Degradable polar hydrophobic ionic polyurethane promotes endothelial and wound healing phenotype of circulating angiogenic cells when co-cultured with monocytes

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Introduction:

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]. The harvest and culture of VSMCs and ECs remain 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 effects of MN in co-culture with CACs on these parameters were investigated.

Materials and 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 films. 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). The data were analyzed using an analysis of variance (one way ANOVA). Significance was defined at a value of $p<0.05$. All data were plotted with the mean ± standard error of the mean (SE).

Results:

Adherent CACs on D-PHI after 1 day were shown to be a mixture of cells with different cell morphologies (round or spindle shaped) whereas at day 7, more cells had spread on the D-PHI surface and made connections between them with extended pseudopodia that are very typical of ECs. The results did not show a significant difference between cell attachment and NO production on both surfaces. Interestingly, the WST assay showed that fibronectin-coated TCPS stimulated the metabolic activity of CACs (absorbance values at 450 nm; 0.11±0.01 at day 1 *vs.* 0.34±0.04 at day 7, $p<0.05$) whereas D-PHI maintained stable activity. Immunoblotting showed decreased CD31 expression when CACs were cultured on fibronectin-coated TCPS, whereas CD31 expression was significantly higher at day 7 on D-PHI when compared to culture on fibronectin-coated TCPS (0.39±0.02 d.u (density units relative to tubulin) on fibronectin-coated TCPS *vs.* 1.24±0.46 d.u on D-PHI at day 7, $p<0.0001$). Cytokine expression showed that the level of TNF-α was significantly lower than the level of IL-10 at day 7 when CACs were cultured on D-PHI (12.63±3.23 pg/mg DNA for IL-10 level *vs.* 1.58±0.46 pg/mg DNA for TNF-α level; $p<0.001$). The co-culture of MN with CACs did not appear to enhance the behavior of the latter cells in terms of cell attachment, growth and NO production. The co-culture confirmed that D-PHI favoured CACs to adopt an endothelial cell-like phenotype and the wound healing phenotype (Fig.1), while decreasing the activation of MN.

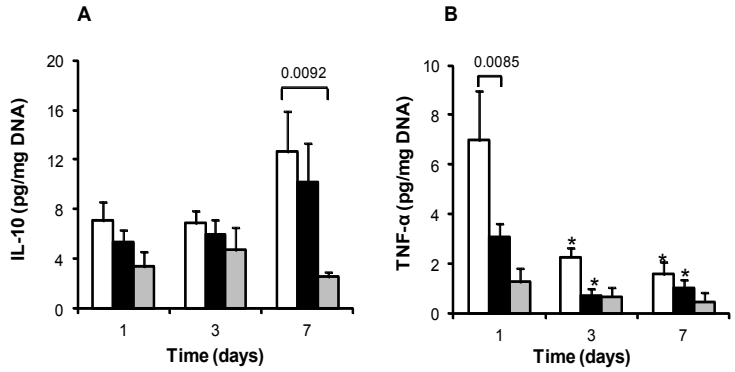

Fig.1: Cytokine release induced by D-PHI surface on CACs cultured with or without MN for 7 days. The D-PHI surface induced a high ratio of IL-10/TNF-α and low TNF-α level expression from MN. *$p<0.05$ *vs.* day 1.

Discussion:

D-PHI selected for an increase endothelial-like cells from CACs over time, and it displayed better behavior on some key benchmarks measured relative to TCPS. These data suggest that D-PHI favors the promotion of a wound healing phenotype, rather than an inflammatory response by the CACs. Moreover, D-PHI was less activating than TCPS for adherent MN. Furthermore, the presence of MN in co-culture with CACs did not appear to be necessary to enhance their activation on D-PHI since CACs maintained their wound healing character without MN. In summary, the results of this study suggest that D-PHI seeded with CACs has the potential to promote functional endothelial-like cells required to tissue engineer a vascular graft.

References:

[1] Battiston K., Biomaterials, 2012; 33(33):8316-8328. [2] Sharifpoor S., Biomacromolecules, 2009 12;10(10):2729-2739.