# Cytokines from monocyte-conditioned medium and their influence on vascular smooth muscle cell phenotype on a degradable polyurethane

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**Introduction:**

Small diameter vascular grafts (<6mm) are prone to failure due to complications arising from thrombogenesis and neointimal hyperplasia (associated with vascular smooth muscle cell (VSMC) migration and proliferation). VSMC phenotype can shift between being contractile vs. synthetic depending on the stimuli that the VSMCs are exposed to [1]. While the contractile phenotype is associated with VSMCs in healthy arteries, for strategies related to the *in vitro* development of tissue-engineered vascular grafts the synthetic phenotype is initially desirable as it is associated with VSMC proliferation and matrix synthesis, which leads to a faster maturation of the vascular tissue *in vitro*. A strategy to modulate VSMC phenotype involves the use of monocytes/macrophages. Monocytes/macrophages are one of the first cell types present following biomaterial implantation [2], and their various secreted cytokines and growth factors can have a significant influence on VSMC response [3]. Previous work has shown that a degradable polar hydrophobic ionic polyurethane (D-PHI) suppresses pro-inflammatory and enhances anti-inflammatory activation from adherent monocytes when compared to well-established biomaterials tissue culture polystyrene (TCPS) and poly(lactide-*co*-glycolide) (PLGA) [4], and that co-cultures of VSMCs and monocytes enhanced VSMC penetration into porous D-PHI scaffolds [5]. The objective of the current study was to investigate the effect of monocyte-released factors on VSMC response and to identify key proteins that may contribute to specific effects on VSMC phenotype observed in VSMC-monocyte co-culture on D-PHI scaffolds.

**Materials and Methods:**

D-PHI scaffolds were prepared by previously established methods [6]. Briefly, a divinyl oligomer (DVO), methyl methacrylate (MMA), and methacrylic acid (MAA) were mixed in a 1:15:5 molar ratio, along with 65 wt% sodium bicarbonate and 10 wt% polyethylene glycol. The material was cured in the presence of the initiator benzoyl peroxide for 24 hr at 110°C. Porogen leaching by sonication resulted in a scaffold with 79±3% porosity. Monocytes were isolated from whole blood obtained from healthy volunteers (University of Toronto ethics approval #22203). D-PHI scaffolds were seeded with human coronary artery smooth muscle cells (Lonza, CC-2583, passage 7-9) in monoculture (100,000 per scaffold), with monocyte-conditioned medium (MCM) derived from monocytes cultured on D-PHI scaffolds, or in co-culture with monocytes (100,000 VSMCs and 200,000 monocytes per scaffold) over a period of 28 days. Cell attachment was assessed by a DNA mass quantification assay. VSMC contractile marker expression was determined by western blotting for α-SMA and calponin. A screen of the composition of the MCM was performed using a cytokine antibody array (RayBiotech). ELISAs were performed for cytokines identified in the array: MCP-1, IL-6, IL-13, and GM-CSF (eBioscience).

**Results:**

Scaffolds seeded with VSMCs and cultured in MCM had significantly greater DNA mass (1668±82 ng) when compared to VSMCs without MCM (1145±53 ng) ($p<0.05$). VSMC mass added to that of monocyte-only cultures was similar to the total DNA mass in co-culture (3206±84 ng) only for VSMCs cultured in MCM (3101±208 ng), but not those cultured without MCM (2587±199 ng). Both MCM (0.30±0.06%, day 28) and co-culture (0.11±0.04%, day 28) suppressed calponin levels compared to those at day 1. A cytokine antibody array was used to screen the composition of the MCM to identify key proteins involved in the modulation of VSMC phenotype observed in the presence of MCM and in co-culture. Two proteins identified of particular interest were MCP-1 and IL-6 for their known influence on VSMC phenotype, which were determined to be present at concentrations in MCM that have been previously shown to affect VSMC growth and migration [7,8]. MCP-1 (5ng/ml) increased DNA mass by 22.1±7.6% ($p<0.05$) for VSMCs on D-PHI scaffolds, while both MCP-1 (66.6±10.3%) and IL-6 (1ng/ml) (69.1±5.6%) had suppressive effects on calponin expression relative to untreated controls. In co-culture a neutralizing antibody for IL-6, but not MCP-1, was shown to suppress the increase in DNA (**Figure 1**) and reverse the suppressive effects on calponin (**Figure 2**) supported in monocyte-VSMC co-culture.

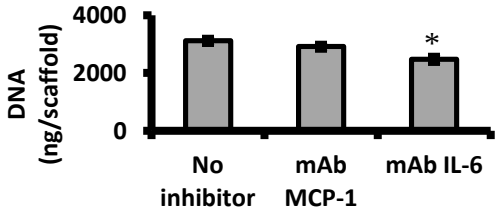


**Figure 1** DNA mass for VSMC-monocyte co-cultures on D-PHI scaffolds after 7 days of culture with or without neutralizing antibodies for MCP-1 and IL-6. n=7-9. Mean±S.E. *$p<0.05$ compared to no treatment.

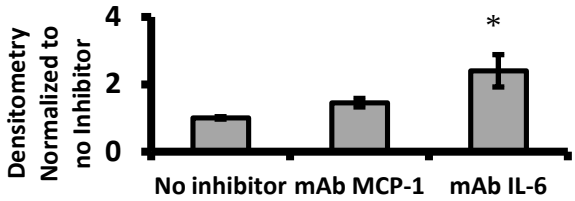


**Figure 2** Calponin expression in VSMC-monocyte co-cultures after 7 days on D-PHI scaffolds. n=6. Mean±S.E. *$p<0.05$ compared to no treatment.

**Discussion:**

MCM and co-culture were shown to modulate VSMC contractile marker expression and growth, with both growth (MCP-1) and contractile marker expression (MCP-1, IL-6) similarly effected in VSMC monocultures by the presence of MCP-1 or IL-6 alone. Neutralizing IL-6 reversed the effects seen in co-culture, suggesting that IL-6 is a protein released by monocytes involved in modulating VSMC phenotype in co-culture on D-PHI.

**References**:

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