Micropatterning Biological Molecules on a Polymer Surface Using Elastomeric Microwells

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This paper describes a new method for micropatterning on planar substrates using poly(dimethylsiloxane) (PDMS) microwells.[1] We have chosen to demonstrate its proof-of-principle by covalently patterning two biomolecules, biotin and a peptide, onto a derivatized polymer from aqueous solution.

The ability to pattern biomolecules on solid substrates has become increasingly important for the development of molecular and cellular biosensors, biomaterials, and genomic and proteomic arrays.[2,3] A diverse array of soft lithography[4] and complementary photolithography[2] techniques have been developed, but not all of these techniques are suitable for patterning biomolecules on surfaces.[5]

The two most commonly used soft lithography techniques for biomolecular patterning are microcontact printing (μCP),[4] and patterning using PDMS flow channels[6,7] Lahiri et al. first demonstrated the covalent patterning of biological ligands onto a COOH-terminated SAM on gold by reactive μCP,[8] and a similar approach to pattern biomolecules was subsequently demonstrated on polymers by Yang et al.[9,10] The primary limitation of reactive μCP, especially on polymers, is the low surface density of the biomolecule immobilized on the surface, due to the small amount of reactant adsorbed on the stamp and the limited contact time of the stamp with the substrate. Patterning using PDMS flow channels circumvents these problems, but it cannot easily create isolated structures because of the necessity of providing fluid channels.

We have developed micropatterning using elastomeric microwell reservoirs (termed Wellpat for brevity) to overcome these limitations in patterning biomolecules. Wellpat involves several steps, as follows (Scheme 1): First, a poly(dimethylsiloxane) (PDMS)[11] stamp with positive relief features was cast from a silicon master with negative relief features. Second, the microwells in PDMS were molded from the elastomeric stamp.[12] Alternatively, the microwells can be directly fabricated by casting PDMS against a silicon or photoresist master with positive relief features.[13] Several shapes of microwells and channels were replica molded from elastomeric PDMS masters (Figure 1), and each mold provided high-resolution microstructures with feature sizes as small as 30 μm.

Scheme 1

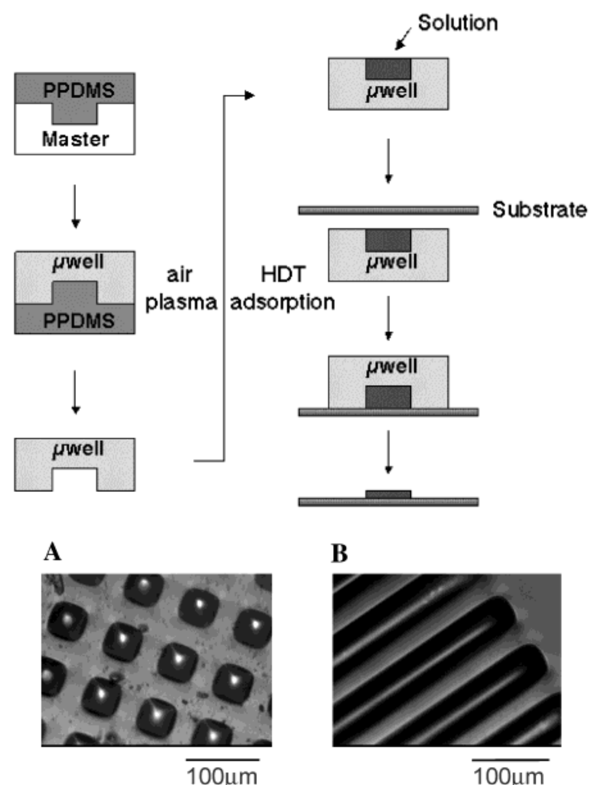

Figure 1. Optical micrographs of PDMS molds filled with an aqueous solution: (a) square microwells, (b) microchannels.

