High-throughput Investigation of Cell Proliferation on Crystalline Polymers

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The results of our investigation of cell proliferation on crystalline polymers will be presented. The goal of the research is to investigate how cells respond to nanometer scale roughness, and crystallizing poly(L-lactic acid) (PLLA) was a convenient system on which to study this.

Polymer films with dimensions 15 mm x 60 mm were prepared by flow-coating chloroform solutions of PLLA onto a silanized silicon wafer. The glass transition temperature of PLLA is 53 °C and the rapid evaporation of the solvent trapped the film in an amorphous state. The samples were placed under vacuum for at least 4 d to allow residual solvent to evaporate. They were then placed on a stage with a temperature gradient from 44 to 100 °C for 4 min to create a gradient in crystallinity, then quickly cooled to room temperature to lock in the gradient.

The morphology of the resulting sample was measured using an atomic force microscope. Along the sample the polymer displayed the usual nucleation-and-growth kinetics associated with crystallization. This created a gradient in roughness that varied from less than 1 nm to approximately 13 nm, which is shown in figure 1.

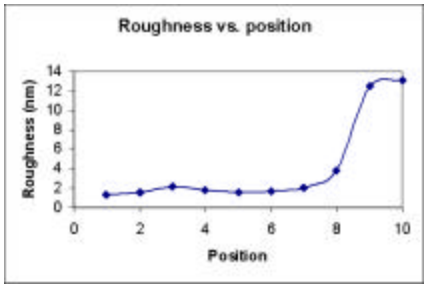

Figure 1: Root-mean-square roughness of the PLLA film as a function of position along the film as measured by atomic force microscopy. The standard uncertainty in these measurements, taken as the standard deviation over multiple experiments is 0.2 nm.

Osteoblastic cells were cultured on the samples for (1, 3, and 5) d to investigate their rate of proliferation as a function of substrate roughness. The cells were cultured in a medium containing 5 % fetal bovine serum at 37 °C. After culturing the samples were rinsed in phosphate buffer solution and fixed in a 2 % formaldehyde solution. The cells were then stained with the fluorescent nuclear stain DAPI.

Proliferation was measured using automated, fluorescence microscopy. A microscope with a translation stage was operated using Image-Pro and the cell number as a function of position was measured using image analysis software to count the number of fluorescing nuclei in each frame. Generally a (10 x 15) grid of images gave reproducible counts across samples and each time point was repeated at least 5 times to validate the results.

The results of this are shown in figure 2 where average total cell number at each position along the library as shown as a function of position.

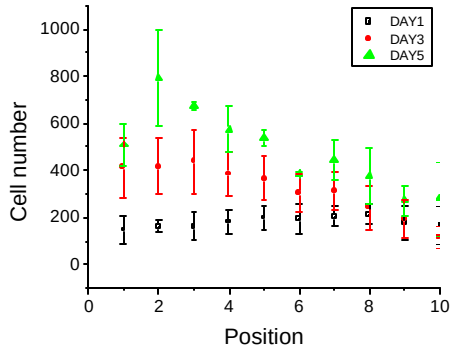

Figure 2: Cell proliferation as a function of library position. The error bars represent one standard deviation and are taken as the standard uncertainty.

It appears that substrate roughness can strongly influence the rate of proliferation of cells. Enzyme-linked immunofluorescent assays were performed to investigate whether there is any significant change in the concentration or conformation of adsorbed attachment proteins such as vitronectin or fibronectin and found none. It appears that these cells are responding strictly to the surface topography.

Disclaimer: Certain equipment and instruments or materials are identified in the paper in order to adequately specify the experimental details. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply the materials are necessarily the best available for the purpose.