Phenotype Characterization of Human Coronary Artery Smooth Muscle Cells Using Cyclic Mechanical Strain

Soroor Sharifpoor[1], Craig A Simmons[1], Rosalind S Labow[2], J Paul Santerre[1]
[1]University of Toronto, Toronto, Canada, [2] University of Ottawa Heart Institute, Ottawa, Canada

Statement of Purpose: Mechanical stimulation is an important feature of vascular smooth muscle cells' (VSMCs) physiological environment. As a result, it has been hypothesized that by using cyclic mechanical strain (CMS), it may be possible to manipulate the phenotypic shift of VSMCs and the expression of their differentiation markers. Studies that support this hypothesis have shown that VSMCs adopt a more contractile phenotype, characterized by reduced DNA synthesis[1] and increased contractile protein expression[2,3] when strained. On the other hand, other work has shown a phenotypic shift towards a more proliferative state[4], which raises the need to increase the field's understanding of the effect of mechanical forces on VSMC behavior. In the current study, a customized bioreactor[5] was used to apply uniaxial CMS to human coronary artery smooth muscle cells (hCASMCs) which were seeded into degradable polar/hydrophobic/ionic polyurethane (D-PHI) scaffolds. This work aims to study the role of CMS in modulating hCASMC proliferation and phenotype.

Methods: Dumbbell-shaped D-PHI scaffolds (23 mm long by 7 mm wide by 4 mm thick) were synthesized following the polymerization of a lysine-based divinyl oligomer as described previously.[5,6] 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%.[5] Adult primary hCASMCs (passages 7-9) were seeded into D-PHI scaffolds (1×10^6 cells/scaffold). At week 0 (after 3 days of static culture), the cell-scaffold system was subjected to uniaxial CMS (10%, 1Hz) for 4 weeks in a customized bioreactor[5]. Static cultures were also continued for 4 weeks. At week 0 and after 1, 2 and 4 weeks of culture, hCASMCs were lysed and the DNA mass was measured. Changes in smooth muscle α-actin (α-SMA), calponin and smooth muscle myosin heavy chain (SM-MHC) expression were measured by immunofluorescence and immunoblotting analysis. Cell and extracellular matrix (ECM) distribution was assessed by hematoxylin-eosin (H&E) histological staining.

Results: Based on DNA analysis (Fig. 1), after 2 weeks of uniaxial strain, a difference ($p>0.05$) in the DNA mass of hCASMCs cultured under static versus CMS conditions was not observed. However, at 4 weeks, statistically more ($p<0.05$) DNA was measured in the CMS samples. Immunoblotting analysis showed that, following 1 and 2 weeks of culture, CMS samples had a statistically lower level of α-SMA, calponin and SM-MHC expression when compared to static samples. However, following 4 weeks of culture, the expression levels of all three contractile proteins in CMS culture had increased to the level of static culture (Fig. 2). H&E staining showed the presence of more cells/ECM deeper within the scaffold following 4 weeks of CMS (Fig.3).

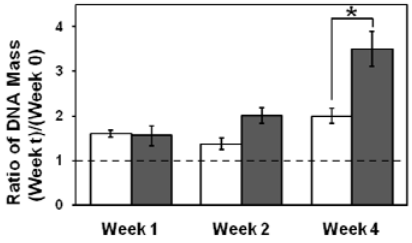

Figure 1. DNA mass of static (white) and CMS (gray) cultures after 1, 2 and 4 weeks relative to the DNA mass at week 0. *Statistically more DNA within CMS samples ($p<0.05$, n=6). Week 0 DNA was 810±64 ng.

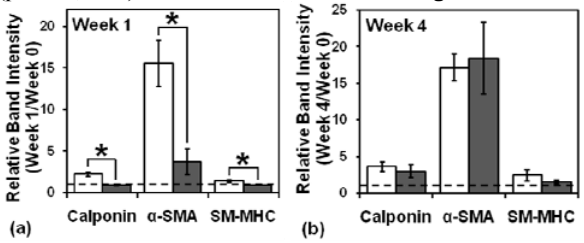

Figure 2. Densitometry analysis of α-SMA, calponin and SM-MHC immunoblots showing relative band intensity ratios at week 1 (a) and week 4 (b) with respect to week 0 for static (white) and CMS (gray) cultures. *Statistically more protein within static samples ($p<0.05$, n=3).

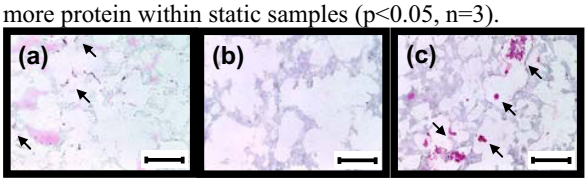

Figure 3. H&E images of hSMC-scaffold samples at week 0 (a) and week 4 for static (b) and CMS (c) cultures from the 1-2 mm zone of D-PHI cross-sections. Arrows: cells/ECM. Scale bars: 250 μm.

Conclusions: The greater DNA mass observed in strained samples after 4 weeks suggests that uniaxial CMS may induce a mitogenic response in hCASMCs. The lower expression of contractile proteins early in the culture period for CMS samples may also indicate a down-regulation of the contractile phenotype in hCASMCs. However, the similar level of protein expression observed at 4 weeks in both culture conditions suggests that while uniaxial CMS does induce a greater DNA mass in hCASMCs after 4 weeks, their contractile phenotype recovers over time. CMS also enhances cell distribution in the scaffold construct. This study emphasizes the importance of implementing timely biomechanical stimuli to regulate VSMC proliferation and phenotype which are essential for vascular 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. Sharifpoor S. Acta Biomater. 2010;6:4218-4228. 6. Sharifpoor S. Biomac. 2009;10:2729-2739.

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