Complex micropatterning of proteins within microfluidic channels

Miju Kim, and Junsang Doh

Abstract—**Microfluidic channels containing protein micropatterned surfaces are useful in many bioanalytical and biological applications. In this study, we developed a new method to integrate microfluidics and protein micropatterning by attaching poly(dimethylsiloxane) (PDMS) microfluidic channels to bio-friendly photoresist films via poly(dopamine) (PDA) adhesive. A bio-friendly photoresist poly(2,2-dimethoxy nitrobenzyl methacrylate-r-methyl methacrylate-r-poly(ethylene glycol) methacrylate) (PDMP) was synthesized and used. By performing microscope projection photolithography (MPP) to the PDMP thin films within PDMS microchannels, complex micropatterns of proteins were successfully generated within microfluidic channels.**

I. Introduction

Protein micropatterned surfaces integrated with microfluidics are useful in many bioanalytical and biological applications including multiplex bioassays, cell separation, and fundamental cell studies [1-4]. Many protein micropatterning methods have been developed [5-10], but most of the methods cannot be performed within microfluidic channels except photochemical patterning [11-14]. Alternatively, microfluidic channels can be directly attached to the protein micropatterned surfaces, but most methods for microfluidic channel bonding require harsh processing conditions such as dehydration and plasma treatment [15-18], which will significantly reduce the activities of proteins. Even for photochemical patterning, bonding microfluidic channels on the surfaces with minimally affecting key properties critical for protein micropatterning and immobilization is not straightforward.

In this study, we used microscope projection photolithography (MPP) based on a bio-friendly photoresist poly(2,2-dimethoxy nitrobenzyl methacrylate-r-methyl methacrylate-r-poly(ethylene glycol) methacrylate) (PDMP) [19, 20] for complex protein micropatterning within microfluidic channels. MPP based on PDMP thin films has been useful for many applications including multi-scale patterning of multiple proteins [21] and dynamic micropatterning of cells [22, 23]. To attach poly(dimethylsiloxane) (PDMS) microfluidic channels on PDMP thin films with minimally altering functionalities of PDMP thin films, we employed muscle-inspired adhesive poly(dopamine) (PDA) coating as a new adhesive for microfluidic channel bonding. Compared with many other methods developed for PDMS bonding to plastic substrates, PDA coating can be achieved under very mild aqueous conditions; nearly all surfaces can be coated just by dipping substrates in an alkaline aqueous solution [24]. This new bonding strategy allowed us to fabricate complex protein micropatterns within microfluidic channels [25].

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II. Materials and Methods

A. Reagents

1,4-dioxane, dopamine hydrochloride, (3-aminopropyl) triethoxysilane (APTES), poly(acrylic acid) solution (PAA, average Mw ~15,000) were purchased from Sigma-Aldrich. Amine-PEO_2–biotin and streptavidin-rhodaimine (SAv-Rhodamine) were purchased from Pierce. SAv-FITC and SAv-Cy5 were purchased from eBioscience. PDMP was synthesized as described elsewhere [20].

B. Poly(dopamine) coating on PDMP surfaces

PDMP surfaces were prepared by spincoating 3% (w/w) PDMP solution in 1,4-dioxane on clean glass coverslips. The PDMP-coated coverslips were baked for overnight to remove residual solvent. The baked-PDMP surfaces were dipped in dopamine solution (2mg/mL in 10 mM Tris-HCl buffer with pH 8.5) for PDA coating. The PDA-coated surfaces were rinsed with DI water, and dried by nitrogen blowing. Water contact angles of PDA-coated PDMP surfaces were measures by using a surface analyzer DSA-100 (Kruss Co.).

C. Protein micropatterning by microscope projection photolithography

A modified Zeiss Axio Observer.Z1 epi-fluorescence microscope with CoolSNAP HQ2 (Photometrics) CCD camera was used for imaging and micropatterning. An XBO 75 W/2 Xenon lamp (75 W; Osram) and DAPI (EX. 365, BS 395, EM BP 445/50), eGFP (EX BP 470/40, BS 495, EM BP 525/50), Cy3 (EX BP 550/25, BS 570, EM BP 605/70), and Cy5 (EX BP 620/60, BS 660, EM BP 770/75) excitation/emission filter sets were used for the illumination and fluorescence imaging. The microscope was controlled automatically using Axiovision 4.6 (Carl Zeiss). The acquired images were analyzed and processed using Methamorph (Universal Imaging, Molecular Devices). Biotinylated coverslips were prepared as described elsewhere [26]. The biotinylated substrates were spincoated with PDMP and further coated with PDA as described above. Then, the PDA/PDMP-coated coverslips were attached to PDMS microchannels, immersed into the PBS, and placed on the microscope stage. The PDA/PDMP-coated coverslips immersed in PBS were exposed using UV through a transparency photomask for 3 min. The PDMP films in the UV exposed region spontaneously dissolved in the PBS and the biotin groups underneath the PDMP films were exposed.

