Combining Human Monocytes in Co-culture with Vascular Smooth Muscle Cells Enhances Vascular Tissue Production under Biomechanical Stimulation

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Statement of Purpose: Developing a tissue engineered construct requires the ability to promote cell growth, cell infiltration, and tissue production within porous 3D scaffolds [1]. While growth factor supplementation is a common strategy used to support these responses, issues with high cost and possible endotoxin contamination can limit the clinical application of this approach [2]. An alternative source of stimulatory growth factors are autologous monocytes and their derived macrophages (MDMs), which when stimulated by an appropriate biomaterial substrate can contribute to cell growth *in vitro* and appropriate tissue regeneration *in vivo* [3]. In vascular tissue engineering, biomechanical stimulation (cyclic strain) can also promote vascular smooth muscle cell (VSMC) growth and tissue production [4]. Attempts to combine the stimulatory effects of growth factor supplementation with biomechanical stimulation, have resulted in some cases in the biomechanical stimulus being mitigated by the growth factor effects [5], indicating the significant challenges of tissue growth regulation. The purpose of this study was to evaluate complementary vs mitigated effects of biomechanical stimulation with monocyte co-culture (as a controlled source of biological effectors) on VSMC growth and tissue production when cultured on an immunomodulatory (i.e. preventing pro-inflammatory monocyte activation) degradable polyurethane (D-PHI).

Methods: Tubular D-PHI scaffolds (3 mm ID, 4 mm OD, 5 mm length) were prepared by polymerizing a divinyl oligomer, methacrylic acid, and methyl methacrylate in a 1:5:15 molar ratio, with sodium bicarbonate and polyethylene glycol as porogens, in the presence of the initiator benzoyl peroxide at 110°C for 24 hr [6]. Porogens were leached from scaffolds by sonication (14 x 2 hr in distilled water), yielding a scaffold with 79 ± 3% porosity [6]. For cell culture, D-PHI scaffolds were seeded with human coronary artery SMCs (Lonza, CC-2583) and monocytes isolated from the peripheral blood of healthy volunteers (U of T ethics approval #22203) in co-culture, as well as appropriate monoculture controls. Scaffolds were either subjected to 4 weeks of static or dynamic culture (10% circumferential strain, 1 Hz) in a custom-designed bioreactor. Following 4 weeks of culture, samples were evaluated for cellularity (immunofluorescence for calponin [IF]), tissue production (collagen I and III, elastin [IF]), phenotype (CD80 and CD86 [M1] and CD163 [M2a] and CD206 [M2c] [IF]), cytokine analysis (FGF-2 [ELISA]), and mechanical testing (elastic modulus [EM], tensile strength [TS]).

Results: After 4 weeks of culture on D-PHI scaffolds, quantification of VSMC scaffold coverage using IF analysis for VSMC contractile marker expression indicated that both dynamic culture and monocyte co-culture increased VSMC number. Furthermore, the combination of dynamic culture and co-culture showed increased VSMC scaffold coverage (**Fig. 1**).

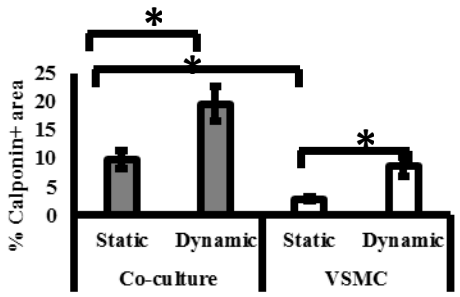

Figure 1 *VSMC scaffold coverage quantified by IF for calponin. n=9. Data are the mean ± S.E. *p<0.05.*

Co-culture resulted in an increase in FGF-2 release, which may have promoted VSMC growth (data not shown). While MDMs expressed both M1 and M2c markers, activation was shown to shift towards a more M2c-like state over time (data not shown), with this state previously shown to support VSMC growth and migration [4].

Table 1 *Mechanical properties. n=7-9. *p<0.05 vs. static M-monocyte, V-VSMC, C-coculture, D-dynamic, S-static*

	VS	VD*	CS	CD*	MS	MD
EM (kPa)	48±7	101±16	70±17	117±10	54±13	67±13
TS (kPa)	19±3	45±7	26±6	51±4	25±5	32±7

Mechanical stimulation of VSMC and co-culture samples increased collagen I and III levels (data not shown) as well as mechanical properties (**Table 1**), with no effect observed for MDMs. However, MDMs were shown to contribute to tissue production in the form of collagen and elastin (51 ± 7 μg/scaffold) (data not shown).

Conclusions: Combining monocyte co-culture and biomechanical stimulation had complementary effects on increasing VSMCs, and biomechanical stimulation further supported tissue deposition and enhanced mechanical properties. MDMs were shown to promote stimulatory growth factor release (FGF-2) from VSMCs, while also being polarized by D-PHI to an M2c-state over time. The latter is conducive to supporting cell/tissue growth. This study highlights the strategic use of MDM-co-culture in an appropriate biomaterial with immunomodulatory character (D-PHI), along with biomechanical stimulation, as an indirect approach to stimulate tissue regeneration.

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