Free paper session 11:New matrials II

F11.1

Cell-loaded spider silk hydrogels as novel inks for biofabrication

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Introduction: Recombinant spider silk proteins are cytocompatible and can be tuned biotechnologically for cell adhesion. These proteins form hydrogels that are physically cross-linked through intermolecular beta-sheet interaction characterized by a fast regain of the initial structure after dispensing. This feature makes them printable using robotic dispensing. Additionally cell-material interactions of these hydrogels can be tuned by introducing RGD motifs to the silk proteins. Using recombinant spider silk hydrogels - both unmodified and functionalized with RGD motifs - we are able to produce cell-free and cell-laden constructs.1
Materials/Methods: Hydrogel were made from recombinant spider silk proteins (eADF4(C16)) based on the repetitive core sequence of dragline silk fibroin 4 (ADF4) of the European garden cross spider (Araneus Diadematus). For the experiments eADF4(C16) and a modification containing an RGD motif eADF4(C16)-RGD was used. Robotic dispensing was performed and patient dermal fibroblast cells were cultured for cell printing and cell seeding. Cell viability was evaluated after printing using live/dead staining.
Results: It could be shown that adhesion of cells seeded onto printed constructs was influenced by the RGD modification with greater adhesion on eADF4(C16)-RGD. The modification of eADF4(C16) with RGD did not influence printability and we were also able to print 3D constructs made from cell-laden recombinant spider silk proteins. A quantification of cell viability 48 h after printing showed an average viability of 70.1±7.6%. The comparison of printed gels with unprinted controls revealed that the printing process did not negatively influence cell viability.
Discussion/Conclusion: Recombinant spider silk proteins are promising novel inks for biofabrication. Physical crosslinks make them dispensable and they rapidly regain structure after release of shear. Due to these characteristics they are printable into defined structures without the need of thickeners or crosslinking additives. However the average viability of cells is lower than in established bioinks (e.g. alginate) and should be improved in further trials. The possibility to tune cell-material interactions by introducing cell-binding motifs makes the material and thus potential applications very versatile.

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F11.2

The crosslinking of hyaluronan controlled by photochemically removable protecting groups

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Aim: The crosslinking of hyaluronan derivatives based on photochemical control.
Methods: Hyaluronan (HA) was chemically modified to photocleavable derivatives by introduction of a photolabile protecting group releasing free amine [1]. Two synthetic strategies to photocurable HA were examined and the products were fully characterized by UV IR and 2D NMR spectroscopy.
Results: The photolysis [2] of appropriately functionalized arylmethyl carbamates of HA produced parent amines which immediately intercrosslinked in the presence of oxidized HA to form hydrogels [3]. The HA derivatives were photolyzed under physiological conditions by use of longwave ultraviolet light (330-370 nm). The photochemical control over the crosslinking was demostrated in temporal (determination of crosslink density) and spatial manner (use of a photomask).
Conclusions: The introduction of photocleavable protecting groups in HA chemistry affords spatial and temporal control over the crosslinking reaction in a polymeric structure. The crosslinked products - biocompatible hydrogels can serve as scaffolds for tissue engineering or regenerative medicine.

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F11.3

Hydroxyapatite containing inks for 3D bone bioprinting

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Aim: 3D printing is an upcoming technology in the tissue engineering field. However there is a lack of materials available for bioprinting. For the manufacturing of osteochondral grafts an osteoconductive material is desired to print an osteointegration layer. Bone cements consisting of calcium phosphate particles mixed with a liquid phase are commonly used for bone engineering. These thick pastes are often difficult to use in 3D extrusion printing because of the separation of the fluid and particle phases an effect known as filter pressing. We aimed to create a formulation for a bone ink which overcomes filter pressing allows good integration to a chondral layer and allows the adherence of stem cells.
Methods: Gellan gum methacrylate (GGMA) and hyaluronic acid methacrylate (HAMA) were synthesized according to established protocols. Hydroxyapatite (HAp) microparticles were blended with the polymer solutions at different particle contents to investigate the influence on the rheological and printing properties. Extrusion printing was performed with a BioFactory equipped with a conical shaped needle with an inner diameter of 610 µm. Bone marrow derived stem cells were cultured on top of discs made from the ink to test its potential for an osteointergration layer.
Results: At a constant 1.5% w/v HAMA increasing the GGMA content from 2% to 4% w/v and increasing HAp content from 20% to 35% w/w led to an increase in ink viscosity at all shear rates. Decreasing the liquid-particle ratio (i.e. increase of the HAp content) made printing impossible for particle contents larger than 20%. GGMA contents of >3% increased the matrix liquid viscosity so filter pressing did not occur and printing was possible. Stem cells cultured on top of this ink formulation adhered well and showed a spread morphology.
Conclusions: We found an ink on 20% Hap 1.5% HAMA and 4% GGMA which has excellent printing properties. Increasing the HAp content above 20% made printing of the ink impossible irrespective of the polymer content. Even at 20% HAp printing was only possible at GGMA concentrations above 3% where the matrix liquid viscosity was high enough to prevent filter pressing. This study furthers our understanding of particle based inks and has important implications for bone engineering.