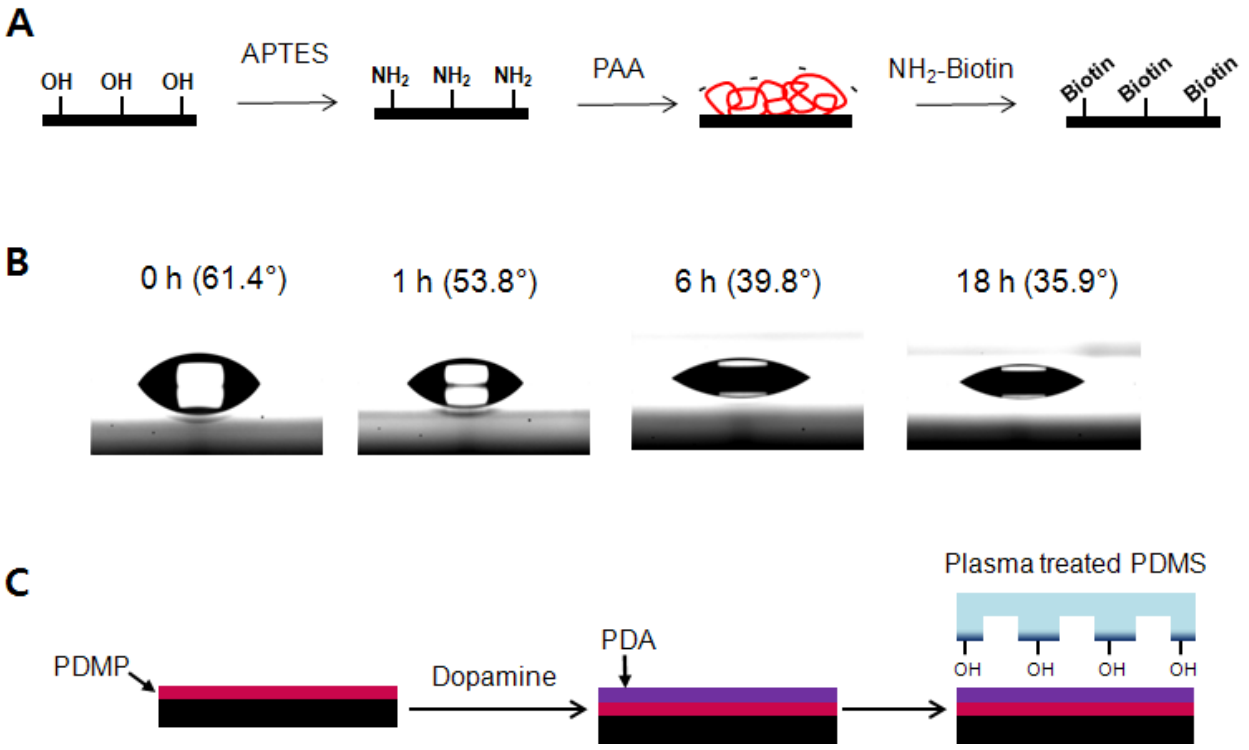

Figure 1. A. Scheme of biotinylation of glass substrates. B. Contact angle change during PDA dip coating. B. Scheme of PDMS microfluidic channel bonding on PDMP surfaces.

The SAv was attached to the exposed biotin groups through incubating the patterned substrates in the SAv-containing PBS solution (5 μg/mL) for 15 min. The unbound SAv was washed away with PBS.

III. RESULTS AND DISCUSSION

A. Attachment of PDMS microfluidic channels on PDMP surfaces via PDA coating

For surface immobilization of proteins, biotin-streptavidin (SAv) interaction was utilized. Biotinylated coverslips prepared by the procedure shown in Fig. 1A were used. The biotinylated coverslips were spincoated with PDMP to form PDMP thin films on the biotinylated substrates. Then, by dipping the PDMP-coated coverslips in dopamine solution, the PDMP thin films were coated with PDA. Since the thickness of PDA films formed on PDMP surfaces depends on the dipping time [24], we varied dipping times and characterized PDA-coated PDMP surfaces by measuring contact angle and checking protein micropatterning capability [25]. As dipping time increased, contact angle significantly decreased (Fig. 1B), indicating surface coverage of PDA increased over time. However, micropatterning capability of PDMP was significantly reduced as PDA films became thicker. We found out that 1 h dip coating was sufficient to provide enough coverage of PDA for bonding while preserving protein micropatterning capability of PDMP thin films.

Then, PDMS channels were attached to the PDMP thin films dip-coated with PDA for 1 h as shown in Fig. 1C. PDMS channels briefly treated with air plasma to generate reactive species on the surfaces were contacted with PDA-coated PDMP for 1 h at 80°C. The bonding strength between PDMS and PDA-coated PDMP surfaces measured by performing pulling tests using a universal testing machine (Instron, model 3343, Instron Corp) was about 60 kPa, which is close to the bonding strengths of chemical glues typically used for PDMS bonding [18]. Moreover, no leaking was observed with a flow rate up to 10 mL/min, and bonding was stable in aqueous environment at least for 10 days [25].

