"Functionalised polyurethane scaffolds mimicking cardiac primitive cell niche microenvironment by additive manufacturing"

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INTRODUCTION: Human cardiac fibroblasts (CFs) have been found to deposit *in vitro* a 2D "biomatrix" (BM) with similar composition to the natural cardiac extracellular matrix (ECM) [1]. The study of the behaviour of human cardiac primitive cells (CPCs) in contact with BM showed that laminin-1 (LN1) promotes the adhesion, viability, proliferation and differentiation of CPCs [1]. Recently, a polyurethane (PU) with elastomeric-like properties was synthesised and scaffolds were prepared by melt-extrusion additive manufacturing (AM) [2]. In this work, PU scaffolds were surface functionalised with LN1 and BM with the aim to reproduce CPC-niche microenvironment. Gelatin (G) was also grafted as a control.

METHODS: PU was synthesized from poly(ε-caprolactone) diol (Mn = 2000 Da), 1,4-budandiisocyanate and L-lysine ethyl ester dihydrochloride [2]. Bi-layered scaffolds with 0°/90° lay-down pattern were prepared by additive-manufacturing technique [2]. Functionalisation was performed by two steps: 1) acrylic acid grafting/polymerization following Argon Plasma treatment; 2) carbodiimide-mediated grafting of LN1, G or solubilised BM (produced by *in vitro* culture of CFs, followed by decellularisation and further BM solubilisation in a pepsin solution). Physicochemical characterisation of the surface coating was performed by XPS, FTIR-ATR, colorimetric tests, static contact angle measurements and ELISA assay. In vitro cell tests were performed using CPCs.

RESULTS: PU scaffolds showed a mean fibre diameter of 152±5 μm and mean spacing of 505±5 μm. FITR-ATR analysis of coated scaffolds showed higher intensity of the absorption bands at 3370 cm-1 (-OH and –NH stretching) and 1650 cm-1 (amide I). Contact angle decreased from 90° for PU to 60-70° for LN1, G and BM coated PU. XPS analysis confirmed the successful surface functionalisation. CPC proliferation on PU-LN1 scaffolds was higher than on PU scaffolds, increasing from 8.2 % on day 7 to 11.8% on day 14. LN1-functionalization also stimulated CPC differentiation into cardiomyocytes, smooth muscle cells and endothelial cells (RT-PCR analysis) (Fig.1). Scaffolds were found to slowly degrade *in vitro* by hydrolytic mechanism, whereas using an enzyme (lipase) degradation occurred in 3 weeks. Subcutaneous implantation of scaffolds in mice showed their tissue compatibility, low inflammatory response and low degradation rate (they were stable after 1 month).

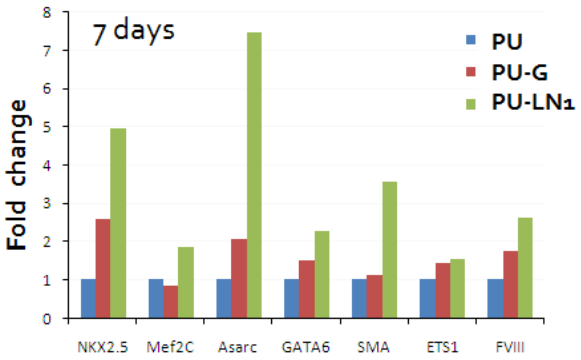

Fig. 1: RT-PCR analysis on CPCs cultures on PU, PU-LN1 and PU-G scaffolds.

DISCUSSION & CONCLUSIONS: PU scaffolds fabricated by AM and surface grafted with LN1 or BM were developed as 3D substrates mimicking CPC niche microenvironment. They could be used as cellularised patches for *in vivo* implantation in myocardial tissue engineering or as *in vitro* models of CPC niches to study CPC behaviour in both normal and pathological conditions.

REFERENCES:

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