Polyelectrolyte Micropatterning Using Agarose Plane Stamp and a Substrate Having Microscale Features on Its Surface

Min Jung Lee, Nae Yoon Lee, Sang Kil Lee,† Sungsu Park, and Youn Sang Kim*

*Division of Nano Sciences, Ewha Womans University, Seoul 120-750, Korea. *E-mail: younskim@ewha.ac.kr*
†School of Chemical and Biological Engineering, Seoul National University, Seoul 150-742, Korea
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We have introduced polyelectrolyte micro-patterning technique employing agarose plane stamp and a hard substrate having microscale features on its surface. With this method, chemically micropatterned surfaces with both positive and negative functionalities were successfully embedded in well-defined microstructures, and selective impartment of charge functionalities was confirmed by patterning bead bearing surface charge. Furthermore, this technique allows highly sensitive immobilization of protein onto targeted surface simply by endowing functionalities, which extends the potential of its use as a tool for high-throughput protein microarray and proteomics. Because plane agarose stamp is free of structures on its surface, there is no concern for pattern collapse, and the combination of agarose plane stamp with patterned substrate is more suited for selective protein patterning compared with adopting surface-patterned agarose stamp with flat substrate. Our technique using agarose plane stamp and a substrate having microscale features on its surface suggests a range of possible applications, including the micropatterning of biofunctionalized copolymer having polyelectrolyte block, immobilization of micro- and nanoparticle with biofunctionalities such as biotin and streptavidine, and establishing optoelectronic microstructures with micro-beads on various surfaces.

Key Words : Micropatterning, Polyelectrolyte, Polymer on polymer stamping, Protein patterning, Surface chemistry

Introduction

Microcontact printing[1-4] and polymer on polymer stamping (POPS)[5] are successful techniques in a range of applications such as electro-optical devices, bio-chemical sensors, and proteomic arrays. Especially, the POPS based on various polyelectrolytes that have water-soluble property and electrostatic charge possesses interesting challenges. POPS has displayed patterning of ultra-thin layer with chemical functions and has presented micropatterning technique[6] of polyelectrolyte multilayer arrays in which functional materials such as nanoparticles could be embedded. Some authors showed various impressive applications with POPS such as micro-beads[7] and microcapsule patterning[8] for optical device, poly(ethylene glycol) (PEG) block copolymer patterning for bio-chemical device and functional multilayer patterning.[9]

Generally, POPS has used the soft mold whose material is based on poly(dimethylsiloxane) (PDMS) which is highly hydrophobic and has poor wetting property of water.[10,11] Although PDMS is a good material for stamp for POPS with its stable surface and low adhesion property, it isn't always the best choice for micropatterning with polyelectrolyte because of its poor wetting and hydrophobic property against water. Micropatterning technique based on PDMS stamp has common failings for water-based material patterning such as protein. In general, they require either fast process or ambient humidity in air for water-based materials.[2] Recently, some groups have suggested surface micropatterning technique that can be used to deliver polar inks (polar chemicals) onto various supports by wet stamp such as agarose stamps.[12] They used the high strength agarose as a stamp, patterned with microstructures, for delivering polar inks (especially, water-based). However, as agarose stamp is fragile, microstructures patterned onto agarose stamp collapse easily in the stamping process. For chemical micropatterning to be successful, it is preferable to use hard substrates having patterned surfaces and flat agarose stamp rather than having a flat substrate and surface-patterned agarose stamp. In this way, polar inks soaked in agarose plane stamp could be transferred selectively onto protruding structures on the hard substrates having patterned surfaces and collapsing of agarose stamp could be prevented. In a previous study,[13] micro- and nanoscale structures on various substrates were successfully fabricated and facilely replicated using UV-curable polymer, and thus fabricated structures could provide firm and concrete physical barriers, which could extend its use toward selective micropatterning of positive and negative surface functionalities.

In this report, we present the POPS technique employing agarose gel as a plane stamp on microstructured polymer substrate having physical barriers and demonstrate chemical micropatterning of nanoscale beads bearing surface charges. In our previous study, we demonstrated physical microstructure patterning techniques using UV-curable polymer on various substrates such as glass, Si wafer and flexible plastic substrate, and have shown successful fabrication of various micro- and nanostructures. As polyelectrolyte can easily be attached on various surfaces simply by treating the surface with O_2 plasma and then dipping into polyelectrolyte

