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# Antibody Layer Fabrication for Protein Chip to Detect *E. coli* O157:H7, Using Microcontact Printing Technique

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**Abstract** An antibody layer was fabricated to detect *Escherichia coli* O157:H7. The micropattern of 16-mercaptohexadecanoic acid (16-MHDA) as alkylthiolate was formed on the gold surface by using the PDMS stamp with microcontact printing (μCP) techniques. In order to form antibody patterns on the template, protein G was chemically bound to the 16-MHDA patterns, and antibody was adsorbed on a self-assembled protein G layer. The formation of the 16-MHDA micropattern, self-assembled protein G layer and antibody pattern on Au substrate was confirmed by surface plasmon resonance (SPR) spectroscopy. Finally, the micropatterning method was applied to fabricate the antibody probe for detection of *E. coli* O157:H7, and monitoring of antigen by using this probe was successfully achieved.

**Key words:** Antibody probe, protein G, mercaptohexadecanoic acid, microcontact printing, micropattern, *E. coli* O157:H7

Biomolecules immobilized on solid surfaces are useful in various areas of science and technology, including biosensors, biochip, chromatography, and diagnostic immunoassay. Biological units should be miniaturized for the integration and multiplication of these systems. Specifically, the patterns of antibodies that are important molecules for the diagnostic immunoassay are essential for the miniaturized immunosensor system. The miniaturization offers several advantages, including cost lowering, integration for simultaneous multicomponent detection, and the efficiency increase of biochemical reactions. Several micropatterning techniques such as photolithography, microspotting, and microcontact printing (μCP) have been used to fabricate the microarray of biomolecules [6, 13, 14]. Among the above-mentioned techniques, the μCP technique is particularly attractive, due to its ability to deposit unlimited patterns and to permit the printing of multiple biomaterials in individual patterns [4].

Using the μCP technique, thiol groups or biomaterials are transferred from a stamp to a gold surface, and they are then attached to the substrate with self-assembly adsorption. Several researchers studied the formation of antibody pattern on a solid surface, using μCP [1, 8]. Based on these studies, the strategies for fabrication of antibody patterns can be categorized into two procedures. One procedure is the direct printing of antibody on a solid surface by using the poly(dimethylsiloxane) (PDMS) stamp. In this system, the binding of antigen to the antibody pattern was confirmed [4]. However, the reproducibility of the amount of adsorbed protein was poor, because proteins are physically adsorbed onto the gold surface. The other procedure is composed of pre-patterning of alkylthiolate on a gold surface and the protein binding on the alkylthiolate pattern. In this procedure, the chemical immobilization of protein onto the alkylthiolate pre-patterning surface results in good repeatability. However, the binding of antigen to the antibody pattern for the immunosensor systems has not yet been studied.

Protein G, a cell wall protein found in most species of *Streptococci*, can be used to construct a well-defined antibody surface. Since protein G specific interacts with the Fc portion of immunoglobulin G (IgG), the paratope of IgG can face the opposite side of the protein G-immobilized solid support [2]. As a result, protein G-mediated antibody immobilization can lead to a highly efficient immunoreaction [9, 10].

In this study, a substrate having the micropatterns of alkylthiolate on gold surface was prepared by the μCP

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technique, and the antibody pattern on the template was fabricated. In addition, the binding of antigen to the antibody micropatterns was studied in the hope of its application to the immunosensor system.

A PDMS stamp was prepared by using the procedure of Xia and Whitesides [15]. A conventionally fabricated silicon master was replicated by pouring liquid prepolymer of PDMS onto the master and then curing it. The micropattern of 16-mercaptohexadecanoic acid (16-MHDA) as alkylthiolate was formed on the gold surface by using the PDMS stamp. The stamp was completely rinsed with ethanol and dried under a flow of nitrogen gas. A drop (200 μl) of 10 mM 16-MHDA in ethanol was placed on the top of the rinsed stamp, enough to cover the surface. A liquid was left for 2 min to allow it to equilibrate with the PDMS surface, and it was then removed quickly under a stream of nitrogen gas, following mild blowing to dissipate the liquid. The inked stamp was placed in contact with the gold substrate for 2 min in order to transfer 16-MHDA from the stamp to the substrate. After removal of the stamp, the gold substrate was submersed into 11-mecaptoundecanol (11-MUOH) for at least 12 h.

