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In vitro models of human cardiac fibrotic tissue based on bioartificial scaffolds

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Cardiomyocytes represent 70-85% of cell population in the adult heart and are the main responsible of the cardiac contractile activity. Myocardial infarction causes the loss of billions of cardiomyocytes and the progressive formation of fibrotic scar. Fibrotic tissue is mechanically stiffer than healthy cardiac tissue, and is mainly populated by cardiac fibroblasts, unable to undergo contraction [2]. *In vitro* models of infarcted tissue represent a key tool to evaluate new therapies for cardiac regeneration. In this work, a model of fibrotic heart tissue was designed and fabricated by culturing human cardiac fibroblasts (HCFs) on bi-dimensional (2D) and three-dimensional (3D) bioartificial scaffolds.

Polycaprolactone (PCL, Mw = 43,000 Da) 2D scaffolds were prepared by electrospinning from chloroform/formic acid (70/30 v/v) solutions to obtain fibrous membranes with both aligned and random morphology. PCL 3D scaffolds with different geometries were produced by melt-extrusion additive manufacturing technique, using a hybrid bio 3D printer (Rokit Invivo). Subsequently, scaffolds were functionalised with gelatin through a mussel-inspired approach based on two steps: (i) 3,4-Dihydroxy-D,L-phenylalanine (DOPA, 2 mg/mL in Tris/HCl 10 mM, pH 8.5) polymerisation on the PCL surface; (ii) incubation in gelatin solution for 16 h [3].

HCFs isolated from human ventricle were cultured at a density of $7x10^4$ and $30x10^4$ cells/cm^2 on 2D and 3D scaffolds, respectively. Their survival, adhesion, proliferation and morphology were investigated through biochemical assays and fluorescence microscopy analyses.

SEM showed that 2D PCL-scaffolds consisted of homogeneous nanofiber membranes, while 3D PCL-scaffolds presented a reproducible interconnected porous structure. QCM-D analysis was performed to follow the functionalisation steps: polyDOPA deposition followed by gelatin grafting. ATR-FTIR and colorimetric assay confirmed successful surface modification for both 2D and 3D scaffolds. Bulk properties did not change after the surface modification, as suggested by unchanged mechanical (tensile stress-strain test) and thermal (DSC analysis) properties. HCFs cultured on gelatin grafted scaffolds showed better attachment and proliferation compared to non-functionalized scaffolds, as well as the deposition of cardiac extracellular matrix (ECM). Moreover, HCF morphology and F-actin expression were investigated as a function of morphology and surface composition.

In conclusion, biomimetic scaffolds able to support the proliferation of HCFs were developed and proposed as promising models of human cardiac fibrotic tissue. The effect of scaffold properties (composition, structure and surface mechanical properties) on the expression of fibroblast markers (α-SMA, Vimentin, DDR2), ECM composition and on direct cardiac reprogramming to cardiomyocytes will be evaluated. Moreover, the obtained models will be validated through the comparison with human cardiac tissue and used to test *in vitro* new cardiac regenerative strategies, e.g. direct cardiac reprogramming.

References

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