Single Cell Migration Speed on Nanofibers is Rho/ROCK and Focal Adhesion Structure Dependent

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Statement of Purpose: The physical environment of a cell affects differentiation, migration and survival. Migration within tissue engineered constructs is critical for the complete cellularization of many types of tissue engineering constructs, including nanofiber constructs made by electrospinning. The focal adhesion is a dense concentration of signalling and scaffolding molecules that forms at the site of transmembrane attachment to the substratum. The physical force across molecules in the focal adhesion can affect signalling site availablility which can be measured using forster resonance energy transfer (FRET) biosensors. Here we compare cell migration speed and focal adhesion activation state when cells are attached to fibers with a range of diameters from 50-1200nm, in order to investigate the effect on the cellular migration activity through focal adhesion signalling and the tension produced across the molecule vinculin.

Methods: Electrospun nanofiber scaffolds are fabricated from PMMA (996kDa). Concentration is varied to control the fiber diameter (0.986 linear correlation coefficient). The fibers are collected on poly(2-hydroxyethyl methacrylate) (polyHEMA) coated glass coverslips. Western blots and immunofluorescence are performed with primary antibodies (CellSignaling) and the appropriate nearIR secondary (LICOR Biosciences). Time lapse photography is conducted with a 10X water dipping objective under DIC with 90 second intervals. Cells (n=5 per fiber diameter, density, or condition) are tracked with the ImageJ Manual Tracking plugin. FRET sensor cDNAs (AddGene) are obtained as kind gifts from the contributors, expanded, transfected into the cell of interest and imaged in fixed cells. The FRET ratio is taken as the ratio of the FRET channel to the DONOR channel. For the Vinculin-Tension Sensor (TS), a threshold was applied to the donor image to approximate the area of the focal adhesions. A Student's t-test is used to compare means, while the Pearson product-moment correlation coefficient is used to determine strength of correlation.

Results: Mouse embryonic fibroblasts (MEFs) have a maximum cell velocity of 194 μm/hr on the 438nm diameter fibers which decreased to 68 μm/hr on the 48nm fibers and 84 μm/hr on the control flat substrate ($p<0.05$, Figure 1). The maturity of focal adhesions has been correlated to migration speed, with the larger mature focal adhesions predicting slower movement, however the Pearson correlation coefficient of 0.39 suggests that focal adhesion length may not be the single determinant of migration speed in a synthetic environment. An alternative hypothesis is that the biochemical activity of the focal adhesion is altered by the fiber diameter. To examine this we compare the activation of Focal Adhesion Kinase (FAK) at residues 397, 576/577 and 925. A Pearson correlation between cell velocity and pFAK 576/577 of 0.872 indicates support for this hypothesis. Active Src 416 also correlates with cell migration speed at a 0.72 coefficient. In further support of focal adhesion signaling, down stream Rho kinases were blocked to show a Rho/ROCK dependence of the nanofiber diameter driven changes in cell migration speed (Figure 1). This was shown with both mouse MEFs and human Mesenchymal Stem Cells (hMSCs).

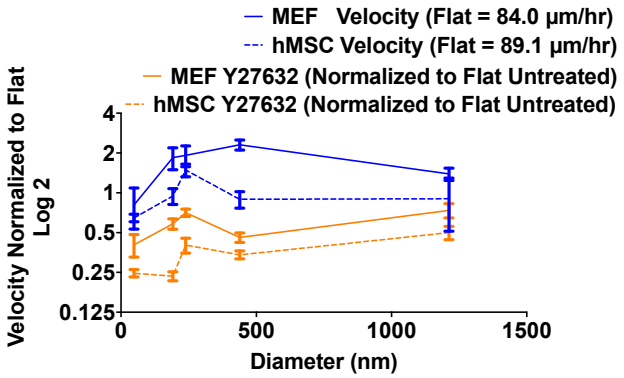

Figure 1. Rho/ROCK Inhibition Slows Migration

A similar shape curve was found for RhoA activation, with all fiber sizes causing at least a 1.3 fold increase over the flat surface as determined by ratiometric imaging of RhoA FRET transfected cells (Figure 2).

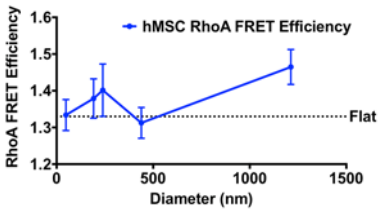

Figure 2. RhoA Activation vs Diameter

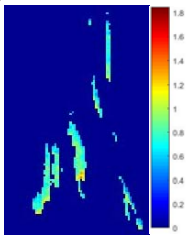

Figure 3. Vinculin-TS

To determine the effect of curvature of the nanofiber surface on the tension formed in the focal adhesion, FRET measurements are made in a Vinculin TS molecule. Preliminary results validate the function of the construct demonstrating gradations of tension within focal adhesions (Figure 3) and an average FRET ratio of 0.57 +/- 0.04 and 0.66 +/- 0.10 for flat and fiber attached focal adhesions within the same cells ($p = 0.061$).

Recent experiments have also begun to investigate a second scaffold parameter, fiber density, on migration speed of cells on a nanofiber scaffold. Migration speed is inversely correlated with the field of view fiber density.

Conclusions: The focal adhesion is a complex concentration of signaling molecules that supports force during attachment and migration to a substrate. While the size of focal adhesions does not correlate well with the observed migration velocity trends, the FAK activation state and recent data on the tension experienced by vinculin offer greater support for the velocity phenotype. Future experiments will build on the scaffold properties explored to provide more insight into the scaffold design on migration potential within tissue engineered constructs.