ENGINEERED MODELS OF FIBROTIC CARDIAC TISSUE AS PREDICTIVE PLATFORMS FOR PRECLINICAL VALIDATION

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Introduction

Myocardial infarction (MI) is the main cause of mortality and morbidity worldwide. After MI, a stiff cardiac fibrotic tissue (CFT) forms, causing a decrease in cardiac ejection fraction [1]. *In vitro* models of pathological cardiac tissue are under development, mainly exploiting cellularized hydrogels, as predictive platforms for preclinical validation of new therapies [2, 3]. However, these models are unable to faithfully reproduce the mechanical properties of CFT. In this work, 2D and 3D models of early-stage human fibrotic tissue were prepared through bioartificial scaffolds with biomimetic architecture, chemical composition and stiffness.

Methods

Polycaprolactone (PCL) was used as scaffold bulk material to reproduce tissue stiffening. CFTs with low and high thickness were engineered from 2D random membranes fabricated by solution electrospinning and 3D square-meshed scaffolds prepared by melt-extrusion additive manufacturing, respectively. Type A Gelatin (G) was grafted on PCL scaffolds surface after 3,4-Dihydroxy-DL-phenylalanine polymerization (PolyDOPA) , to obtain biomimetic properties. Scaffolds physico-chemical properties were thoroughly investigated. Ventricular human cardiac fibroblasts (v-HCFs) were cultured on scaffolds up to 3 weeks. Immunofluorescence analysis and two-photon microscopy were used to evaluate the activation into fibrotic cell phenotype and the deposition of pathological cardiac ECM on scaffolds.

Results

Electrospun 2D scaffolds random mats showed defect-free nanofibers with 127 ± 33 nm diameter and < 1 µm average pore size. 3D PCL scaffolds (0.7 mm thickness) with square mesh size of 150 µm showed high shape fidelity and porosity degree. Scaffold stiffness was higher than healthy cardiac tissue as measured by surface AFM analysis in wet and dry conditions. Immunostaining showed that scaffold surface mechanical properties and architecture triggered the activation of myofibroblast phenotype and fibrotic-like ECM deposition. Moreover, SEM and two-photon excitation fluorescence showed ECM homogeneous distribution on 2D and 3D scaffolds.

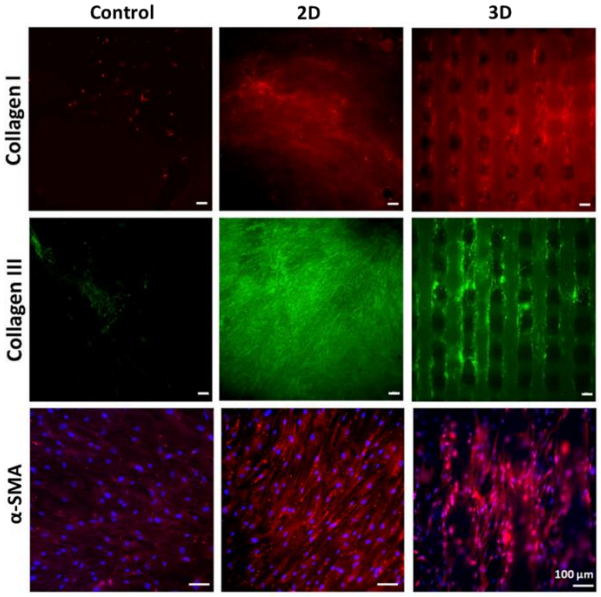

Figure 1: Immunostaining for Collagen -I, -III and α-SMA on v-HCFs cultured for 3 weeks on PCL/polyDOPA/G scaffolds and G-coated glass (control). Nuclei were stained in blue with DAPI.

Discussion

2D and 3D bioartificial PCL scaffolds surface functionalized with polyDOPA/G were prepared, provided with CFT-like surface composition and stiffness. They supported long-term culture of v-HCFs, and triggered their fibrotic activation and pathological ECM deposition. Such *in vitro* models can reproduce patient-specific features of human cardiac fibrosis for the testing of new regenerative therapies.

References

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Acknowledgements

This project is funded by the European Research Council under European Union's Horizon 2020 research and innovation programme grant agreement No-772168.