Poster session 1: Application for Biofabrication – engineerd tissue

P1.1

3D bioprinted composite hydrogels accelerate endochondral bone formation in-vivo

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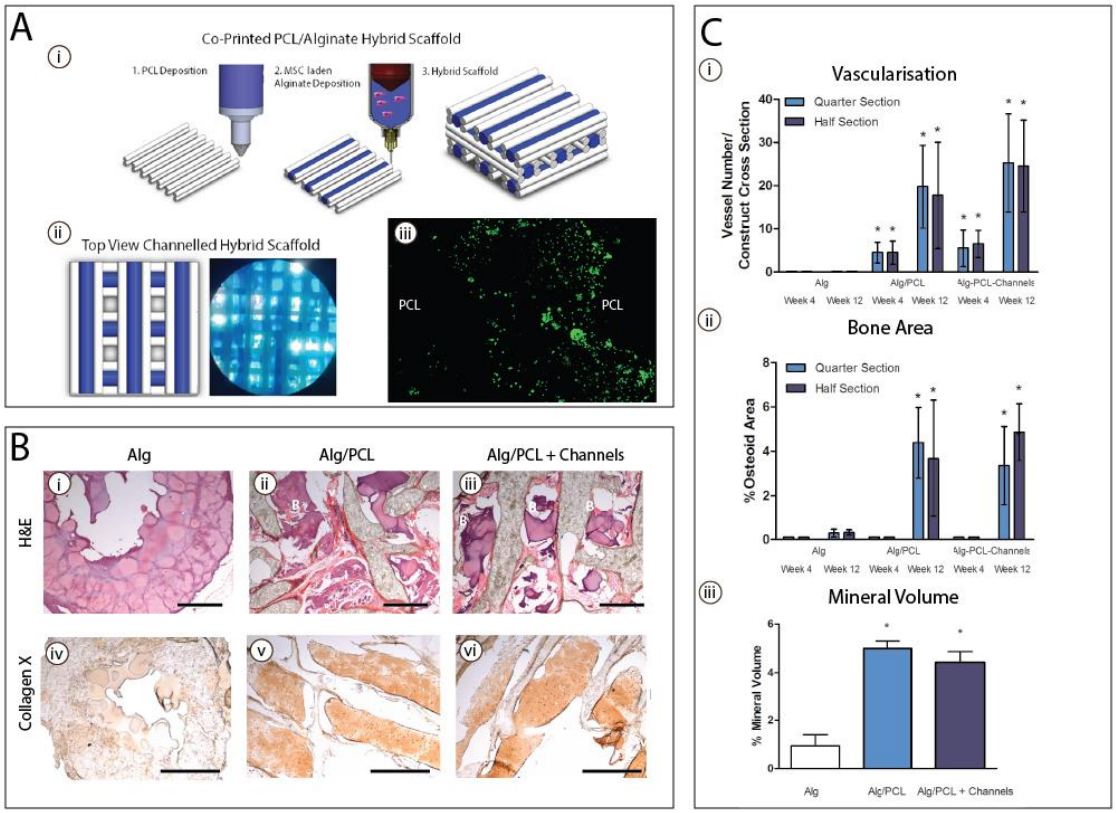
Introduction: Cartilage tissues engineered using mesenchymal stem cells (MSCs) form bone in-vivo by executing an endochondral programme offering a promising route for bone regeneration. However bone formation and vascularisation typically occur within peripheral regions of these tissues an issue that will become exacerbated when scaling up to larger clinically sized bone defects. In addition the relatively poor initial mechanical properties of the cartilaginous tissues make them unsuitable for use in a load bearing defect.
Aim: The objectives of the study were to use 3D bioprinting to engineer composite hydrogels that are 1) capable of supporting bone formation and vascularisation within core regions 2) mechanically functional to support load bearing within a large bone defect.
Methods: Composite hydrogels were engineered by bioprinting a polycaprolactone (PCL) structure alongside an MSC laden RGD-γ irradiated alginate hydrogel (compressive modulus 160X greater with PCL incorporation). The hydrogel was deposited within every second fibre spacing to create a network of channels to accelerate vascularisation within core regions. Small and large (8X Volume) constructs were bioprinted; chondrogenically primed in-vitro and implanted subcutaneously for 12 weeks to assess their capacity to support endochondral bone formation in-vivo. Solid MSC laden hydrogels without channels or PCL were implanted as a control.
Results: After 12 weeks in-vivo composite hydrogels supported significantly higher levels of bone formation and vascularisation compared to the control solid hydrogel. (Coll X H&E μCT). Bone formation and vascularisation occurred within core regions of the composites but remained confined to peripheral regions of the hydrogel alone group. Comparable levels of bone formation occurred in the larger composite grafts indicating scalability.
Conclusion: This study demonstrates that 3D bioprinting can be used to engineer composite hydrogels with enhanced biological and mechanical functionality suitable for scalable endochondral bone regeneration strategies.



