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

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INTRODUCTION

Myocardial infarction (MI) is the main cause of morbidity and mortality. After MI, heart undergoes phenotypic changes leading to cardiac fibrosis and progressive heart failure. Nowadays, the only available therapy allowing the recovery of cardiac function is heart transplantation, while new advanced regenerative therapies are under investigation[1]. *In vitro* testing platforms able to mimic the biophysical behavior of pathological human cardiac tissue could reduce *in vivo* animal trials following 3Rs principle. Previous literature reported *in vitro* cardiac tissue models based on natural hydrogels, which are limited by the lack of structural cues and fast degradation rate. Herein, stretchable scaffolds, provided with biomimetic biochemical and biophysical cues were designed for the *in vitro* modelling of mechanically-stimulated fibrotic cardiac tissue.

EXPERIMENTAL METHODS

Scaffolds with parallel-wavy fibers pattern were fabricated by melt-extrusion additive manufacturing (MEAM) from polycaprolactone (PCL) and analyzed by SEM. Scaffold pores were filled with gelatin methacrylate (GelMA) hydrogels, mimicking extracellular matrix (ECM)-like microenvironment[2]. PCL scaffolds were surface functionalized with poly(4-Dihydroxy-DL-phenylalanine) (polyDOPA) to improve interfacial adhesion with GelMA hydrogels. GelMA hydrogels with different concentrations were initially prepared, using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as photoinitiator. Photorheology allowed to define the optimal hydrogel curing protocol. Human cardiac fibroblasts (HCFs, PromoCell) were embedded in GelMA hydrogels (cell density: $5 \cdot 10^6$ cells/ml) 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. Further *in vitro* cell tests on PCL/GelMA scaffolds are ongoing.

RESULTS AND DISCUSSION

MEAM processing parameters were optimized to fabricate scaffolds with high shape fidelity, as demonstrated by their morphological analysis by optical microscopy and SEM (Fig 1a-b). Mechanical properties of PCL/GelMA scaffolds (Fig 1c) were tailored by PCL fibre diameter, mesh geometry, and GelMA hydrogel concentration in order to mimic the stiffness (1-9 MPa)[3] and maximum elastic deformation (15-22%)[4] of human cardiac fibrotic tissue.

PCL/GelMA scaffold stretchability and integrity were preserved after both static and cyclic tensile tests.

HCFs viability tests on GelMA hydrogels showed that the addition of LAP and UV curing parameters did not exert cytotoxicity effects. Phalloidin and α-SMA staining showed homogeneous cell distribution in GelMA hydrogels and HCFs acquisition of a fibrotic phenotype.

Immunofluorescence analysis performed after 1 and 2 weeks culture time showed the secretion of collagen Type I and IV in cell-laden GelMA hydrogels, suggesting fibrotic ECM deposition. *In vitro* cell tests on PCL/GelMA scaffolds are in progress.

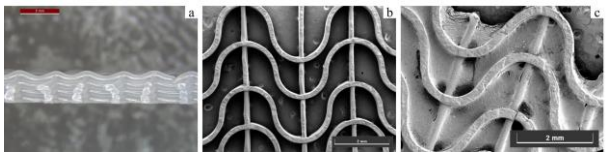

Fig.1 a) Optical and b) SEM images (40x) of PCL scaffold. c) SEM image of PCL/GelMA scaffold (37x).

CONCLUSION

In this work stretchable PCL/GelMA scaffolds were designed for the *in vitro* engineering of post-infarct human cardiac tissue. Different fibrotic conditions could be mimicked by tailoring the concentration of GelMA hydrogel filling PCL scaffolds. In the future, cells will be cultured on stretchable PCL/GelMA scaffolds in dynamic conditions (bioreactor), to get innovative predictive *in vitro* models of human cardiac fibrotic tissue. The models will allow preclinical validation of advanced cardiac regenerative strategies[1], by long-term testing under mechanical stimulation.

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