Complex Three-Dimensional Nanometer-Scale Architectures for Tissue Engineering

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ABSTRACT

Fine control of nanostructures promise to enhance our ability to understand the fundamentals of cell behavior by mimicking the inherently nanoscopic dimensions of the cell from the nanometer scale topographies of the extracellular matrix (ECM) to the actual chemical species at the cell-matrix interface. A potentially powerful tool to produce nanometer scale topography may be realized by using the lithographic technique known as Proximity-field nanoPatterning (PnP).[1,2] Complex three-dimensional nanometer scale architectures were prepared with the conventional photopolymer SU-8. Several different 3D architectures were produced. To assess potential cytotoxicity of the materials, Bovine Aortic Endothelial Cells (BAECs) were seeded onto planar as well as 3D structures. After 10 hours, cell attachment and spreading was evaluated.

INTRODUCTION

Cell growth and expression have been shown to respond to surface topology.[3] Many techniques exist for creating nanopatterned two-dimensional surfaces including electron beam lithography, near-UV photolithography, nanoimprint lithography, self-assembly, phase separation, and colloidal lithography.[4,5] Fewer techniques exist to create truly three-dimensional patterned structures.[5] The technique of Proximity field nanoPatterning (PnP) was developed by John A. Rogers at UIUC..[1,2] Briefly, a polydimethysiloxane (PDMS) elastomer phase mask is placed on the surface of spincast photoresist. The PDMS phase mask is patterned in x, y, like a grating, and also in z, with dimensions roughly equal to the exposure wavelength. The phase mask is held in place to the angstrom level by generalized adhesion forces (primarily van der Waals forces), without applying external pressure. The surface relief on the phase mask modulates the phase of transmitted light by a significant fraction of π and in a spatial geometry defined by the layout in x and y, by the depth of the features in z, and by the index of refraction of the PDMS relative to the underlying material. Passage of light through this phase mask generates a complex 3D light intensity distribution by the spatial phase modulation and can be described by diffraction (Abbe theory)[6] and the Talbot effect[7] (self-imaging). This 3D distribution of light intensity patterns extends from the near-surface region of the phase mask (< $\lambda_{exposure}$) to the proximity region below the phase mask surface, several millimeters away. The underlying resist is thus exposed in certain regions of high intensity (> burn threshold of the resist) and not exposed in regions of low intensity (< burn threshold of the resist). The light intensity pattern is limited only by the areal size of the phase mask and the diameter and coherence of the exposure source. Therefore, once the mask has been produced and fabrication parameters have been determined, PnP can be used to create large scale complex 3D architectures on the bench top in minutes with minimal capital expense.

SU-8 photoresist was used for PnP development because it is an epoxy-based negative tone resist and can support patterns with high aspect ratios and 3D structures. As the fabrication parameters for this system are known, we began exploring the use of these SU-8 materials in cellular systems. SU-8 is an epoxy resin which contains a photo-acid generator (PAG), usually an iodonium salt that, upon exposure to an appropriate exposure wavelength, generates acid which catalyzes the cross-linking of the expoxide functionalities in SU-8. The chemical structure and examples of SU-8 PnP 3D nanostructures are shown below.

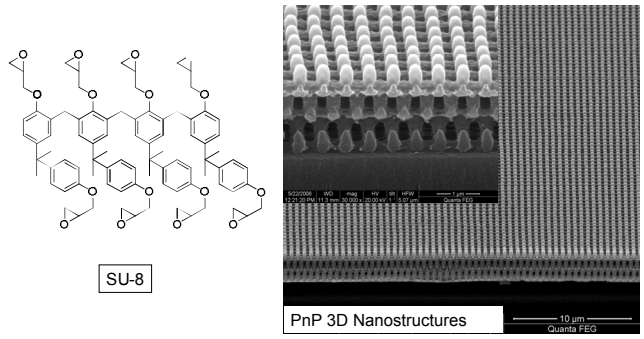

Figure 1.: SU-8 chemical structure and examples of cubic square array of posts PnP 3D nanostrucutures fabricated with SU-8.

The PnP technique provided a unique opportunity to evaluate cellular behavior on large-area complex 3D ordered structures with a variety of geometries (cubic, hexagonal, quasicrytal). BAECs were used to assess cell attachment, spreading, and proliferation on four different SU-8 3D architectures as well as on planar films.

EXPERIMENTAL

3D Structure formation in SU-8. PDMS phase masks were created by applying Sylgard mixed according to directions (Dow Corning, Midland MI) to a master mold.

SU-8 (Microchem Corp., Newton MA) was spun cast against a silicon substrate and the elastomeric phase mask was applied to the SU-8 by simply placing it on the surface of the photopolymer. The surface of the mask was exposed to collimated light (λ = 365 nm), creating 3D light intensity distributions through the thickness of the spincast SU-8. After exposure, the phase mask was removed simply by peeling it back from the surface. Finally, the photopolymer was baked and developed, leaving behind the 3D nanostructure.

To create SU-8 planar films, SU-8 was spun cast onto silicon wafers and flood exposed using 365 nm wavelength light, followed by baking and development to ensure similar surface chemistries of the SU-8 materials..