A) i. Schematic demonstrating 3D bioprinting of composite PCL/MSC laden hydrogels ii. Top view of composite structure, alginate (blue) deposited within PCL structure (darker blue) demonstrating network of channels pore size (300-400µm) iii. Live dead staining of MSCs within hydrogel post printing B) H&E (i-iii) and collagen X (iv-vi) staining of groups after 12 weeks of in-vivo implantation, b; bone v; vessel formation (C) Histomorphometric quantification of bone formation (i) and vascularisation (ii) and µCT quantification of mineral volume (iii) after 12 weeks of in-vivo implantation

P1.2

construction of a novel tissue engineered corneal scaffold by combination cell three dimension printing and plastic compression

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Aim: The aim of this study is to construct a tissue engineered cornea for use in the replacement of dysfunctional corneal tissue which would resolve the problem of the shortage of corneal graft material.
Methods: First the keratocytes were mixed with collagen/gelatin/ alginate through the 3D (three-dimension) printer (based on the principle of rapid prototyping) the mixed biomaterials was printed layer by layer to construct the 3D hydrogel scaffolds. Second through the controllable compression technology (mechanical compression combined with capillary principle) to compress the 3D hydrogel and used the low concentration of calcium chloride to crosslink the compressed scaffold. Finally seeded the isolated corneal epithelial stem cells on scaffold and used cytokines to promote the cells to adhere proliferate and stratify on the scaffold.
Results: Under the optimal printing parameters (taper needle with a diameter of 0.2 mm surrounding temperature of 5℃ and material temperature of 30℃) the minimum printed line was 370μm and the cell viability was 90%. After plastic compression the mechanical property of printed scaffold increased. The seeded epithelial cells grew and stratified on the scaffold with corneal immune phenotype (Keratin 3 expressed in superficial epithelium).
Conclusions: This study provides the first line of evidence that the keratocytes printed scaffold can adequately support limbal epithelial cell expansion stratification and differentiation and have potential application in tissue engineering cornea.

P1.3

Defect-specific large format tissue engineered bone implants

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Aim: Craniomaxillofacial (CMF) injuries make up 26% of US battlefield injuries in recent conflicts(1) 10% of US emergency room trauma(2) and over 400 000 reconstructive surgical procedures in the US annually. In many of these cases there is a shortage of bone graft stock. A tissue engineering approach accommodating the geometric complexity of the CMF skeleton may provide a new treatment option.
Methods: Poly(propylene fumarate) (PPF) scaffolds were designed to fill a 4 cm mandibular segmental defect in a canine model. Scaffolds with Schoen's Gyroid pore geometry with 125-200 um struts (Figure 1A) were 3D printed on an EnvisionTEC (Dearborn MI) Perfactory® P3 to insure tissue infusion and complete resorption in a 3-4 month bone healing window. Scaffold resorption is necessary for remodeling of the healing bone wound site into strong bone. Human bone marrow-derived mesenchymal stem cells (hMSCs) were purchased (Rooster Bio Frederick MD) because they are known to be safe (i.e. non-immunogenic[3]) as MSCs or early stage osteoblasts for allogeneic or xenogeneic therapies. The seeding density of hMSCs needed to bring about full coating of the scaffold during a one week growth factor regimen of 5 ng/ml FGF2 40 ng/ml PDGF-bb and 20 ng/ml EGF was determined. Once coated with hMSCs a two week differentiation regime of 27 ng/ml BMP-7 and osteogenic media containing dexamethasone (10^-7 M) β-glycerophosphate (10 mM) and ascorbic acid (50 µg/ml) is used to bring about secretion and mineralization of bone extracellular matrix (ECM).
Results: Histological analysis of alizarin red S staining and assay alkaline phosphatase (ALP) staining and assay and Scanning Electron Microscopy (SEM) documents the production of a scaffold that is well coated with bone ECM.
Conclusions: Resorbable PPF scaffolds coated with bone ECM may be integrated in CMF injury sites in much the same way as an autologous bone graft.

