Pattern Generation of Cells on a Polymeric Surface Using Surface Functionalization and Microcontact Printing

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It has been a great interest to generate patterns of biologically-active molecules (*e.g.*, biotin, DNA, saccharides, peptides, and proteins) and cells on solid substrates because of the applications in biosensors, modulation of cell-substrate interactions in biomaterials and tissue engineering, neuroelectronics, high-throughput screening, and microarrays.[1,2] Among the methods for generating two-dimensional patterns on solid substrates, a soft lithographic technique called microcontact printing (μCP) has intensively been used to generate patterns of self-assembled monolayers (SAMs) and been applied to pattern generation of biomolecules and cells based on the patterns of the SAMs.[3] Although the technique of μCP is simple to perform in ordinary chemistry and biology research laboratories and does not require any special equipments or apparatus, its use has largely been restricted to the SAMs on gold, silver, or silicon oxide until recently,[4] which limits utility as biomaterials. Several researchers recently reported the application of μCP to polymeric surfaces.[5] Chilkoti and collaborators patterned biotin onto the surface of various polymers including poly(ethylene terephthalate) (PET), poly(ethylene) (PE), polystyrene (PS), and poly(methylmethacrylate) (PMMA). They functionalized the polymer surfaces chemically, presented pentafluorophenyl ester groups (which are reactive to primary amine groups) on the surfaces, and performed μCP of amine-terminated biotin onto the activated surfaces.[6] Another approach was also developed to overcome the substrate limitation of μCP. Utilizing chemical vapor deposition (CVD) of polymers, Langer and collaborators first coated various solid substrates with functionalized poly(*p*-xylylene)s (parylenes) the backbone of which contained chemically-reactive functional groups such as pentafluorophenyl esters and carboxylic anhydrides, and patterned the polymer surfaces with endothelial cells by a sequential deposition of biotin, streptavidin, biotin-conjugated anti-α_5-integrin, and endothelial cells.[7]

Herein we describe a convenient procedure for patterning PS surfaces by printing amine-terminated compounds. The PS surface was chemically modified to present SAMs of alkylsiloxanes terminating in reactive carboxylic anhydride groups. A combination of surface functionalization and μCP makes it possible to extend the ability of patterning surfaces with μCP from gold, silver, and silicon to polymers (and other substrates). The procedure used in this paper involves simple four steps: (1) preparation of vinyl-terminated SAMs of siloxanes; (2) oxidation of the terminal vinyl groups into carboxylic acid groups; (3) formation of reactive SAMs that present interchain carboxylic anhydrides;[8] (4) pattern delivery to the reactive SAMs using μCP.

PDMS prepolymer (Sylgard 184, Dow Corning Corp) was cast against a microfabricated photoresist master. Curing the prepolymer (at 60 °C for 6 hours) and peeling it away from the master provided a negative replica of the two-dimensional pattern of the photoresists. The negative replica of PDMS was used as a stamp. Before casting the PDMS prepolymer, the master was pre-treated with (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane (United Chemical Technologies, Inc.) for 1 hour under vacuum at room temperature to functionalize the surface with a fluorocarbon. This functionalization aided in release of the PDMS stamp from the master.

Figure 1 shows a schematic representation of the procedure for patterning amine-terminated compounds on the PS surface. Before forming SAMs of 6-hex-1-enyltrichlorosilane (Gelest, Inc.) on the PS, we oxidized a PS Petri dish in an oxygen plasma for 1 minute. Plasma treatment introduces a variety of oxidized functional groups such as hydroxyl, carbonyl, and carboxyl groups into the outer few layers of the polymer film.[9] Vinyl-terminated SAMs were assembled by placing the oxidized PS Petri dish in a vacuum desiccator containing 6-hex-1-enyltrichlorosilane. The terminal vinyl groups were then oxidized (by using 0.5 mM of $KMnO_4$, 14.7 mM of $NaIO_4$, and 3 mM of K_2CO_3, for 24 h) to carboxylic acid groups.[10] Reaction of the carboxylic acid groups with trifluoroacetic anhydride (0.1 M) and triethylamine (0.2 M) in DMF (10 mL) gave the SAMs of siloxanes that presented interchain carboxylic anhydride groups. Undecylamine was mirocontact-printed onto the substrate presenting interchain carboxylic anhydride groups. The substrate was then immersed in a DMF solution of poly(ethylene glycol)amine (methoxy-PEG-NH_2 (MW 5000); Shearwater Polymers) (10 mM). We used the ethylene glycol-terminated amine to render underivatized regions resistant to cells because ethylene glycol is known to be the most effective functional group in minimizing non-specific adsorption of proteins, cells, and bacteria.[11]

