"Bioartificial" scaffolds for the development of *in vitro* models of human cardiac fibrotic tissue

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Introduction

Heart failure is a global pathological condition affecting approximately 26 million people worldwide (1). Cardiac contractile activity is performed by cardiomyocytes, muscular cells that occupy 70-85% of the adult heart (2). When obstruction of coronary arteries occurs, cardiomyocytes in the left ventricle die, with the progressive formation of a fibrotic scar. Fibrotic tissue is mechanically stiffer than healthy cardiac tissue, and is mainly populated by cardiac fibroblasts, unable to undergo contraction (3). In this work, a model of fibrotic heart was designed by culturing human cardiac fibroblasts on bioartificial scaffolds with aligned or random morphology. Scaffolds were found to mimic the morphological and biological features (e.g. cell-cell and cell-extracellular matrix (ECM) interactions) of infarcted cardiac tissue. *In vitro* models of infarcted tissue represent a key tool in evaluating new therapies for cardiac regeneration.

Experimental part

Polycaprolactone (PCL) scaffolds were prepared by electrospinning (Linari Engineering) from chloroform/formic acid (70/30 v/v) solutions (flow rate: 1.5 mL/min; voltage: 20 kV; needle-collector distance: 20 cm) to obtain fibrous membranes with both aligned and random morphology, as well as high surface-to-volume ratio for subsequent functionalisation with gelatin. Gelatin was grafted through a mussel-inspired approach based on two steps: (i) initial 3,4-Dihydroxy-D,L-phenylalanine (DOPA) polymerisation (2 mg/mL DOPA solution in Tris/HCl 10 mM buffer at pH 8.5) on the fibre surface, followed by (ii) gelatin grafting through dipping in gelatin solution for 16 hours (4).
Samples were characterized after each functionalization step by FTIR-ATR and XPS analyses, SEM for morphological characterisation and tensile mechanical tests. PolyDOPA coating formation and gelatin grafting were monitored in real-time by using quartz crystal microbalance with dissipation technique. Cardiac fibroblasts isolated from human ventricle (PromoCell) were cultured onto the substrates at a cell density of $7x10^4$ cells/cm^2, testing their adhesion and morphology. Expression of common fibroblast markers (α-SMA, Vimentin, DDR2) was evaluated by immunofluorescence and western blot analyses as a function of scaffold morphology. Finally, the expression of typical proteins of cardiac ECM on the scaffolds was also investigated.

Results

Porous PCL membranes with nanosized fibres were prepared (Figure 1).

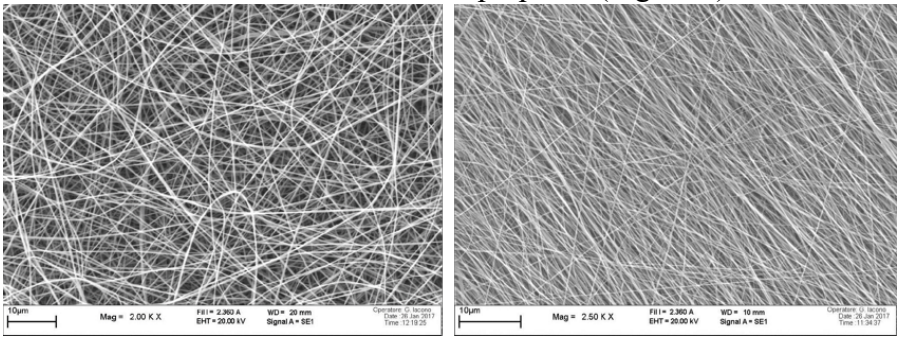

Figure 1. Esemplary SEM images of electrospun PCL membranes: (left) random; (right) aligned.

Surface modification with polyDOPA did not significantly affect the fibrous morphology and the tensile mechanical behaviour of the corresponding unfunctionalized scaffolds.
On the other hand, gelatin grafting slightly increased fibre size. Successful gelatin grafting was demonstrated by FTIR-ATR and QCM analyses. Gelatin grafting favoured attachment and proliferation of cardiac fibroblasts, as well as the deposition of cardiac ECM respect to control scaffolds. The effect of fibrous morphology on cardiac ECM deposition was investigated.

Conclusions

Bioartificial and biomimetic scaffolds able to support the proliferation of cardiac fibroblasts were developed and proposed as promising models of human cardiac fibrotic tissue, for testing new cardiac regenerative strategies, e.g. direct cardiac reprogramming. In the future the effect of scaffold properties (composition, structure and surface mechanical properties by modulating coating stiffness by gelatin crosslinking) on direct cardiac reprogramming from fibroblasts to cardiomyocytes will be evaluated.

References

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Figure 2: Biorecar project logo.