Combinatorial Characterization of Osteoblast Attachment on Micropatterned Polyurethanes

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Statement of Purpose: Understanding the exact mechanism of cell-biomaterial interaction is one of the main objectives in biomaterial research. Surface roughness, geometric spacing of adhesive and non-adhesive area, and surface mechanical properties have been studied for decades to understand their influence on cell behavior and cell fate upon adhesion [1,2,3,4]. Research investigating the link between chemical and physical surface properties and cellular response is important for future medical applications, especially for surface-sensitive applications such as bone repair. This research will have a wide impact in understanding the effects of polymer synthesis and processing on cellular engineering for adhesion-dependent cells. We are using phase separated materials to allow patterning in 2D structure to be extended further into 3D structure.

Combinatorial Micropatterned Polyurethanes Preparation Methods: Poly(ethylene glycol) (**PEG**, M_w=2,000, Sigma-Aldrich), poly(caprolactone) (**PCL** M_w=80,000, M_w/ M_n=1.43, Aldrich) were used create two-dimensional libraries of composition and annealing temperature (Φ/T), prepared on 22mm×22mm cleaned and etched silicon chips [5]. 4,4 Methylene bis-phenyl diisocyanate (**MDI,** Sigma-Aldrich) and **Pluracol ®** (Mw=430, BASF) in Tetrahydrofuran (**THF,** EMD) were used as spacer/crosslinker. **Standard cell culture and assays** were followed for culturing mouse osteoblast-like cells (MC3T3-E1 subclone 4 from ATCC, Rockville, MD, passage 6 or 7) seeded onto combinatorial chips of surface lateral patterns at a density of 5,000 cells/cm^2 for 1 d. **Image Acquisition:** Surface lateral and cell density were studied by crossed-polarized and fluorescent microscopy (multi-channel, automated). Typically, for a 22mm×22mm library, at 10× magnification rate, 324 images from distinct locations (1,200μm×1000μm) were acquired. **Computation and Analysis**: Quantitative descriptions of surface lateral patterns and cell proliferation were attained by image processing with the ImageJ (NIH) and Matlab™.

Results/Discussion: Surface patterning has been utilized to elucidate cell-biomaterial interaction by managing the chemical contrast between the 'adhesive patterns' (PCL-rich-phase) and the 'non-adhesive background' (PEG-rich-phase). In this research, PEG is used to reduce non-specific adsorption of protein, and hence reduce cell adhesion. PCL is relatively more hidrophobic compared to PEG and is used as the adhesive phase. A subset of the library is shown in Fig 1. Cell-to-PCL feature local analysis was done to the whole library to extract any preferential cell attachment to PCL feature properties. Enhancement of cell attachment was shown in some combinations of PCL-feature-to-cell distance and PCL-feature area as shown in Figure 2.

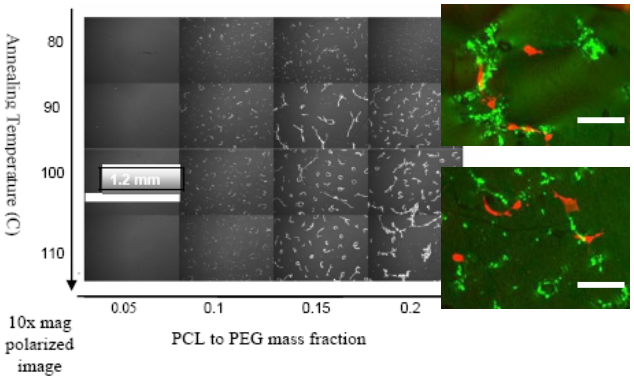

Figure 1. Surface Patterns on a PEG-MDI-Pluracol/PCL Library. (white: PCL phase; each image size: 1189μm×966μm). *Inserts:* Cell attachment (red, actin) on PCL features (green) – (*Reference to Fig. 2)* rectangular area (top) and triangular area (bottom). Bar = 100 μm.

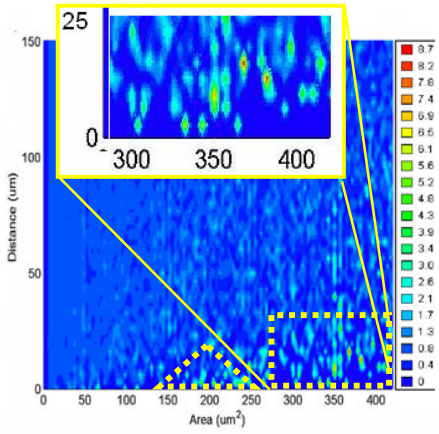

Figure 2. Surface plot of adhesion enhancement observed within distance (μm) from a PCL- feature of area (μm2), compared to random cell attachment event.

Conclusions: Combinatorial surface lateral patterns library has been developed through polyurethane blend micropatterning. Certain size and spacing of the patterns have been shown to give apparent enhancement on cell adhesion density.

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