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Strategies to evaluate alginate based bioinks applying extrusion printing for biofabrication

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Abstract: For extrusion bioprinting, tailored bioinks combining tissue-like mechanical properties, 3D printability and high biocompatibility are needed. The strategy of this project includes the precise characterization of the synthesis route and material properties, description of the printing process, as well as computing of flow and shear stress profile inside the printing needle. Furthermore, in vitro analyses using lentiviral fluorescent reporter cells were performed, which is a novel method that has so far hardly been used in biofabrication. The evaluation of the hydrogel and printing process for successful biofabrication is a multi-stage process and in our case demonstrated that ADA-GEL is a promising bioink.

I. Introduction

Various additive manufacturing technologies have been used to produce three-dimensional (3D) complex bio-artificial structures, while the extrusion process is currently probably the most widely used technology [1]. For this bioprinting technology, tailored bioinks combining tissue-like mechanical properties, 3D printability and high biocompatibility are needed. Sodium alginate, a well-known natural polymer, is a favorable material in biofabrication because of its high biocompatibility, mild gelation behavior at room temperature and tuneable properties. An obvious disadvantage of alginate is its poor ability to support cell attachment due to the lack of efficient sites for cell adhesion.

The aim of this work was to improve alginate from brown algae by oxidation to alginate dialdehyde (ADA) in order to increase its binding affinity to gelatine containing arginine-glycine-aspartic acid (RGD) binding sequences [2-3]. The strategy of this project also includes the characterization of the hydrogel synthesis, materials properties, description of the printing process by grid structure test (GST) and filament fusion test (FFT), computation of the pressure-flow relationship of shear-thinning bioinks as well as determination of the flow and shear stress profiles inside the printing needle. Furthermore, in vitro analyses using lentiviral fluorescent reporter cells were performed, which is a novel method that has so far hardly been used in biofabrication.

II. Material and methods

Pure alginate (PH176, approval as pharmaceutical excipient, JRS PHARMA GmbH & Co. KG, Germany) solution with a concentration of 2 % (w/v) was used as a reference bioink. Covalently crosslinked alginate-dialdehyde (ADA) gelatine (GEL) hydrogel was synthesized similar to the method described by Sarker et al [3]. ADA was prepared by the oxidation of alginate using sodium (meta) periodate under the complete absence of light. The final suspension was dialyzed for five days and lyophilized afterwards, in order to obtain pure ADA as dry product. For the preparation of the bioink hydrogel, an aqueous gelatine (Type A, derived from porcine skin, gel strength 300) solution was added into an ADA solution and covalently crosslinked.

Lentiviral, fluorescent reporter cells stably expressing the FUCCI cell cycle sensor to monitor cell proliferation or farnesylated tdTomato protein (plasmamembrane-labeling) to monitor cell morphology were generated using NIH/3T3 fibroblasts as parental cells [4]. Transduced cells were embedded in 2% alginate and 2.5% ADA-GEL at $1x10^6$ cells/ml and analyzed at different time points after extrusion printing.

ADA-GEL constructs were fabricated at room temperature using a bioprinter. For the evaluation, the same set up was used: nozzle diameter (200 µm), pressure (15-45 kPa), geometry and amount of layers. By using a 0/90° lay-down-pattern a 4 layered biofabricated construct was produced layer by layer, and afterwards printing quality was analysed (GST). For FFT, meandering pattern was designed, consisting of parallel strands with a total length of 20 mm and decreasing strut distance. In order to model shear stress and residence time profiles, different models were tested. In rheological shear-flow sweeps of ADA-GEL, no Plateau region at low shear rates could be observed. Thus, a power-law model was used to fit the data and model the profiles, as described by *Paxton et al.* [5]. Contrary to this, a clear plateau region could be observed for alginate solutions. Thus, in this case a Careau-Yasuda model was used to fit the rheological shear-flow sweeps and further to model the profiles with the help of the following online tool: https://bio.physik.fau.de/flow_webpage/flow.html

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III. Results and discussion

The successful, reproducible use of bioinks requires a valid characterization of material properties. The materials used in this study were examined with regard to molecular weight, mannuronic/guluronic acid ratio, degree of oxidation and cross-linking, and rheological properties. In addition to standard cytotoxicity tests (data not shown), we used NIH/3T3-derived FUCCI cell cycle sensor and tdTomato-Farnesyl reporter cells to biologically evaluate both inks. While the fraction of proliferating cells (green) was comparable in 3D alginate and ADA-GEL after 48 h of culture, it significantly dropped in alginate, but only slightly in ADA-GEL after 96 h (Fig. 1A, B). In addition, cells were able to elongate and spread in ADA-GEL, but not alginate (Fig. 1C, D).

