Protein Array Fabricated by Micro-Contact Printing Method for Detection of Marker Protein to Genetically Modified Organisms

W. Lee[*], S. H. Paek[**] and J. W. Choi[*, ***]

[*]Interdisciplinary Program of Integrated Biotechnology, 1 Shinsoo-dong, Mapo-gu, Seoul 121-742, Korea, biolee@mail.sogang.ac.kr
[**]Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea, shpaek@korea.ac.kr
[***]Department of Chemical and Biomolecular Engineering, Sogang University, 1 Shinsoo-dong, Mapo-gu, Seoul 121-742, Korea, jwchoi@ccs.sogang.ac.kr

ABSTRACT

For the past decade, microarray technology has evolved from genomics to proteomics. This trend offers several advantages. In small volumes, biochemical reactions may not be diffusion-limited and may thus be more efficient; less reagent and sample solution are used, lowering costs per test. In this study, micro-contact printing (μCP) technique was applied to the formation of protein pattern. In μCP, master and poly(dimethylsiloxane) (PDMS) stamp were fabricated sequentially. The fabricated PDMS stamp was coated with a solution containing target material for patterning, and brought in contact with Au substrate. By using the fragmented antibody, the antibody was immobilized on the Au surface in an oriented way. The formation of pattern was visually investigated with fluorescence microscopy. As the concentration of target protein decreased, the fluorescence intensity was also decreased, of which the detection limit was 100 pg/mL of human serum albumin (HSA). The layer formation and pattern feature was maintained throughout this study. Experimental results suggest that the protein array fabricated by?the proposed method could be successfully applied to the measurement of various proteins.

Keywords: micro-contact printing, poly(dimethylsiloxane), protein array, fluorescence microscopy, sandwich type immunoassay

1 INTRODUCTION

For the past decade, microarray technology has evolved from genomics to proteomics. This trend offers several advantages. In small volumes, biochemical reactions may not be diffusion-limited and may thus be more efficient; less reagent and sample solution are used, lowering costs per test [1, 2]. Minituarized assays can be done quickly and simultaneously in large numbers; small scales can be the key to the realization of certain type of assays, e.g., where laminar flow in capillaries is required. Several techniques have been examined for creating micron-level two-dimensional arrays of biomolecules on surfaces, including the use of conventional microarrayer, piezoelectric dispenser, and self-assembled monolayer [3-5].

In this respect, DNA-based arrays have become the leading technology for high integration and miniaturization of bioassays. It can be organized from precursors using photolithography, pin arrayer printing robots, or inkjet printers [6, 7]. But there is need to develop another technique that allows the immobilization of biologically active proteins such as enzyme and antibodies due to their easily fragile nature and activity loss by random orientation. As a consequence, proteins have to be delivered to surfaces as functional entities in a patterned fashion in order to produce arrays.

Over the past several years, microcontact printing (uCP) technique has been developed to transfer alkanethiols onto Au substrate, of which the application has been expanded to protein patterning, nanoparticle pattern formation, and patterned cell adhesion [8-10]. In uCP method, a poly(dimethylsiloxane) (PDMS) stamp is coated with a solution containing target material for patterning, and brought in contact with a functionalized solid substrate such as glass, silicon, or gold. Because of the low cost of fabrication as well as the simplicity of transferring target material to substrates, the uCP technique is versatile and very attractive for diverse application.

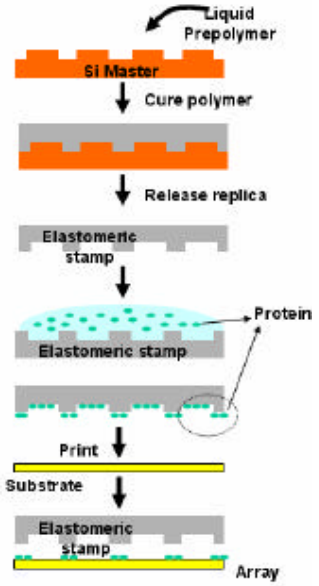


Fig. 1. Schematic description of microcontact printing for protein array fabrication

In this study, the previous effort with respect to oriented antibody immobilization is expanded into the organization of protein pattern. Fig. 1 shows the schematic description for protein patterning based on μCP technique. On the patterned surface, the proposed assay system is visualized by fluorescence labeled secondary antibody. Because the size of patterned protein is dependent on the pixel size of stamp, the protein pattern size can be minituarized into nanoscale.

2 MATERIALS AND METHODS

2.1 Materials

All solutions were prepared with Millipore (Milli-Q) water. Human serum albumin (HSA) and fluorescence isothiocynate (FITC)-labeled HSA was purchased from Sigma (St. Louis, MO, USA) and AbCam (UK), respectively. Monoclonal and polyclonal antibody against human serum albumin (HSA) was purchased from Sigma, respectively. Cy3 labeled antibody against rabbit IgG was utilized as secondary antibody and purchased from Amersham Biochemistry (UK). Antibody was immobilized on the Gold (Au) surface which was precleaned with piranha solution. Other chemicals used in this study were obtained as reagent grade.

