Free paper session 8: Scaffold-based approaches III

F8.1

3D hydrogel scaffolds for cartilage tissue engineering

Alicja Kosik, Wojciech Świeszkowksi
Warsaw University of Technology, Warsaw, Poland

Scaffolds for tissue reconstruction and regeneration must have appropriate structural and functional properties. Recently hydrogels have become attractive materials applied to the repair different tissues and organs. Biodegradable and biocompatible hydrogels are crosslinked networks of hydrophilic polymers that have the capacity to retain large volumes of water. A particular advantage of high water content is easier transport of important nutrients inside the structure of the scaffold. Moreover the hydrogels possess mechanical properties very similar to the soft tissues. One group of the hydrogels that have shown excellent potential in variety of biomedical applications are alginates. Traditionally alginate hydrogels have been crosslinked via printing alginate solution into CACl2 bath or mixing them before printing.
In this work we proposed to use a coaxial dispensing system. This system make possible to deposit a solid filament by the extruding at the same time the polymeric and the crosslinking solution. The special design coaxial needle included the inner core and outer shell was used to fabricate scaffolds with controllable speed of crosslinking. We use poly-L-lysine (PLL) to modify structure of alginate. The adhesion is interpreted simple as the interaction between the polyanionic cell surface and the polycationic layer of absorbed polylysine. In the study three stage crosslinking: physical electrostatical and chemical were used.

F8.2

A novel method using 3D printing to maintain implant shape for ear cartilage reconstruction

Dafydd Owen Visscher, Ernst Jan Bos, Marco Helder, Paul van Zuijlen
VUmc, Amsterdam, Netherlands

Aim: Reconstruction of auricular cartilage for patients with congenital deficiency or traumatic injury of the ear is a major challenge in the field of plastic and reconstructive surgery. In recent years tissue engineering has shown promising results for reconstruction of cartilage. However two key challenges in the field of auricular tissue engineering are preservation of adequate auricular shape and fabrication of biologically and mechanically adequate cartilage. In this study we developed and tested a combined construct consisting of a porous 3D-printed PCL mold encapsulating a collagen I/III backbone scaffold and hyaluronan hydrogel for form-retention and auricular cartilage tissue engineering.
Methods: Goat ear chondrocytes (C) perichondrocytes (P) and adipose-tissue-derived mesenchymal stem cells (ASC) were culture-expanded encapsulated in hydrogel either isolated or in a combination of C-ASC (20:80 ratio) P-ASC (20:80 ratio) or C-P (20:80 ratio) and cultured in Polycaprolactone (PCL)-encaged collagen I/III scaffolds at 37°C for 28 days. The constructs were analyzed with Alcian Blue multi-photon laser scanning microscopy (MLSM) and macroscopic imaging
Results: Results showed that encaging the scaffolds with 3D-printed porous PCL molds completely prevented scaffold contraction after 28 days of in vitro culturing (Fig. 1). Histological and MLSM analysis confirmed glycosaminoglycan and collagen production in most but not all scaffolds. Less extracellular matrix formation was seen in scaffolds seeded with ASC alone and C-ASC scaffolds.
Conclusion: This study shows that a combined construct consisting of a 3D-printed PCL mold collagen scaffold and hydrogel can support combinations of different cell types to produce auricular cartilage with adequate biological properties.

