Patterning Inside Microfluidic Channels using a Soft Lithographic Method

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1. Introduction

The control of surface properties and spatial presentation of function molecules within a microfluidic channel is important for the development of diagnostic assays, microreactor, and for performing fundamental studies of cell biology and fluid mechanics. Here, we present a simple technique, applicable to many soft lithographic methods, to create robust microfluidic channels with precise control over the spatial properties of the substrate. In this approach, the patterned regions were protected from oxygen plasma by controlling the dimensions of the poly(dimethylsiloxane) (PDMS) mold as well as the sequence of fabrication steps. The approach was used to pattern a non-biofouling polyethylene glycol (PEG)-based copolymer or the polysaccharide hyaluronic acid (HA) within microfluidic channels. These non-biofouling patterns were then used to fabricate arrays of fibronectin (FN) and bovine serum albumin (BSA) as well as mammalian cells. In addition, further control over the deposition of multiple proteins onto multiple or individual patterns was achieved using laminar flow. Also, cells that were patterned within channels remained viable and capable of performing intracellular reactions and could be potentially lysed for analysis.

2. Experimental section

Fabrication of patterned microfluidic channels

We generated patterned surfaces using microcontact printing and molding to demonstrate the versatility of the approach to pattern microchannels with various soft lithographic techniques.1 HA films were prepared by spin-coating (Model CB 15, Headaway Research Inc.) a solution containing 5 mg HA/mL of distilled water onto silicon dioxide substrates (glass slides or wafers) at 1500 rpm for 15 s. Immediately after coating, a plasma cleaned PDMS stamp with negative features was brought into conformal contact with the stamp and left to be dried for 12 h at room temperature. The patterned surfaces were then washed with PBS to remove the non-chemisorbed HA from the surface.1

To synthesize poly(TMSMA-r-PEGMA), PEGMA and TMSMA and AIBN were dissolved in tetrahydrofuran at a molar ratio of 1.0 : 1.0 : 0.01 and degassed for 20 min and were reacted using free radical polymerization at 70 °C for 24 h. The solvent was then evaporated leaving behind a viscous liquid. The synthesized poly(TMSMA-r-PEGMA) was used to pattern surfaces using both micromolding and microcontact printing. To pattern using micromolding, glass slides were plasma cleaned for 3 min and the poly(TMSMA-r-PEGMA) solution (10 mg/mL in MeOH) was spin-coated onto each glass slide (1000 rpm for 10 s). A PDMS stamp was then immediately placed in conformal contact with the spin coated surface and left undisturbed for 1 h. To pattern the PEG-based copolymer using microcontact printing, the PDMS mold was plasma cleaned for 3 min and subsequently a few drops of a solution of 10 mg/mL of polymer in MeOH was placed on the stamp. To generate a uniform coating on the PDMS stamps, the mold was either spin coated at 1000 rpm for 10 s or air dried until a thin film remained. The pattern on the PDMS mold was then transferred onto the substrate by firmly pressing the mold and the substrate together. All patterns were cured at 110 °C for 15 min. A schematic presentation is shown in scheme 1.

3. Results

Patterned substrates within microfluidic channels

Channels were fabricated with PEG-based copolymer or HA patterns. The pattern edges in these images were clearly visible which provided an easy way to detect pattern fidelity and to align the channel. To characterize the non-biofouling properties of these patterned microfluidic channels, protein adsorption experiments were performed by flowing FITC-BSA or TR-BSA or FN through the channels. Fluorescent images in Fig. 1 are representative protein patterning images for various tested conditions. Both PEG-based polymer as well as HA showed excellent protein resistance for BSA (95±2% and 97±3% respectively) and FN (96±3% and 95±3% relative to bare glass) within the channels.

Patterning of cells within microfluidic channels

To examine the potential of the patterned microfluidic channels for generating cellular arrays within microfluidic channels, we fabricated patterned microfluidic channels using both HA and PEG-based copolymer (Fig. 2). Prior

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to cell seeding, a solution of FN was flowed through the channels for 15 min. As previously shown, the FN selectively adsorbs to the exposed regions forming strong anchoring sites for cells. A cell suspension of NIH-3T3 was then flowed in the channels. A cell suspension of 5 x 107 cells/mL was found to be optimum to form cellular monolayers or arrays. Concentrations of <1 x 107 cells/mL did not form confluent cell layers while concentrations >1 x 108 cells/mL clogged the channels.

