소프트 리소그라피를 이용한 표면 및 마이크로 채널 내 리피드 이중층 패터닝 방법

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Soft lithographic patterning of supported lipid bilayers onto a surface and inside microfluidic channels

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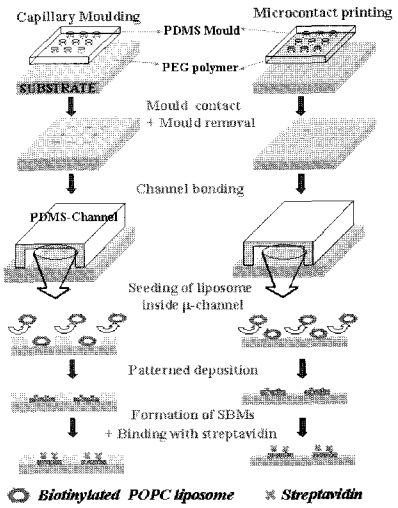
1. Introduction

Recently, supported lipid bilayer (SLB) membranes have attracted considerable attention as a biomimetic platform for various applications such as fundamental biological research of cell-membrane, lipid-assisted bioassay and biosensor.[1,2] In particular, the micropatterning of SLBs has been used to study cell behavior on controlled surfaces, and to fabricate lipid-assisted biochips such as DNA-chips, and to study lipid activity kinetics[1,3,4].

Micro/nanopatterning of SLBs within microfluidic devices is a prerequisite for the development of high-throughput biosensors and for performing chip-based studies of cellular interactions based on lipid bilayers. A number of strategies were demonstrated to pattern lipid bilayers inside fluidic channels such as microfluidic flow patterning[5] and polymer lift-off[6]. Microfluidic flow patterning, which utilizes laminar flowing streams to pattern within microfluidic channels, is a powerful method to obtain microarrays with varying composition but is limited to generating geometrical patterns in the shape of the laminarly flowing streams with pattern sizes on the order of a few tens of micrometers. Polymer lift-off is also an elegant way to fabricate micropatterned SLBs in a controlled fashion but has limitations due to the potential toxicity of the photoinitiator, the need for expensive equipment and the difficulty in patterning the surface without modifying the surface topography. Thus, the development of simple and economically viable method for patterning flat substrates and inside microfluidic channels with pattern size ranging from a few tens of micrometers to sub-micrometer is potentially of great benefit.

2. Experimental method



Scheme 1. A schematic diagram for patterning supported bilayer membranes (SBMs) onto glass substrate and inside microfluidic channels eitherby using capillary moulding or microcontact printing with PEG-based polymers.

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Fabrication of the microstructures within microfluidic channels

To fabricate microstructures by capillary moulding, a few drops of 5% (w/v) PEG copolymer [poly(TMSMA-r-PEGMA)] solutions in water were placed on glass substrate. To make conformal contact, a patterned PDMS mould was carefully placed onto the surface and then the sample was stored overnight at room temperature to allow evaporation of water for complete evaporation of the solvent. To fabricate microstructures by microcontact printing, a patterned PDMS stamp was plasma cleaned for 1 min (60W, PDC-32G, Harrick Scientific Inc.) to ensure proper cleaning and to increase wettability. After pretreatment, the PDMS mould was inked with 1%(w/v) solution of the comb polymer in a 50:50(v/v) H2O/ethanol mixture and placed directly onto substrate. The stamp was left for 30 s and peeled off (Scheme 1). To complete the device fabrication both for capillary moulding and microcontact printing, a PDMS mould with the features of the microfluidic channel and a patterned glass slide were plasma cleaned for 45 s (60 W, PDC-32G)without disturbing the PDMS stamp used for patterning (i.e., in conformal contact with the substrate) (Scheme 1). After plasma treatment, the PDMS stamp was peeled off from the substrate and the microfluidic mould was aligned and brought in conformal contact with the substrate and firmly pressed to form an irreversible seal. Fluids were driven through the channels using a SP200i syringe pump (World Precision Instruments, Sarasota, FL) that was connected to the device using polyethylene tubing (BD, Franklin Lakes, NJ).