The PDMS molds were oxidized with an air plasma (80 W, 1 min, Plasmod, March Instruments) to make the surface uniformly hydrophilic. Next, the top surface of the stamp was contact-printed with hexadecanethiol (HDT) using a flat, oxidized PDMS stamp and allowed to dry, which selectively rendered the area between the wells hydrophobic.[14] The difference in wettability between the wells (hydrophilic) and the region between wells (hydrophobic) introduced by this step enables easy filling and confinement of an aqueous solution containing the biomolecule of interest to the microwells during the filling step (Scheme 1).

A poly(ethylene terephthalate) (PET) film (Dupont Inc.) was chemically derivatized to introduce COOH groups, as described previously.[9] The surface COOH groups were then reacted with *N*-hydroxysuccinimide (NHS) (0.2 M) and 1-ethyl-3-(dimethylamino)propyl carbodiimide (EDAC) (0.1 M) in distilled water. The NHS-ester-functionalized surface was dried under nitrogen and used immediately for micropatterning biomolecules.

We covalently patterned a cell-adhesive peptide[15] by Wellpat using two strategies: (1) patterning biotin on the surface and then using the streptavidin−biotin system[16] to subsequently pattern a

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(5) This is because of the specific requirements imposed by biomolecules (especially proteins) upon the patterning process; these are patterning, which must be accomplished from aqueous solution, and exposure of the biomolecule to: (a) the air−water interface, (b) organic solvents, or (c) high temperature, which must be minimized to avoid denaturation.
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(13) The two-step process is more laborious than directly casting from a microfabricated silicon master, but it reduces the use of the silicon master and thereby significantly decreases the cost associated with micropatterning.
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biotinylated peptide; (2) direct, covalent patterning of the peptide onto the derivatized PET surface.[17] In separate experiments, 100 μL of a 10 mM solution of EZ-Link biotin-PEO-LC-amine[18] ((+)-biotinyl-3,6,9-trioxaundecanediamine, Pierce) (biotin-NH_2) in phosphate buffer or gly-arg-gly-asp-ser-pro-lys(tetramethyl rhodamine)NH_2 in phosphate buffer (GRGDSP(K-TMR)NH_2) were pipetted onto the PDMS microwells, and the droplets that remained on the hydrophobic surface between the microwells were blown away with a stream of nitrogen gas. The derivatized polymer substrate was then brought into physical contact with the PDMS microwells (Figure 1).[19] The entire assembly was inverted, which brought the solution into contact with the surface. The reaction between biotin-NH_2 (or GRGDSP(K-TMR)NH_2) and the NHS esters on the surface of PET was allowed to proceed for 30 min at room temperature, following which the substrate was lifted away from the PDMS mold, and extensively washed in ethanol.

After patterning biotin-NH_2 on PET by Wellpat, the substrate was incubated with 0.1 μM unlabeled streptavidin or Alexa488-labeled streptavidin in 10 mM HEPES, 0.02%(v/v) Tween 20 detergent, for 30 min at room temperature. The samples were washed exhaustively in buffer, and confocal fluorescence microscopy was used to examine each stage of the patterning of the biotinylated peptide. Spatially well-resolved patterns of streptavidin were observed (Figure 2a) with a signal-to-noise ratio (S/N) in the patterns (pattern intensity/background intensity) of 10 ± 0.9 (Figure 2b), which is significantly greater than the S/N of ~5 that is typically obtained by reactive μCP on PET.[20] We believe that the higher contrast of streptavidin patterns obtained by Wellpat compared to μCP of biotin-NH_2 on PET[9,10] results from the longer contact time of the substrate with biotin-NH_2 than is possible with μCP, thereby providing a higher surface density of immobilized biotin.

