Alginate Dialdehyde-Gelatin based inks for bioprinting in cardiac tissue engineering

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Keywords – Alginate, gelatin, bioink, in vitro cardiac models

INTRODUCTION

Cardiovascular diseases represent a tremendous social and economic worldwide burden. New effective therapies are highly demanded as alternatives to heart transplantation, the only therapeutic option in case of end stage heart failure. Scalable and reproducible bioprinted constructs can be fabricated as therapeutic patches for cardiac regeneration or the development of *in vitro* models of human cardiac tissue for preclinical validation of new advanced approaches [1].

Alginate (Alg)-based bioinks have been widely investigated thanks to their tunable properties and cost-effectiveness. Ionic crosslinking of Alg ink via pH-triggered release of calcium ions from insoluble calcium salts (internal ionic crosslinking mechanism) allows the 3D printing of uniform self-supporting fibers without the need for post-crosslinking or the use of support materials [2]. However, Alg presents limited *in vivo* degradability and poor cell adhesion. To overcome these hurdles, herein oxidized alginate (ADA) and gelatin (Gel) were combined with Alg.

In this study, novel Alg-ADA-Gel hydrogels based on internal gelation mechanism were optimized for applications as bioinks for *in vitro* cardiac tissue engineering.

MATERIALS AND METHODS

ADA was prepared by partial oxidation of Alg via sodium metaperiodate and characterized through MAS Solid-State NMR spectroscopy and via the iodine–starch test to determine the degree of oxidation [3]. Initially, Alg-ADA hydrogels were optimized varying the final calcium ions concentration (0.1-3%w/v) and Alg:ADA weight ratio (%w/w) (from 100:0 to 50:50). Then, Gel was added as additional component in Alg-ADA hydrogels, varying the polymer weight ratios of (Alg+ADA):Gel (%w/w) from 100:0 to 70:30, while keeping constant the final polymer concentration (%w/v). Rheological characterization through time sweep, amplitude sweep and shear rate analysis was performed. *In vitro* degradation studies were conducted in phosphate buffered saline (PBS) at 37°C for 21 days. *In vitro* cytocompatibility studies of Alg-ADA-Gel hydrogels were performed using adult human cardiac fibroblasts (AHCFs) according to ISO 10993. Finally, AHCFs were cultured on Alg-ADA-Gel hydrogels for 1 and 7 days, and cell behaviour was assessed by fluorescence microscopy.

RESULTS AND DISCUSSION

ADA was produced with an overall yield of 75% w/w and an oxidation degree of 25%. ^{13}C MAS NMR analyses of ADA confirmed the successful formation of free reactive aldehyde groups. Firstly, the composition of Alg-ADA hydrogels was optimized to obtain viscoelastic properties close to those of cardiac tissue. Alg-ADA hydrogels showed rheological properties suitable for 3D bioprinting and dependent on ink stabilization time, due to the progressive release of calcium ions over time. Moreover, the presence of ADA increased the degradation rate of hydrogels, with optimized Alg-ADA samples showing higher weight loss (42%) compared to Alg-samples (25%) after 21 days.

Then, Gel was incorporated into Alg-ADA at a proper amount to support AHCF adhesion. Shear thinning inks with tunable viscoelastic properties (G’ 650-1300 Pa) were obtained by varying Gel concentration. *In vitro* cell tests showed the cytocompatibility of Alg-ADA-Gel hydrogels. Moreover, the presence of Gel significantly increased AHCFs adhesion compared to Alg-ADA hydrogels.

CONCLUSIONS

Novel Alg-ADA-Gel inks with internal gelation mechanism were optimised to provide suitable properties for bioprinting. Cell loading in the developed inks is currently in progress to bio-print constructs to be exploited in cardiac tissue modeling or treatment.

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ACKNOWLEDGEMENTS

This project is supported from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (BIORECAR GA N° 772168). PoC Instrument project POLHYGENTH (Compagnia di San Paolo – Fondazione Links) is also acknowledged.