Generation of Laminin Micropatterns on Biocompatible Substrates Using Microscale Plasma-Initiated Patterning

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Statement of Purpose: Microscale patterning of biomolecules is used in a wide range of biological and medical applications, including biosensors, tissue engineering and immunoassays.[1,2] Current methods to micropattern biomolecules include photolithography,[2,3] microcontact printing,[3,4] microfluidic networks,[3,5] and micromolding.[3,6] Each technique has relative advantages and limitations in terms of cost-efficiency, ease, reproducibility and applicability to specific ink/substrate combinations. A novel micropatterning method, termed microscale plasma-initiated patterning (PIP),[7] has recently been developed to create various biomolecular micropatterns (e.g., poly-L-lysine, bovine serum albumin and anti-rabbit immunoglobulin G) on a wide range of biocompatible polymeric substrates.[7] In this work, the versatility of PIP is further extended and demonstrated by the generation of laminin micropatterns on several biocompatible substrates (e.g., glass, polydimethylsiloxane (PDMS), polyethylene (PE), poly(methyl methacrylate) (PMMA) and nylon).

Methods: Substrates were patterned with laminin following the procedure outlined in Figure 1.

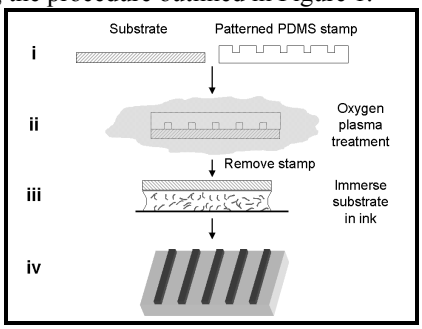

Figure 1

This procedure utilizes a striped, patterned PDMS stamp to preferentially expose and protect areas of the underlying substrate to oxygen plasma. The patterned PDMS stamp, consisting of parallel lanes (10-50 m widths) separated by spaces of various micron-sized widths, was placed into contact with the substrate, and the entire unit exposed to oxygen plasma (50 W, 660 mTorr, 300 s). Once plasma-treated, the stamp was removed and the substrate immediately immersed in a few drops of a laminin solution in phosphate buffered saline (100 g/mL) for 60 s at room temperature. Following immersion, the substrates were gently swirled in 20 mL of deionized water for approximately 10 s. Adsorbed laminin molecules were fluorescently tagged as follows: substrates were incubated for 1 h in a primary antibody solution of rabbit anti-laminin, rinsed three times in PBS, incubated for 1 h in a secondary antibody solution of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G and rinsed in PBS an additional three times. Laminin micropatterns were air-dried and visualized using confocal laser scanning microscopy.

Results/Discussion: As shown in the fluorescent micrographs (Figure 2, scale bar indicates 50 m), plasma-treating the substrates in the fashion outlined in Figure 1 preferentially increased the hydrophilicity of the exposed substrate regions to produce distinct patterns on the substrate with different relative hydrophilicities determined by the PDMS-stamp pattern.

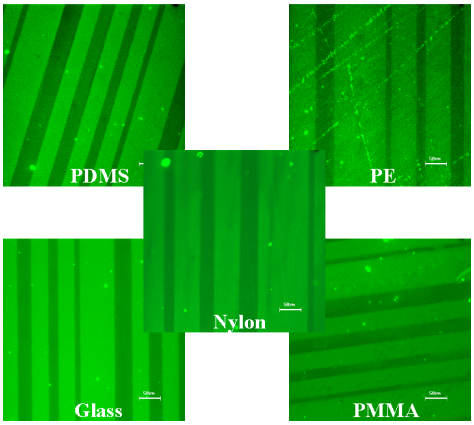

Figure 2

Once formed, these distinct regions exhibited varying affinities for the laminin molecules in solution, thereby creating micropatterns by the preferential attachment of laminin molecules to either the plasma-exposed or plasma-protected substrate regions. As shown, the striped patterns were well resolved from the underlying substrate, exhibited uniform laminin distribution within the pattern and had lateral dimensions in good agreement with stamp features.

Conclusions: These results successfully expand the applicability of PIP to include laminin micropatterning on various biocompatible substrates. The inherent simplicity demonstrated by this method makes PIP an attractive and reliable alternative to other micropatterning methods currently available.

References:

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