Cellular reactions and lyses within microfluidic channel

To analyze the potential of this patterning approach for various analytical applications, we tested the ability of the immobilized cells to carry out enzymatic reactions using ethidium homodimer and calcein-AM molecules. The membrane permeable calcein-AM enters all cells, and is enzymatically converted to green-fluorescent calcein in the cytoplasm. Cells with an intact plasma membrane (viable cells) retain calcein, and thus fluoresce green. Only cells with a compromised plasma membrane (dead cells) take up ethidium homodimer (seen as a red dye). Thus, we were able to analyze the viability and functionality of these cells within the channels.

4. Conclusion

In conclusion, a technique was developed to fabricate stable microfluidic channels with precise control over the spatial patterning of the substrate. In this technique, the patterned regions were protected from oxygen plasma by controlling the dimensions of the PDMS mold as well as the sequence of fabrication steps. Proteins were immobilized with precision on the substrate of the microfluidic channels. In addition, laminar flows were used to control the adsorption of multiple proteins within particular regions of multiple or individual patterns within the channels. Fibroblasts were patterned within the channels through adhesion to FN coated regions. The cells remained viable and performed enzymatic reactions and could be lysed to potentially release intracellular components (i.e. proteins).

5. References

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(2) A. Khademhosseini, K. Y. Suh, S. Jon, G. J. Chen, G. Eng, J. Yeh, and R. Langer, Anal. Chem. 76, 3645 (2004).

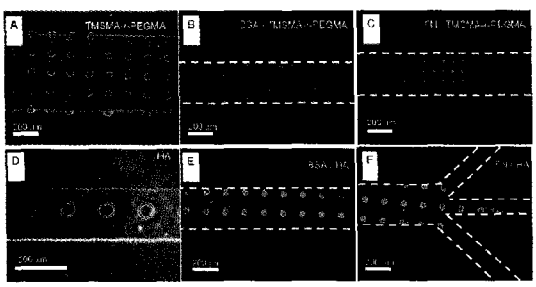
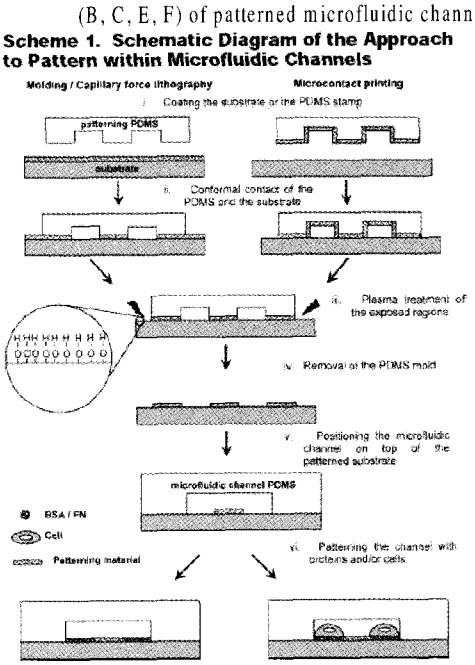


Figure 1. Light micrograph (A, D) and fluorescent images (B, C, E, F) of patterned microfluidic channels



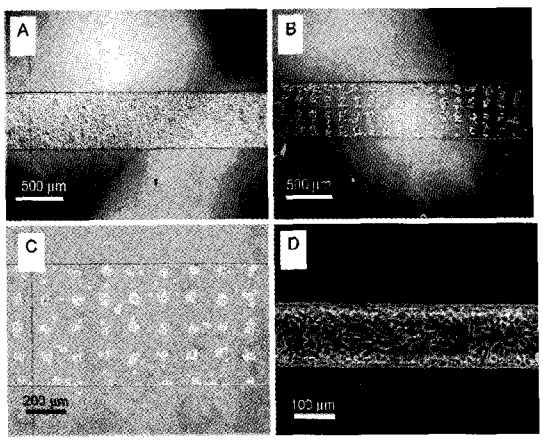


Figure 2. NIH-3T3 fibroblast adhesion and patterning within microfluidic channels at t = 0 h (A) and after 6 h (B-D). (A) shows the initial cell density within the microfluidic channels while (B) and (C) represent the cell patterned channels on poly(TMSMA-r-PEGMA) and HA respectively. (D) represents fibroblast adhesion to non-patterned microchannels.