Micropatterned Interfacial Polymer Layers for Directing Cellular Response

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Statement of Purpose: It has been previously proposed that providing haptotactic or chemotactic gradients to the growing axons can potentially solve the issue of failure of axons to exit biomaterial bridges for spinal cord injury and lead to successful nerve regeneration.[1] Using surface-initiated Living-Radical PhotoPolymerization (si-LRPP) we have formed gradient and patterned interfacial layers using hydrophilic monomers such as methacrylic acid (MAA) and poly(ethylene glycol) monoacrylate (pPEGMA), The effects of exposure time, light intensity, photoinitiator concentration and monomer concentration on the kinetics of polymer brush formation and resulting surface chemistry have been studied for both systems. By using a dynamic photomask, polymer brush gradients have been created and verified using Atomic Force Microscopy (AFM). Furthermore, by using photomasks we have created two-dimensional patterns of grafted polymer brushes that enable spatial control over desirable cell-material interactions. By combining patterns of different polymer brush chemistries with gradient brushes conjugated with bioactive molecules, surfaces can be created to spatially direct cellular responses such as adhesion, growth, and migration.[2]

Methods: Grafting studies were conducted on two distinct substrates to demonstrate versatility of the si-LRPP process. Polyurethane (PU): Solution of hexa-functional urethane diacrylate (CN975, Sartomer, Exton PA), triethylene glycol dimethacrylate (crosslinker), tetraethylthiuram disulfide (TED, photoiniferter), initiator (Irgacure 651, Ciba, Tarrytown, NY) is injected between two glass slides and exposed to 365nm UV light at 25mW/cm^2 for 600s. Glass Substrates: A monolayer of photoiniferter *N*,*N*-(Dimethylamino)-dithiocarbamoyl benzyl(trimethoxy)silane (SBDC) is attached to piranha treated and flame dried glass.[3] Grafting: Substrates covered with monomer solution (MAA, PEGMA (MW =375) or PEGMA (MW = 3000)) are exposed to 365nm UV light at an intensity of 25mW/cm^2. Surface grafting is characterized using contact angle (CA) to measure wettability and UV spectroscopy to measure extent of cationic dye adsorbed to anionic polymer grafts. Patterned and gradient layers are characterized using AFM and fluorescent dye (dansylcadaverine) conjugation. NIH 3T3 fibroblasts are cultured in serum-containing media on micro-patterned polymer layers at a density of 20000 cells/well and observed using optical microscopy.

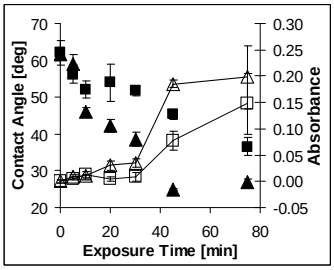

Figure 1. Effect of exposure time, & increase of light intensity from 6.25 (■,□) to 25mW/cm^2 (▲,△) on CA (■,▲) and dye absorbance (□,△).

Results/Discussion:
Increasing UV exposure time from 0 to 75 min results in a larger extent of polymer grafting as indicated by the decrease in CA and increase in dye absorbance values with exposure time in Figure 1. Furthermore, an increase in light intensity leads to much faster attainment of final CA and dye absorbance values (Figure 1) indicating higher grafting rates. Polymer brush thickness gradients of pMAA were created as described previously[3] using an opaque movable mask. AFM height data (□) and intensity profile scan (–) of a fluorescently tagged brush confirm gradient formation (Figure 2). The slope of the gradient can be easily controlled by changing the mask velocity.[4] Next to spatially control cell adhesion, we grafted pMAA and pPEGMA patterns using high resolution photomasks. NIH 3T3 cell cultured on pPEGMA 375 patterns adhere only in the PEG free areas (Figure 3a) indicated by the black regions of the photomask in Figure 3b.

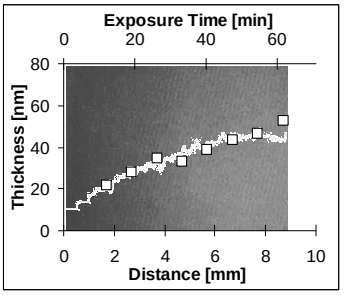

Figure 2. The thickness (□) and fluorescent intensity (–) overlaid on an image of a conjugated brush layer indicate a gradient structure.

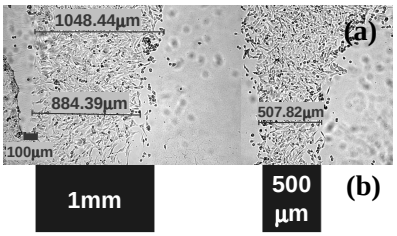

Figure 3. Day 4 image of patterned pPEGMA 375 surface showing cells do not adhere in regions grafted with PEG.

Conclusions: **V**arious polymers such as pMAA, PEG 375 and PEG 3000 have been successfully grafted on PU and glass surfaces with nanometer control over brush thickness. By using photomasks, surface density and thickness gradients as well as high resolution patterns have also been obtained. Cell culture on PEG 375 patterned surfaces indicate spatial control over cell adhesion. Future plans include conjugation of neuron specific proteins to direct neural cell growth.

References:
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