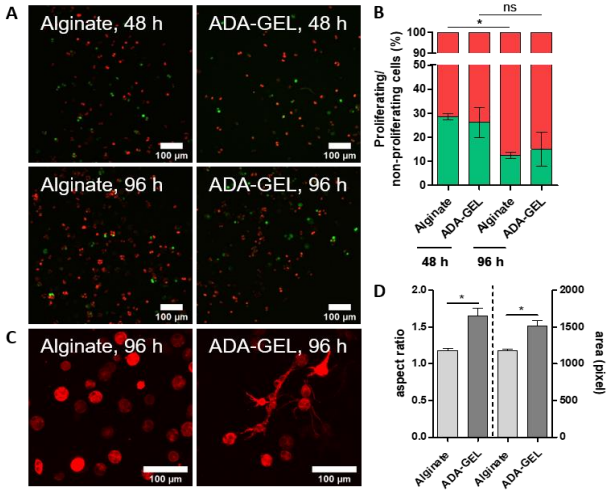

*Figure 1: Confocal microscopy analysis of cell proliferation (A, B; FUCCI, green: prolif. cells, red: non-prolif. cells) and morphology (C, D; tdTomato-Farnesyl) of NIH/3T3-reporter cells in 3D alginate vs. ADA-GEL. * p < 0.05.*

These results demonstrate that the adhesive motives present in ADA-GEL significantly improve cell adhesion, spreading and proliferation and further exemplify the applicability of live cell fluorescence microscopy approaches to monitor cells in 3D biofabrication.

The printing assessments showed significantly increased uniformity and accuracy of ADA-GEL constructs, compared to those made from alginate (Fig. 2).

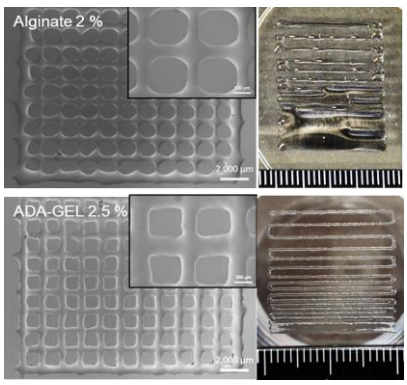

Figure 2: GST- and FFT-based evaluation of printability for alginate and ADA-GEL.

The FFT analysis demonstrated a higher resolution for ADA-GEL bioink compared to alginate. Images in A and C show maximum projections of 3D confocal scans. * p < 0.05.

The maximum shear stress for ADA-GEL was just below 100 Pa and around 115 Pa for alginate (Fig. 3). Both inks displayed residence times below 10 s for the bulk of the hydrogel. However, ADA-GEL showed a "plug-flow" pattern with residence times below 5 s over large parts of the needle radius, compared to alginate with considerably higher residence times in the needle periphery. These data suggest that shear stress induced damages might be lower with ADA-GEL.

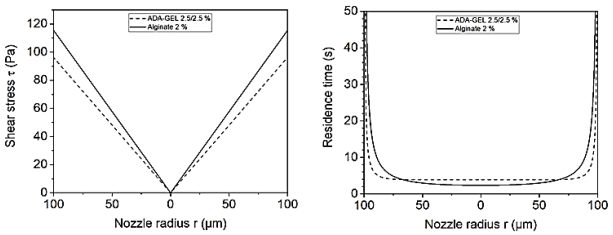

Figure 3: Computed shear stress and residence time profiles for alginate and ADA-GEL. ADA-GEL: p = 0.25 bar; alginate: p = 0.3 bar; Nozzle size for both: length = 1.3 cm; radius = 100 µm.

IV. Conclusions

Chemical analysis of the bioink, and characterisation of the cell-material interaction and printability as well as shape fidelity provide information for a successful biofabrication approach. Here, the methods used confirmed that ADA-GEL is a promising bioink. In future, we will correlate these results with the assessment of the long-term cell activity to identify, analyse and modulate molecular mechanisms that govern cell behaviour in such ADA-based bioinks.

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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.

References

[1] Groll, J., et al. (2018). "A definition of bioinks and their distinction from biomaterial inks." Biofabrication, 11(1), 013001.
[2] Detsch, R., Sarker, B., Zehnder, T., Frank, G., and Boccaccini, A. R. (2015). "Advanced alginate-based hydrogels." Materials Today, 18(10), 590–591.
[3] Sarker, B., et al.. (2014). "Fabrication of alginate–gelatin crosslinked hydrogel microcapsules and evaluation of the microstructure and physico-chemical properties." Journal of Materials Chemistry B, 2(11), 1470.
[4] Koh, S.-B., et al. (2017). "A quantitative FastFUCCI assay defines cell cycle dynamics at a single-cell level." Journal of Cell Science, 130(2), 512–520.
[5] Paxton, N., Smolan, W., Böck, T., Melchels, F., Groll, J., and Jungst, T. (2017). "Proposal to assess printability of bioinks for extrusion-based bioprinting and evaluation of rheological properties governing bioprintability." Biofabrication, 9(4), 044107.