The role of substrate rigidity in epithelial to mesenchymal transitions (EMT); implications in fibrotic responses

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Statement of Purpose: Biomaterials, whether synthetic or natural, often elicit fibrotic responses following implantation, which can lead to a multitude of problems including device failure and host rejection. Fibrotic responses are characterized by excessive deposition of extracellular matrix (ECM). Biomaterial-induced fibrosis is best known as the foreign body capsule and consists of a thick collagen capsule surrounding the biomaterial. The mechanisms initiating fibrotic responses are poorly understood, yet evidence implicates epithelial to mesenchymal transitions (EMT) as a possible mechanism contributing to the onset and progression of fibrotic reactions [1]. EMT in a tissue would result in cell differentiation from epithelial cell phenotypes, such as endothelium, epithlium, etc., into synthetic fibroblasts and/or myofibroblasts, which would further perpetuate fibrotic responses through the generation of additional ECM. Evidence also suggests that substrate rigidity plays a role in modulating cell differentiation, with cells differentiating more effectively on substrates which are similar in rigidity to the tissue of their natural surroundings [2]. To design effective biomaterials, we must understand the underlying mechanisms of fibrosis and the role of substrate compliance on the activation of these processes. In this study we were interested in exploring the mechanics of substrate rigidity as a potential mechanism regulating epithelial cell phenotype and its potential for driving EMT. We hypothesized that as substrate rigidity increases, epithelial cells will undergo EMT in a Rho GTPase dependent fashion.

Methods: To determine the role of substrate rigidity on EMT events, poly-acrylamide (PA) gels of varying bis concentrations were created. PA gel solutions are produced by combining acrylamide and bis to final concentrations of 10% acrylamide, and 0.3%, 0.07%, 0.13%, or 0.26% bis and then polymerized by the addition of ammonium persulfate and N,N,N',N'-tetramethylethylenediamine (0.05% final concentration each). The gels were allowed to polymerize overnight at room temperature. The relationship between Young's modulus of the gel and bis concentration was determined macroscopically as previously described by Engler et al [3] and by indentation Atomic Force Microscopy. Because PA is anti-adhesive, fibronectin is attached to the surface using the heterobifunctional crosslinker sulfosuccinimidyl-6-(4'-azido-2'nitrophenylamino) hexanoate (sulfo-SANPAH; Pierce Chemical Co.) Freshly isolated primary epithelial cells were cultured on PA gels of varying rigidities. Activation of Rho GTPases was performed by standard Rhotekin, PAK, and WISP pulldown assays and epithelial proliferation and EMT was assessed at 24, 48, and 96 hours by an Alamar Blue assay and western blotting for E-cadherin (ECad, epithelial cells) and vimentin (fibroblasts), respectively. EMT events were also analyzed at after 1 week in culture by PCR for various epithelial and mesenchymal markers including, spc and E-Cadherin (epithelial cell) and vimentin and prolyl-4-hydroxylase (p4h, mesenchymal cells). To further investigate the role of Rho GTPases in biomaterial-mediated EMT, cells were analyzed in the presence of pharmacological inhibitors.

Results: As expected, PA gel rigidity was found to increase linearly with increasing bis concentration (Figure 1). Fibronectin ligand density was verified as constant on the different surfaces using standard protein analysis and a modified ELISA. Intracellular Rho GTPase activity was up regulated on substrates with increasing stiffness and epithelial cell proliferation was found to increase proportionally. Expression of epithelial markers was found to decrease, while expression of mesenchymal markers was found to increase with increasing rigidity.

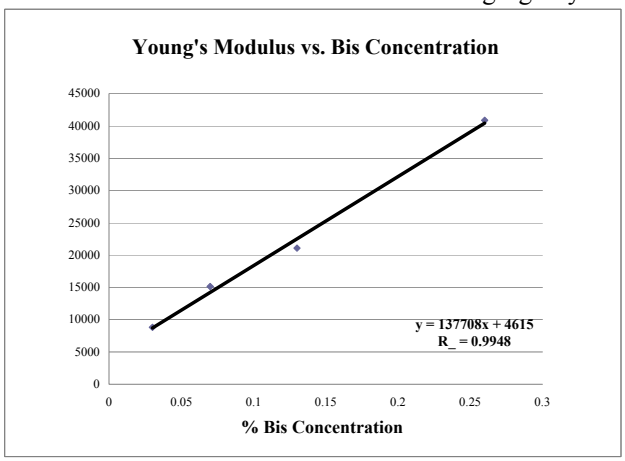

Figure 1. Young's modulus as a function of bis concentration

Conclusions: These data suggest that substrate rigidity modulates EMT events, with epithelial cells cultured on substrates of higher rigidities differentiating into cells with fibroblast characteristics. The increase in proliferation observed with increasing substrate rigidity may be partly explained by EMT since fibroblasts are usually more proliferative than alveolar epithelial cells, yet further studies are necessary to fully understand these responses. The exact mechanism(s) of the effect of substrate rigidity on epithelial cell behavior remains to be elucidated, however, our studies indicate that increasing rigidity does appear to induce EMT. These effects on cell responses need to be considered as a critical design constraint when developing biomaterial technologies.

References:

[1] Willis, B.C., et al. Am J Pathol, 2005. **166**(5): p. 1321-1332.
[2] Engler, A.J., et al. J Cell Biol, 2004. **166**(6): p. 877-87.
[3] Engler, A.J., et al. Cell, 2006. **126**(4): p. 677-89.