Probing Complex Cell Behavior on Individual Polystyrene Nanofibers

Zimberlin, J.A., Weiger, M.C., Cicerone, M.T.
National Institute of Standards and Technology, Polymers Division

Statement of Purpose: For normal tissue function, individual cells must sense and integrate numerous chemical and mechanical cues from their surrounding 3D environment. How cells accomplish this is not understood, but methods are being developed to distinguish how these cues are sensed. Currently, understanding cellular response mechanisms to nano- and micro-scale 3D structures has become an area of great scientific interest. Various studies have demonstrated that cell affinity and orientation are influenced by the diameter of electrospun nanofibers, possibly because internal mechanisms are tuned to respond to fibers in the extracellular matrix of similar curvature. In order to develop an understanding of how and why cells are responsive to nanotopological features, we have investigated the effect of small diameter fibers on cell affinity and orientation as well as the spatial localization of cytoskeletal components with respect to singular nanofibers. This study was accomplished by culturing MC3T3-E1 osteoblasts on tissue culture (TC) polystyrene (PS) substrates containing a low surface density of PS nanofibers, and quantify the distribution of cells on and off the fibers.

Methods: *Nanofiber formation*. A PS solution with a mass fraction of 10 % was prepared from TCPS (Corning), by dissolving in dimethylformamide. The solution was filtered with a 0.20 μm syringe filter. The fluorescent dye, Rhodamine 123 (Sigma) was added to the PS solution at a concentration of 20 μg/mL to enable visualization of the fibers by fluorescence microscopy. PS nanofibers, with a diameter of approximately 1 μm, were formed using a custom electro-spinning setup. Briefly, the PS solution was drawn into a 1 mL syringe fitted with a 22 gauge needle which was then mounted onto a syringe pump. An electric field of 15 kV was applied between a PS substrate and the syringe needle (a separation distance of 17.5 cm) as the syringe pump dispensed the PS solution at a rate of 1 mL/hr.

Cell Culture. All cell studies were performed using cell passages below 20 and cultured at 37 ^{o}C and 5 % CO_2. MC3T3-E1 mouse calvaria-derived osteoprogenitor cell line were cultured in αMEM (Lonza) with 10 % fetal bovine serum (FBS) (Invitrogen/Gibco) and 1 % kanamycin (Sigma).

Cell Response Experiments. MC3T3-E1 osteoblasts were plated on the PS nanofiber substrates and allowed to spread for 3, 6, 12 or 24 hr before cell fixation and immunochemical analysis of cytoskeletal structures. Cell motility and spreading behavior was monitored by taking sequential brightfield images on a Nikon Eclipse TE300 inverted microscope every 2-6 hr over a 48 hr period. The cell samples were stored in an incubator at 37 ^{o}C when they were not being imaged.

Immunochemical Analysis. Cell samples were fixed using a 3.7 % formaldehyde solution in buffer with a soft fixing for 10 min and a final fixing step for 10 min. After washing twice with dulbecco's phosphate buffered solution [PBS], the cells were permeabilized with permeabilization solution for 10 min. The cells were washed two times with PBS and blocked for 1 hr with an antibody dilution. Fluorescent cell images were captured with epi-fluoresence excitation (480 nm for Rhodamine 123 and 540 nm for vinculin and actin) with a Nikon Eclipse TE300 inverted microscope.

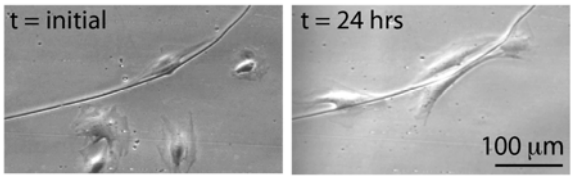

Figure 1. Phase contrast images of preferential adherence of cells to nanofibers.

Results: During cell affinity experiments, we observed that over a two day period 80 % (16/20) of the cells remained adhered to the nanofibers, while 20 % (4/20) migrated over and away from them. Further, 10 additional cells to the original 20 approached the nanofibers and spread on them. An example of a cell approaching a fiber and spreading on it is seen in Figure 1. Cytoskeletal staining of actin after both 3 hr and 3 days of cell culture show significant alignment of the stress fibers to the nanofibers, as seen in Figure 2.

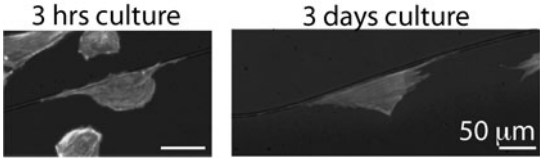

Figure 2. Actin stress fiber alignment with nanofiber.

Conclusions: In summary, our preliminary results show that the cells prefer to adhere to the PS nanofibers, rather than to the flat substrate. The cells are responsive to singular topographical features (nanofibers) on an otherwise flat substrate. The cells preferentially adhere to the fibers and seldom move away from them once they have come into contact with them. This response to nanotopography is also observed in the cytoskeletal orientation of actin stress bundles in the direction of the nanofibers. A more quantitative study of both cell adherence to the nanofibers and cytoskeletal orientation relative to the fibers is being carried out as well as the effect of varying fiber diameter on these cellular responses.

References:

Sun, T. Biotech. and Bioeng. 2007; 97: 1318-1328.
Tian, F. J. of Biomed. Mat. Res. Pt. A. 2008; 84A: 291-299.
Bashur, C. A. Biomat. 2006; 27: 5681-5688.