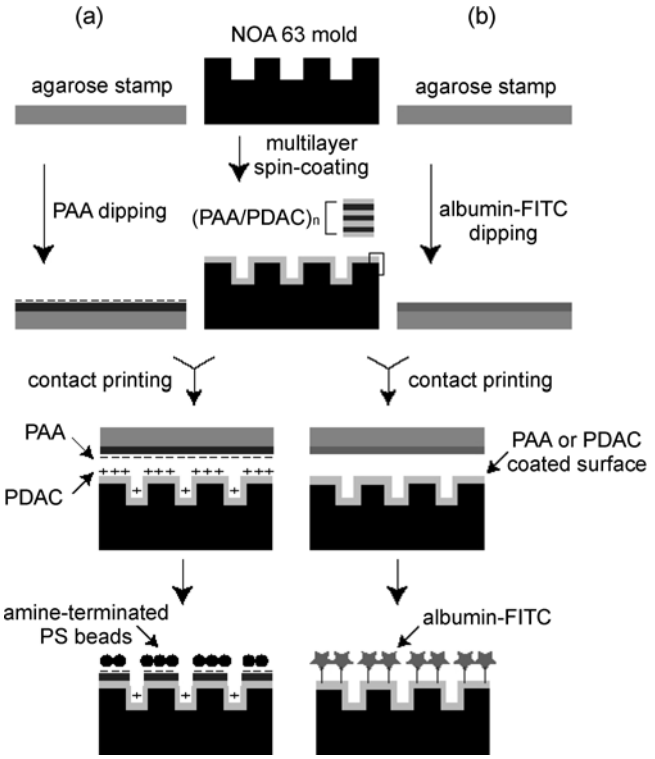

Figure 1. Schematic illustrations demonstrating polymer on polymer stamping (POPS) technique for (a) polyelectrolyte transfer onto polymer microstructure for selective bead patterning and (b) protein patterning.

solution, surface charge and property on microstructures can be easily controlled. We used agarose as a stamp material for POPS to transfer polyelectrolyte. As agarose possesses stable surface and non-toxic properties, it is a very popular material to be employed for various biological applications. In addition, it is a preferable material for use as a stamp for multiple-use because of its excellent maintenance of humidity in the ink soaking process.

Experimental Sections

Figure 1 schematically illustrates the overall process for POPS technique for the chemical micropatterning on micro-structured polymer substrate using agarose gel as a plane stamp. First, polymer microstructure was fabricated by dropping UV-curable prepolymer (NOA 63, Norland Company, NJ, USA) on a substrate and stamping with PDMS stamp having microscale features on its surface. This assembly of prepolymer and PDMS stamp was then cured under UV light (λ = 365 nm) for 30 min using UV lamp (Philips, TL 8W) and the PDMS stamp was peeled off. This UV-curable polymer (NOA 63)[14-16] originally developed as an optical adhesive has excellent optical properties over a wide spectral range (transmission above 98% in a wavelength range from 360 to 1,260 nm) and strong mechanical properties (1,655 MPa). This polymer structure fabricated on a substrate is highly resistant to swelling in various organic solvents such as methanol, toluene, and trichloroethylene. This high resistance in organic solvent becomes advantageous especially in the process of surface treatments. Next, agarose stamp was made by dissolving 3-5 wt% of agarose powder in deionized water (DI water) followed by degassing and melting through heating. Hot agarose solution was poured against a plane petri-dish and cooled down at room temperature. After gelation, agarose plane stamp was peeled off and cut into pieces of about 2 cm $\times$ 2 cm $\times$ 0.5 cm in size.

Figure 1a demonstrates procedures for bead patterning on surfaces selectively modified with positive charges. Prior to spin-coating positively charged polyelectrolyte solution, the polymer structure fabricated on a substrate was briefly exposed to O_2 plasma (1 torr, 25 W, 20 s) to give a negative charge on top of polymer microstructures. After surface treatment with O_2 plasma, 0.5 wt% aqueous solution of poly(diallyldimethylammonium chloride) (PDAC, MW 100,000-200,000) was spin-coated at 3,000 rpm for 40 sec to impart positive charge and washed thoroughly with DI water. We dipped the agarose plane stamp in 0.5 wt% aqueous solution of poly(acrylic acid) (PAA, MW 100,000), a negative polyelectrolyte, for approximately 10 min. After drying with N_2, the agarose stamp was heated on a hot plate at 40 °C for 10 min to eliminate excess water on the agarose stamp surface. The agarose stamp came into conformal contact with the polymer microstructure for 1 min and polar ink was transferred onto the polymer microstructures through electrostatic interaction. After peeling off the agarose stamp, the surface of the microstructures became oppositely charged. In this case, the surfaces of protruding microstructures possessed negative charges owing to the carboxylic group of PAA, and the rest of the surfaces possessed positive charges owing to PDAC. To obtain positive charge on the very top layer of the protruding microstructures, we dipped the positively treated polymer microstructures in aqueous PAA solution again, washed with DI water, and made contact with a new agarose plane stamp which was dipped in aqueous PDAC solution. In this way, the outermost layer of the protruding surface was replaced with positive charge of PDAC and positively charged bead was directed to bind inside the concave structures (data not shown).

