Substrate Rigidity Modulates EMT: Implications in Biomaterials-associated Fibrosis

Ashley Carson Brown, Vincent F. Fiore, Justin Chen, and Thomas H. Barker.
Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Statement of Purpose: Biomaterials often elicit fibrotic responses, which are characterized by excessive deposition of extracellular matrix (ECM) and hardening of the surrounding tissue. Biomaterial-induced fibrosis, best known as the foreign body capsule, consists of a thick collagen capsule surrounding the biomaterial. While a significant number of recent findings have highlighted the influence of mechanical properties, such as stiffness, in influencing cell phenotype [1], it remains unclear what, if any, contribution reduced tissue compliance has on the onset, regulation, and futher progression of phenotypes associated with fibrotic responses. To design effective biomaterials, we must understand the underlying mechanisms of fibrosis and the role of substrate compliance on the activation of these processes. During the progression of fibrotic responses, there is a significant contribution of synthetic, fibrotic mesenchymal cells derived from the resident epithelial cell population due to epithelial to mesenchymal transitions (EMT) [2]. EMT in a tissue results in cell differentiation from epithelial cell phenotypes into synthetic fibroblasts and/or myofibroblasts, which would further perpetuate fibrotic responses through the generation of additional ECM. A growing body of evidence has identified transforming growth factor beta (TGFβ) as a primary contributer to the onset and progression of EMT. Interestingly, recent studies by Wipff *et al.* demostrate that fibroblast activation of TGFβ increases on increasingly rigid substrates, leading to greater myofibroblast differentiation on stiff, but not compliant substrates [3]. Based on the known role of TGFβ in induction of EMT and these recent findings highlighting the role of substrate rigidity in TGFβ activation, we hypothesized that increases in substrate rigidity would modulate cell contractility, leading to TGFβ activation and subsequently EMT.

Methods: To determine the role of substrate rigidity on EMT events, poly-acrylamide (PA) gels of varying bis concentrations were generated to model a range of tissue stiffness values *in vitro* from 2kPa to 32kPa. Because PA is anti-adhesive, fibronectin was attached to the surface using the heterobifunctional crosslinker sulfosuccinimidyl -6-(4'-azido-2' nitrophenyl-amino)hexanoate (sulfo-SANPAH; Pierce Chemical Co.) RLE-6TN epithelial cells were cultured on PA gels of varying rigidities and EMT responses characterized after 5 days in culture through analysis of E-cadherin (epithelial marker) and α-smooth muscle actin (mesenchymal marker) protein expression and localization, cell spreading, and stress fiber formation/alignment. To investigate the role of cell contractility in rigidity-mediated EMT, cells were analyzed in the presence of 10μM Y-27632 Rho associated kinase (ROCK) inhibitor. Furthermore, TGFβ activation was determined through gene expression analysis of the TGFβ response gene plasminogen activator inhibitor-1 (Pai-1).

Results: Epithelial cells maintained a normal morphology, expression of epithelial markers, and cell-cell contacts as characterized through E-cadherin immunofluorescence staining when cultured on lower rigidity substrates. Conversely, cells cultured on increasingly rigid substrates displayed a more mesenchymal morphology, a loss of cell-cell contacts, acquisition of mesechymal markers, and α-SMA stress fiber formation. These differences were abrogated upon the inhibition of ROCK, suggesting cell contractility at least partially mediates stiffness enhanced EMT. Expression of the TGFβ-responsive gene Pai-1 was found to significantly increase ($p<0.01$) on the two highest rigidity substrates analyzed compared to the lowest rigidity substrate, and this response was normalized upon the addition of Y-27632 (Figure 1). Futhermore, as a control, the addition of 5 ng/ml active TGFβ to cells cultured on the lowest rigidity substrate induced EMT indicating a dominant role for TGFβ in the observed responses.

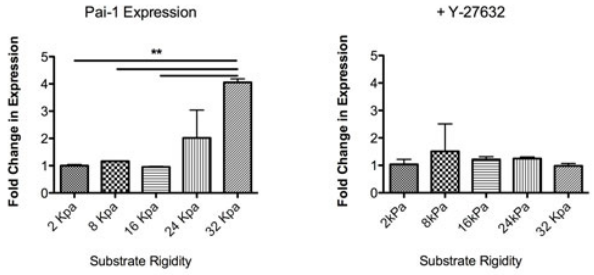

Figure 1. Pai-1 Expression in Response to Increasing Substrate Rigidity

Conclusions: These data imply that substrate rigidity modulates EMT events, with epithelial cells cultured on substrates of higher rigidities acquiring mesenchymal phenotypes and increasingly expressing the TGFβ response gene, Pai-1. Rigidity-mediated EMT and increases in Pai-1 expression were abrogated upon the inhibition of ROCK, while addition of exogenous active TGFβ to cells on low rigidity substrates induced EMT and Pai-1 expression, suggesting cell contractility at least partially mediates stiffness enhanced EMT, through increasing activation of TGFβ. These results link material rigidity to fibrotic responses and highlight the importance of considering elastic modulus as a critical design constraint when developing biomaterial technologies.

References:

[1] Engler, A.J., et al. Cell, 2006. 126(4): p. 677-89.
[2] Willis, B.C., et al. Am J Pathol, 2005. 166(5): p. 1321-1332.
[3] Wipff, P.J., et al. J Cell Biol, 2007. 179(6): p. 1311-1323.