An unlabeled streptavidin pattern was next incubated with 0.1 μM biotin-GRGDSP(K-TMR) (10 mM HEPES, 0.02%(v/v) Tween 20 detergent) resulting in the formation of a peptide micropattern (Figure 2c). The S/N in the pattern was 7 ± 0.5 (Figure 2d). The feasibility of direct micropatterning of a GRGDSPK(TMR)-NH_2 peptide was also investigated by reaction between the NH_2 moiety and the NHS ester on the surface (Figure 2e). Although patterning was successful, the S/N of the peptide patterns created by covalent coupling was 5 ± 0.8, which is significantly lower than the S/N of 7 ± 0.5 ($P < 0.001$, unpaired t-test) obtained for its biotin−conjugate patterned using the streptavidin−biotin system, We believe that the lower S/N obtained by covalent patterning is due to the limited reactivity of the amine moiety, caused by steric hindrance from the adjacent dye molecule.

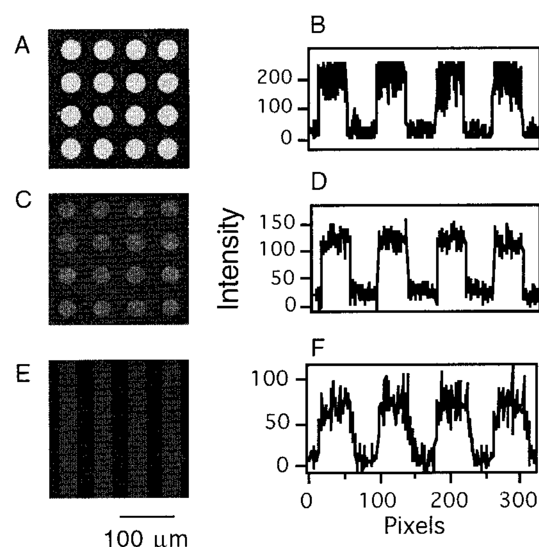

Figure 2. Micropatterns fabricated by Wellpat on COOH-derivatized PET: (a) biotin−NH_2/Alexa488-streptavidin; (c) biotin−NH_2/streptavidin/biotin−GRGDSP(K-TMR); (e) covalent patterning of GRGDSP(K-TMR)-NH_2; (b) (d), and (f) are line profiles of the micropatterns in (a), (c), and (e), respectively.

In summary, we have demonstrated the feasibility of a new micropatterning method, Wellpat, which has several attractive features: (1) Patterning is achieved from aqueous solution. (2) Wellpat is a flexible method in the choice of substrate; a wide variety of substrates including polymers, as shown here, and (by obvious extension) SAMs on gold or glass should be amenable to patterning by Wellpat. (3) Wellpat also enables the concentration of the biomolecule and its contact time with the surface to be varied over a wide range. This is especially advantageous compared to μCP, where the concentration of the patterning species cannot be significantly varied. (4) Compared with μCP, low-molecular weight PDMS is not left behind in the patterned regions, which we have observed occurs during μCP.[10] (5) Unlike patterning with PDMS flow channels, isolated structures (e.g., circles and squares) can be easily fabricated by Wellpat. (6) The spatially delimited wettability of the microwells enables easy filling and confinement of an aqueous solution to the microwells. (7) Wellpat can be interfaced with piezoelectric or inkjet dispensers to simultaneously create patterns of many different biomolecules; this feature will add to the complexity but is attractive because it will enable the spatial resolution to be dictated by the size of the microwells, which is easily controlled, and not by droplet spreading. These studies are currently in progress.

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(17) Both biotin−GRGDSP(K-TMR) and GRGDSP(K-TMR)NH_2 were synthesized by Anaspec Inc. (San Jose, CA) and were of over 90% purity. Tetramethyl rhodamine (TMR) was covalently attached to the amine moiety in the lys (K) residue during solid-phase synthesis of each peptide.

(18) This reagent is a long-chain (LC) derivative of biotin with a poly(ethylene oxide) (PEO) spacer (22.9 Å spacer length).

(19) The elastomeric nature of PDMS is critical in providing a leak-proof seal between the microwell mold and the substrate.

(20) In a control experiment, where a biotin micropattern was incubated with Alexa488-labeled streptavidin that had been presaturated with free biotin, no patterns were observed by fluorescence microscopy, which confirmed that the formation of streptavidin patterns was caused by molecular recognition between the protein and micropatterned biotin on the surface.