2.2 Fabrication of Mask and Master

The chromium mask was purchased and prepared by Micro Image Co., ltd (Korea). Before mask fabrication, array of the squares with a dimension of 5 μm × 5 μm was organized by computer added design (CAD). Masters, which contained a positive relief of the stamp mold, were manufactured at the nanofabrication center in Korea Institute of Science and Technology (KIST). These masters were made by first spin coating of a positive photoresist, AZ 7220 from Shipley (Marlborough, MA), on a silicon wafer. Irradiation with UV light through a chrominium mask was used to render the exposed photoresist soluble, which was then removed by washing with the developer LDD26 from Shipley. Two different stamp configurations were used for these experiments. One consisted of the embossing configuration with 5 μm × 5 μm square pattern, and the other consisted of engraved configuration.

2.3 Stamp Fabrication

PDMS stamps are fabricated by casting and curing Sylgard 184 (Dow Corning. Midland, MI, USA) an elastomeric polymer, against silicon master with 3 um-thick features made by photolithography. A prepolymer and curing reagent was mixed with a volume ration of 10 : 1, and the mixture was poured onto silicon master. And then the viscous polymer solution was degassed in the vacuum station (Bernant, IL, USA) for 2 hr. After curing at 60℃ overnight, the elastomeric stamp bearing the negative pattern of the master was peeled off, sonicated with 70% ethanol for washing, and dried under nitrogen blowing.

2.4 Microcontact Printing: Sample Preparation

Because the surface property of the prepared PDMS elastomer is hydrophobic, proteins for fabrication through μCP can be easily adsorbed on the PDMS surface. This process of loading proteins on the stamp surface has been referred to be as "inking". After inking on the PDMS surface, it was incubated for approximately 1 minute, and the stamp was dried using slide centrifuge (Fisher, UK). The Au surface was contact onto the dried PDMS stamp. The mechanism of transfer has not been known at this time, but the most probable explanation is that binding affinity between the thiolate molecules and gold surface will be stronger than the adhesion force between the PDMS surface and the thiolated molecules [11, 12]. After 30 seconds, the solid substrate fabricated with PDMS stamp was released and applied for the further processing.

2.5 Analysis

One of the most common techniques used for sensing binding on an immunological array is to use a secondary reagent such a second antibody or antigen that is fluorescent labeled. Patterns of fluourescent labeled specific secondary antibody were used in this work and were detected by fluorescence microscopy (Leica, Germany) equipped with charge-coupled device (CCD). A mercury lamp with optical filter at 488 nm and 530 nm was used to induce fluorescence of fluoresceine isothiocyanate (FITC) and Cy3 functional groups conjugated to anti-IgG molecules.

3 RESULTS AND DISCUSSION

Fig. 2 shows the fabricated chromium mask, master mold developed by photolithography, and PDMS stamp molded by the master.

AZ7220 photoresist was selected for definite aspect ratio of the fabricated stamp. The chosen photoresist was coated on the p-type silicon wafer and baked softly at 65℃ and 100℃ in series. Then, exposure and development was carried out for 4 sec. and 1 min., respectively. When the process time was much more than the designated value, the undercut phenomena happened on the overall region. Through the development, patterned master was produced through the development. Through the micrograph of the Cr mask and fabricated master as shown in Fig. 3, it is expected to produce a delicate PDMS stamp, which was

confirmed through the microscopic view of contact region (square feature) with a dimension of 5 μm × 5 μm.

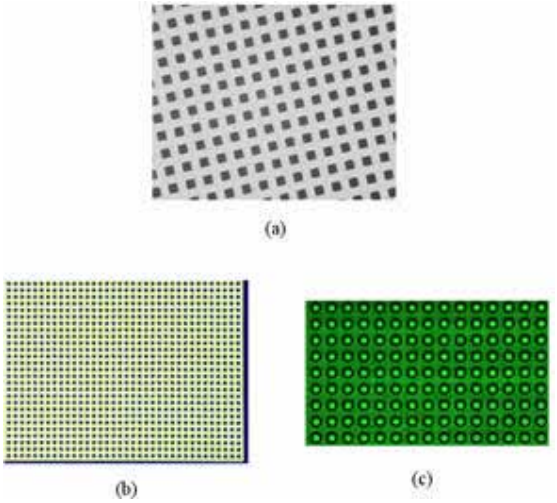
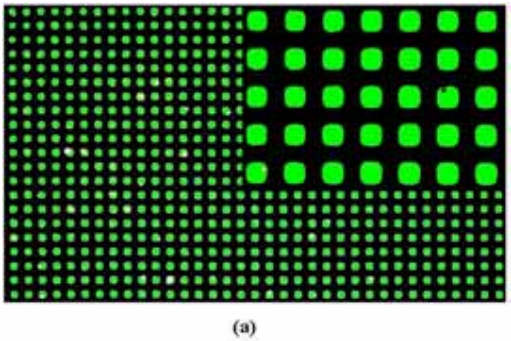


Fig. 2. Micrographs of (a) patterned Cr mask, (b) fabricated Si master, and (c) Fabricated PDMS stamp. The dimension of square is 5 μm × 5 μm, respectively.

