In vitro models of human pathological cardiac tissue via bioartificial scaffolds

In vitro models of human pathological cardiac tissue *via* bioartificial scaffolds

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1. Introduction

Heart failure is a global pathological condition affecting approximately 26 million people worldwide[1]. After myocardial failure, heart undergoes phenotypic changes with cardiomyocyte death, fibroblast invasion and the progressive formation of a fibrotic scar[2].
In vitro models of infarcted tissue represent a key tool to evaluate new therapies for cardiac regeneration. In the development of an *in vitro* model of cardiac tissue, the complexity of *in vivo* tissue physiology should be reproduced, such as cell adhesion, proliferation and spatial alignment[3].

2. Aim

To design and fabricate a model of fibrotic heart, based on bi-dimensional (**2D**) and three-dimensional (**3D**) bioartificial scaffolds, with aligned or random morphology, able to:

- **mimic morphological and biological features, e.g. cell-extracellular matrix (ECM) interactions) of infarcted cardiac tissue**
- **sustain human cardiac fibroblasts (HCFs) culture**

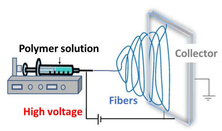
2. Materials and Methods

2.1 Scaffolds

Scaffolds based on polycaprolactone (**PCL**, Mw = 43000 Da) were prepared by 2 techniques:

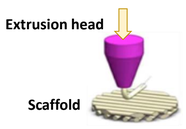
2D membranes - Elettrospinning

- ✓ Solution concentration: 20% w/v
- ✓ Solvent: 70% Chloroform, 30% Formic acid v/v
- ✓ Flow rate: 0.5 mL/h
- ✓ Voltage: 15 kV



3D scaffolds- Melt extrusion printing

- ✓ Temperature: 120°C
- ✓ Nozzle: 200 µm
- ✓ Speed rate : 5-13 mm/s
- ✓ Pressure: 350-600 kPa



2.2 Surface modification

1. 3,4-Dihydroxy-DL-phenylalanine **D,L-DOPA** (Sigma-Aldrich) treament: polyDOPA coating

Tris/HCl pH 8.5
D,L-DOPA 2 mg/mL
7 h, RT
under agitation

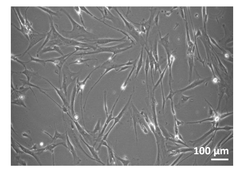
DL- DOPA

2. Type A **gelatin** from porcine skin (Sigma-Aldrich) treatment: polyDOPA/gelatin coating

Tris/HCl pH 8.5
Gelatin 2 mg/mL
16 h, RT

Gelatin

2.3 Cell source



Human Cardiac Fibroblasts (**HCFs,** PromoCell), isolated from human ventricle, were seeded at the following cell densities:

- ✓ 2D: $7x10^4$ cells/cm^2
- ✓ 3D: 2 x 10^5 cells/cm^2

Cells were analysed for viability with Resazurin dye assay (Sigma-Aldrich) and imaged through Phalloidin staining (Life Technologies).

ECM proteins were stained through immunofluorescence after scaffold decellularization treatment (Triton 0.25% e NH_4OH 10 mM in PBS).

3. Results

3.1 Scaffold fabrication

2D scaffold fibrous membranes, characterized by SEM, showed homogeneous nanofibers with few defects (Figure 1A). The average diameter was 130 ± 30 nm (Figure 1B), while major pore area is 0.021-0.66 µm^2 (Figure 1C).

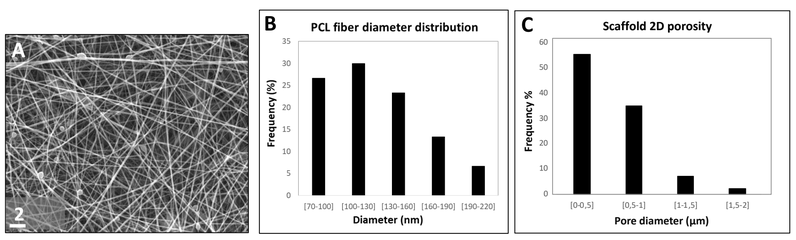
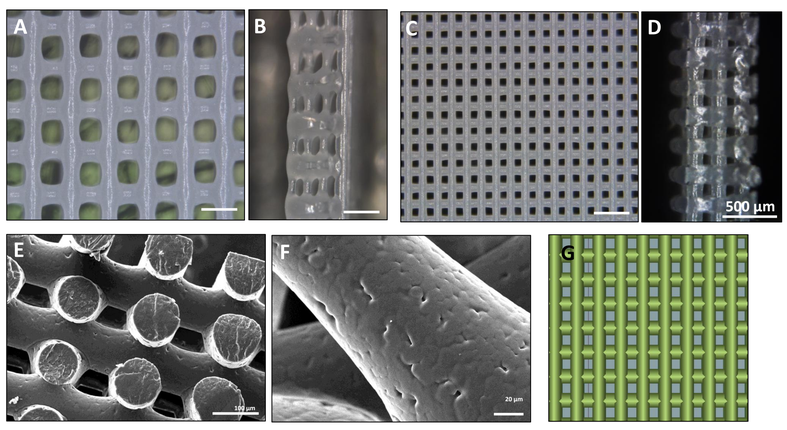