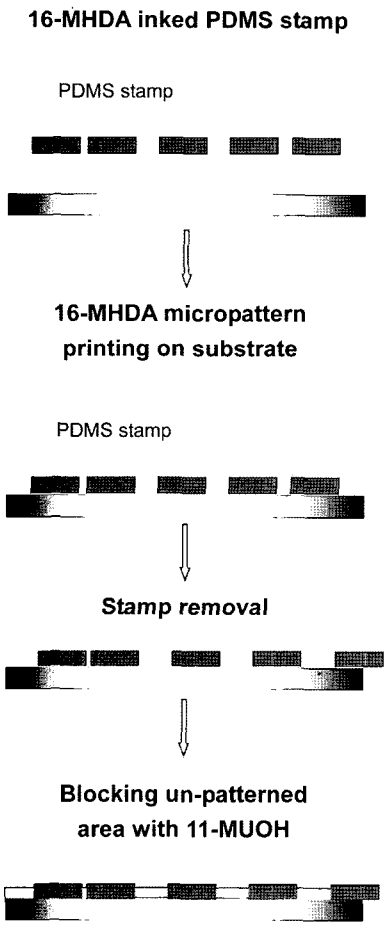



**Fig. 1.** Schematic description of microcontact printing method.

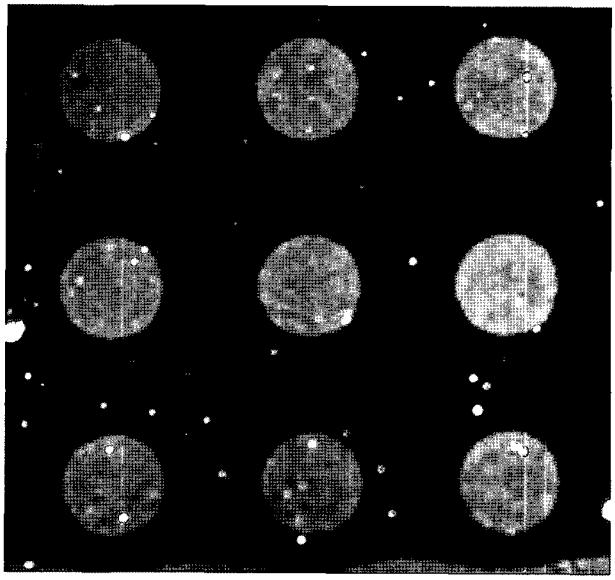


**Fig. 2.** Fluorescence images of FITC-labeled protein G (100 μg/ml) spot bound to the template, prepared by using the microcontact printing technique.

The substrate with the micropatterns of 16-MHDA was completed by washing with an ethanol and water solution. The schematic procedure of the microcontact printing method is described in Fig. 1.

In order to form antibody patterns on the template, protein G (Prozyme Inc., CA, U.S.A.), which could bind the Fc domain of an antibody, was chemically bound to the 16-MHDA patterns. Thus, the template was treated with 10% 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) in water/ethanol (10/1, v/v) solution for 2 h at room temperature, after which FITC-labeled protein G (100 μg/ml) was placed on the template for 2 h. After washing the template with 10 mM phosphate buffer (PBS, pH 7.4) containing 0.1% Tween 20 detergent, the fluorescent image of micopatterns (Fig. 2) was acquired, using fluorescence microscopy (DML/HCS, Leica Microsystems Wetzar GmbH, Germany). Based on this fluorescence image, the formation of the micropattern could be confirmed. The diameter of each pattern was 60 μm and the span was 50 μm. It is known that the pattern in micrometer or sub-micrometer scale could be formed by using μCP techniques [7]. Whereas the terminal carboxyl group of 16-MHDA is esterficated through EDAC treatment and can covalently bind the lysine residue of protein G, the terminal hydroxyl group of 11-MUOH nonspecifically binds proteins. Therefore, protein G was bound to only the 16-MHDA micropattern after washing with the detergent. In the experiment with dodecanthiol instead of 11-MUOH, protein G remained in the outer area of the micropattern after treatment with Tween 20. Whereas the 11-MUOH-coated surface with a terminal hydroxyl group is hydrophilic, the dodecanethiol-coated

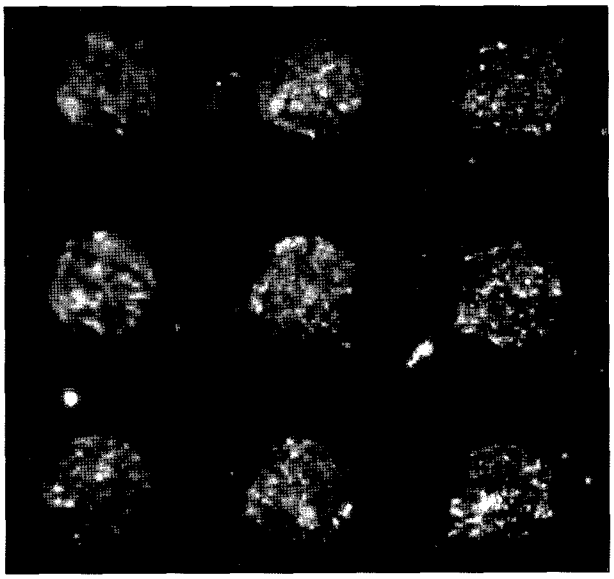


**Fig. 3.** Fluorescence image of the binding of FITC-labeled insulin to the antibody pattern immobilized on a protein G micropattern.

