Evaluation of a monocyte/vascular endothelial cell co-culture on a vinyl-based polyurethane designed for vascular graft generation

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Statement of Purpose:
Tissue regeneration alternatives for peripheral vascular disease are actively being investigated; however, few studies have probed the role of the wound healing monocyte-derived macrophage. A vinyl-polycarbonate-urethane (VPU-0) has been designed with specific properties for vascular graft generation (1). Two of the cell types of primary importance in vascular repair are the differentiating monocyte (MC) and endothelial cell (EC). A goal in tissue regeneration is to ensure that MC have an anti-inflammatory, wound-healing phenotype that will aid in tissue repair thus affecting the desired growth and phenotype of EC and vascular smooth muscle cells (2).The establishment of a successful co-culture of EC and MC on VPU-0 will allow for an evaluation of the effects of the surface chemistry on these cell types. In this study, the influence of VPU-0 on differentiating MC and EC separately and together was assessed by measuring cell attachment, EC growth and esterase activity (EA) (a marker of activation and degradative potential) with comparison to tissue culture polystyrene (TCPS) as a control surface.

Methods:
A divinyl oligomer (DVO) was synthesized by mixing 2-hydroxyethylmethacrylate, poly(hexamethylene carbonate) diol and lysine diisocyanate in a 2:1:2 molar ratio with dibutyltin dilaurate catalyst at 50°C overnight. The VPU-0 films consisted of DVO, methacrylic acid and methyl methacrylate at a 1:5:15 molar 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 (purchased from Lonza) and MC isolated from whole blood as previously described (3) were seeded in a 1:3 ratio onto VPU-0 films and wells of a TCPS plate. Cells were cultured for 48h in a 50:50 mixture of RPMI-1640 and EC growth medium (Lonza). The cell lysates were assayed for DNA, EA (1 unit=nmol/min p-nitrophenol released from p-nitrophenylbutyrate) and the EC surface receptor CD31 by immunoblotting analysis as described previously (3,4). In addition, cell growth was measured after 48h of cell material interaction by a 4h incubation with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) in the culture medium above (5mg/mL). The reaction was terminated and the plate read at 570 nm (Sigma procedure).

Results and Discussion: Both MC and EC possessed degradative potential on TCPS and VPU-0 as measured by EA, which was present when cells were cultured alone or together in co-culture (Fig. 1). While there was no significant difference between the surfaces, it was important to note that EA was present in cells on VPU-0 for the ultimate degradation and resorption of an implanted graft. When EA for MC and EC alone was compared to the co-culture activity (set to 100%; EA range= 1.4-3.5 units/mL lysate for VPU-0 vs 1.0-3.1 for TCPS), the contributing activity from each cell type was additive regardless of the ratio of cells remaining at 48h). Results for MTT (growth of EC) and DNA (cell attachment of both cell types) assays showed a comparable trend. Values for the co-culture were similar to the additive results of each cell type cultured alone (data not shown). This indicated that both cell types were able to remain viable, that ECs were capable of growth and that both expressed EA on this surface. Fig 2 shows an immunoblot with lysates from MC, EC and the co-culture. Only the ECs, whether alone or in co-culture, expressed the EC specific cell surface marker, CD31. The immunoblot banding intensity suggests an increase in CD31 in the co-culture. This confirmed that the MC did not affect the EC's ability to maintain their phenotype while in co-culture on VPU-0.

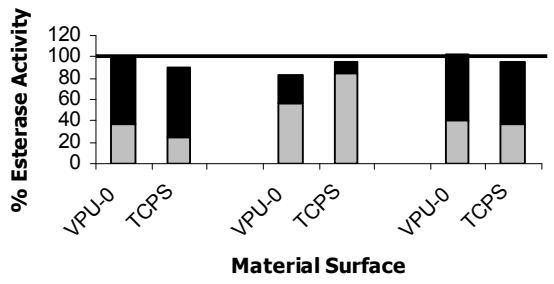

Fig. 1: EA of MC (black) and EC (grey) as a percentage of total EA assayed in co-culture (solid line=100%) for 3 separate trials on VPU-0 and TCPS.

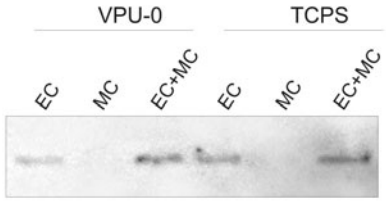

Fig. 2: Immunoblot showing CD31 marker in EC culture and EC + MC co-culture on VPU-0 or TCPS.

Conclusion: This study showed that a co-culture of MC and EC can successfully be established on VPU-0 where EC growth and cell attachment of MC are mutually exclusive and do not inhibit or alter the EC phenotype or the MC degradative potential. Future studies will involve the measurement of EA, DNA, and cell growth at later time points and determine if the EC are non- thrombogenic, vasodilatory and express appropriate angiogenic markers.

References: 1. Sharifpoor S. *et al*., WBC, May 2008 2. Lazarov-Spiegler O *et al*., FASEB J. 1996 10:1296. 3. Labow RS, *et al*. Biomater. 2002;23:3969. 4. Matheson LA, *et al*. J Cell Physiol. 2004;199:1.

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