Figure 1. *2D scaffold characterization. A) SEM analysis. B and C) were obtained by ImageJ software analysis.*

3D scaffolds with different pore sizes (Figure 2A-D) and highly interconnected porosities (Figure 2E) were produced using the CAD model showed in Figure 2G. Table reported in Figure 2H summarize geometrical characteristics of the 2 type of scaffold showed in A-B and C-D.



H

Scaffold	Filament distance	Pore Area (top view)	Pore Area (side view)	Scaffold porosity
A-B	350 µm	77 ± 8 ·10^3 µm^2	11 ± 3 ·10^3 µm^2	59%
C-D	150 µm	13 ± 2 ·10^3 µm^2	7 ± 1 ·10^3 µm^2	46%

Figure 2. A-D) *Microscopy images of surface and section of 3D PCL scaffolds with pore dimension of 350 µm (A-B) and 150 µm (C-D). E-F) SEM images of PCL filament interconnection and filament surface. G) CAD model drawing for the generation of the G-code used for scaffold printing. H) Geometrical characteristics measured by image analysis and porosity values determined by gravimetric analysis.*

3.2 Functionalization Characterization

polyDOPA coating growth on PCL was followed through QCM-D analysis (Figure 3), resulting in a rigid layer (dissipation value did not significantly change during DOPA polymerization) while the frequency shift (Δf) was proportional to mass change. On the contrary, gelatin coating deposition caused a Δf decrease and ΔD increase.

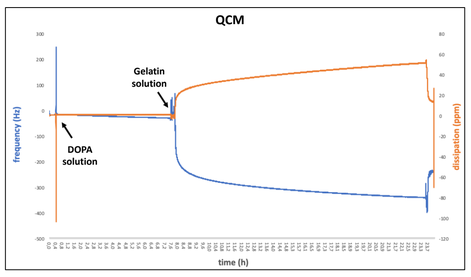


Figure 3. *QCM-D analysis, during DOPA and gelatin deposition. Arrows indicate time of reagent addition.*

After gelatin functionalization, ATR-FTIR spectrum showed the typical adsorption bands of gelatin: amide I (1653 cm^{-1}), amide II bands (1544 cm^{-1}), and a wide band centered at 3300 cm^{-1} referred to the N-H and O-H stretching vibrations (Figure 4).

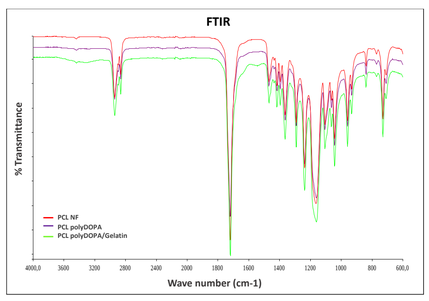
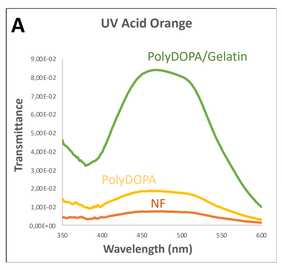


Figure 4. *ATR-FTIR spectrum of not functionalized PCL (PCL NF), PCL functionalized with polyDOPA (PCL polyDOPA) and PCL functionalized with polyDOPA/gelatin (PCL polyDOPA/gelatin).*

Gelatin amount was quantified through Acid Orange assay (Figure 5) and was about 9.3 units/cm^2.



