Time dependent Alginate Dialdehyde-Gelatin bioinks for cardiac regeneration

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Cardiovascular diseases represent an unmet global clinical challenge due to heart tissue limited intrinsic regenerative capacities. Scalable and reproducible 3D bioprinted cell-laden constructs are a promising approach as therapeutic patches for cardiac regeneration or as *in vitro* models for new drug preclinical discovery and validation.
Alginate (Alg)-based bioinks have been widely investigated thanks to Alg tunable properties and cost effectiveness. Alg internal ionic gelation mechanism allows to obtain homogeneous self-standing 3D printed filaments without the use of support baths or post-printing crosslinking treatments. Herein, oxidized alginate (ADA), Alg and gelatin (Gel) were blended to obtain hydrogels able to support cell adhesion and having controlled *in vivo* degradability. In detail, novel Alg-ADA-Gel bioinks were optimized exploiting internal crosslinking and bioprinted with adult human cardiac fibroblasts (AHCFs) for perspective applications in *in vitro* cardiac tissue engineering.
Alg-ADA bioink composition was tailored to achieve cardiac tissue-like viscoelastic properties by varying polymer weight ratio and calcium ions concentration. Gel incorporation into Alg-ADA was then optimized to support AHCF adhesion, varying (Alg-ADA):Gel (%w/w) ratios from 100:0 to 75:25. Alg-ADA-Gel showed tunable viscoelastic properties (G' 650-1300 Pa) and degradation profile (40-80% weight loss after 21 days in PBS) by varying Gel concentration. Alg-ADA-Gel printability was investigated over time, showing a time-dependent shear thinning due to the gradual pH-triggered release of calcium ions over time. Finally, 3D AHCF-laden Alg-ADA-Gel bioinks could be successfully printed and the samples with the highest gelatin content (25% w/v) allowed AHCFs adhesion after 24 hours of incubation.
In conclusion, novel Alg-ADA-Gel inks based on internal gelation mechanism were optimised to provide time-dependant crosslinking features suitable for bioprinting. AHCFs-loading in the optimized inks allowed to print 3D cell-laden constructs with potential application for cardiac tissue modeling or regeneration.

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