Time-dependent natural polymer-based bioinks for cardiac tissue engineering

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Introduction:

Cardiovascular diseases represent a worldwide social and economic challenge, due to heart tissue limited regenerative capabilities. 3D bioprinted cell-laden constructs are a promising approach to develop patches for cardiac regeneration or in vitro models for new drug preclinical discovery and validation [1].

Alginate (Alg) is widely studied as ink material thanks to its cost-effectiveness and tunable features. Alg internal ionic gelation mechanism allows to obtain homogeneous self-standing 3D printed filaments without employing support baths or post-printing crosslinking treatments [2]. 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 an internal crosslinking mechanism and bioprinted with adult human cardiac fibroblasts (AHCF) for *in vitro* cardiac tissue engineering.

Methods:

Alg/ADA bioink composition was optimized varying polymer weight ratio and calcium ion concentration. Hydrogels were prepared with different (Alg+ADA):Gel (%w/w) ratios from 100:0 to 70:30, and characterised by rheological analysis and *in vitro* stability in PBS at 37°C. The printability of Alg/ADA/Gel hydrogels was assessed through optical imaging. Finally, cell viability of AHCFs-laden Alg/ADA/Gel bioinks was analysed by fluorescence microscopy.

Results:

Alg/ADA hydrogels composition was tailored to achieve cardiac tissue-like viscoelastic properties. The introduction of ADA allowed to achieve higher degradation rate (40% weight loss) compared to Alg samples (25% weight loss) after 21 days in PBS. Gel incorporation into Alg/ADA produced inks with tuneable viscoelastic properties (G' 650-1300 Pa) by varying Gel concentration. Alg/ADA/Gel hydrogels showed a shear thinning behaviour suitable for 3D bioprinting and dependent on ink stabilization time, due to the gradual pH-triggered release of calcium ions over time. AHCF-laden Alg/ADA/Gel bioinks could be successfully printed producing scaffolds with high shape fidelity and good cell viability, indicating biocompatibility of the bioinks and cell-friendly printing conditions.

Conclusions:

Novel Alg/ADA/Gel bioinks 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 modelling or regeneration.

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References: 1. Paoletti, et al. Cells, 7: 114, 2018; 2. Sardelli, et al., Soft Matter, 17: 8105-8117, 2021.