Figure 1b demonstrates protein patterning procedures. Surfaces of protruding structures on polymer microstructure was coated first with either PAA or PDAC since proteins bind well on both negatively charged and positively charged surfaces.[17] Agarose plane stamp was dipped in 1.1 mg/mL fluorescein isothiocyanate-conjugated bovine albumin (albumin-FITC) solution, prepared by dissolving in phos-phate-buffered saline (PBS, pH 7.4), for 10 min and dried by N_2 blowing. The agarose stamp came into conformal contact with the polymer microstructure for 1 min to transfer protein solution onto the polymer microstructures.

Results and Discussion

In POPS process, the water holding capacity of the

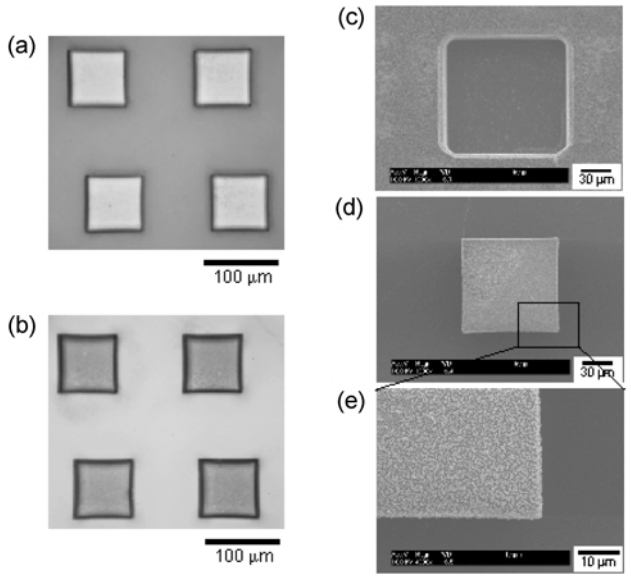

Figure 2. The optical microscopy (OM) images (a and b) and scanning electron microscopy (SEM) images (c-e) of amine-terminated PS beads selectively immobilized on negatively charged concave structures (a and c) and negatively charged protruding structures (b, d, e) of polymer microstructures.

material to be used as a stamp becomes a critical factor because the ability to soak polar ink and continuously release it determines its capacity as a stamp. Figures 2a and b are optical microscopy images which show the results of polyelectrolyte (PDAC) transfer from agarose stamp to negatively charged PAA onto the surface of microstructures. Slurry of amine-terminated polystyrene (PS) bead, approximately 400 nm in sizes, was immobilized. The beads were selectively patterned with high selectivity in accordance with surface charges. On the other hand, PDMS stamp turned out to be unsuitable for transferring polar chemicals such as polyelectrolyte because of its poor wetting and hydrophobic property against water (data not shown). The water holding capacity of PDMS and agarose were calculated by weighing them before and after soaking in DI water for 10 min. The water holding capacities of PDMS and agarose were measured to be approximately 0.046 and 4.16 wt% respectively, which fits well with our results. High humidity maintenance of agarose plane stamp facilitated easy transfer of polyelectrolyte onto the polymer microstructure. These results suggest that water holding capacity of the stamp is important in transferring water-soluble polyelectrolyte from the stamp to the microstructures, and agarose plane stamp turned out to be a highly suitable material as a stamp. Recently, some authors reported on the O_2 plasma treatment of PDMS mold surface for improved attachment of polyelectrolyte in POPS process.[6] However, O_2 plasma treatment of PDMS stamp may cause its surface to become very brittle and easily breakable.

Figure 2c shows the scanning electron microscopy (SEM) image of positively charged bead selectively patterned on negatively charged, concave structures, whereas Figure 2d