Patterning of biotinylated lipid vesicles and biotin-streptavidin binding

A few drops of biotinylated liposome vesicles dissolved in PBS (pH = 7.4) at 100 mM were evenly distributed onto the patterned PEG substrates and incubated at room temperature for 40 min, and then the sample was rinsed thoroughly with PBS. After that, Alexa Fluor 488 conjugated streptavidin dissolved in PBS (pH 7.4) at 50 g/mL was stained onto thesurface patterned lipid membrane at room temperature for 40 min and the sample was rinsed with PBS several times. To generate the lipid bilayer membrane micropatterns inside microfluidic channels, the solution of biotinylated lipid vesicles was seeded through the patterned microfluidic channel for 30 min at a flow rate of 5 L/min. For measuring biotin-streptavidin binding, a solution of streptavidin dissolved in PBS (pH 7.4) at 50 g/mL was run through the channel for 45 min additively. All patterned surfaces were analyzed using an inverted fluorescent microscope (IX71, Olympus). All staining experiments were performed three to five times to ensure the reliability of the data. Fluorescent images were taken and quantified using Image-pro plus 5.1 (Olympus).

3. Results and discussion

Patterning of lipid vesicles on glass substrate

To pattern lipid vesicles on glass substrate, PEG microstructures were fabricated either by using microcontact printing or capillary moulding methods (scheme 1). For microcontact printing, a PDMS mould was inked with the methacrylate based comb polymer containing pendent oligoethylene glycol side chains. The microstructures formed by microcontact printing were reported to be very stable in water and could be exploited to spatially control adhesion and proliferation of biological species such as cells and proteins.28 For capillary moulding, a PDMS mould was placed onto a drop-dispensed solution of the PEG-based random copolymer [poly(TMSMA-r-PEGMA)]. The moulded PEG structures formed robust microstructures after the evaporation of the solvent. It was observed that the mobility of the two polymers was different on glass substrate; the comb polymer was not quite mobile while in contact with the substrate surface presumably due to the presence of methacrylate backbones that could be absorbed to the surface. As a result, the use of the comb polymer in capillary

moulding was not successful. Similarly, the PEG copolymer was relatively mobile at the time of contact because of low viscositysuch that the microcontact printing method was difficult to handle. For these reasons, the two polymers were used for different patterning methods.

The three-dimensional and cross-sectional atomic force microscopy(AFM) images shown in Figures 1(a-b) indicated that the substrate surface was completely exposed with good edge definition when patterned by contact printing. The height of the printed PEG layer was ~13 nm which is higher than a few nanometers generally obtained for self-assembled monolayers due to high concentration and viscosity of the PEG comb polymer. The microstructures shown in Figures 1(c-d) could also be generated with the clear surface exposure. The height of the microstructure was ~294 nm, much higher than that for microcontact printing due to the fact that the capillary moulding involves a higher amount of the PEG copolymer. This increased height could act as a physical barrier to regulate the diffusion of adsorbed lipid bilayers as shown shortly.

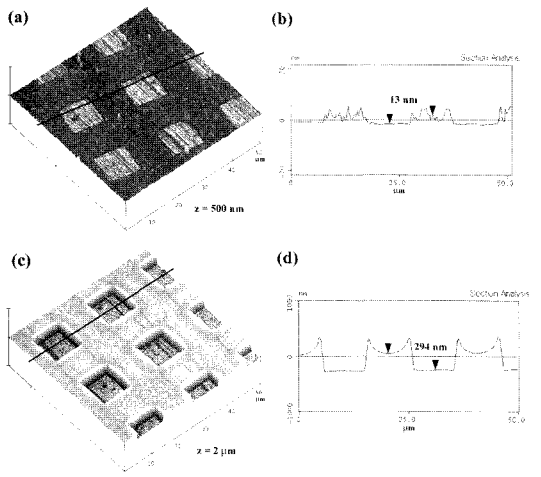

Figure 1. Three-dimensional and cross-sectional atomic force microscopy (AFM) images of the patterned PEG surfaces using microcontact printing (a-b) and capillary moulding (c-d). The box size was 10 μm.

Fabrication of microfluidic channels with patterned SLBs

Microfluidic devices with arrays of SLB membranes were created by irreversible sealing of a PDMS channel onto the pre-patterned glass substrate with proper alignment (Scheme 1). In this process, the covered mould during the moulding process was left for protecting the patterned surface during oxygen plasma treatment. After surface treatment by plasma, the covered PDMS mould was carefully detachedfrom glass substrate, and the PDMS channel mould was subsequently bonded to the substrate in such a way that the patterned region was included in the channel.