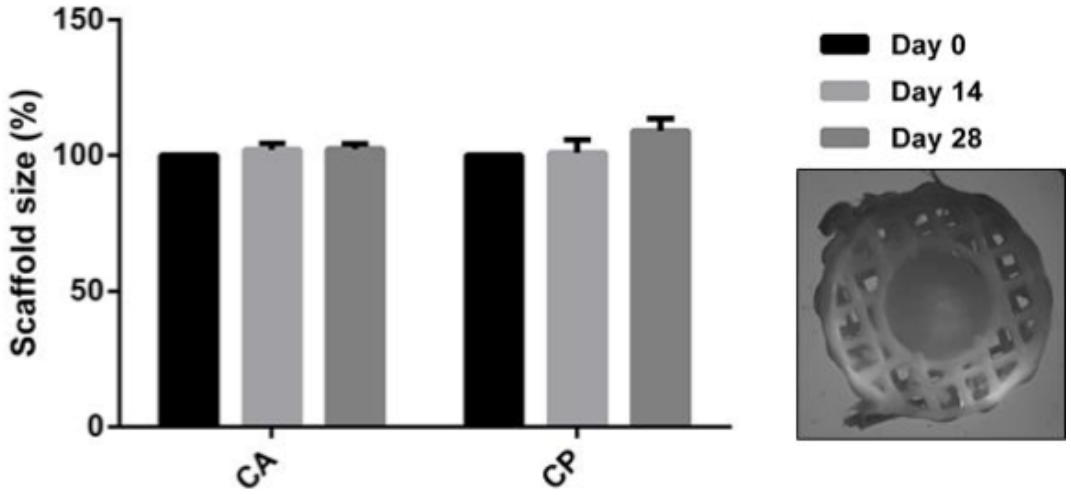

Figure 1. In vitro scaffold contraction of collagen I/III scaffolds in combination with hyaluronan hydrogel encapsulated in 3D printed PCL scaffolds. After 14 days and 28 days of in vitro culturing none of the hydrogel-collagen I/III scaffold groups showed any change in scaffold size. Picture shows macroscopic

morphology of PCL mold with scaffold located centrally. CA: chrondrocytes-adipose stem cells (1:4), CP: chrondrocytes-perichondrocytes (1:4). n=3 for both groups.

F8.3

Reinforced gelatin hydrogels for cartilage repair: from in vitro testing to implantation in the equine knee joint

Jetze Visser, Ferry Melchels, Irina Mancini, Harold Brommer, Paul Dalton, Wouter Dhert, Daniel Saris, Dietmar Hutmacher, Rene van Weeren, Jos Malda
University Medical Center Utrecht, Utrecht, Netherlands

Purpose: Hydrogels for cartilage repair are currently unable to meet simultaneously the mechanical and biological requirements for successful outcomes. We developed composite constructs based on gelatin methacrylamide (GelMA) as a suitable biological environment and highly structured poly(ε-caprolactone) (PCL) scaffolds for mechanical reinforcement to better approach the mechanics of articular cartilage.
Materials and Methods: GelMA hydrogels were reinforced with defined microfiber scaffolds that were fabricated from medical-grade PCL using a 3D-printing technique termed melt electrospinning writing. The stiffness and elasticity of reinforced hydrogels was analyzed and the chondrogenic potential of embedded human chondrocytes (n=6) was evaluated after an in vitro loading regime in a bioreactor system. A surgical technique for implanting and crosslinking the reinforced gels into cartilage defects was subsequently developed in cadaveric equine knee joints and a pilot study was performed in one Shetland pony (two defects in one joint) to evaluate surgical feasibility and implant fixation.
Results: The reinforced hydrogels approached the elasticity and dynamic stiffness of articular cartilage. While PCL scaffolds and GelMA hydrogels are both equally soft the stiffness synergistically increased for fiber-reinforced hydrogels up to 54-fold to 405 ± 67.5 kPa when using a 93% porous PCL scaffold. Human chondrocytes embedded in the GelMA/PCL composites were viable and were more responsive to an in vitro physiological loading regime in terms of ACAN and COL1A1 gene expression than chondrocytes in GelMA only. After two weeks the reinforced gel was still in place in both cartilage defects in the pony as observed macroscopically and histologically.
Conclusion: The stiffness of gelatin hydrogels was significantly increased through reinforcement with 3D-printed scaffolds approaching the mechanical properties of articular cartilage. Preliminary data from the equine model are encouraging and show good short-term fixation. A comparative long-term study of (reinforced) hydrogels as cell carriers for cartilage repair in equine stifle joints is currently ongoing.

F8.4

Biofabrication of reinforced 3D-constructs using pre-cross-linked two-component hydrogels

Kristel Boere, Maarten Blokzijl, Jetze Visser, Elder Linssen, Jos Malda, Wim Hennink, Tina Vermonden
Department of Orthopedics, UMC Utrecht, Utrecht, Netherlands

