The Effect of Substrate on the Mechanical State of the Cell

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Statement of Purpose:

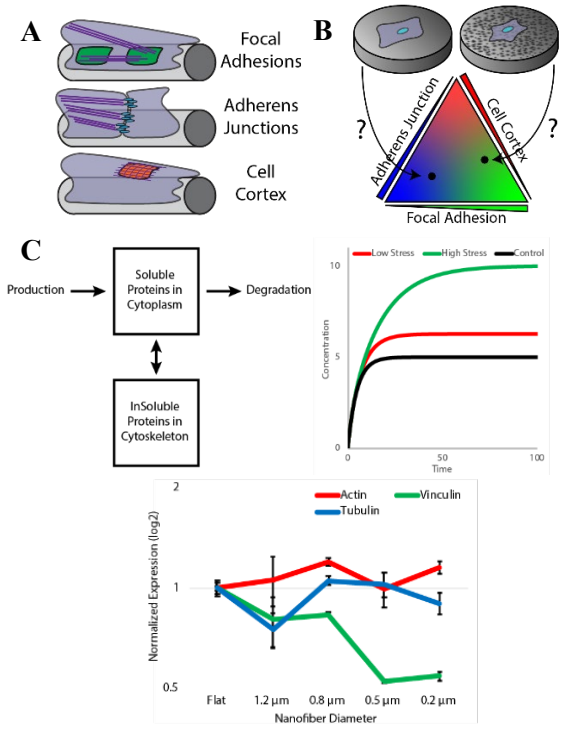
Cells respond to different surfaces through changes in morphology, gene expression, migration, and many additional characteristics [1]. These changes reflect the cell's adaptation to the surface. The surface can provide cues through mechanosensitive signaling pathways, often which begin at cell attachment sites such as focal adhesions or adherens junctions [2]. Control over the downstream effects of these signaling pathways would allow for control over a variety of cellular processes, many of which are extremely complex (i.e. stem cell differentiation). However, the focal adhesions and adherens junctions responsible for sensing and communicating stimuli that trigger these cascades are highly dynamic and transient and signal strength is dependent upon complex size and abundance [2]. Therefore, it would be beneficial to understand what surfaces favor which type of cell attachments. In this work, we investigate the differences in the relative amounts of each mechanosensitive complex induced by various nanofiber surfaces.

Methods:

We coated glass coverslips with poly(2-hydroxyethyl methacrylate) and then electrospun poly(methyl methacrylate) nanofibers with diameters of 0.2, 0.5, 0.8, 1.2 μm. Human mesenchymal stem cells (hMSCs) were seeded on the fibers and coated glass coverslips (control) and grown in α-MEM at 37°C in a 5% CO_2 atmosphere. Cells were lysed from the nanofiber substrates using tissue protein extraction reagent and total protein was quantified with the precision red advanced protein assay. Relative amounts of mechanical proteins actin, tubulin, and vinculin were measured by western blotting. In addition to western blotting, we will fix and immunostain cells on each nanofiber substrate and use fluorescence microscopy to visually assess actin, tubulin, and vinculin localization and abundance. Following qualitative immunostaining, we will conduct in-cell western assays to quantitate relative protein numbers.

Results:

hMSCs were successfully cultured on nanofibers and control glass coverslips. Following western blotting of the sample lysates, results demonstrated the two cytoskeletal proteins actin and tubulin remained at constant levels independent of fiber diameter. However, vinculin, which localizes to focal adhesion complexes, decreased in abundance with decreasing fiber diameter.



A) Cells can transmit forces to external environment through three complexes. The relative distribution of force to all three complexes establishes cell mechanical state.

B) Cells may exhibit higher proportions of certain mechanosensitive complexes dependent on surface topography.

C) Model predictions indicate proteins actively under high stress in the cytoskeleton accumulate in the cell. Western blotting confirmed this model, showing a decrease in vinculin in concert with a decrease in fiber diameter.

Conclusions:

Greater understanding of the interactions between surface features and mechanosensitive complexes (i.e. focal adhesions) is an important step in rationalizing biomaterial design. By intelligently selecting materials with particular properties, we can trigger certain signaling pathways and improve control over processes such as differentiation and tissue formation. Future studies will focus on further investigation of which surface characteristics favor which structural complexes and the resulting downstream effects.

References:

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[3] Kanchanawong P. Nature. 2010;468:580-584