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Advanced alginate-based hydrogels

Hydrogels with specific surface structures for biofabrication applications

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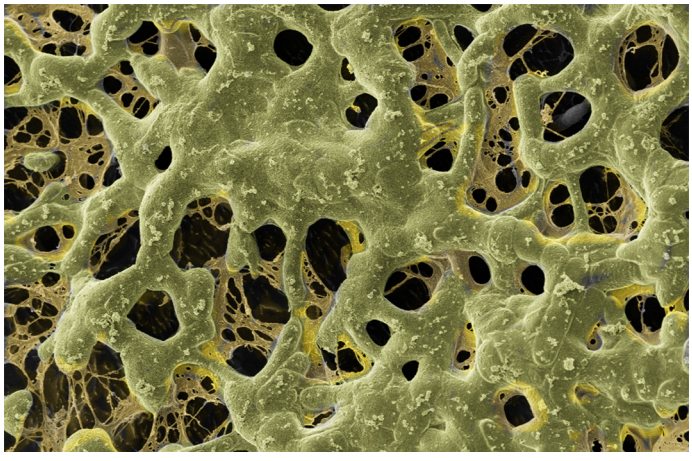

The search for suitable three-dimensional (3D) scaffolds analogous to the natural extracellular matrices is a central task in the field of tissue engineering (TE). These scaffolds should deliver cells to the desired site in the patient's body supporting the formation of new tissue [1]. Various hydrogels have recently been used to mimic the extracellular matrix of several tissues; however the adaptation of materials properties and scaffolds geometry for TE remains a challenge. In this regard, 3D additive manufacturing (AM) technologies can be exploited to mimic natural tissue structures. Utilizing the layer by layer printing approach, this technology enables the production of constructs with complex structures or intricate external and internal geometries in biofabrication strategies [2]. AM techniques such as bioplotting have the advantage of relatively free design of scaffold structures regarding their 3D architecture as well as the accurate positioning of different materials, cell types and bioactive substances. One aspect of 3D bioplotting techniques is the development of novel hydrogels as suitable bioinks. Hydrogels are hydrophilic polymers of natural or synthetic origin. The appropriate hydrogels for this application should exhibit controllable swelling and degradation kinetics, as well as adjustable mechanical properties, tailored chemical and physical structure, crosslinking density, diffusivity and porosity [3]. Especially, the supply of oxygen and nutrients throughout the hydrogel depends on the porosity, pore diameter and pore interconnectivity, which are decisive parameters affecting also cell growth and proliferation in the 3D matrix. A novel hydrogel system based on oxidized alginate covalently crosslinked with gelatin (ADA-GEL) has been recently developed [4] and utilized for biofabrication approaches to design tissue engineering scaffolds, in which cell growth, proliferation and migration were observed to be promoted [4]. The aim of this study was to characterize the microstructure of ADA-GEL hydrogel in order to establish the correlation between hydrogel microstructure and cell growth behavior.

ADA-GEL hydrogel was synthesized as described by Sarker et al. [4]. Briefly, alginate di-aldehyde (ADA) was synthesized by controlled oxidation of sodium alginate and afterwards the suspension was dialyzed against ultrapure water for 7 days. The ADA-GEL hydrogel was prepared by 5% (w/v) aqueous solution of gelatine dropped slowly into an ADA solution (5% (w/v)) in phosphate buffered saline (PBS) with a pH of 7.4 under stirring. For the bioplotting of the ADA-GEL hydrogel a moveable bioplotter, in 3-axis configuration (type BioScaffolder 2.1, GeSIM, Großerkmannsdorf, Germany) was used. For the plotting of the hydrogel a micro-nozzle with an inner diameter of 200 μm was chosen to reach a high resolution. The design and the dimensions of the plotting geometries were defined over the 'ScaffoldGenerator software' of the bioplotter. Simple lines and grid-like, square