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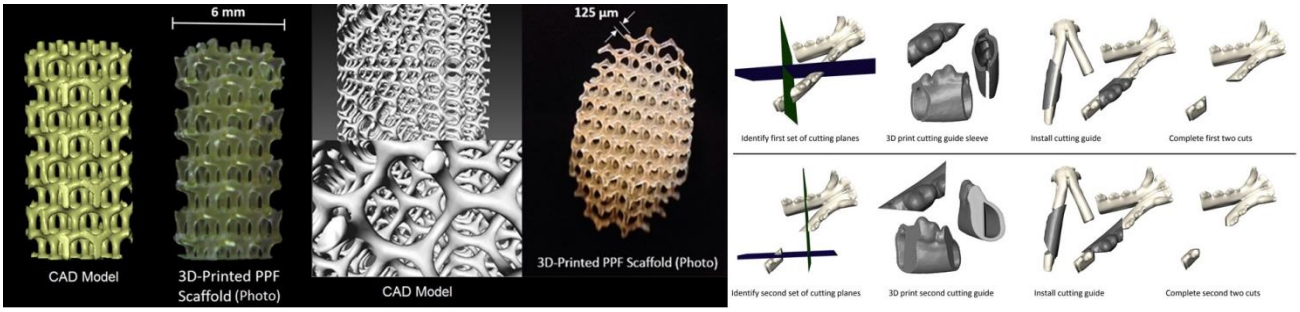

Figure 1: Mandibular Tissue Engineered Bone Implants: Canine mandibular segmental defect repair model. (1A) An EnvisionTEC Perfactory® P3 3D printer is used to render poly(propylene fumarate) (PPF) scaffolds with a Schoen Gyroid pore geometry. (1B) Cutting guides are prepared to create the mandibular defect site in a mandibular segmental defect model.

P1.5

Development of bio-inks for the construction of bone tissue equivalents by extrusion-based bioprinting

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Background & Aim: Biofabrication techniques provide the opportunity to construct tissue equivalents with defined size and geometry combined with accurate positioning of cells and matrix components. Still the search for a material that is processable with the used printing technique and at the same time supports the function of the tissue specific cells is ongoing. Therefore a bio-ink was developed which is based on methacrylated gelatin (GM) and hydroxyapatite (HAp) in order to support osteogenic differentiation of stem cells for the construction of bone tissue equivalents.
Methods: Human adipose-derived stem cells (hASCs) were resuspended in bio-inks based on UV-curable GM modified by addition of HAp nano-particles which resemble the inorganic phase of natural bone. Using an extrusion-based bioprinter 3-dimensional cell-laden hydrogels were built up in layers with varying compositions of the bioink. The gels were then cultured in osteogenic medium for up to 21 days. The change in mechanical properties of the gels was analysed by measuring their storage module G'. The extent of differentiation in the individual layers was assessed by paraffin-sectioning of the gels and staining of the sections for collagen type I and other components of the extracellular matrix (ECM).
Results: The deposition of the bio-ink in the form of filaments as well as the buildup of layer-by-layer constructs worked well and resulted in structurally defined hydrogels that were stable under culture conditions. The addition of HAp to the bio-ink resulted in a significant increase in the gels' storage module compared to the gels made of pure GM. After 21 days of culture in medium supplemented with osteogenic factors the cells in the HAp-containing gels showed osteogenic differentiation to a greater extent compared to the cells in the GM-control gels as shown by measurements of mechanical properties. These results as well as the staining of ECM components illustrate the enhancement of the cells' matrix deposition by additional HAp in the hydrogels.
Conclusion: We could show the successful development of a bio-ink that is processable with extrusion-based bioprinting-techniques and allows the buildup of cell-laden hydrogels which can support and reinforce the osteogenic differentiation of encapsulated ASCs.

