3D bioartificial stretchable scaffolds for an *in vitro* model of fibrotic cardiac tissue

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INTRODUCTION

Cardiovascular diseases, including myocardial infarction, are the main cause of mortality worldwide. Cardiac fibrosis typically arises after myocardial infarction, causing progressive heart failure. Heart transplantation still represents the only available therapy for end-stage heart failure. Hence, new advanced regenerative approaches are under investigation to reduce or revert cardiac fibrosis [1] and *in vitro* human cardiac fibrotic tissue models can support their preclinical development and reduce *in vivo* animal trials in agreement with 3Rs principle. Herein, stretchable scaffolds, able to mimic the biophysical and biochemical behaviour of fibrotic human cardiac tissue, were designed to develop *in vitro* testing platforms.

MATERIALS AND METHODS

Polycaprolactone (PCL)-based scaffolds with parallel-wavy fibers were designed by means of ordinary mechanical methodologies and verified through finite element analysis (FEA). They were fabricated by melt-extrusion additive manufacturing (MEAM) (Fig. 1 a-b). Scaffold pores were filled with gelatin methacrylate (GelMA) hydrogels, mimicking extracellular matrix-like microenvironment (Fig. 1c) [2]. GelMA hydrogels with different concentrations were prepared using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as photoinitiator (Fig. 2). PCL scaffolds were surface functionalized with poly(4-Dihydroxy-DL-phenylalanine) (polyDOPA) to improve interfacial adhesion with GelMA hydrogels. Photorheology allowed to select the optimal hydrogel curing protocol preserving biocompatibility. Human cardiac fibroblasts (PromoCell) were encapsulated in GelMA hydrogels and cultured up to 2 weeks. Cell viability (resazurin assay) and myofibroblast protein expressions were analyzed. GraphPad Prism® software was employed for analysis of variance (ANOVA). Static and cyclic tensile tests were performed on PCL/GelMA scaffolds.

RESULTS AND DISCUSSION

Stretchable PCL scaffolds with high shape fidelity were obtained by optimizing MEAM processing parameters (Fig. 1a-b). Analytic and FEA analyses allowed the validation of the experimental results. Mechanical properties of PCL/GelMA scaffolds (Fig. 1c) were tuned by PCL scaffold geometry, and GelMA hydrogel concentration in order to mimic the stiffness (1-9 MPa) [3] and maximum elastic strain (15-22%) [4] of human cardiac fibrotic tissue. GelMA hydrogel pores sizes increased by reducing GelMA concentration (Fig. 2). PCL/GelMA scaffolds preserved stretchability and integrity after both static and cyclic tensile tests. Cardiac fibroblasts viability tests on GelMA hydrogels showed their biocompatibility. DAPI, Phalloidin and α-SMA staining showed homogeneous cell distribution inside GelMA hydrogels.

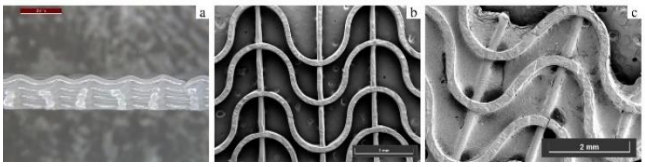

Figure 1. a) Optical and b) SEM images (40x) of PCL scaffold. c) SEM image of PCL/GelMA scaffold (37x).

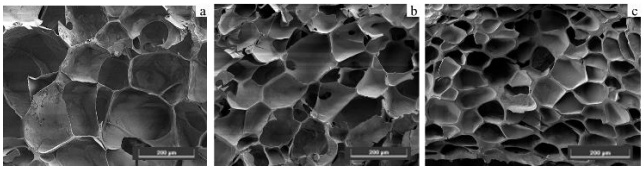

Figure 2. SEM images (280x) of a) GelMA hydrogel at 5% w/v, b) 7% w/v and c) 10% w/v.

CONCLUSIONS

Stretchable PCL/GelMA scaffolds were designed for cardiac cells culture, to reproduce post-infarct human cardiac tissue at different severity degrees tailoring the stiffness of GelMA hydrogels. Such innovative predictive *in vitro* models of human cardiac fibrotic tissue will be exploited for preclinical validation of advanced cardiac regenerative strategies [1], under mechanical stimulation.

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