Degradable polar hydrophobic ionic polyurethane promotes endothelial and wound healing phenotype of circulating angiogenic cells

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Statement of Purpose:

Vascular graft materials pose several design challenges, which include the management of the microenvironment effects and the maintenance of cell biology. A degradable polar hydrophobic ionic polyurethane (D-PHI) material has demonstrated unique cytokine expression properties from adherent monocytes (MN) [1, 2]. This led to favorable characteristics in *in vitro* studies related to relevant cells involved tissue regeneration, including; MN, vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) [3]. The harvest and culture of VSMCs and ECs remains a challenge for the seeding/formation of vascular grafts. Circulating angiogenic cells (CACs) from blood have been shown to stimulate neovascularization and endothelial repair. Hence, the objective of this current study was to assess the effect of D-PHI on CAC adhesion growth and function. The capacity of D-PHI to modulate CAC maturation into an endothelial phenotype and cytokine expression was determined. As well, the effect of MN in co-culture with CACs on these parameters was investigated.

Methods:

D-PHI films were prepared using a protocol modified from Sharifpoor et *al.* [2]. Divinyl oligomer (DVO) was synthesized by mixing hydroxyethylmethacrylate, polyhexamethylene carbonate diol, and lysine diisocyanate in a 2:1:2 ratio in dimethylacetamide (solvent) with the catalyst dibutyltin dilaurate. The D-PHI films were made by combining DVO, methacrylic acid and methyl methacrylate at a ratio of 1:5:15 with initiator benzoyl peroxide at 0.003 mol/mol vinyl group. Total peripheral blood mononuclear cells (PBMCs) were isolated from human blood. PBMCs were cultured on fibronectin-coated tissue culture polystyrene (TCPS) for 4 days to obtain CACs and cultured on TCPS for 1 day to obtain MN. In the first phase, CACs were seeded on fibronectin-coated TCPS and on D-PHI film. Next, CACs were co-cultured with MN (ratio 1:1) on D-PHI. Cell attachment was assessed using DNA analysis and scanning electron microscopy (SEM), growth by WST assay, cell function by nitric oxide (NO) production, endothelial differentiation using immunoblotting (CD31), and cytokine expression by ELISA assays for TNF-α (pro-inflammatory) and IL-10 (anti-inflammatory).

Results:

Adherent CACs on D-PHI after 1 day were shown to have a round morphology, typical of MN, whereas at day 7 cells had extended pseudopodia, typical of endothelial progenitor cells (EPCs). The results did not show a significant difference between cell attachment and NO production on both surfaces. Interestingly, the metabolic activity of CACs increased on fibronectin-coated TCPS whereas it remained similar on D-PHI suggesting that the fibronectin-coated TCPS surface stimulated cytokine/ protein release from MN which stimulated the metabolic activity of EPCs. Immunoblotting showed decreased CD31 expression when CACs were cultured on fibronectin-coated TCPS, whereas CD31 expression increased when cells were seeded on D-PHI. These data suggested that the D-PHI surface supported greater cell retention and allowed the endothelial maturation of CACs. Moreover, cytokine expression showed that the D-PHI material supported a higher ratio of IL-10/TNF-α after day 3 which has previously been associated with a transition to a wound healing phenotype. The co-culture of CACs with MN confirmed that the D-PHI surface favored the endothelial maturation of CACs and the wound healing phenotype (Fig.1), while also decreasing the activation of MN.

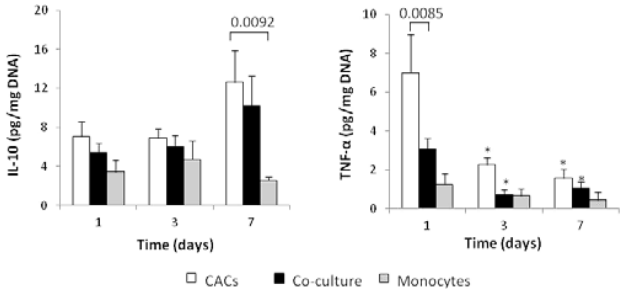

Fig.1: Cytokine release induced by D-PHI surface on CACs cultured with or without MN for 7 days. The D-PHI surface induced a high ratio of IL-10/TNF-α and low TNF-α level expression from MN.

Conclusion:

D-PHI was shown to favor the endothelial maturation of CACs when compared to fibronectin-coated TCPS while inducing a wound healing phenotype. Moreover, the D-PHI surface reduced the activation of MN. In summary, the data suggest that D-PHI material loaded with CACs has the potential to support vascular tissue regeneration.

References:

[1] Battiston K., Biomaterials, 2012; 33(33):8316-8328. [2] Sharifpoor S., Biomacromolecules, 2009 12;10(10):2729-2739. [3] McBane J., Biomaterials 2011; 32(26):6034-6044.