P1.6

Calcium phosphate cement (CPC) core/shell scaffolds for bone tissue engineering

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Core/shell structured scaffolds are being extensively studied for their applications in tissue engineering; especially as drug delivery systems. Combination of two materials in a single entity (as a core and shell) opens up the possibility to integrate various sensitive components such as growth factors or living cells during the fabrication process. A hard shell could provide protection to the sensitive components when loaded in the core apart from providing structural stability to the scaffold. Hydrogels are most commonly used materials for core/shell fabrication; for drug delivery applications usually a high concentrated material is used which enables controlled released of the loaded growth factor by diffusion.
In our present work using a 3D plotting system with two dispensing units and coaxial needles we have fabricated core/shell scaffolds consisting of CPC as the shell and various hydrogels as the core (Fig. 1A). After fabrication of the scaffolds they were hardened in two different ways i.e. setting in water and setting in humid environment (Fig. 1B&C) – the latter is useful to avoid leaching of loaded growth factors. The setting regime had a strong impact on the physical properties such as porosity and mechanical strength. The intrinsic advantage of 3D plotting to fabricate scaffolds at physiological conditions enabled us to incorporate growth factors in CPC core/shell scaffolds. For growth factor delivery studies the core materials were loaded with BSA or VEGF and were set in both ways. Measurement of their release over a period of 14 days revealed that water-set scaffolds exhibiting a higher porosity released higher protein amounts (Fig. 1D). Bioactivity of released growth factor was confirmed by bioactivity assay and cell culture experiments. In conclusion the investigated core/shell design is a promising strategy to include functionality into CPC scaffolds.
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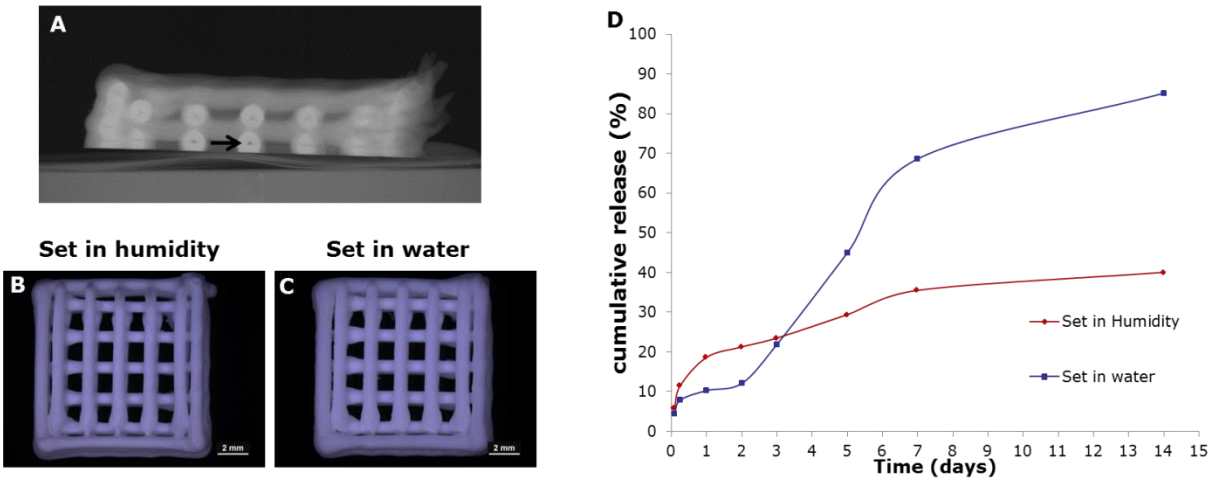

Figure 1: (A) µCT image showing the core (alginate; arrow) and shell (CPC). (B&C) CPC core shell scaffolds set in humidity and set in water. Release of BSA from both scaffold types (D).

