Proliferation and Phenotype of Human Coronary Artery Smooth Muscle Cells in Polyurethane Porous Scaffolds under Cyclic Mechanical Strain

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Statement of Purpose: Biomechanical stresses are important considerations in the physiological environment that regulate vascular smooth muscle cell (VSMC) function. Several *in vitro* studies have shown that when VSMCs are subjected to cyclic mechanical strain (CMS) at physiological levels (5-10% strain), they either adopt a more contractile phenotype (reduced cell proliferation[1] and increased expression of contractile proteins[2,3]) or become more proliferative in nature[4]. These conflicting results have been attributed to the variation in cell type, cell species, strain conditions and supporting matrix properties, suggesting that mechanical stimulation modulates cell behavior differently depending on the experimental conditions used.[5] The current study uses a customized bioreactor to apply uniaxial CMS to human coronary artery smooth muscle cells (hSMCs) seeded into degradable polar/hydrophobic/ionic polyurethane (D-PHI) scaffolds. This work aims to study the role of CMS in modulating hSMCs proliferation and phenotype.

Methods: Dumbbell-shaped D-PHI scaffolds (23 mm long by 7 mm wide by 4 mm thick) were synthesized based on a modification of a previously described protocol.[6] A lysine-based divinyl oligomer was polymerized with methacrylic acid and methyl methacrylate monomers (molar ratio of 1:5:15, respectively) in the presence of benzoyl peroxide initiator (0.003 mol/mol vinyl group). A double porogen system consisting of sodium bicarbonate particles (65 wt%, ~90% between 105-420μm) and polyethylene glycol (10 wt%, 600Da) was used to confer macroporosity and microporosity to the scaffolds, respectively. The porosity of the final material was 79 ± 3% as measured by fluid displacement.[6] Adult primary hSMCs (passages 7-9) were seeded at a density of 1×10^6 cells/scaffold onto the upper surface of D-PHI scaffolds. At week 0 (after 3 days of static culture), the cell-scaffold system was subjected to uniaxial CMS (10%, 1Hz) for 2 weeks in a customized bioreactor. Static cultures were also continued for 2 weeks. At week 0 and after 2 weeks of culture, the cell-scaffold samples were fixed and processed for histology (Trichrome and Hematoxylin-Eosin (H&E) staining) and scanning electron microscopy (SEM). Scaffold mechanical properties following cell growth in both culture conditions were also measured.

Results: Low magnification SEM images showed more hSMCs in both static and CMS cultures at 2 weeks when compared to week 0 samples. While it was difficult to ascertain differences in the cell populations amongst the two culture conditions, strained hSMCs appeared to have more cell-cell contact, forming large sheets of cells on D-PHI scaffolds (data not shown). Furthermore, higher density of fibrillar structures were observed in the CMS samples (Fig.1c), suggesting more matrix production from strained hSMCs. Based on H&E staining, CMS cultures also showed improved cell migration and more uniform distribution of cells in the D-PHI scaffold (Fig.2). Preliminary mechanical testing on 2-week static cultures showed a 6× increase in the elastic modulus of D-PHI scaffolds over cell-free samples, with no significant difference in the elongation-at-yield (~ 0.45).

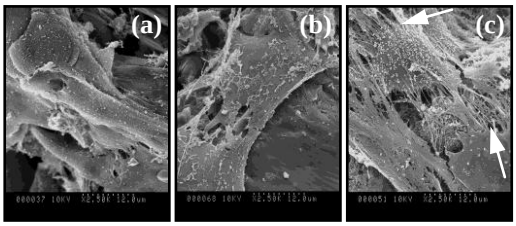

Figure 1. SEM of hSMC-seeded D-PHI scaffolds at week 0 (a) and after 2 weeks of static (b) and CMS culture (c). Arrows: fibrillar structures. (2500× magnification)

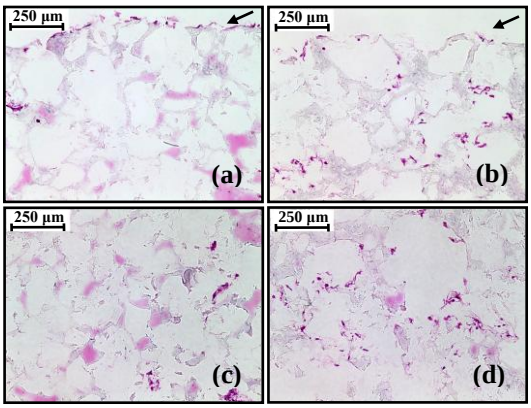

Figure 2. H&E images of hSMC static (a,c) and CMS (b,d) cultures after 2 weeks. Images were taken from the 0-1 mm upper (a,b) and 1-2 mm central (c,d) zones of D-PHI cross-sections. Arrows: scaffold upper surface.

Conclusions: The greater production of fibrillar matrix-like structures suggests that uniaxial CMS may promote a more proliferative phenotype in hSMCs. CMS also appears to enhance cell migration and distribution in the scaffold constructs. However, to better understand the effect of CMS on hSMCs, immunohistochemical and immunofluorescence analysis of smooth muscle α-actin, calponin and smooth muscle myosin heavy chain is currently being investigated. Furthermore, mechanical testing will be completed on strained samples to confirm the role of mechanical stimulation in increasing matrix production. This study emphasizes the importance of implementing appropriate biomechanical stimuli to regulate VSMC proliferation and phenotype during *in vitro* tissue regeneration.

References: 1. Hipper A. Eur J Physiol. 2000;440:19-27. 2. Tock J. Biochem Biophys Res Commun. 2003; 301:1116-1121. 3. Qu MJ. J Vasc Res. 2007; 44:345-353. 4. Stegemann JP. Ann Biomed Eng. 2003; 31:391-402. 5. Kakisis JD. Endothelium. 2004;11:17-28. 6. Sharifpoor S. Biomacromolecules. 2009;10:2729-2739.

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