How do cells translate biomaterial properties into changes in gene expression?

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Statement of Purpose: Many medical devices fail due to material-host interactions, in particular inflammation. However, there is a material in our bodies that for the most part works well for decades. That material is the basement membrane which is a material made of proteins and proteoglycans to which vascular endothelial cells adhere in normal blood vessels. These endothelial cells receive cues from the basement membrane and their other surroundings that cause them to respond by not recruiting inflammatory cells or platelets except in pathological situations. What is it about this material that elicits desirable responses from endothelial cells whereas synthetic materials elicit undesirable responses? Can doctors use that knowledge to trick cells into responding to synthetic materials in a desirable way? To answer these questions, it is essential to understand how these cells sense biomaterial properties and convert those sensations into gene regulation. In this study we systematically control the stiffness and protein composition of a material and then study the responses of human umbilical vein endothelial cells that are brought into contact with those materials.

Methods: We measure the activity of four signaling kinases (JNK, IKK, Akt, and ERK1/2) for cells in contact with materials of which we have varied stiffness and protein composition. Briefly, polyacrylamide gels with varying percentage of bis-polyacrylamide are polymerized, activated with Sulpho-SANPAH (Pierce), and incubated with an extracellular matrix (ECM) protein. Human umbilical vein endothelial cells (HUVECs) (Cascade Biologics) are brought into contact with the surfaces, frozen at the desired time point, lysed, and the lysate is immunoprecipitated (IP) with an antibody for a kinase of interest (JNK, IKK, Akt, and ERK1/2). The IP surface is exposed to substrate for the kinase in the presence of P32-labeled and unlabeled ATP, filtered through a vacuum filter plate, then the filters are analyzed by liquid scintillation. We also measure the expression of several genes by fluorescence activated cell sorting (ICAM-1 and E-selectin), ELISA (tissue factor), and competitive binding (prostacyclin).

Results: HUVECs cultured on substrates with varying mechanical compliance (2 kPa vs. 12 kPa) exhibit differences in intracellular signaling in the IKK, JNK, and ERK pathways but no statistical differences in the Akt pathway (Figure 1). These differences in mechanical compliance translate into increased numbers of HUVECs expressing detectable numbers of E-selectin. In this case, the less stiff gels (2kPa) show increased percent shift (FACS) of E-selectin expression when compared to HUVECs cultured on 12kPa gels.

We have also studied the effect of varying ECM on intracellular signaling. 10 different ECM compositions were contacted with HUVECs and the activity of 4 kinases was measured at several time points and a mean,

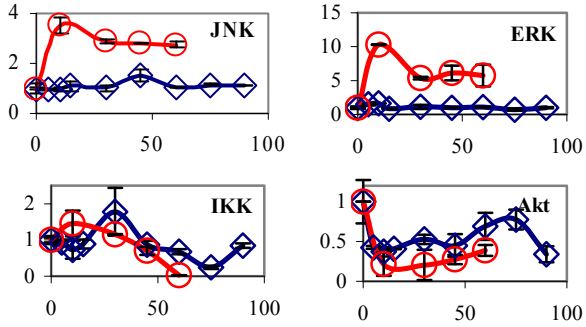

Figure 1. Kinase assay data (y-axis, relative fluorescence) for HUVECs cultured on 2kPa (blue) and 12kPa (red) gels conjugated with collagen I for various times (x-axis in minutes).

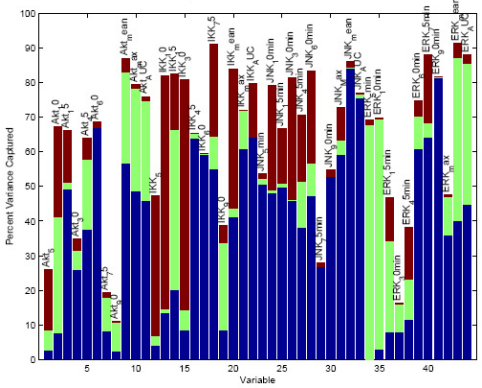

Figure 2. Principal component (PC) analysis of effect of ECM on intracellular signaling. Percent of variance captured by PC1 – blue, PC2 – green, PC 3 – brown for each signal time point, mean, max, and AUC.

maximum, and area under the curve (AUC) were calculated for each. Principal component analysis shows that JNK is the most strongly affected, ERK and Akt second, and IKK last. JNK correlates with tissue culture plastic and gelatin and negatively with chondroitin sulfate, vitronectin, and fibronectin. ERK correlates with vitronectin, and Akt correlates with chondroitin sulfate and heparan sulfate. Finally IKK correlates with laminin and matrigel.

Conclusions: These results establish correlations between mechanical properties and intracellular signaling (JNK and ERK) as well as between ECM composition and intracellular signaling (JNK with tissue culture plastic and gelatin, IKK with matrigel and laminin, et cetera). Current work focuses on further correlating these intracellular signaling responses to cell behavior such as recruitment of inflammation. Preliminary results here indicate that JNK and ERK activation may inhibit expression of E-selectin.

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