The performance of fabricated PDMS stamp was evaluated by the fluorescence microscopy. The anti-monkey IgG labeled with FITC was utilized as ink of the stamp and then transferred to the Au surface. Fig. 3(a) shows the fabricated protein pattern. The ink was successfully transferred to the solid surface, and the size of transferred feature was maintained on the overall region. Fig. 3(b) shows the microscopic view of fluorescence generated by HSA-FITC bound on the fabricated protein pattern with Mab against HSA. As the concentration of antigen decreased, the fluorescence intensity decreased accordingly. When the concentration of HSA below 1 μg/mL was applied to the fabricated pattern, it could not be observed through the fluorescence microscopy.



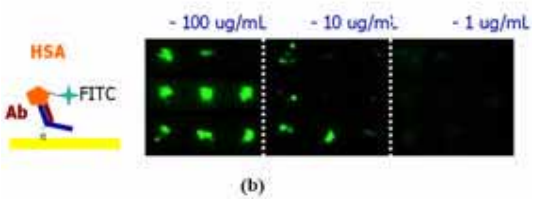


Fig. 3. Fluorescence micrographs of (a) pattern of FITC-labeled antibody fabricated by uCP method and (b) FITC-labeled human serum albumin bound on the fabricated antibody pattern

With the patterned antibody surface, antibody-antigen interaction was investigated based on fluorescence microscopy. Fig. 4 shows the microscopic view of fluorescence generated by labeled antigen (HSA) and labeled secondary antibody, respectively. Antibody fragment was utilized for oriented immobilization. When HSA labeled FITC was introduced on the fabricated biosurface, the feature of protein pattern was main tained as shown in Fig. 4. As ever, when the sandwich type immunoassay was implemented on the protein array as shown in Fig. 4, the square type shape was also maintained with a dimension of 5 μm × 5 μm although there was nonspecific binding region in the fabricated surface at the concentration above 1 μg/mL. When 100 pg/mL of HSA was applied to the proposed sandwich type assay, the fluorescence generated by secondary antibody labeled with Cy3 was still observed as shown in Fig 4. Therefore, it could be concluded that the detection limit of the fabricated protein array based on sandwich type immunoassay was determined to be 100 pg/mL of HSA.

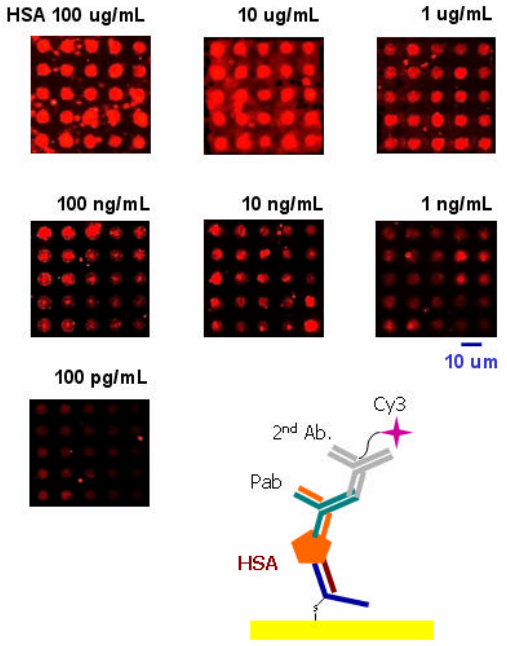


Fig. 4. Fluorescence micrographs of sandwich type immunoassay using Cy3-labeled secondary antibody with respect to HSA concentration.

4 CONCLUSION

Protein array was fabricated based on the μCP method. Under the optimal fabrication condition for the production of master mold, PDMS stamp was successfully fabricated with definite aspect ratio. On the fabricated pattern, protein immobilization and antibody-antigen interaction was several washing procedure was applied to the patterned surface, the shape of protein pattern (square) was maintained on the overall region. The experimental results showed the proposed assay system can be implemented on the fabricated protein chip for multi-target analysis.

ACKNOWLEDGEMENT

This research was supported by Korea Research Foundation Grant (KRF-2002-005-D00003).

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