Statement of Purpose: The availability of an adhesive surface to a cell modulates the cellular response in different ways. One interesting way to manipulate adhesive area is to alter the topographical cues (others possibilities are surface ligand density and stiffness of the surface). Previous studies showed that surface topographies play critical roles in differentiating osteoblasts independent of chemical factors [1]. While adhering to certain topographies, osteoblasts undergo drastic morphological changes including enhanced focal adhesions, stress fiber formation and increased contractility (upon formation of acto-myosin complexes). RhoA GTPase signaling cascade is activated upon contractility (upon formation of acto-myosin complexes).

Methods: Poly(methylmethacrylate) (PMMA) nanofibers were produced using electrospinning. The average diameters of fibers were measured to be 850 nm. As a material control 2% PMMA solution was spin coated onto a glass cover slip. MC3T3/E1 cells were seeded on either flat PMMA coated or fiber coated cover slips. The cell morphology was observed using immunofluorescence imaging. A modified probe Atomic Force Microscopy (AFM) was used to measure the mechanical properties of the cells growing on fibrous and flat PMMA substrates. In this method the cellular stiffening due to cytoskeleton rearrangement results in viscosity changes which were measured by the amount of deflection of the cantilever tip that the force applied by the substrates into spring deflection (Fig 2. C). Following the previously published modified Hertz model [4];

$$\delta = 3F(1-v^2)4ER^2/3$$

where $\delta$ is the deformation of the sample, F is the applied force, v is the Poisson’s ratio (0.5), E is the elastic modulus and R is the radius of the sphere. In order to investigate the cellular growth and differentiation signaling ERK1/2, p38 and JNK/SAPK semi-quantitative western blot was performed with inhibitors of cytoskeletal tension related to RhoA GTPase signaling. We also examined directly RhoA GTPase activity using a RhoA activity assay (Cytoskeleton Inc). In order to test osteogenic differentiation ALP staining was performed using SigmaKit No85 after 2 week incubation on experimental substrates.

Results: Previous studies from our group show that (2) growth and differentiation signals of MAPK signaling cascade change significantly when fibers diameters reach 800 nm. We hypothesized that the observed changes could be due to increased cytoskeletal stiffness along a fiber which an individual cell is adhered to. We first measured the cell stiffness and found a cell on a fiber was three times stiffer than a cell on a flat surface. We further investigated whether the stiffness is due to RhoA $\rightarrow$ ROCK (Rho associated coiled coil kinase) $\rightarrow$ MLCK pathway. When a ROCK inhibitor was added the stiffness dropped to 2.75 MPa approximately 40 percent less than a cell on a flat substrate and 5 times less than a cell on a fiber (Table 1).

Table 1. The stiffness values of individual cells adhering to flat PMMA or fiber coated surfaces.

<table>
<thead>
<tr>
<th></th>
<th>Cells on PMMA</th>
<th>Cells on glass slide</th>
<th>Cells on fibers with Y27632</th>
<th>Cells on fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.62 MPa</td>
<td>7.13 MPa</td>
<td>15.3 MPa</td>
<td>2.75 MPa</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>3.62 MPa</td>
<td>1.92 MPa</td>
<td>5.89 MPa</td>
<td>1.39 MPa</td>
</tr>
</tbody>
</table>

Figure 1. Relative changes in p38 phosphorylation in response to ROCK inhibitor (Y27) and MyosinIIa inhibitors on flat (PMMA) vs fiber coated surfaces.

Conclusions: RhoA involvement in initial adhesion to extracellular matrix like topographies have significant effects on growth and differentiation signals on primary osteoblast cells.

References: