Specific cytokines released by monocytes cultured on a degradable polyurethane (D-PHI) influence VSMC response

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Statement of Purpose: Following the implantation of a biomaterial, monocytes play a critical role directing the subsequent cellular and wound healing response [1]. These effects are orchestrated through a combination of monocyte-released factors [2] and direct cell-cell contact between monocytes and other cell types [3]. Previous work evaluating the use of a degradable polar hydrophobic ionic polyurethane (D-PHI) for vascular tissue engineering applications indicated its ability to support both an anti-inflammatory monocyte state [4] while also supporting growth and a contractile vascular smooth muscle cell (VSMC) phenotype [5]. Subsequent work with monocytes in co-culture with VSMCs showed enhanced VSMC growth and infiltration into porous D-PHI scaffolds relative to VSMCs alone [5]. The present work aims to determine if there is a defined relationship between D-PHI-specific interactions with monocytes and the cytokines they subsequently release, which could in turn promote VSMC growth and migration.

Methods: D-PHI scaffolds were prepared by previously established methods [6]. Monocytes were isolated from whole blood from healthy volunteers (University of Toronto ethics approval #22203). Human VSMCs (Lonza, CC-2583, passage 7-9) were seeded in 50:50 RPMI:DMEM medium in monoculture (100,000 VSMCs) with or without monocyte conditioned medium (MCM) from monocytes cultured on D-PHI scaffolds, or in direct co-culture with monocytes (100,000 VSMCs, 200,000 monocytes). MCM was prepared by taking supernatants from monocyte-only cultures every 24 hr and separating any non-soluble components by centrifugation (2000*g*, 5 min). Cultures were assessed for cell attachment (DNA mass quantification), VSMC phenotype (western blotting for calponin, α-SMA), and cellular infiltration (H&E). A screen of the composition of the MCM was determined from a cytokine antibody array (RayBiotech). MCP-1 and IL-6 (PeproTech) (target cytokines identified using the array) were quantified by ELISAs (eBioscience).

Results: VSMCs cultured in MCM on D-PHI scaffolds had significantly greater DNA mass at day 28 (1668±82 ng) than VSMCs without MCM (1145±53 ng) ($p<0.05$). By day 28, both MCM (0.30±0.06) and co-culture (0.11±0.04) similarly down-regulated the expression of the contractile phenotypic marker calponin relative to their respective level of calponin expression at day 1. To further elucidate the possible contribution of monocyte-released factors on VSMC growth and phenotype, a cytokine antibody array was used to identify key proteins present in MCM. Among these proteins, MCP-1 and IL-6 were of specific interest due to their known ability to modulate VSMC response [7,8]. IL-6 and MCP-1 were determined to be present at concentrations shown to be relevant for inducing VSMC migration and growth [7,8], with different release profiles (**Fig. 1**).

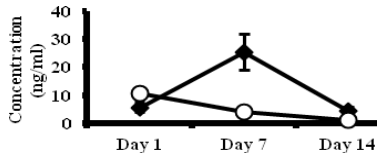

Figure 1 Release profile for MCP-1 (black diamonds) and IL-6 (white circles) when monocytes were seeded on D-PHI scaffolds. n=3 from three donors. Mean ± S.E.

VSMCs were subsequently cultured on D-PHI scaffolds for 7 days and treated with MCP-1 and IL-6 at doses representative of those present when monocytes were cultured on D-PHI (**Fig. 1**). MCP-1 was shown to have a modest but positive effect on DNA mass (**Fig. 2**), whereas both MCP-1 and IL-6 were shown to suppress α-SMA and calponin expression (**Fig. 2**).

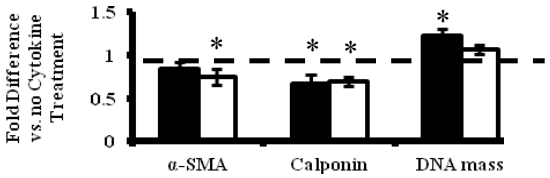

Figure 2 Levels of DNA mass and calponin and α-SMA expression for VSMCs cultured on D-PHI scaffolds for 7 days and treated with MCP-1 (5ng/ml) (black) or IL-6 (1ng/ml) (white) relative to no treatment. n=6 (WB) or 9 (DNA). Mean ± S.E. * $p<0.05$ compared to no treatment.

These results suggest that MCP-1 and IL-6 are involved in influencing VSMC response when monocytes are co-cultured with VSMCs on D-PHI.

Conclusions: MCM was shown to have a significant effect on VSMC growth and contractile phenotype. MCP-1 and IL-6 were present at concentrations relevant for contributing to the effects observed with MCM, and when supplemented in medium were subsequently confirmed to produce these effects when VSMCs were cultured on D-PHI scaffolds. Future inhibition studies to neutralize released MCP-1 and IL-6 in co-cultures of monocytes and VSMCs will be carried out to confirm the role of monocyte-released cytokines in effects previously observed in co-culture.

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Acknowledgements: CIHR grant #230762, Cell Signals (Battiston), Ontario Graduate Scholarship (Battiston).