotropic ultrafine fiber basal structures and microscale superimposed isotropic orientations emulating the features of TM extracellular matrix. Scanning electron and confocal laser scanning microscopy highlighted that MSCs were viable adhered and colonized the scaffolds directionally according to the predesigned patterns. In OC scaffolds the osteo-differentiated hMSCs showed good cell viability upregulation of bone markers and appropriate level of mineralization detected via microCT. The acoustic properties of OC constructs resulted superior to those of commercial prostheses for sound pressures ranges of 50-100 dB and frequencies of 250-8.000 Hz.
Conclusions: Our findings showed that these TM and OC fabricated scaffolds allowed hMSC adhesion and differentiation. TM superimposed architectural pathways directed cell growth thus appearing promising candidates for functional membrane replacement. HMSCs cultured on OC scaffolds were able to differentiate in vitro into osteoblasts producing bone extracellular matrix. The cellularized OC constructs showed acoustic response superior to that of commercial prostheses. Further studies should assess hMSC differentiation into TM fibroblasts producing collagenous fibers along circular and radiate directions.

References:
1. Mota C et al. Biofabrication 2015 May 7;7(2):025005.

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