

Abstract

Investigating inter-layer crosslinking of alginate-gelatine hydrogels to modify layer adhesion

A. Behrends^{1*}, L. Evers¹, A. C. Dell¹, R. Leonov¹, D. Wendt¹, Th. Friedrich¹ and T. M. Buzug¹

¹ Fraunhofer Research Institution for Individualized and Cell-Based Medical Engineering, Luebeck, Germany * Corresponding author, email: <u>andre.behrends@imte.fraunhofer.de</u>

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Alginate-gelatin hydrogels are widely employed as bioink components in bioprinting due to their ability to mimic tissuelike functionalities. To enhance the structural integrity of bioprinted objects made from these hydrogels, ionic crosslinking is typically performed using calcium chloride after the entire object has been printed [1-4]. This study investigates the effects of inter-layer crosslinking applied between the deposition of successive printing layers on layer adhesion.

Test structures were printed using a commercial bioprinter (CELLINK BIO X6, CELLINK, Gothenburg, Sweden). The test structures consist of two-layer rectangular shapes measuring 30 mm by 30 mm. After extruding the first layer of hydrogel, we printed calcium chloride as a crosslinker using drop-on-demand printing. Subsequently, the second layer of hydrogel was extruded using a pneumatic printhead. We varied key parameters, including calcium chloride concentration, volume of crosslinker deposited per unit area, and the time interval between crosslinker application and the extrusion of the second layer.

Preliminary findings indicate that inter-layer crosslinking improves structural integrity, potentially increasing stiffness and reducing delamination under stress. However, in some instances, reduced inter-layer adhesion may be beneficial—such as simplifying the removal of support structures. These findings emphasize the dual functional potential of controlled crosslinking in bioprinted constructs, highlighting its role in achieving enhanced performance during cell culture or implantation.

AUTHOR'S STATEMENT

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REFERENCES

- [1] A. GhavamiNejad, N. Ashammakhi, X. Y. Wu, et al., *Crosslinking Strategies for 3D Bioprinting of Polymeric Hydrogels*, Small, 16(35):e2002931, 2020, doi: 10.1002/smll.202002931.
- [2] M. B. Łabowska, K. Cierluk, A. M. Jankowska, et al., A Review on the Adaption of Alginate-Gelatin Hydrogels for 3D Cultures and Bioprinting, Materials (Basel). 14(4):858, 2021, doi: 10.3390/ma14040858.
- [3] A. A. Soufivand, J. Faber, J. Hinrichsen, et al., *Multilayer 3D bioprinting and complex mechanical properties of alginate-gelatin mesostructures*, Sci Rep, 13, 11253, 2023, doi: 10.1038/s41598-023-38323-2.
- [4] D. Kang, Z. Liu, C. Qian, et al., 3D bioprinting of a gelatin-alginate hydrogel for tissue-engineered hair follicle regeneration, Acta Biomaterialia, 165, pp. 19-30, 2023, doi: 10.1016/j.actbio.2022.03.011.