Free paper session 3: Engineerd tissues and organs I

F3.1

Bioprinting de novo cartilage with extracellular matrix-based bioink

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Aim: Bioprinting is an emerging technology in personalized medicine capable of producing patient specific grafts. Current polymeric bioinks lack the complexity of the native extracellular matrix (ECM) which contains a vast array of growth factors and matrix molecules. To address this shortcoming an ECM-based bioink was developed.
Methods: Articular cartilage particles were mixed with gellan gum and alginate to form a bioink further combined with chondrocytes in 6 millions/ml concentration. Rheological mechanical and biological properties of the bioink were characterized.
Results: Patient specific cartilage grafts based on clinical imaging data were printed (Fig.1). The bioink illustrated shear thinning behavior and had tensile modulus of 116kPa ± 5.6kPa after crosslinking with cells. Cell viability remained high (>80%) after printing and statistically significant increase in DNA was measured at day 21. Furthermore when supplemented with transforming growth factor (TGF-β3) a substantial increase in cartilage ECM components including collagen type 2 and glycosaminoglycans were observed after 3 and 8 weeks in culture.
Discussion: The presented bioink consists of components that are already in medical use thus avoiding the extensive regulatory hurdles faced by many other bioinks. ECM particles from any tissue source can be printed in similar manner thus enabling the reconstruction of tissue analogues for printing multi-tissue organs. This represents an important step towards clinically-applied 3D bioprinting of organs.

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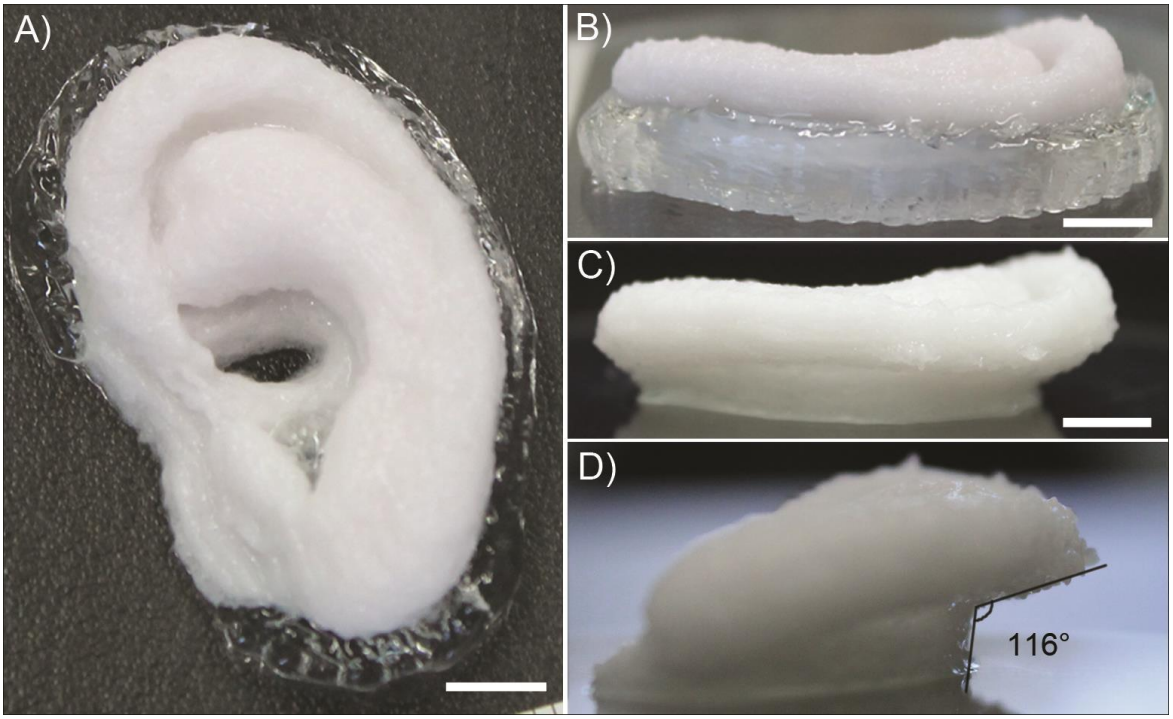

Fig.1 Pinna shape after printing A-B) and after removal of the support C-D). Scale 5mm.