To demonstrate the ability of the microfluidic channels formed here to act as lipid based-bioassay and analytical tools, biotin-streptavidin bindings were analyzed using a fluorescence microscope. To analyze biotin-streptavidin binding within microfluidic devices, biotinylated lipid vesicles were labeled with the fluorochrome DiI, and Alexa 488-conjugated streptavidin was prepared as a receptor. As shown in Figures 2 (a-c), the lipid bilayer membrane was formed by fusion of patterned lipid vesicles onto pre-located regions of the substrate. Also, streptavidin was selectively deposited with the biotinylated lipid bilayer membrane (Figures 2 (d-f)), suggesting that the biotinylated lipid membrane could act as a platform for a wide range of applications such as bioassay-chips and biosensors using antigen-antibody interactions.

The reason why the moulding process was mostly used to pattern inside microfluidic channels is to minimize the diffusion of adsorbed lipid layers. As shown in Figures 2 (g-h), the adsorbed SLB membranes appeared to migrate across the boundary between the exposed surface and the adjacent microcontact printed PEG layer. In comparison, there was no significant diffusion of the lipid membrane when patterned by microcontact printing without flow, indicating that the destruction of the pattern was medicated by some flow-induced migration of

the SLB membranes. On the contrary, the moulded microstructures were effective in restricting the diffusion of the lipid for ~11 hrs in a continuous stream of streptavidin solution probably due to the increased height of the microstructures. After ~11 hrs the lipid layer started to diffuse to the adjacent regions. The stability on a surface was almost the same with that within the microchannel, suggesting that the flow does not affect the stability of the SLB membranes substantially. In all the microfluidic experiments, a simple Y-shaped channel was used as shown in Figure 2(i), which could be expanded other types of microchannels with proper handling.

4. Conclusions

We have presented two soft lithographic methods for patterning SLBs onto flat substrates and inside microfluidic channels.

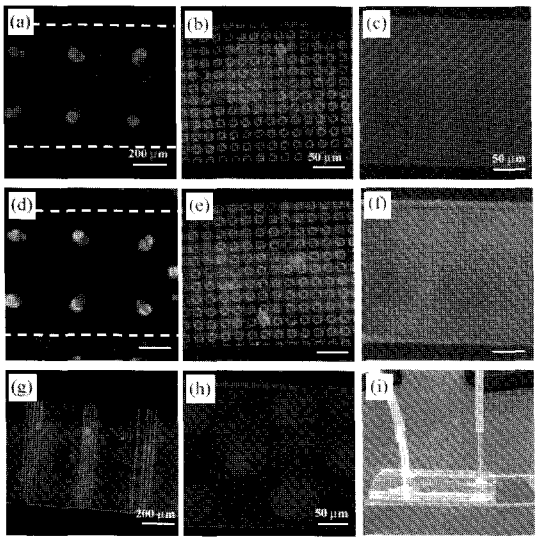

Figure 2. (a-c) Fluorescent images of the patterned biotinylated SLBs using capillary moulding: (a) 70 μm wells, (b) 1 μm square strips, and (c) 5 μm boxes. (d-f) Fluorescent images of the same regions in (a-c) after conjugation with streptavidin. (g-h) Fluorescent images of the patterned biotinylated SLBs using microcontact printing: (g) 10 μm lanes and (h) 70 μm wells. (i) A simple Y-shaped channel used in the experimen

We have presented two soft lithographic methods for patterning SLBs onto flat substrates and inside microfluidic channels. Microcontact printing and capillarymoulding methods were used to create robust microstructures of the PEG-based polymers, which acted as resistant layers against non-specific adhesion of lipid vesicles. Both methods could be used to fabricate the patterned PEG surfaces with the substrate surface clearly exposed whereas the capillary moulding approach turned out to be more efficient in regulating the adhesion and migration of the lipid vesicles. It is hoped that this simple method provides an alternative platform for the fabrication of lipid-based immunoassay chips and a useful tool for research of lipid membrane within microfluidic devices applicable for high-throughput applications.

Acknowledgments

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