Cellular Attachment. BAECs were seeded onto polymer four 3D architectures, planar films, and Tissue Culture Polystyrene (TCPS, positive control) at a density of 2.5 x 10^4 cells/cm^2 and cultured under standard conditions. After 10 hours, samples were fixed, dehydrated in ethanol, and visualized with SEM (Hitachi S-5200, Pleasanton, CA). Attached cell number was determined after 10 and 72 hours using CellTiter-Glo® Luminescent Cell Viability Assay (Promega Corp., Madiosn, WI) which measures the quantity of ATP in viable, metabolically active cells. Samples are measured and compared to a standard curve made with known cell numbers.

RESULTS AND DISCUSSION

Using the PnP process, four different 3D nano-structures were fabricated in SU-8 including cubic (square post), hexagonal, square holes, and penrose quasi-crystal (cubic square posts shown in Figure 1). At 10 hours, there were fewer cells attached to SU-8 compared to the TCPS controls, indicating that overall, untreated SU-8 is a less attractive surface for cell attachment than TCPS. There was no significant difference in the number of cells attached to any of the 3D architectures compared to the planar film

controls (Figure 2). Visualization with SEM, however, showed varied degrees of spreading of the cells on the different surfaces (Figure 2). On the planar surfaces and cubic/square post architectures, cells spread well, with many extensions probing the surface. The other architectures, showed varying degrees of spreading with minimal to no cellular extensions.

After 3 days in culture, variations in cell number were observed between the different treatment groups (Figure 3). As seen at 10 hours, the TCPS had significantly more cells than any of the SU-8 samples. The cubic/square post structures, which supported well spread cells at 10 hours, had the least number of cells at day 3, with significantly fewer cells than the square holed and quasi-crystal structures and planar films.

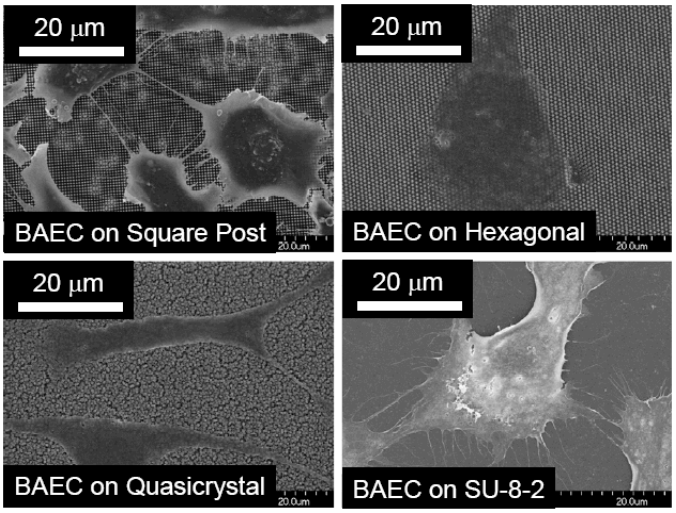

Figure 2. BAEC cell attachment and spreading on 3D architectures created with PnP using the conventional photoresist SU-8.

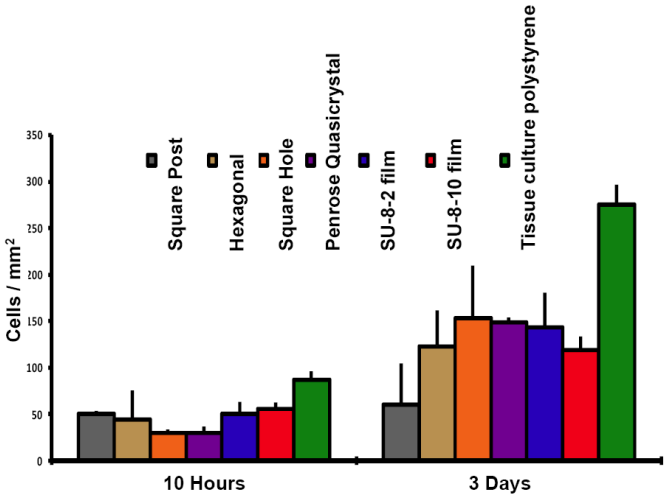

Figure 3. BAEC cell number on SU-8-10 PnP patterned structures compared to SU-8-2 and SU-8-10 planar films and tissue culture polystyrene positive controls. n = 5 for all treatments.

CONCLUSIONS

PnP lithography was used to fabricate 3D nanostructures in the photopolymer SU-8. Bovine Aortic Endothelial cells were seeded onto the 3D structures, SU-8 planar films, and TCPS controls. Cell attachment and spreading was assessed at 10 hours and 3 days. Attachment did not vary between the SU-8 formulations at 8 hours, indicating that the nano-topography of the 3D architectures did not influence cell attachment. After 3 days in culture, however, differences were detected between the cell numbers on the cubic/square post and all other treatments. These preliminary results suggest that the 3D architecture influences cellular rate of proliferation. Future work will explore effects of nano-topography on cellular gene expression. Additionally, fabrication of 3D nanostructures by PnP using biocompatible and/or biodegradable photopolymers will be investigated. .

AKNOWLEDGEMENTS

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