Taken together, a new bonding strategy based on PDA adhesive to attach PDMS microfluidic channels to functional surfaces with minimal alteration of the surface functionalities was successfully developed.

B. Complex protein micropatterning on surfaces within PDMS microchannels

Protein micropatterning within microfluidic channels were achieved by the procedure schematically shown in Fig. 2A. PDMP thin films attached with PDMS microfluidic channels were filled with PBS and mounted on a microscope stage. Then, a transparency film photomask was inserted into a field diaphragm of a fluorescence microscope and light is illuminated through DAPI excitation filter. Light-exposed areas were spontaneously dissolved in PBS, resulting in exposure of biotin groups underneath PDMP thin films. Selective immobilization of SAv on the light-illuminated areas can be achieved by adding SAv solution into the microfluidic channels. By repeating these procedures,

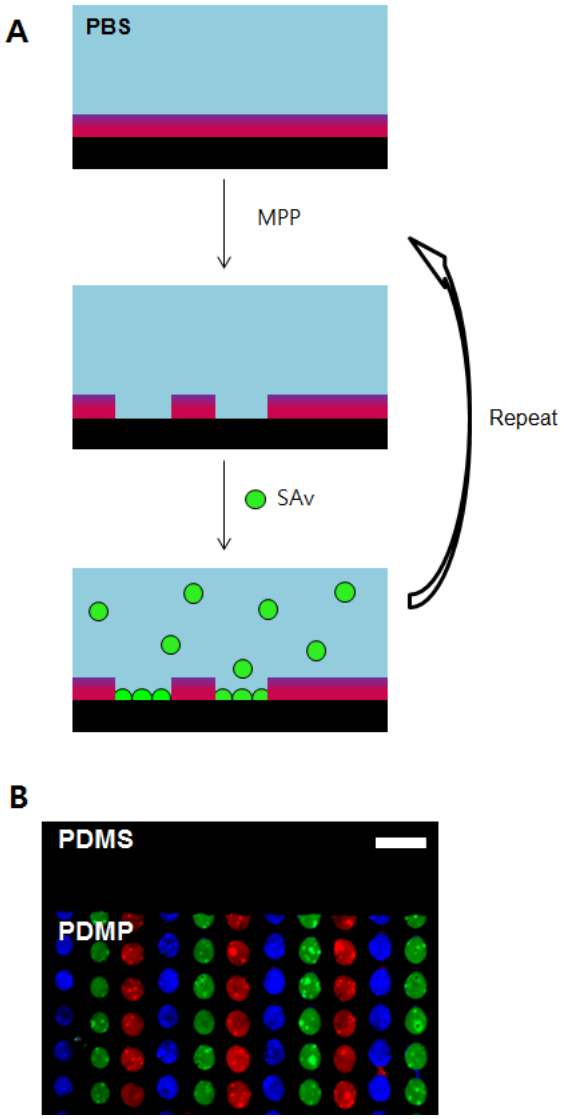

Figure 2. A. Scheme of micropatterning of multiple streptavidins (SAvs). B. Representative fluorescence overlay images of multiple SAvs with three different fluorophores, FITC, Rhodamine, and Cy5. Scale bar: 50 μm.

multi-SAv micropatterning within microfluidic channels can be achieved.

A representative overlay fluorescence image of successfully fabricated surfaces containing SAvs labeled with three different fluorophores (FITC, Rhodamine, and Cy5) is shown in Fig. 2B. Importantly, no fluorescence signal was detected in PDMS-bonded area, meaning leaking did not occur during micropatterning.

One of the advantages of microfluidics is the capability to generate complex concentration gradient of chemicals within microfluidic channels. Complex concentration gradient of solution in microfluidic channels can be transferred onto the surfaces [25], resulting in complex surface micropatterning. To test this possibility, gradient microchannels with serial dilution of solutions were fabricated [27] and attached on the surfaces coated with PDMP thin films. By generating a square array of circles by MPP and immersing micropatterned surfaces into gradient flow containing various concentrations of fluorophore-labeled SAv, gradient surface micropatterns was successfully fabricated [25].

In this way, complex micropatterns of SAvs were fabricated within microchannels. This method can be readily extended to the patterning of multiple distinct biotinylated proteins, which are either commercially available or easily prepared in the laboratory using commercially available reagents, by simply adding incubation steps with biotinylated proteins after each SAv deposition.

IV. Conclusion

We demonstrated a new method for the fabrication of complex protein micropatterned surfaces inside microfluidics by attaching PDMS microfluidic channels to a bio-friendly photoresist PDMP. The technique developed in this study will be useful for multiplex bioassays and fundamental cell biology.

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