Interaction of Vascular Smooth Muscle Cells with High-Porosity Vinyl Polycarbonate-Urethane Scaffolds under Cyclic Mechanical Strain

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Statement of Purpose: Arterial media vascular smooth muscle cells (VSMC) are exposed to circumferential cyclic strain (5-10%).[1,2] Previous *in vitro* studies have shown that in the absence of mechanical stimuli, VSMC experience a shift towards a more synthetic phenotype and a reduction in contractile protein expression, similar to the phenotypic shift observed during atherosclerosis.[3,4] However, in other studies, the application of mechanical strain increased cell proliferation but did not change the expression of smooth muscle α-actin (α-SMA) contractile protein.[5] While the importance of mechanical strain on VSMC behavior is undeniable, further investigation is required to successfully control and manipulate cell behavior in the presence of mechanical stimulation. To mimic the biomechanical conditions *in vivo*, a cyclic mechanical strain (CMS) bioreactor was designed and constructed to apply uniaxial CMS to high-porosity vinyl polycarbonate-urethane (VPU) scaffolds which were seeded with an A10 embryonic aortic rat cell line. The effect of CMS on VSMC behavior was investigated through the measurement of DNA content.

Methods: Dumbbell-shaped VPU scaffolds (23 mm long by 3 mm thick) were synthesized by reacting a lysine-based divinyl oligomer with methacrylic acid and methyl methacrylate monomers in a molar ratio of 1:5:15. The polymerization reaction was carried out in the presence of benzoyl peroxide initiator (0.003 mol/mol vinyl group) and a double porogen system was used to confer macroporosity and microporosity to the scaffolds (sodium bicarbonate particles (65 wt%, ~90% between 105-420μm) and polyethylene glycol (10 wt%, 600Da), respectively). The porosity of the final material (VPU-75) was 79 ± 3% as measured by fluid displacement.[6] To study scaffold strain profiles and to predict its behavior in response to uniaxial stretching, finite element analysis (FEA) using SolidWorks® (COSMOSWorks®) was performed. In this preliminary work, A10 cells were used between passages 2-3 and were maintained in DMEM supplemented with 4mM L-glutamine, 10% fetal bovine serum and 2% penicillin-streptomycin in a humidified atmosphere in 5% CO_2 at 37°C. 15 ml of a VSMC suspension ($1x10^5$cells/ml in DMEM) were transferred onto the upper surface of the scaffolds. At day 0 (after 3 days of static culture), the cell-scaffold system was subjected to uniaxial CMS (10%, 1Hz) for 7 days in a customized bioreactor. Static cultures were also continued for 7 days. At day 0 and after 1, 3 and 7 days of CMS/static culture, VSMC were lysed and DNA content was measured. Qualitative analysis using scanning electron microscopy (SEM) was also performed.

Results: FEA showed that upon a 2 mm uniaxial displacement in the x-direction the scaffold rectangular section (SRS) is subjected to a 10% homogenous x-strain (Fig.1A) and a -2% homogenous y-strain (Fig.1B). Thus, the VSMC in the SRS will be subjected to physiologically relevant strains. The maximum x-strain was observed in a very small region near the posts (18%), and was lower than the scaffold's elongation-at-yield (~30%).

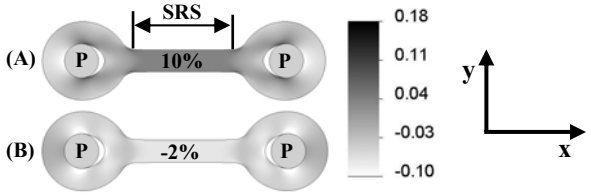

Figure 2. x (A) and y (B) strain profiles after a 2 mm displacement in the x-direction. P: Posts in the bioreactor.

Based on DNA analysis (Fig.2), after 3 and 7 days of CMS, there was a statistically significant increase in DNA content within the SRS. SEM showed more cells in CMS cultures confirming the DNA results (data not shown).

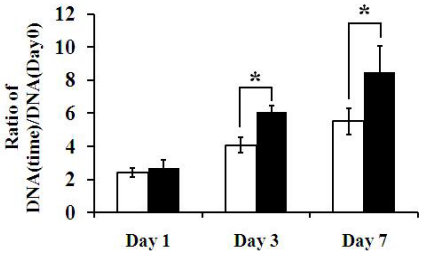

Figure 2. DNA of static (white) and CMS (black) cultures after 1, 3 and 7 days relative to DNA at Day 0 (SRS cells). *Statistically more DNA within CMS samples ($p<0.05$). Day 0 DNA was 4.9±0.2 ng/μl.

Conclusions: The increased DNA content and the greater cell density observed in CMS cultures suggest that uniaxial CMS may promote a more synthetic phenotype in A10 cells. However, to better understand the effect of CMS on VSMC further work is required. Specifically the effect of CMS on the expression of α-SMA and calponin is currently being investigated. Also future studies will explore the effect of CMS on human VSMC. This study emphasizes the importance of understanding and implementing the appropriate biomechanical factors for regulating VSMC proliferation and phenotype expression during *in vitro* tissue regeneration

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Acknowledgement:NSERC/CIHR-Grant #337246/83459