Effect of material surface on monocyte and smooth muscle cell co-cultures

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Introduction: The wound healing monocyte-derived macrophage (MDM) is an important component of the tissue regeneration process that is characterized by a decrease in pro-inflammatory cytokines (1); however the effect of the wound healing MDM on vascular tissue engineering has not been thoroughly investigated. A recent study suggested that monocytes could affect endothelial cell proliferation twenty fold whereas vascular smooth muscle cell (VSMC) proliferation was only increased 1.9 fold (2). A critical element of tissue regeneration is the selection and design of scaffold materials. Given the importance of the MDM control in tissue regeneration it would therefore be important to design scaffold materials on the basis of their ability to induce a pro-wound healing state from MDM. Given that recent work has shown that a vinyl-lysine diisocyanate polycarbonate-urethane (VPU-0) generated increased IL-10 (pro-wound-healing) and reduced TNF-α (pro-inflammatory) from monocytes (3), the goal of this work was to study a co-culture system to assess the ability of VPU-0 to induce a wound-healing MDM that could influence VSMC function as assessed by cell adhesion and number.

Materials and Methods: Divinyl oligomer (DVO) was synthesized by mixing hydroxyethylmethacrylate (HEMA), polyhexamethylene carbonate diol (PCN) and lysine diisocyanate (LDI) in a 2:1:2 molar ratio in dimethylacetamide (solvent) with the catalyst dibutyltin dilaurate at 50°C overnight. The VPU-0 films were made by combining DVO, methacrylic acid and methyl methacrylate at a molar ratio of 1:5:15 with benzoyl peroxide initiator. The solution was pipetted into a polypropylene 96 well plate and then cured at 110°C overnight. SMGM®-2 cryopreserved human coronary smooth muscle cells (Lonza-Clonetics) (cultured in DMEM with 10% FBS and 2% penicillin/streptomycin at passages 5-9) were seeded at a density of 20,000 cells per well (20V). Human monocytes were isolated as previously described (4) and plated the next day at a density of 20,000 (20M), 40,000 (40M) or 80,000 (80M) cells per well in 200µL of 10% fetal bovine serum in RPMI medium or half DMEM and half RPMI for the co-culture. After 24 hours and 7 days cell adhesion was tested by centrifuging plates inverted at 1000xg for 5 minutes prior to cell lysis. Regular cell attachment was tested by centrifuging plates upright. Previously inverted centrifugation for short times has been found to be a good test for cell adhesion (5). Cells were lysed with 120µL of 0.05% Triton X-100 lysis buffer and assayed for DNA. VPU-0 materials were compared to tissue culture polystyrene (TCPS).

Results and Discussion: Monocyte adherence to VPU-0 was significantly greater than monocyte adherence to TCPS at cell densities of 40M and 80M (948ng/well vs 314 ng.well (40M) and 1250 ng/well vs 610 ng/well (80M) after an upright spin, $p<0.0001$). When adhesion was tested by inverted centrifugation this significance only held true for the plates with 80M (484 ng/well vs 208 ng/well, $p=0.018$). There was no significant difference in VSMC adherence in mono-culture or adherence at the cell density of 20M and VSMCs for the upright or inverted plates (Fig. 1a and b). Co-cultures with 40M and 80M on VPU-0 had significantly more DNA than those on TCPS when spun upright (Fig 1a) and this difference held true when 80M and 20V were co-cultured and centrifuged under inverted conditions (Fig 1b). This suggests that monocytes can induce more cell attachment to VPU-0 when in sufficiently high concentrations, e.g. 4:1 monocytes:VSMCs.

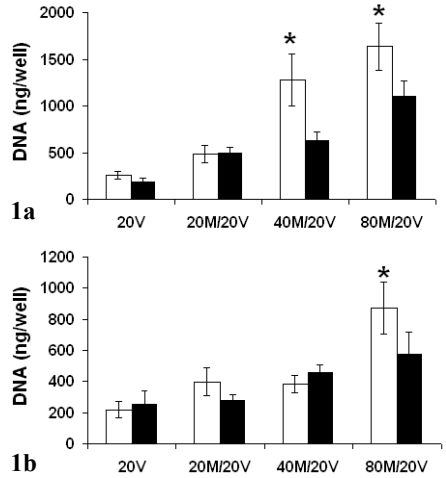

Figure 1: VSMCs (V) and co-cultures (M/V) were seeded on VPU-0 (white) or TCPS (black) for 24 hours and then centrifuged (a) right side up or (b) inverted prior to DNA analysis. Mean DNA (ng) per well was reported (n=3 +/- standard error). *Significantly higher DNA than on TCPS ((a) $p<0.0001$(40M/20V), $p=0.0004$(80M/20V), and (b) $p=0.014$ (80M/20V)).

Samples at seven days showed similar trends in terms of monocyte adhesion; however, the co-culture material difference was no longer significant (data not shown). These data possibly suggests that the maximum confluence was reached earlier for the VPU-0 samples whereas it took longer for this to occur when the cells were on TCPS. VSMC sample DNA values increased as a function of time, indicating a proliferative state.

Significance: VPU-0 may be a useful building block for tissue regeneration applications since both monocytes and VSMC showed good initial attachment when co-cultured. On-going studies are now looking at cell markers and cytokine profiling of the co-culture system to aid in the characterization of the VSMC and MDM phenotype, as well as looking at three dimensional scaffold cultures to assess cell infiltration, and tissue formation.

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