Profiling cell-biomaterial interactions via high content imaging on roughness gradients of polymer blends

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Statement of Purpose: We propose an iterative approach for the accelerated development and optimization of application-specific biomaterials using combinatorial biomaterial synthesis and high-content cellular assays. Fluorescently engineered cells were cultured on polymer substrates with a controlled gradient of roughness to ascertain the effect of nanometer changes in surface topography on cell morphogenesis. The proposed approach is novel in that it utilizes both high content imaging of cells and high throughput material fabrication platforms to simultaneously explore the effects of surface topography and chemistry on cell-biomaterial interactions.

Methods: Tyrosine derived degradable poly (DTE carbonate) and poly (DTO carbonate) were synthesized as previously described[1]. Compositional variations of blends of these two degradable polymers were subjected to linearly decreasing temperatures, leading to spatial gradients of surface roughness over regions of uniform composition[2]. Saos-2 cells engineered to express a group of GFP fusion proteins (e.g. GFP-farnesylation, GFP-actin, GFP-actinin, GFP-paxillin, GFP-Rac1, GFP-Rho) were cultured on the roughness gradient substrates, incubated for 24h at 37°C and imaged in real-time, without fixation, within a temperature controlled POC chamber retrofitted on the motorized stage of a Leica TCS SP2 confocal laser scanning microscope (CLSM) (Leica Microsystems Inc. Exton, PA). Tile-scanned CLSM images were obtained at low (10X) magnification over a 2.5x4.5mm region. Single cell imaging allowed the quantification of morphologically based cell descriptors (Image Pro Plus, Silver Spring, MD) and functional data (e.g. cell attachment and spreading). Individual descriptors of cell cytoskeletal morphology and cellular functions were compared as function of location on the roughness gradient (and therefore a function of local topography) utilizing ANOVA with Tukey's post-hoc test (SPSS Inc. Chicago, IL).

Results/Discussion: Our data indicates that differences in the surface roughness along the polymer gradient blends are strong determinants of cell spreading behavior. However, this study demonstrates a quantitative methodology by which a continuous gradient of surface topographies may be studied with simultaneous control of chemistry. Roughness gradients in blends of 50/50(wt/wt) p(DTE carbonate)/p(DTO carbonate) elicited elevated levels of cell attachment and pronounced changes in the intracellular molecular descriptors for cytoskeletal organization and focal adhesions at an intermediate roughness level (RMS=20±3nm). Intermediate surface roughness resulted in increased cell spreading, increased number and length of actin stress fibers and increased number and strength of focal adhesions (as determined by integrated optical density). This indicates that while increasing roughness leads to increased surface area, which may promote cell attachment, once surface features became too large, cell attachment is inhibited. This may be attributed to cooperative contributions of surface topography, surface ligand density and protein adsorption and conformation. Thus, the optimal surface contours are likely those that provide for increased attachment area, without extreme features that possibly inhibit protein and extracellular matrix and adsorption.

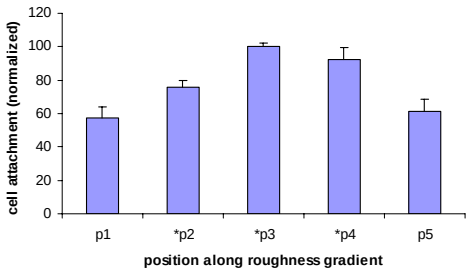

Figure 1. Cell adhesion at various positions on roughness gradients

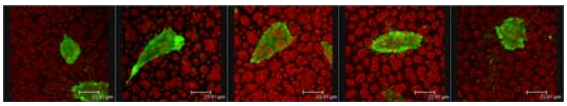

Figure 2. SAOS2 cell morphology along roughness gradients

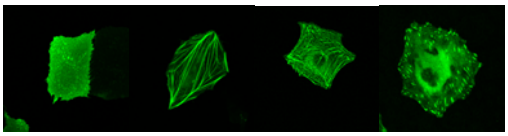

Figure 3. GFP engineered SAOS2 cells. (Images from left to right: GFP-f, GFP-actin, GFP-actinin and GFP-paxillin).

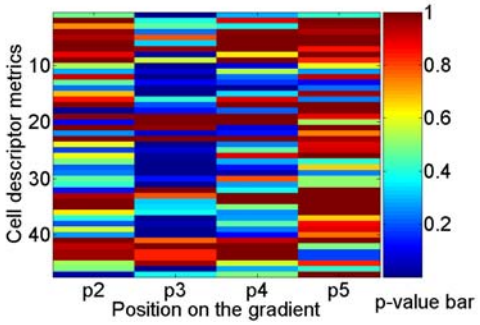

Fig. 4. Color-coded p-values of cell descriptors relative to controls are depicted for positions along the roughness gradient.

Conclusions: Our study demonstrates the feasibility of obtaining quantitative cell descriptors via in-situ imaging of polymer gradient substrates. Furthermore, the data captures the quantitative extent to which surface topography can alter the qualitative nature of cell adhesive responsiveness to polymer substrates through integrin-actin cytoskeleton adhesion complex pathways. Future work includes extending this approach for other GFP-reporter based descriptors on a wider range of combinatorially engineered polymers and in 3-D.

References:

1. Bourke, SL. Adv Drug Deliv Rev 2003(55)447-466
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