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F11.4

Mapping the biofabrication window of gelatin methacrylamide and gellan gum hybrid hydrogels

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Aim: The layered tissue organization of articular cartilage can be mimicked in tissue-engineered constructs by stratified bioprinting of cell-laden hydrogels. A promising hydrogel for this purpose is gelatin methacrylamide (gelMA) as it supports cartilage-like tissue formation by chondrocytes [1] and is suitable for bioprinting [2]. Recently the bioprinting properties of gelMA were improved by the addition of gellan gum (gellan) [3]. However the optimal proportions of both polymers in the bioink formulation are unknown for the purpose of cartilage biofabrication. Therefore the aim of this study is to evaluate bioinks with multiple gelMA/gellan concentrations and ratios for their printability mechanical properties and chondrogenic potential of embedded chondrocytes.
Methods: Multiple gelMA and gellan concentrations (3-25% gelMA in combination with 0-1.5% gellan) were evaluated to find the optimal range for bioprinting: i.e. concentrations that allow reproducible strand formation and incorporation of cells at 15-37°C. Rheology measurements were performed to measure the viscosity and yield stress and hydrogels within the printing range were further evaluated with unconfined compression tests to determine the Young's modulus. Finally cartilage-like tissue formation by equine chondrocytes (n=3) in vitro was evaluated over a period of 42 days.
Results: A wide concentration range of gelMA/gellan hydrogels appeared to be suitable for bioprinting. In general the addition of gellan (0.5-1%) induced a yield stress and improved the quality of filaments enabling a reduction of the gelMA concentration to 3%. The addition of gellan or an increase in total polymer concentration increased construct stiffness. Although all evaluated concentrations supported cartilage-like tissue formation the highest GAG/DNA levels were measured in 10/0-0.5% gelMA/gellan groups. Histology revealed a homogeneous distribution of matrix molecules in samples with low total polymer concentrations (<11%) while confined matrix clusters were found in samples with higher polymer concentrations (≥11%).
Conclusions: The addition of gellan to gelMA hydrogels improves their bioprinting capabilities increases overall construct stiffness and supports cartilage formation of chondrocytes. However relatively high gellan to gelMA ratios (1:10) compromised cartilage matrix production and high total polymer concentrations hampered matrix distribution.
To conclude 10/0.5% gelMA/gellan allows us to bioprint constructs with a high shape-fidelity while maintaining excellent cartilage-matrix production of chondrocytes. Therefore this hybrid hydrogel is currently further evaluated for the use of cartilage biofabrication.

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F11.5

Crosslinked Poly (trimethylene carbonate) Structures with Icariin-loaded Poly(ε-caprolactone) Microspheres prepared by Stereolithography

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Aim: Fractures of the orbital floor and wall that are too large for spontaneous healing (>1 cm2) require medical implants that restore the orbital volume and promote de novo bone formation[1]. Here we prepare fitting patient specific implants containing a controlled release system for osteopromotive icariin[2] by stereolithography.
Methods: Three-armed poly(trimethylene carbonate) (PTMC) and poly(ε-caprolactone) (PCL) were formed by ring-opening polymerization. PTMC was functionalized with methacrylic anhydride to form a photo-crosslinkable macromer (PTMC-MA). Icariin-loaded PCL microspheres were prepared using a solid-in-oil-in-water (s-o-w) emulsion technique. Size distribution of the microspheres was analyzed by scanning electron microscopy. Drug loading and drug release into phosphate buffered saline with 0.5% (w/v) Tween 80 was studied by UV-Vis spectroscopy. Composite implants of photo-crosslinked PTMC with the microspheres were prepared by stereolithography. The resin consisted of PTMC-MA microspheres propylene carbonate diluent Lucirin TPO-L photo-initiator and Orasol Orange G dye. This was processed by a commercially available stereolithograph[3]. The resulting structures were analyzed by scanning electron microscopy.
Results: By adjusting processing parameters in the s-o-w technique the size of the microspheres was controlled down to 40 µm. These sizes are compatible with the high resolution of stereolithography. The drug-release from the particles was determined by their size and the drug-loading. Between 55 and 100% of the drug-load was released in 7 days. Scanning electron microscopy images of photo-crosslinked PTMC-MA structures with icariin-loaded PCL microspheres showed that the microspheres were homogeneously distributed and well-embedded in the matrix. They were also present on the structure surface.
Conclusions: We have prepared icariin-releasing PCL microspheres and their size was controlled to match the resolution of stereolithography. The drug release rate was furthermore controlled. Additionally icariin-loaded microspheres were incorporated in photo-crosslinked PTMC structures prepared by stereolithography. This allows for the fabrication of icariin-releasing PTMC implants for orbital floor reconstruction.

