Evaluation of a Degradable Polar Hydrophobic Ionic Polyurethane Designed for Vascular Graft Generation in an *in vitro* Endothelial and Monocyte Cell Co-culture

[1,2]Sarah McDonald, [2,3]Loren A. Matheson, [4]Joanne McBane, [4]Soroor Sharifpoor , [4]J. Paul Santerre, [1,2]Rosalind S. Labow
[1]University of Ottawa, Ottawa, ON, Canada, [2]University of Ottawa Heart Institute, ON, Canada, [3]University of Saskatchewan, Saskatoon, SK, Canada, [4]University of Toronto, Toronto, ON, Canada

Introduction: A new degradable polar hydrophobic ionic polyurethane (D-PHI) with suitable mechanical properties (1) for the the generation of a vascular graft was evaluated for cell biocompatibility and growth. Following implantation of a device, either a wound healing or an inflammatory response is initiated by macrophages (MDM) at the implant site. This determines the cytokine profile exposed to all cells in the local environment. Recently when monocytes (Mono) were cultured on D-PHI *in vitro*, the material promoted differentiation to a MDM wound healing phenotype relative to cells cultured on tissue culture polystyrene (TCPS), since cells on D-PHI released significantly more IL-10 than either IL-1β or TNF-α over time (2). Endothelial cells (EC) play a central role in the formation of a functional blood vessel and their phenotype and proliferation are in part regulated by Mono (3). Since Mono are always present at an implant site, the objective of this study was to determine if EC are able to proliferate on D-PHI in the presence of Mono while maintaining their functional phenotype.

Methods: A divinyl oligomer (DVO) was synthesized by mixing 2-hydroxyethylmethacrylate poly-(hexamethylene carbonate) diol and lysine diisocyanate in a 2:1:2 ratio with dibutyltin dilaurate catalyst at 50°C overnight (1). The D-PHI films consisted of DVO, methacrylic acid and methyl methacrylate at a 1:5:15 ratio with benzoyl peroxide initiator. Following curing at 110°C, the films were removed and placed in a 96-well TCPS plate. Human coronary artery ECs (Lonza) and Mono isolated from whole blood (2) were seeded in a 1:3 ratio onto D-PHI films and the wells of a TCPS plate. Cells were cultured for 48 hrs in a 50:50 mixture of RPMI-1640 and EC growth medium (Lonza). The cell lysates were assayed for the cell surface receptor CD31 by immunoblotting analysis and supernatants for IL-10 and TNF-α using ELISA. Cell lysates were also assayed for protein concentration. Cell growth was measured after 48hrs, 96hrs, and 7 days of cell material interaction by incubating with the cell proliferation reagent WST for 4h. The optical density was read @ 450nm.

Results: As seen in Fig 1, ECs maintained their CD31 marker when cultured alone but had significantly more CD31 in the co-culture with Mono. CD31 levels are indicative of adherent, functional ECs that have formed a confluent layer (4). The WST growth assay results demonstrated that D-PHI viability increased ~7fold from 48hrs to 96hrs and >10 fold by 7 days. ELISAs for TNF-α (inflammatory) and IL-10 (anti-inflammatory) were conducted on the supernatants from Mono, EC, and the co-culture allowing for an assessment of the inflammatory response of the different cell types over time (Fig 2). Both cytokines decreased over time; however, IL-10 was consistently higher than TNF- α which was absent from the co-culture at 7 days.

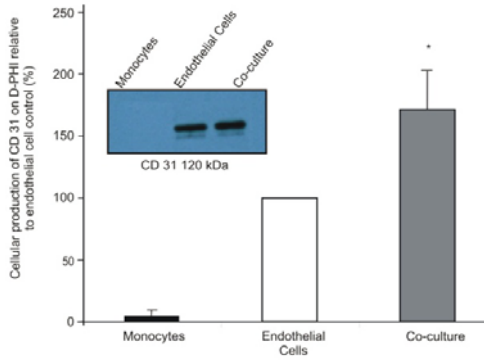

Figure 1: Immunoblotting analysis of the expression of CD31 after 7 days in culture. CD31 was significantly higher in the co-culture as compared to EC alone (*).

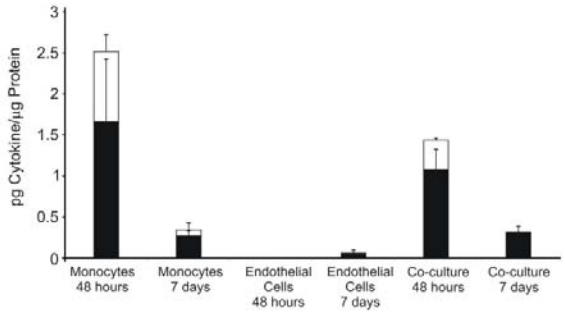

Figure 2: TNF-α (white) and IL-10 (black) present in cell supernatants and normalized to protein at 48 h and 7 days assayed by ELISA.

Conclusion: *In vitro,* D-PHI films allowed for EC proliferation either alone or in co-culture with Mono. The co-culture promoted a functional EC layer while decreasing the pro-inflammatory cytokine TNF-α. Direct contact of Mono with EC was necessary in order to exert their beneficial effect (3) and may be due in part to the activation of EC *N*-terminal–methionylated RANTES (Met-RANTES), a key factor in the S phase of EC cell division (3). For this to occur Mono need to be non-activated which D-PHI promotes (2). These data suggest that D-PHI is a suitable material for promoting the desired functional phenotype of EC for tissue engineering a vascular graft. Future work will further examine the interaction of the EC-Mono co-culture with smooth muscle cells and the non-thrombogenicity of the EC layer.

References: (1) Sharifpoor S et al. Biomac 2009; 10:2729-39 **(2)** McBane et al. Biomater, 2009; 30:5497-5504 (3) Schubert SY et al. Arterio thromb vasc biol 2008;28:97-104. (4) Gulati R et al. Circ Res 2003;93:1023-1025.

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