B

PCL sample	Absorbance	NH_2 Concentration [ug/mL]	NH_2 density [10^6 units/cm^2]
NF	0.02 ± 0.01	0.32 ± 0.15	0.84 ± 0.41
PolyDOPA	0.04 ± 0.03	0.92 ± 0.90	2.42 ± 2.40
PolyDOPA/Gelatin	0.08 ± 0.01	1.72 ± 0.20	4.50 ± 0.53

Figure 5. *UV analysis of Acid Orange assay and amino groups quantification. UV-Vis spectra (A) and gelatin density (B).*

3.3 Cell viability, proliferation and ECM release

HCFs were cultured on NF and functionalized 2D and 3D scaffolds.
For all scaffolds, polyDOPA and polyDOPA/gelatin conditions showed improved cell attachment (% of viable cells on the scaffolds after 24 h), compared to NF (Figure 6).

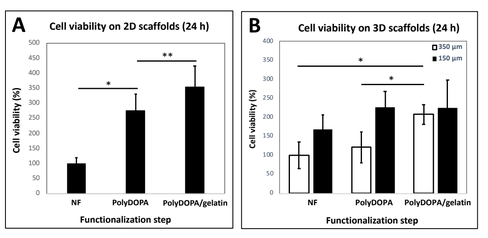


Figure 6. *Resazurin assay on HCFs on PCL scaffolds after 24 h culture. A) Viability on 2D PCL scaffolds. % referrers to NF scaffolds. B) Viability on 3D scaffolds with pore size of 350 µm and 150 µm. % referrers to NF scaffolds-350 µm pore size.* * p < 0.05; ** p < 0.005

After 7 days of culture, polyDOPA/gelatin condition was able to sustain cell culture and proliferation, as shown after Phalloidin staining (Figure 7).

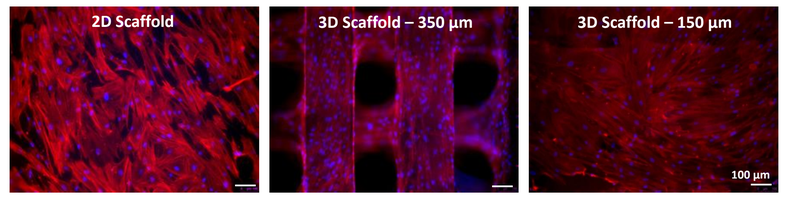


Figure 7. *Phalloidin staining after 7 days culture, on 2D scaffolds and 3D scaffold with pore size 350 µm and 150 µm, all functionalized with polyDOPA/Gelatin. Red: F-actin; blue: nuclei.*

After 21 days of culture, scaffold were decellularized and stained for the detection of typical cardiac ECM proteins, revealing the deposition of Fibronectin and Collagen IV (Figure 8).

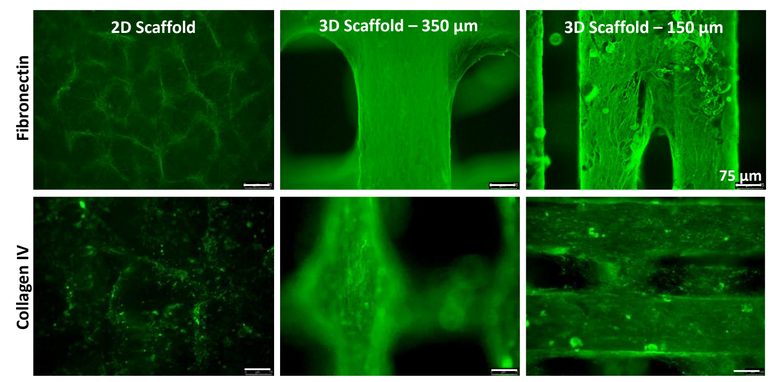


Figure 8. *Immunostaining on decellularized 2D and 3D scaffold (pore size 350 µm and 150 µm), all functionalized with polyDOPA/Gelatin. Top: Fibronectin, Bottom: Collagen IV.*

4. Conclusions and Future Developments

- ✓ Bioartificial scaffolds able to support HCF adhesion and proliferation were developed and proposed as models of human cardiac fibrotic tissue.
- ✓ Future work involves the evaluation of the effect of scaffold properties (composition, structure and surface mechanical properties) on cell proliferation, and on the expression of fibroblast markers and extracellular matrix proteins.
- ✓ The developed models will be exploited for the in vitro testing of new cardiac regenerative strategies.

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Acknowledgments

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