P1.7

Personalised bio-printing tissue engineered cell constructs for nasal reconstruction

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Aims: Complete nasal reconstruction after tumour removal is a challenging surgery that frequently does not fulfil the aesthetic expectation and functional outcome the patient desires. Tissue engineering of complete nasal cartilage constructs with matched patient facial contours and the mechanical properties of natural cartilage present a near perfect treatment ideal. Our method aims to create a personalised bioprinted composite for nasal reconstruction mimicking the mechanical properties and architecture of nasal cartilage. The composite consists of polycaprolactone to provide structural support with a thermoresponsive and UV crosslinkable hydrogel gelatin methacrylate (GelMA) which acts as a cell carrier.
Methods: The 3D model was designed using the CT scan of the patient with a medical imaging software package. The 3D model was printed using an extrusion deposition system. Briefly polycaprolactone was melted at 74˚C and then extruded through at 23G needle at a deposition speed of 16mm/s. Ovine chondrocytes were isolated from articular cartilage. After 5 days in culture chondrocytes were encapsulated in three different concentrations of GelMA and bioprinted at different temperatures. Scaffolds were UV cross-linked for 60 sec.
Results: A personalised construct with clinically relevant size and shape for nose reconstruction was 3D printed using polycaprolactone. A 32% porosity was observed in the microCT analysis of the structure. Bioprinted chondrocytes encapsulated in GelMA showed an average of 80% viability. Secretion of ECM and increment in the strength of the hydrogels was observed after 14 days of culture.
Discussion and Conclusions:
This bioprinting platform allows us to make an accurate personalised and biodegradable scaffold. This structure was of a clinically relevant size and has been developed to guide suturing of cartilage grafts during surgery. Bioprinted chondrocytes incorporated into the scaffold during the printing process were shown to remain viable and functional following 35 days in culture. Extracellular matrix secretion was observed alongside remodelling and subsequent improvement of the scaffold structural properties.

References:
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Acknowledgements:
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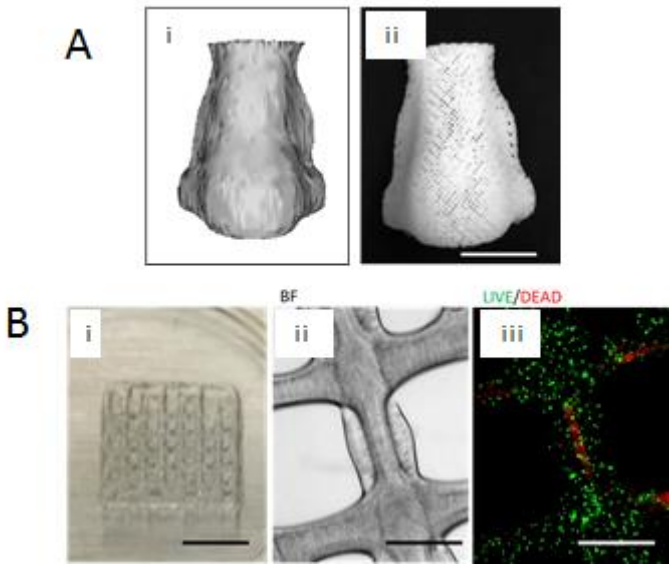

A)Personalised 3D printed nose model. i) 3D model design ii) Polycaprolactone 3D printed porous nose. Scale bar represents 2cm. B) Bioprinted GelMA/chondrocytes construct. i) Macroscopic image of the construct. Scale bar represents 5mm. ii and iii) Representative images showing viability and distribution of the chondrocytes within the hydrogel. Scale bar represents 500µm