F3.2

Computational-informed design and biofabrication of 3D spatially patterned constructs for bone tissue engineering

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Aim: In this work we present the computational-informed design of patterned bone tissue engineering (TE) constructs as well as show proof-of-concept for bioprinting of the 3D patterned designs of cells within a hydrogel matrix.
Methods: A previously validated in silico model of bone regeneration [1] was used to investigate the influence of particular stem cell patterns in a TE construct on the predicted bone healing outcome in a 4 mm sized murine femoral defect. Eight patterns each defined as a linear cell density gradient were evaluated for two different total average cell densities (Table 1). The most promising patterns were biofabricated with a micro-extrusion based bioprinter [2] using 3% low gelling temperature agarose mixed with different densities of NIH 3T3 cells (0.1-0.5-1-5 x105 cells/ml). After bioprinting the constructs were stained with Calcein-AM and Propidium Iodide and imaged by confocal microscopy.
Results: For a total average cell density of 50 000 cells/ml three different patterns predicted complete healing of the bone defect (Table 1). The optimized patterned constructs were successfully bioprinted and retained 90-95% cell viability after printing.
Conclusions: The in silico results clearly show that patterned constructs are able to enhance bone regeneration compared to uniform constructs depending on the gradient direction and total average cell density. Bioprinting such cell gradient constructs is novel and this work indicated that the optimized gradient pattern could be successfully bioprinted. As such the implementation of in silico design appears to be a promising strategy to optimize TE constructs in the face of the large amount of degrees of freedom created by the recent advances in biomanufacturing.

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References:
[1] Carlier A. et al. (2012) PLoS Comput Biol 8:e1002724
[2] Kucukgul C. et al. (2015) Biotech and Bioeng DOI:10.1002/bit.25493

	total average cell density = 50 000 cells/ml	total average cell density = 500 000 cells/ml
uniform	62.17%	49.93%
↑↑ ↓↓	59.31%	53.76%
↖↗ ↙↘	87.41%	49.36%
←→ ←→	100% (*)	57.03%
↙↘ ↖↗	99.12% (*)	51.81%
↓↓ ↑↑	92.12% (*)	52.08%
↘↙ ↗↖	74.14%	53.12%
→← →←	66.44%	64.38%
↗↖ ↘↙	72.21%	52.46%

Table 1: Predicted amount of bone formation after 90 days. The arrows show the direction of the gradient (from low to high cell density) in the cylindrical construct with respect to the long bone axis, with ↑ being perpendicular, → parallel and ↙ oblique to the axis. (*) indicates the patterns that result in complete healing of the segmental defect.

F3.3

Rapid formation of the bone marrow-like tissue

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To elucidate the mechanism of hematopoiesis and bone marrow (BM) diseases the realization of BM three-dimensional (3D) culture system is very important. Because more than 90% of the BM cells are composed of non-adherent hematopoietic cells it is difficult to organize the dispersed BM cells with the conventional aggregation methods e.g. hanging drop method and rotary shaker culture. The objective of this study is reproducing the BM-like tissue from dispersed-state BM cells in vitro. We reported a method of forming aggregates using methylcellulose (MC) medium (Kojima et al. Biomaterials 2012). This medium can make a 3D aggregation state rapidly regardless of adhesive property of the cells. When this method applied to BM cells they gathered in 10 minutes and formed tissue-like spheroids within 24h. The formed BM-like tissue was possible to remove from MC medium on the Day 1. Although the cell density of the BM-like tissue is slightly low sections of the organoids were similar to those of the BM tissue obtained from the mouse femur. By hematoxylin-eosin staining the blood cells were maintained in the BM-like tissue for 3 days. LIVE/DEAD assay showed that the cell viability of the BM-like tissue was about 60% after 3 days culture. By staining with anti-CD68 antibody macrophages in the BM-like tissue were found at the same frequency as those in the intact BM tissue. It was confirmed that mesenchymal cells for example CXCL12+ reticular cells PDGFRα+ cells and vimentin+ cells were maintained in the BM-like aggregates. CXCL12+PDGFRα+ cells were probably CXCL12-abundant reticular (CAR) cells playing pivotal role in hematopoietic niche. These results indicated that the method using MC medium was suitable to reconstitute the BM-like tissue.

