Biomaterials - Natural Polymers

S03-01

Heparin-based cryogel materials - a versatile platform for biomedical applications

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Question New approaches for regenerative therapies require advanced customized scaffolds in order to modulate cell behaviour in vitro or in vivo and/or to deliver bioactive molecules in a controlled and desired manner. In this context this work aimed to tailor and test a recently developed modular biohybrid cryogel material consisting of 4-armed poly(ethylene glycol) (starPEG) and heparin [1] for different biomedical applications. Methods Chemical crosslinking was combined with cryogelation technology and subsequent lyophilisation, either within templates [1] or in a water-in-oil emulsion [2] to obtain starPEG-heparin cryogel scaffolds and microcarriers. To control biomolecular properties of the cryogels, the heparin component was used for a range of effective secondary biofunctionalization schemes (covalent attachment of cell adhesion ligands and reversible binding of heparin-affine growth factors). Results The sponge-like materials are soft but very tough. Their high porosity provides seeded cells a large surface area for attachment, and a three-dimensional, extracellular matrix-mimicking environment while reducing shear stress during injection. We present: a) Cryogel scaffolds for co-housing pancreatic islets and mesenchymal stem cells in extra-hepatic sites for the treatment of diabetes type 1 b) Cryogel scaffolds as in vivo bioreactors for the production of bispecific antibodies for cancer immunotherapy c) Cryogel microcarriers as injectable platform for cell transplantation and growth factor delivery d) Cryogel discs as gentle transfer tool for fragile tissue Conclusion Summarizing, customized heparin-based cryogels are promising candidates for various biomedical applications.

References

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[2] B. Newland et al., *Small* 11 (2015) 5047 Acknowledgements We thank the Wellcome Trust and the Deutsche Forschungsgemeinschaft for funding and the groups of Prof. M. Bachmann and Prof. E. Bonifacio, TU Dresden, for collaboration.

S03-02

Learning from nature - hyaluronan/collagen hydrogels maintain growth factor activity and stimulate skin repair

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Question

There is a high need for novel biomaterials to treat multi-morbid patients with reduced healing capacities. Non-immunogenic hyaluronan (HA)-based hydrogels can be adjusted in their biofunctionality via the incorporation of collagen and sulfated glycosaminoglycans. Especially sulfated HA (sHA) modulates the bioactivity of several signaling proteins and stimulates endothelial cells and fibroblasts [1, 2]. Hence, this study aimed to investigate biomimetic HA/collagen-hydrogels as carrier for heparin-binding epidermal-growth-factor-like growth factor (HB-EGF) and as 3D microenvironments for fibroblasts and keratinocytes.

Methods

Hydrogels were prepared by crosslinking HA and sHA acrylates in the presence of collagen followed by lyophilization [2, 3]. Their HB-EGF release profiles were examined via ELISA. Fibroblasts and keratinocytes served to analyze the biological effects of the gel composition and of hydrogel-released HB-EGF. Wound closure was studied in a porcine ear skin culture model.

Results

Hydrogels with sHA show a retarded HB-EGF release suggesting that sHA controls the interaction with this protein. Even after 3 days at 37°C, HB-EGF released from sHA-containing gels stimulates keratinocyte migration and fibroblasts to express the keratinocyte stimulating hepatocyte growth factor. This indicates that HB-EGF remains biologically active. In addition, sHA-containing gels with or without HB-EGF significantly enhance skin wound closure compared to gels without sHA or non-treated wounds.

Conclusion

Bioinspired HA/collagen hydrogels are able to stimulate fibroblasts, keratinocytes and wound repair, which might translate into an improved healing response of injured skin tissues.

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S03-03

Elastin-based Materials for Biomedical Applications

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Upon wounding the fibrous structure of the extracellular matrix is disrupted and altered. Treating wounds with protein scaffolds supports the reconstitution of the tissue structure and improves the overall process of healing. Therefore the fabrication of novel biocompatible and absorbable biomaterials is a need for current biomedical applications. Elastin has unique characteristics, such as a very low Young's modulus and a high proteolytic resistance, which are beneficial for innovative protein-based biomaterials. We therefore developed and considerably characterized novel fibrous and sponge-like biomaterials based on isolated elastin and demonstrated the use of pyrrolochinoline quinone (PQQ) for inducing native cross-links in recombinant elastin-like materials.