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structures with an edge length of 15 mm were plotted to generate test specimens. The pressure and the plotting speed were adjusted considering the experiment in a range between 55 and 60 kPa and 20 mm/s, respectively. The number of struts plotted over the edge length as well as the number of layers in *z*-direction were adjusted. The syringe was filled with the hydrogel and processed into six well culture plates (VWR, Germany), which were placed in a holder on the static plotter platform. Ionic gelation was performed using 0.1 M $CaCl_2$ solution for 10 min immediately after plotting. The processing temperature was set to 37 °C by heating the cartridge containing the hydrogel-cell mixture to prevent the gelling of the gelatine and to confirm a cell-friendly condition. Afterwards the samples were washed with deionized water to eliminate the adhered $CaCl_2$ solution from the surface of scaffolds. To evaluate the internal structure of the ADA-GEL hydrogel scaffolds cryo-scanning electron microscopy (cryo-SEM) was performed (Zeiss Auriga, Zeiss, Jena, Germany) equipped with a cryo system (PP3010T, Quorum Technologies Ltd, UK). For this analysis the bioplotted scaffolds were rapidly frozen in liquid nitrogen at −180°C and subsequently transferred to the preparation chamber where sublimation etching was utilized to enhance the structure details. After this the samples were sputtered with a thin platinum layer using the integrated sputter coater. Then the sample was transferred into the SEM chamber and placed on a cold stage at −179°C. The images were taken under this condition.

As shown elsewhere [4], ADA was synthesized by periodate oxidation of alginate which facilitates crosslinking with gelatin through Schiff's base formation between the free amino groups of gelatin and the available aldehyde groups of ADA. The bioplotted hydrogel scaffolds exhibited pore sizes of 300–500 μm and strut diameters of 500–900 μm with a calculated total porosity varying between 37 and 44 vol%, as described in our previous study [5]. Employing cryo-SEM technique, further essential evidence about the sub-microstructure of the hydrogel can be gained. The image displayed on this issue's cover shows a cryo-SEM image of the surface structure of ADA-GEL hydrogel. By applying a magnification of 10k× the surface morphology of the hydrogel exhibiting porosity is detectable. Analyzing further cyro-SEM images ($N = 30$), the diameter of the pores was measured, which is in the range of 20–1600 nm. Interestingly, it was not possible to analyze the microstructure of hydrogels from SEM images [4–6] when the hydrogels were supercritically dried. In this context it has to be mentioned that the mesh size of dense alginate hydrogels ranges between 5 and 20 nm [3,7], which cannot be analyzed in the present case, as the samples in this study were sputtered. We hypothesize that the generated alginate based hydrogel structure exhibits also pores in the mentioned dimension.

Moreover, the plotted ADA-GEL hydrogel constructs exhibit a hierarchy of pore sizes from the nano to the micrometer range. The biological properties of these hydrogels were studied by comparing the viability and morphology of MG-63 osteosarcoma cells, encapsulated in gelatin and RGD-modified alginate, as reported elsewhere [6]. After 4 days of incubation, cells formed extensive cortical protrusions and after 2 weeks they proliferated, migrated, and formed cellular networks through the ADA-GEL material. These results indicate that the present hydrogel offers adequate pore structures for cellular development. In addition, cell activity was found to be doubled after a couple of days, indicating that ADA-GEL is a promising material for biofabrication [4]. As previously shown, embedded cells with high activity indicated that the material can be processed in a defined, biocompatible, cell-friendly manner by bioplotting [5].

Designing materials that can promote cell adhesion and migration starts with the understanding of cell-material interactions in 3D. The results of this study, in particular the characterization of the pore structure, confirm that ADA-GEL hydrogel represents a promising matrix to support and promote the growth and repair of natural tissues and it is a suitable bioink for the development of scaffolds by biofabrication.

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Further reading

[1] R. Langer, D.A. Tirrell, Nature 428 (2004) 487–492. http://dx.doi.org/10.1038/nature02388.
[2] T. Billiet, et al. Biomaterials 33 (2012) 6020–6041. http://dx.doi.org/10.1016/j.biomaterials.2012.04.050.
[3] D. Seliktar, Science (80–) 336 (2012) 1124–1128. http://dx.doi.org/10.1126/science.1214804.
[4] B. Sarker, et al. J Mater Chem B 2 (2014) 1470 http://dx.doi.org/10.1039/c3tb21509a.
[5] T. Zehnder, et al. Biofabrication 7 (2015) 1–12. http://dx.doi.org/10.1088/1758-5090/7/2/025001.
[6] A. Grigore, et al. Tissue Eng Part A 20 (2014) 2140–2150. http://dx.doi.org/10.1089/ten.tea.2013.0416.
[7] J. Klein, et al. Eur J Appl Microbiol Biotechnol 18 (1983) 86–91. http://dx.doi.org/10.1007/BF00500829.