Human epidermoid carcinoma A431 cells were grown in

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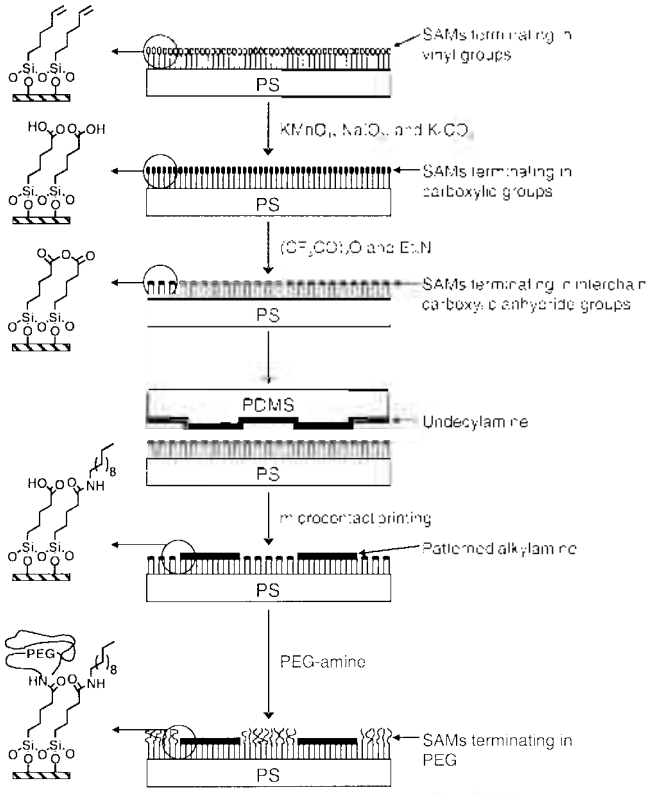

Figure 1. A schematic representation of surface functionalization and microcontact printing.

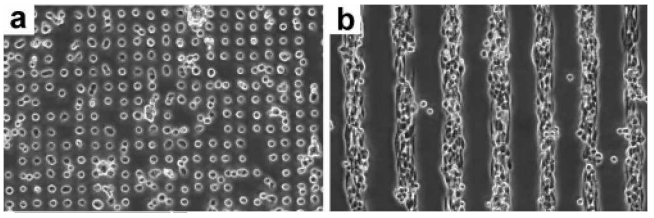

Figure 2. Optical micrographs of patterned human epidermal carcinoma cells on (a) circles (10 μm in diameter) and (b) lines (50 μm in width).

Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and cultured at 37 °C under humidified 10% CO_2. About 2 mL of a cell suspension (1×10^5 cells/mL) was seeded onto the patterned substrate. Figure 2a shows 10-μm-circles (where undecylamine was printed) separated by 10 μm and Figure 2b shows cell pattern of 50-μm-lines separated by 50 μm. A single cell is isolated in the 10-μm-circle and the shape of the deposited cells is roughly round (Figure 2a), while the shapes of cells confined in the 50-μm-lines are either round or oval. Four or five cells are deposited in the cross-section of 50-μm-lines and interestingly the cells deposited at the edge of the lines form highly-elongated oval shapes (Figure 2b).

In summary, we demonstrated a pattern generation of cells on the polystyrene surface with a combination of surface functionalization and microcontact printing. The method demonstrated here could be applicable to a wide variety of polymers that are amenable to surface modification. Although we demonstrated a generation of cell patterns based on the simple deposition of cells onto the methyl-terminated, hydrophobic surfaces and the inhibition of cell deposition onto the ethylene glycol-terminated surfaces, the same chemical transformation–formation of amide bonds by coupling between carboxylic anhydride and amine group–could be utilized to generate patterns of biomolecules and cells based on biospecific interactions.

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