Mature elastin was isolated from porcine aorta and partially hydrolyzed to obtain soluble α - and κ -elastin. Protein-based fibrous materials were fabricated by electrospinning from elastin blends and stabilized by chemical cross-linking. Sponge-like biomaterials were produced by lyophilization of hydrogels. In an alternative approach employing recombinant tropoelastin, we induced native cross-links using PQQ, which was successfully covalently immobilized onto magnetic sepharose beads and thus can be recycled.

We could successfully fabricate fibrous elastin-based materials and established a scalable process for the production of nonwoven biomaterials. Furthermore, we produced protein sponges from α -elastin/gelatin composite hydrogels by freeze drying. The microstructure and porosity of the materials was investigated by scanning electron microscopy and nano- or microscale X-ray computed tomography. Mechanical properties were determined by tensile testing and atomic force microscopy.

The here presented methods and materials allow the fabrication of innovative and tunable elastin-based biomaterials for biomedical applications, in particular for cutting-edge wound dressings or implant coatings.

S03-04

Designable ECM-Matrix Protein ToolBox - Creation of Elastine-Like Protein (ELP)-Based 2D & 3D Material Systems with Adjustment of E-modulus and Epitope Modification

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Designing biomaterials facilitating cell-instructive environments is the goal of our effort in creating protein-based extracellular matrix-protein systems. They mainly consist of elastine-like proteins (ELPs) as basic scaffold for this design approach. Since ELP-sequences designed are close analogs of the human elastine sequences they share their advantageous properties such as non-immunogenicity, stimulus responsive functional switches and adjustable elasticity.

Access to such protein-based systems is enabled via our complex set of molecular biotechnological tools, for example a patented protein-assembler platform consisting of a method to assemble the genes of highly repetitive matrix proteins including epitope organization via our "One Vector Tool-Box Platform" (OVTP), a photochemical crosslink system allowing to precisely adjust the mechanical properties of the matrix-protein hydrogels within the complete physiologically relevant range, tuned bioprocess engineering from small and medium scale matrix protein production and ECM epitope incorporation into these materials. Currently we are combining this protein modification scheme with innovative types of 3D printing with in situ cross linking forming 3D-objects from the micrometer to the centimeter scale. The recent systems focus is: I) cell-instructing ECM-component hydrogels providing defined chemically and physically, as well as spatially engineered and organized and mechanically adjustable ECM-mimicry, II) large volume tissue regeneration utilizing growth chambers and porous biopolymer scaffolds and III) mechano-active protein-based materials.

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S03-05

Bone sialoprotein functionalised collagen gels enhance angiogenesis and osteogenesis

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Introduction

Bone sialoprotein (BSP) plays an essential role in bone mineralisation and remodelling. Due to its collagen-binding motif, BSP linking to collagen gels is a suitable approach [1]. Therefore, the aim of this study was to combine BSP with collagen gels and evaluate the effects *in vitro* on gene expression and angiogenic behaviour and *in vivo* on bone regeneration.

Methods

BSP release kinetics in collagen gels were measured over 7 days using fluorescein-coupled BSP. Human osteoblasts (hOBs) and human umbilical vein endothelial cells (HUVECs) were seeded as mono- or co-culture into collagen gels with or without BSP addition for evaluation of cell viability (Alamarblue assay) and osteoblast gene expression (qPCR). Moreover, angiogenesis was analysed with spheroids in the sprouting assay. *In vivo*, two 5 mm borehole defects were set in the rat calvarial, followed by implantation of no, pure, BSP or BMP-7 functionalised collagen gels. New bone formation was analysed after 3 and 8 weeks via μ CT and histology. MICROFIL® perfusion was used to visualise vessel formation after 8 weeks.

Results

A continuous BSP release was observed during the first seven days. Cell viability was significantly enhanced in collagen gels with low BSP concentration for HUVECs as well as for hOBs. BSP treated collagen gels enhances SP7, OPN and RUNX2 expression of hOBs after 4 days. Sprout formation was significantly enhanced in low concentrated BSP collagen gels. *In vivo*, new bone formation appeared as follows: BMP-7 gels > high BSP-concentrated gels > pure collagen gels > low BSP-concentrated gels > no treatment.

Conclusion

In vitro, BSP functionalised collagen gels enhance cell viability and sprout formation in lower concentrations, while higher BSP concentrations enhance osteoblast gene expression. A concentration dependent effect was also observed *in vivo*.

References

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