Fibronectin Fibrillogenesis Allows Cells to Overcome Mechanical Cues

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Statement of Purpose: Cells respond to mechanical cues from the substrate to which they are attached. These mechanical cues have been shown to play a role in cell migration, proliferation, differentiation, and survival. Increased force generation in response to substrate rigidity is one such response, and assembly of extracellular matrix (ECM) fibrils is another. Fibronectin (FN), a dimeric glycoprotein that comprises *de novo* ECM, binds to cells through interactions of $\alpha_5\beta_1$ integrin on the cell surface with the $III_{9\text{-}10}$ domains of FN (1,2). Cellular contractions stretch FN exposing cryptic sites for binding of other FN molecules as well as other ECM proteins. FN thereby allows for cellular growth, proliferation, and migration. Here we hypothesize that the assembly of FN fibrils on a biomaterial creates a mechanism through which cells can supplant substrate mechanical cues. We will test this hypothesis by plating human embryonic lung fibroblasts on surfaces of varying stiffness, and subsequently inhibit FN fibrillogenesis with a bacterial protein fragment.

Methods: Microfabricated pillar arrays (mPAs) consist of cantilever pillars organized in an array that deflect in response to cell-generated forces. mPAs also serve as a scaffold for FN fibrillogenesis (3). mPAs are fabricated from polydimethylsiloxane (PDMS) using a master silicon wafer to form the negative mold, which is then used to form the mPA (4). FN is micro-contact printed onto the tops of the pillars using wild-type FN. Cells were plated at a density of 100,000 cells per mL onto mPAs and incubated for 24 hrs overnight. Cells were plated onto mPAs of varying heights. Height is inversely related to stiffness: as pillar height decreases, stiffness increases. Four outputs were measured for each cell: traction force, cell size, nuclear size, and FN assembly. Cell images were captured using a fluorescence microscope. Cellular traction forces, cell size, nucleus size, and FN fibril area were quantified using an original MATLAB image-processing algorithm. To block FN assembly, cells were incubated with 10 μM of the functional upstream domain (FUD) of the protein adhesion F1 found in the bacteria S. pyogenes. This domain has previously been shown to inhibit FN assembly.

Results: Cells were plated on pillars with stiffness ranging from 12 nN/μm to 75 nN/μm. As cells were plated onto posts of increasing stiffness, they generated larger forces. On each stiffness, cells assembled FN fibrils; however, larger fibrils were seen only on softer surfaces, suggesting that cells use FN assembly as a means of overcoming mechanical signals. On each stiffness, cells spread to similar, larger sizes, in addition to a proportionate nuclear size. To determine the effects of FN fibrillogenesis in these processes, we inhibited assembly with FUD. Results indicate that cell spreading and nuclear size were significantly reduced in the absence of FN fibril formation.

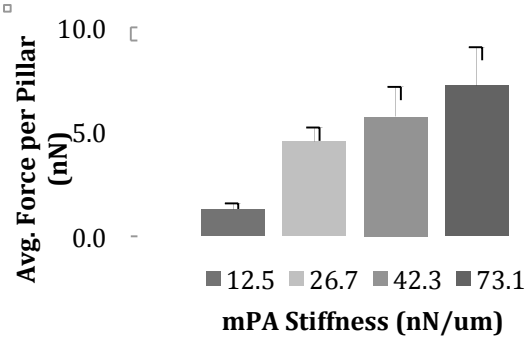

Figure 1. The relationship between substrate stiffness and average force per pillar. The average force vector per pillar was calculated for each cell by summing total force magnitude and dividing by the number of cell-occupied pillars. Forces increased with increasing substrate stiffness. There exists a clear linear relationship between substrate stiffness and forces generated.

Conclusions: Here we will present data that demonstrates a critical role for FN fibrillogenesis as a modulator of mechanical signaling. We have shown that lung fibroblasts assemble FN fibrils on all stiffness surfaces, yet assemble larger fibrils only on softer surfaces. On these softer surfaces, cells generate less force, but are still able to spread to sizes comparable to cells plated on stiffer surfaces. Nuclear size is also comparable across all stiffness surfaces. When FN fibril assembly is inhibited, lung fibroblasts respond to mechanical stimuli in a manner that is more consistent with published mechanobiology literature; that is to say they spread poorly on softer surfaces, and have correspondingly smaller nuclei. This suggests that cellular assembly of FN fibrils provides cells with a mechanism to subvert the influence of mechanical signals. In this way, the study and control of ECM assembly is critical to biomaterial design, particularly in arenas where mechanical signals are being exploited to drive cell function.

References:

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