F3.4

Patterning of tissue spheroids biofabricated from human fibroblasts on the surface of electrospun polyurethane matrix using 3D bioprinter

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Organ printing is a computer-aided additive biofabrication of functional three-dimentional human tissue and organ constructs according to digital model using tissue spheroids as building blocks. The fundamental biological principle of organ printing technology is a phenomenon of tissue fusion. Closely placed tissue spheroids undergo tissue fusion process driven by surface tension forces. In order to ensure tissue fusion during post-printing tissue spheroids must be placed and maintained close to each other. We report here that tissue spheroids biofabricated from human fibroblasts could be placed and maintained on the surface of biocompatible electrospun polyurethane matrix using 3D bioprinter according to desirable pattern. The patterned tissue spheroids attached to polyurethane matrix during several hours and completely spreaded during sereval days. Tissue constructions biofabricated by spreading of patterned tissue spheroids on the biocompatible electrospun polyurethane matrix is a novel technological platform for 3D bioprinting of human tissue and organs.

F3.5

3D plotting of a biphasic scaffold consisting of a calcium phosphate cement and a growth factor loaded hydrogel blend

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Based on our work about 3D plotting of a calcium phosphate cement (CPC) paste under mild conditions* we consider 3D plotting to be suitable to include biological components such as growth factors or even cells in calcium phosphate-based scaffolds during fabrication. In this work we explored one strategy to integrate growth factors: we applied multichannel plotting to build up biphasic scaffolds consisting of CPC and hydrogel strands. VEGF as model growth factor was loaded into a highly concentrated alginate-gellan gum hydrogel blend immediately prior to plotting. The scaffold design was optimized to ensure stable integration of the hydrogel strands into the CPC structure and to provide a gradient structure of the hydrogel within the scaffold to control local release of VEGF. Multichannel plotting yielded biphasic scaffolds with stable integrated hydrogel strands. Biologically active VEGF was released for at least seven days proven by a clear stimulation of endothelial cell proliferation and tube formation in medium which was collected as supernatant from the scaffolds at various time points of incubation. Micro-CT revealed open macroporosity for both pure CPC and biphasic CPC/hydrogel scaffolds (figure 1B). Even after swelling of the hydrogel microscopic images indicated an open porosity. The scaffold design and minimal feature size could get downscaled to a suitable size for a rat femur defect with a filigree arrangement of the strands (figure 1A). The mechanical properties of both the pure CPC and the biphasic scaffolds are not significantly different comparing Young's moduli. A first in vivo study was performed to investigate the suitability for bone defect healing in the rat femur defect. The biocompatibility of the scaffold was proven new bone formation was observed.
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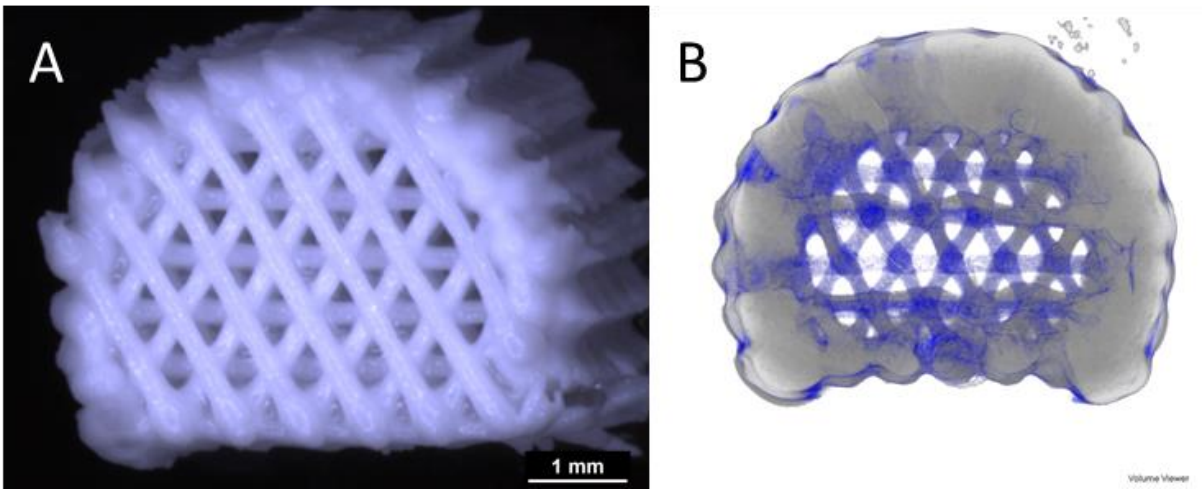
*Lode J Tissue Eng Reg Med 2014 8 682



A) CPC-scaffold for in vivo tests with the interface to the bone. B) µCT-section of a biphasic scaffold consisting of CPC (white) and hydrogel (blue).