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F11.6

3D Powder printing of magnesium phosphate bone cements with strontium substitution

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Aim: After traumata or tumor resections the irregular shape especially for cranio- and maxillofacial implants is a major challenge in bioceramic implant fabrication. 3D powder printing is one of the most promising additive manufacturing techniques enabling the fabrication of patient specific ceramic implants matching mechanical and biological functionality. In this study we employed a fast degrading magnesium phosphate cement [1] in which magnesium ions were substituted by strontium ions. This substitution seems to be a promising way to promote proliferation and differentiation of osteoblast precursor cells [2] and therefore supports tissue ingrowth.
Methods: $Mg_{3-x}Sr_x(PO_4)_2$ (x=0 0.5 1) powder supplemented with 5 % (hydroxypropyl)methylcellulose was 3D printed with water as binder followed by sintering at 1100 °C. Sintered samples were compared with post-hardened ones which partially converted into struvite ($MgNH_4PO_4 \cdot 6H_2O$) after immersion in 3.5 M ammonium phosphate solution. Mechanical testing was performed accompanied by characterization with XRD SEM and µCT device. The ion concentration of degrading cements in surrounding media was detected by inductively coupled plasma - mass spectroscopy. Biological evaluation was performed with osteoblast cell lines MG63 and hFOB.
Results: Strontium substitution and phase conversion after post-hardening was proved by XRD SEM and µCT. Mechanical properties of post-hardened samples were in the range of those for cancellous bone with a maximum compressive strength of 36.7 MPa and a Weibull modulus of 4.3 - 8.8. Passive degradation was high for all cements and strontium release of approx. 127 µg/ml was detected over 10 days. The high degradation rate however reduced cell attachment during in vitro tests especially for strontium substituted cements.
Conclusion: Material properties could be maintained after strontium substitution leading to good printing results with a reliability of sintered and post-hardened samples comparable to hand-made ceramics. In vitro tests did not prove an enhanced osteoblast proliferation or activity due to the rapid dissolution leading to a reduced cell attachment. However the high degradation rate indicates the suitability of this material as a biodegradable bone implant.

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F11.7

Novel crosslinked gelatin microspheres for cell and drug delivery to be used in bio-printing processes

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Aim: Cell and drug carriers help obtaining a homogeneous distribution into a printed matrix offering to cells an adherent substrate during the printing process and allowing the on-purpose selection of drugs to be released. The aim of this work was to obtain size-controlled and reproducible microspheres and to optimize the cell seeding/drug loading procedures. Then we aim to investigate the feasibility of a bioprinting process by insertion of cell- and/or drug-loaded microspheres into an in situ polymerizable hydrogel matrix.
Methods: The microspheres were prepared by crosslinking gelatin type A with methylene-bis acrylamide via a patented mild Michael-type addition [1]. Innovatively crosslinking was performed at 40°C in a water/oil emulsion under stirring with simultaneous formation of microspheres. The protocol was optimized to prevent microspheres aggregation. Microspheres morphology was examined by SEM and diameters calculated from SEM images by ImageJ software. Weight and size variation were analyzed in culture medium at 37°C up to complete degradation by introducing the microspheres into tea filters. The crosslinking degree was analyzed by titration (ninhydrin assay) of free NH2 groups. The drug release in water at 37°C was tested in a dissolution apparatus after swelling crosslinked gelatin samples in a hydrophilic model-molecule (i.e. caffeine) solution. In vitro cytotoxicity was investigated using L929 fibroblast cell line and different approaches were tested to optimize the cell seeding and adhesion to microspheres by the use of PDMS-coated tissue culture multiwell plates.
Results: In the swollen state the 80% of microspheres had a diameter in the 50 - 200 µm range; larger sizes were sieved off by a low-pressure device purposely set up. A weight increase up to 1100% was observed during the first 6 h whereas degradation started after 3-5 days. The crosslinking degree of gelatin after microspheres synthesis resulted in the range 73 - 86%. Crosslinked gelatin was shown to release the 100% of loaded caffeine in the first hours. In vitro cell tests demonstrated the absence of cytotoxicity. Cell adhesion and proliferation onto gelatin microspheres was enhanced by the use of PDMS-coated plastics with cells showing the typical elongated morphology.
Conclusions: Gelatin microspheres proved to be suitable as cell carriers and capable of loading and releasing model drugs therefore adequate for the combined aim of cell and drug delivery.
Cell- and/or drug-loaded microspheres introduced in a hydrogel matrix during a bio-printing process is presently under investigation.

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