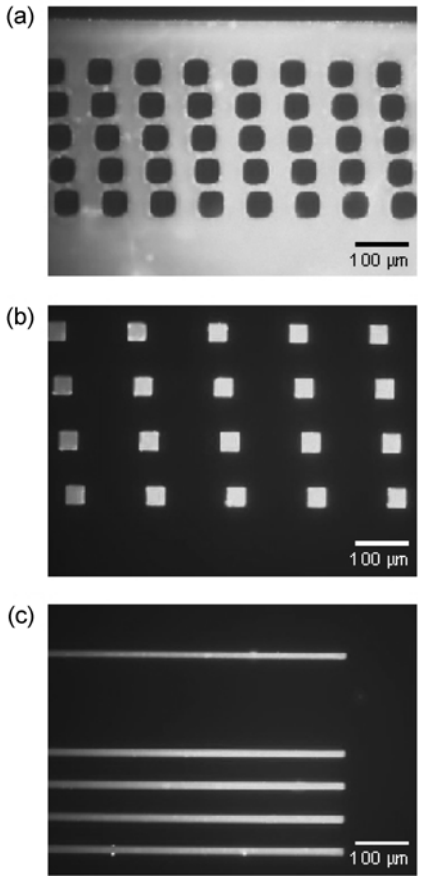

Figure 3. The fluorescent microscopy images of albumin-FITC on PDAC-coated microstructure surfaces when (a) square shapes were concave structures, (b) square shapes were protruding, convex structures, and (c) microchannels were protruding, convex structures.

represents the SEM image of bead patterned on negatively charged, protruding surfaces on polymer microstructures. Except for a few beads nonspecifically bound on the positively charged surface, most of them were neatly patterned on oppositely charged surfaces as clearly demonstrated in the enlarged photo (Fig. 2e).

Figure 3 shows fluorescent microscopy images demonstrating the selective protein patterning on PDAC-coated surfaces. Clear difference in the intensities of fluorescence indicates successful and highly selective PDAC coating on directed surfaces. Although protein binding was successful for both PDAC and PAA-coated surfaces, protein binding was more selective for positively charged surfaces coated with PDAC probably because the protein solution we used was negatively charged under our experimental condition; the isoelectric point (pI) of the protein was 4.7 and the pH of PBS buffer was 7.4. To examine the potential of this agarose plane stamp for multiple-use, one agarose plane stamp, soaked in protein solution for 10 min, was consecutively stamped six times on six polymer microstructures coated with PDAC on the very top layer and fluorescence intensities of patterned proteins were compared in accordance with the order of stamping. As observed, fluorescence

intensities of patterned protein were almost identical regardless of the order of stamping, which strongly indicates that protein patterning was highly reproducible despite several consecutive uses, extending the potential and efficiency of this agarose stamp for multiple-use with single soaking.

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References

1. Xia, Y.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 550.
2. Bernard, A.; Renault, J. P.; Michel, B.; Bosshard, H. R.; Delamarche, E. *Adv. Mater.* **2000**, *12*, 1067.
3. Chen, C. S.; Mrksich, M.; Huang, S.; Whitesides, G. M.; Ingber, D. E. *Science* **1997**, *276*, 1425.
4. Wu, X. C.; Bittner, A. M.; Kern, K. *Adv. Mater.* **2004**, *16*, 413.
5. Hammond, P. T. *Adv. Mater.* **2004**, *16*, 1271.
6. Park, J.; Hammond, P. T. *Adv. Mater.* **2004**, *16*, 520.
7. Zheng, H.; Lee, I.; Rubner, M. F.; Hammond, P. T. *Adv. Mater.* **2002**, *14*, 569.
8. Feng, J.; Wang, B.; Gao, C.; Shen, J. *Adv. Mater.* **2004**, *16*, 1940.
9. Yang, S. Y.; Lee, D.; Cohen, R. E.; Rubner, M. F. *Langmuir* **2004**, *20*, 5978.
10. Park, S. H.; Lim, T. W.; Yang, D.-Y.; Kong, H. J.; Kim, R.-H.; Kim, K.-S.; Lee, K.-S. *Bull. Korean Chem. Soc.* **2004**, *25*, 1119.
11. Ng, J. M. K.; Gitlin, I.; Stroock, A. D.; Whitesides, G. M. *Electrophoresis* **2002**, *23*, 3461.
12. Campbell, C. J.; Smoukov, S. K.; Bishop, K. J. M.; Grzybowski, B. A. *Langmuir* **2005**, *21*, 2637.
13. Kim, Y. S.; Baek, S. J.; Hammond, P. T. *Adv. Mater.* **2004**, *16*, 581.
14. Cazorla, O.; Lacampagne, A.; Fauconnier, J.; Vassort, G. *Brit. J. Pharmacol.* **2003**, *139*, 99.
15. Zauscher, S.; Klingenberg, D. J. *J. Colloid Interf. Sci.* **2000**, *229*, 497.
16. Mogensen, K. B.; Petersen, N. J.; Hübner, J.; Kutter, J. P. *Electrophoresis* **2001**, *22*, 3930.
17. Houska, M.; Brynda, E. *J. Colloid Interf. Sci.* **1997**, *188*, 243.