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Abstract

Marine biopolymers for DLP-bioprinting of vital bone constructs

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Osteoarthritis is a degenerative cartilage disease influenced by aging, mechanical stress, and inflammation. As the disease progresses, bone tissue is affected, leading to osteochondral defects. Traditional, systemically applied therapies often show limited effectiveness, highlighting the need for more targeted treatment options. Hydrogels serve as suitable materials for the fabrication of vital implants via digital light processing (DLP) bioprinting. This technique enables precise 3D printing of constructs loaded with cells, bioactive factors or photoactive delivery systems, through UV-mediated hydrogel gelation. The biophysical properties of marine biopolymers may offer biophysical advantages for this technology, but their potential has not been explored yet. In this study, we evaluated the use of marine polymer-based hydrogels for DLP bioprinting of cell-laden 3D constructs. Hydrogels were composed of methacrylated alginate combined with varying concentrations of jellyfish collagen. Bioinks included photoinitiators and a photoabsorber, as reported elsewhere[1]. Osteogenic cells were mixed with the bioinks and printed using a Lumen X printer at 37 °C. The designed constructs (height: 3.64 mm, diameter: 7 mm) included a interconnective grid of 500 µm pores and some larger pores with 2000 µm and were post-crosslinked with 50 mM $CaCl_2$. We assessed cellular viability and morphology using Calcein-AM/Hoechst staining, focal adhesion kinase/actin labeling, followed by confocal or scanning electron microscopy, and DNA quantification over 14 days. Pure methacrylated alginate showed excellent printability in agreement with the 3D model. Additional $CaCl_2$ crosslinking was however essential to maintain the structural stability of the constructs. Nevertheless, pure aliginate lacks RGD binding sites mediating cell adhesion. Thus, alginate constructs profit from combination with other extracellular matrix proteins, such as marine collagen with a lower melting temperature compared to mammalian collagens which gelate above 20°C. Calcein-AM staining, DNA quantification, and SEM indicated cellular viability over 14 days in marine collagen containing constructs. Our findings indicate that marine collagen is beneficial for cell adhesion and supports the viability of cells in the investigated time frame. In addition, further experiments are on the way to assessing the impact of marine collagen on osteogenic differentiation. Overall, due to its lower melting point marine collagen may serve as a promising alternative to mammalian collagen enabling bioprinting at physiological temperatures.

Author's statement

Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee. Acknowledgments: We thank BMBF WIR!-BlueHealthTech-BlueBioPol (FKZ 03WIR6207A.BMBF) for financially supporting this work and Ocean Basis for the supply of marine collagen.

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