Effect of Substrate Composition and Compliance on Endothelial Expression of ICAM-1 and E-Selectin

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Abstract — **One of the major limitations of current biomaterials is their recruitment of inflammation which leads to scar tissue encapsulation. Natural materials such as blood vessels, however, do not recruit inflammation unless in a pathological state. Two factors thought to be important to maintaining endothelial cells in an anti-inflammatory state are the extracellular matrix (ECM) proteins and the mechanical compliance of the substrate to which they adhere.[1] In this study we create poly(acrylamide) gels with differing compliance which are cross-linked to one or more ECM molecules to examine the effect of substrate composition and compliance on human umbilical vascular endothelial cell (HUVEC) expression of leukocyte adhesion receptors ICAM-1 and E-Selectin, using fluorescence activated cell sorting (FACS).**

Keywords — **biomaterials, inflammation, endothelium.**

METHODS: HUVECs are grown on polyacrylamide gels that are fabricated and crosslinked to ECM molecules using a method described by Putnam *et al.*[2] Briefly, polyacrylamide and BIS-polyacrylamide are mixed, varying the percentage of BIS-polyacrylamide to change gel compliance. The polyacrylamide is gelled, activated with Sulpho-SANPAH (Pierce), and incubated overnight in a solution of ECM protein to covalently bind the ECM molecule to the gel. HUVECs are seeded and cultured for 2-3 days in order to reach confluence monolayer. After a confluent monolayer is obtained the cells are removed from the substrate using a PBS/0.5 M EDTA solution. Each cell sample is counted and incubated for 1 hr with a PE conjugated primary antibody to either ICAM-1 (CD54, Becton, Dickerson) or E-selectin (CD62E, Becton, Dickerson). After washing and fixing, FACS is performed to quantify expression of ICAM-1 and E-selectin.

Results/Discussion: Characterization of ICAM-1 and E-Selectin by HUVECs cultured on different substrates is performed by FACS analysis. Results in figure 1 (A) show that HUVECs cultured on laminin surfaces express significantly more ICAM-1 than cells cultured on matrigel or collagen (p<0.05, ANOVA). The expression is represented by the geometric mean of fluorescence of the cells that demonstrated a greater fluorescence than control (determined by analyzing cells not incubated with the fluorescent antibody). This is an indication of how many ICAM-1 molecules are present on the surface of the ICAM-1 activated cells. Figure 1 (B) shows the percent of cells that are shifted into the E-selectin positive region of the FACS plot. This value is an indication of the percentage of cells with greater fluorescence than unlabeled controls. The shifted cells express a detectable amount of E-selectin molecules; whereas, unshifted cells do not express a detectable number of E-selectin molecules. This is expected since non-activated endothelial cells are not thought to express E-selectin. Figure 1 (B) demonstrates that a greater number of cells are activated (express a detectable level of E-selectin) after being cultured on the softest gel (p<0.005).

ECM Comp.			
Collagen	9.388±0.744	9.614±0.920	8.894±0.614
Matrigel	9.654±0.994	9.241±1.900	9.920±1.039
Laminin	10.387±1.595*	9.871±1.774*	10.689±1.391*
BIS %	0.03	0.115	0.26

B **Percent cell shift of E-Selectin**

ECM Comp.			
Collagen	21.175±9.545*	8.330±1.760	8.413±1.594
Matrigel	30.118±22.85*	17.800±2.965	17.585±10.31
Laminin	29.438±17.18*	14.515±2.303	14.258±1.641
BIS %	0.03	0.115	0.26

Figure 1. (A) Geometric mean of ICAM-1 positive cells cultured on collagen I, laminin, and matrigel at varying substrate stiffness. Asterisks represents statistical significance, $p < 0.05$ (laminin surfaces show greater expression, n=6). (B) Percent of cells that are E-Selectin positive (n=2).

Conclusions: These data suggest that soft laminin surfaces would recruit more leukocytes and that stiff collagen surfaces would recruit fewer leukocytes. Future work will include directly measuring leukocyte adhesion in a flow environment and measuring the intracellular signals mediating the effect surface composition and compliance have on gene regulation.

Acknowledgements: This work was funded by NIH grants R21 EB004386 and K22 DE014846.

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