Combining cyclic mechanical strain and monocyte co-culture enhances collagen production and vascular smooth muscle cell infiltration into a porous degradable polyurethane scaffold

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Statement of Purpose: Strategies for tissue engineering a vascular graft aim to promote cell growth, obtain a uniform cell distribution within the tissue engineered construct, and to promote the contractile phenotypic state of the vascular smooth muscle cell (VSMC). Biomechanical stress is an important regulator of VSMC function and has been shown to significantly enhance VSMC growth and migration [1]. Another important regulator of VSMC function *in vivo* is the monocyte/macrophage, where monocytes are an important cell type present following biomaterial implantation. These cells have been shown to be positive contributors to vascular graft remodeling depending on their activation state within the M1/M2 spectrum [2,3]. VSMC-monocyte co-culture strategies are thus an attractive option for regulating VSMC response on biomaterial scaffolds. Such systems have been shown to promote VSMC migration [4]. The objective of this study was to evaluate the effect of dynamic mechanical strain when combined with monocyte co-culture, on extracellular matrix (ECM) production and VSMC infiltration into a degradable polar hydrophobic ionic polyurethane (D-PHI) scaffold.

Methods: D-PHI scaffolds were prepared by mixing a divinyl oligomer (DVO), methacrylic acid (MAA), and methyl methacrylate (MMA) in a 1:5:15 molar ratio along with the initiator benzoyl peroxide (0.032 mol/mol vinyl group), as well as polyethylene glycol (10 wt%) and sodium bicarbonate (65 wt%) as porogens [5]. The resulting mixture was packed into dumbbell-shaped Teflon molds and cured for 24 hr at 110ºC, followed by 14 x 2 hr periods of sonication for porogen leaching. The resulting scaffolds were seeded with monocytes isolated from the whole blood of healthy volunteers (University of Toronto ethics approval #22203) (1,000,000/scaffold), human coronary artery smooth muscle cells (VSMCs, Lonza, CC-2583) (500,000/scaffold) or monocytes and VSMCs in co-culture (1,000,000 monocytes + 500,000 VSMCs). Seeded scaffolds were cultured for up to 4 weeks under static conditions, or cultured under dynamic conditions in a custom-designed bioreactor that applied 10% uniaxial strain at a frequency of 1 Hz [5], after which the formed tissue in the scaffolds was digested with a papain solution for 48 hr at 65ºC. The cells within the scaffold were analyzed for total DNA mass, cellular infiltration (H&E), ECM production (OH-Pro and GAG quantification), as well as monocyte/macrophage polarization (immunofluorescence [IF] for CD80, CD86 [M1 markers] vs. CD163, CD206 [M2 markers]).

Results: After 4 weeks of culture on D-PHI scaffolds, the VSMC-monocyte co-culture supported an increase in DNA mass relative to the sum of VSMC and monocyte monocultures for both static (6636±1249 vs. 4545±827 ng/scaffold) and dynamic (6522±1497 vs. 4564±660 ng/scaffold) conditions. H&E analysis indicated greater cellular infiltration into the middle portion of the scaffolds with the combination of co-culture and biomechanical stimulation (**Figure 1**). The combined co-culture-dynamic condition similarly resulted in the greatest amount of collagen content in scaffolds after 4 weeks vs. monocultures (**Figure 2)**, while co-culture enhanced GAG content vs. monoculture conditions ($p<0.05$), with no effects of dynamic vs. static culture (data not shown).

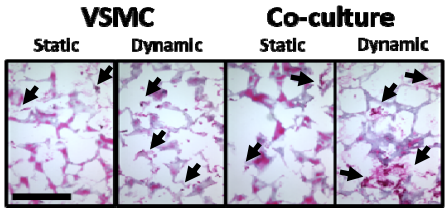

Figure 1 H&E stained sections of D-PHI scaffolds 4 weeks post-seeding. Scale bar is applicable to all images and represents 500 µm. Arrows indicate cells.

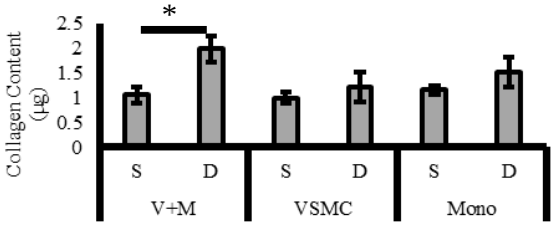

Figure 2 Collagen content for scaffolds seeded with both VSMCs and monocytes (V+M) or with only VSMCs or monocytes (Mono) after 4 weeks under dynamic (D) or static (S) culture. N=5-6. mean ± S.E. * $p<0.05$.

IF analysis of M1/M2 marker expression indicated no change in the ratio of CD80/CD163 or CD86/CD206 over time, suggesting no shift towards pro-inflammatory MDM polarization over the 4 weeks of culture (data not shown). The latter isssue is a common challenge of many synthetic biomaterials, such as PLGA [6].

Conclusions: The combination of biomechanical stimulation and monocyte co-culture supported enhanced cellular infiltration into porous D-PHI scaffolds while also promoting enhanced collagen production relative to static co-culture conditions. This study supports the importance of combining optimized co-culture strategies with bioreactor systems as a means to enhance cell and tissue growth for tissue engineering strategies.

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