Aim: Within the field of biofabrication there is an ever increasing need for novel hydrogels that can act as bioinks. Here we present the evaluation of a new two-component bioink for 3D-bioprinting capable of forming large highly defined constructs. Furthermore we demonstrate its application in combination with a printed covalently grafted reinforcing polymer network.
Methods: Hydrogels were prepared from cysteine functionalized thermo-responsive polymers (PNC) mixed with poly(ethylene)glycol (PEG) or hyaluronic acid (HA) cross linkers both functionalized with N-hydroxysuccinimide (NHS) moieties with a total polymer concentration of 11.3 and 9.1%wt. respectively. Crosslinking was achieved via a chemo selective reaction known as oxo-ester mediated native chemical ligation and was allowed to proceed for 30 minutes prior to 3D printing. To mimic the flow and setting of the hydrogel during the 3D-printing process rheological experiments were performed using a cone and plate equipped rheometer. To create reinforced constructs we utilized covalent grafting of the developed hydrogel to a printed grid formed from NHS functionalized thermoplastic polycaprolactone derivative. Cell viability of chondrocytes encapsulated within the hydrogels was quantified using live/dead staining.
Results: 3D-printed hydrogel constructs were successfully prepared resembling grid-like structures hollow cones and a model representing a femoral condyle achieving a porosity of 47.9 ± 2.3%. It was found that the cross-linking reaction continued after extrusion resulting in mechanically stable hydrogels that exhibit a storage modulus of 9 kPa after 3 hours. Rheological measurements underscored the ability to extrude the hydrogels as well as their rapid recovery after applied shear forces. Creep-recovery tests showed that covalent grafting of the developed hydrogel to a thermoplastic material resulted in an increase in the torque required to achieve plastic deformation when compared to an absence of covalent linkages. Printed thermoplastic constructs infused with hyaluronic acid containing gels showed high cell viability of chondrocytes further illustrating their potential use as bioink.
Conclusions: The 3D-printing of these pre-cross-linked two-component hydrogels allows for the creation of constructs with good shape fidelity. Additionally covalently linking the hydrogel to modified thermoplastic fibers further improves mechanical properties as well.

F8.5

Biofabrication of tissue engineered cartilage constructs via an automated 3D micro-tissue assembly system

Naveen Mekhileri, Ben Schon, Khoon Lim, Gary Hooper, Tim Woodfield
University of Otago Christchurch, Christchurch, New Zealand

Aims: Long term repair of damaged articular cartilage represents a major challenge [1]. Combining high-throughput microtissue fabrication methods with 3D printed scaffolds as bottom-up approaches for tissue engineering are emerging strategies. Furthermore these strategies promote cell-cell/niche interactions as well as cell differentiation capacity [3]. However few technologies have been developed to automate the fabrication and precise assembly of microtissues within mechanically stable 3D Printed scaffolds [2]. We aimed to develop a 3D microtissue assembly system for fabricating large complex tissue-engineered constructs with high seeding efficiency without adversely affecting cell viability.
Methods: An automated microtissue assembly system consisting of a fluidic-based singularisation and injection modules was developed and incorporated into a commercial 3D bioprinter (SYS-ENG Germany). The singularisation module processes hundreds of microtissues and delivers individual microtissues to an injection module (via custom LabView control software) for insertion into specific locations in a 3D plotted scaffold. Human nasal chondrocytes were isolated and Ø1mm microtissues formed using a high-throughput 96-well plate format using chondrogenic media [3]. Singularisation efficiency was determined by the number of microtissues successfully singularized and injected (n=100). Bright-field microscopy measured physical dimensions of microtissues and live/dead and trypan blue exclusion assays were used to quantify cell viability (n=4).
Results: Singularisation efficiency was 97%±6.6 demonstrating the capability of the optimised fluidic-based system for individual microtissue singularisation and delivery. There was no significant difference in size and shape ($p>0.05$) or viability (live/dead and trypan blue assay) of microtissues before and after automated singularisation and injection. Furthermore 3D plotted PEGT/PBT polymer scaffolds (1mm fiber spacing) were fabricated [1 3] and a bi-layered tissue constructs containing predifferentiated chondrogenic microtissues were successfully assembled. Culture of 3D assembled microtissues has demonstrated rapid fusion and chondrogenic differentiation (GAG/DNA collagen II) in human chondrocytes and MSCs.
Conclusion: We demonstrate a novel and efficient system for the automated assembly of predifferentiated microtissues in 3D plotted scaffolds without deforming or significantly affecting microtissue viability. This technology paves a pathway for biofabricating large assembled tissues with complex 3D architecture and of clinically relevant size and shape.

Acknowledgements:
We acknowledge funding from the Royal Society of New Zealand Rutherford Discovery Fellowship (TW).

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