In *vitro* Model of Vascular Healing in the Presence of Biomaterials

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Introduction*:* Endothelialized synthetic vascular grafts suffer from difficulties in endothelial cell (EC) retention, and tissue engineered vascular constructs that mimic nature are early in their development. Also, stents can fail due to intimal hyperplasia, and themselves induce an inflammatory and thrombotic response. Central to advancing these strategies is an understanding of EC/blood/biomaterial interactions. Following static culture, biomaterial activated monocytes were shown to induce a proinflammatory EC phenotype [1], and biomaterial-activated monocytes and neutrophils differentially induced a procoagulant EC phenotype [2]. Due to the impact of smooth muscle cells (SMCs) on vascular healing processes, the objective of this work was to elucidate the influence of biomaterial-induced activation of leukocytes on EC *and* SMC phenotype, as well as the response to EC/SMC cross-talk in complimentary co-culture systems. Coordinated EC/SMC co-culture systems with divergent SMC phenotypes were developed, characterized, and utilized to examine the impact of presence and phenotype of SMCs on EC response to biomaterial-pretreated leukocytes in static culture.

Methods: Polyethylene teraphalate transwell filters (0.4μm pores, BD Biosciences) were incubated in MCDB 131 (Mediatech Cellgro) with 5% (v/v) FBS containing 1% (v/v) HyQ Pen-Strep (Mediatech Cellgro), 1% (v/v) L-glutamine, 2ng/mL hFGF-b (PeproTech), and 0.5 ng/ml hEGF (Gibco) (secretory HASMC media) overnight to enhance cell attachment. Filters were then inverted and human aortic SMCs (HASMCs) (0, 15000, or 90000 cells/cm^2) were dripped onto filter and allowed to adhere for 2h at 37°C. Filters were returned to their wells and covered in secretory HASMC media. *More secretory phenotype*: Human aortic ECs (HAEC; Clonetics) (40,000 cells/cm^2) were added to the inner compartment. *Less secretory phenotype*: HASMCs were incubated overnight in secretory HASMC media. Media was then replaced with forced contractile media (lacks FBS and growth factors) for 48h. Then, media was replaced with secretory HASMC media containing only 2% FBS (quiescent HASMC media). HAECs (40,000 cells/cm^2) were added to the inner compartment. All plates were incubated at 37°C for 48h prior to assay. Resulting HASMC phenotypes were examined as compared to forced contractile HASMCs at 48h or 72h after initiation of culture: ELISAs for IL-8 and MCP-1 secretion, immunocytochemsitry for α-smooth muscle actin, and flow cytometry for proinflammatory (ICAM-1 and VCAM-1), procoagulant (tissue factor (TF)), and anticoagulant (thrombomodulin) marker expression. Peripheral human whole blood was collected into 50U/mL pre-heparinized syringes, and monocytes and PMNs were isolated with CD14 and CD15 positive selection (Miltenyi Biotec), respectively. Isolated monocytes or PMNs were mixed with polystyrene (45 um, Polysciences, Inc) beads at 54*10^4 beads/ml. The leukocyte/bead mixtures and controls (untreated or fMLP-treated leukocytes) were agitated for 2 hours on a hematology mixer, and applied to EC or SMC monolayers or the EC surface of EC/SMC co-cultures for 5 or 24h. Flow cytometry for proinflammatory (ICAM-1, VCAM-1, E-selectin), procoagulant (TF) or anticoagulant (thrombomodulin) marker expression was assessed on both ECs and SMCs, and EC proliferation was assessed in a flow cytometric BrdU assay.

Results/Discussion: More and less secretory HASMCs had divergent phenotypes at 48h, but were beginning to revert to a more secretory phenotype by 72h. *ECs in monoculture or co-culture with SMCs*: E-selectin, ICAM-1, VCAM-1, and TF, as well as thrombomodulin shedding were upregulated on ECs following incubation with fMLP or biomaterial-pretreated monocytes (but not PMNs). The presence of more secretory SMCs in co-culture limited EC proliferation, and further increased expression of all markers (except thrombomodulin, which had subdued shedding). The presence of less secretory SMCs subdued E-selectin and TF expression, as well as thrombomodulin shedding did not impact ICAM-1 expression, and increased VCAM-1 expression compared to monocultured ECs. *SMCs in monoculture:* Biomaterial-pretreated monocytes or PMNs increased the proinflammatory/ procoagulant phenotype of more secretory HASMCs, and induced a proinflammatory/ procoagulant phenotype in initially forced contractile HASMCs above the presence of serum-containing media alone.

Conclusions: These studies show the ability (though time limited) to control the phenotypic state of cultured HASMCs using specific co-culture methods. The presence of more secretory HASMCs in general enhanced HAEC activation in response to biomaterial-pretreated monocytes, while the presence of less secretory HASMCs suppressed HAEC activation in response to biomaterial-pretreated monocytes (and PMNs to a small degree). Inconsistencies in trends for VCAM-1 and thrombomodulin expression may be due to reversion of less secretory HASMCs to more secretory HASMCs by the 24h time point. Additionally, biomaterial-pretreated monocytes and PMNs amplified a HASMC phenotypic shift away from a more quiescent state. It is likely that the compounding effect of secretory SMCs and biomaterial-activated leukocytes are responsible for altered vascular wound healing upon implantation of stents or vascular grafts. Understanding the specific signals causing these effects, or signals delivered by contractile SMCs that limit these effects help to provide design criteria for development of devices or grafts capable of long term patency.

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References:

[1]Lester & Babensee. J Biomed Mater Res 2003;64A:397.
[2]Rose & Babensee. J Biomed Mater Res 2005;72A:269.