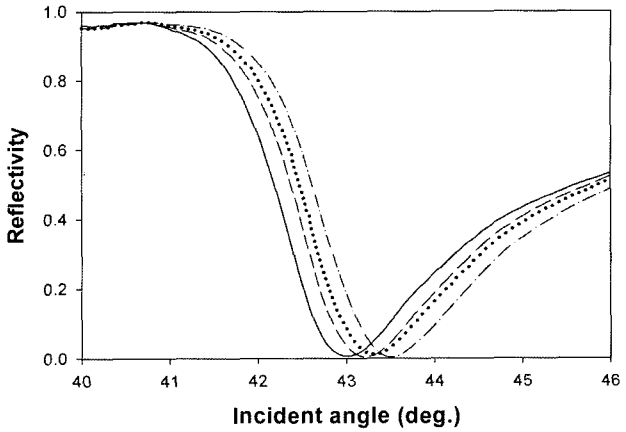


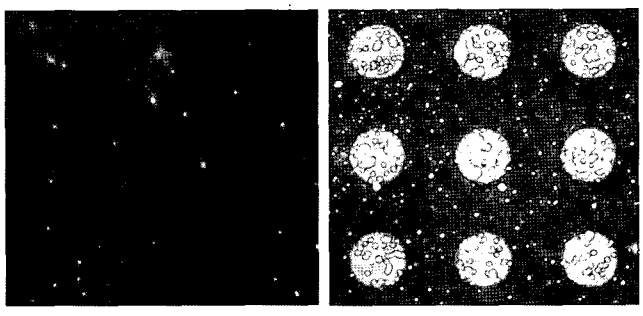

**Fig. 4.** The changes of SPR curve by serial adsorption of the 16-MHDA, protein G, and Mab on a gold surface.
Solid line, gold surface; dashed line, 16-MHDA layer; dotted line, protein G layer; dash-dot line, Mab layer.

surface with a methyl group is hydrophobic. Hydrophobic interactions might occur between the methyl group of dodecanethiol and protein G. Indeed, physical adsorption of protein by hydrophobic interaction has previously been reported [3, 11]. Therefore, the application of 11-MUOH was necessary for the protein pattern formation.

The binding of antigen to antibody pattern formed by the μCP technique was also investigated. Polyclonal antibodies (Sigma Co., MO, U.S.A.) against insulin and the FITC-labeled insulin were used to confirm the binding of antigen to the antibody pattern formed by the μCP technique. Figure 3 shows the fluorescent image on which the FITC-labeled insulin was found to bind the antibody pattern: The antibody was immobilized on the protein G bound to 16-MHDA pattern formed by the μCP technique. As shown in Fig. 3, the antigen was bound to the antibody pattern and the nonspecific binding was not significant. These results, therefore, confirm that antigen-antibody binding can successfully be achieved on the micropattern prepared by the μCP technique.

Finally, the antibody probe for detection of *Escherichia coli* O157:H7 (ATCC 43895) was fabricated, using the μCP technique. Monoclonal antibody (Mab) against *E. coli* O157:H7, which gave a single precipitin arc with *E. coli* O157:H7 LPS and its O polysaccharide, was purchased from Fitzerald Industries International, Inc. (Cat. No. 10-E03, MA, U.S.A.). As 16-MHDA, protein G, and Mab were adsorbed onto the gold surface, the SPR curve changed, as shown in Fig. 4. The SPR angle of the bare gold surface was 43.020±0.03. After 16-MHDA and protein G were adsorbed on the gold surface, the SPR angle was shifted to 43.220±0.03 and 43.320±0.03, respectively. It is known that the shifts of SPR angle result from the adsorption of dielectric materials on the gold surface [5]. Furthermore, the SPR angle was significantly shifted from 43.320±0.03 to 43.490±0.03 on immobilization of Mab on the self-assembled protein G layer, thus confirming the binding interaction between self-assembled protein G layer and antibody molecules.

The binding of *E. coli* O157:H7 to the antibody pattern was monitored by using an FITC-labeled secondary antibody (Biogenesis Ltd., NH, U.S.A.). The antibody probe was fabricated by immobilization of Mab on the protein G covalently bound to the 16-MHDA that was formed on the gold surface by the μCP technique. A sample solution containing *E. coli* O157:H7 was applied to the antibody probe for 2 h to achieve the antigen-secondary antibody reaction. Figure 5 shows the fluorescence image of the antibody probe after the reaction: Although some fluorescence was generated in the blocked area from nonspecific binding, the binding of *E. coli* O157:H7 to the



A B

**Fig. 5.** Fluorescence image of the antibody probe for the detection of *E. coli* O157:H7 (A) with control solution (0 CFU/ml), (B) with sample solution ($10^7$ CFU/ml).

antibody probe (Fig. 5B) could sufficiently be detected, compared with the control experiment (Fig. 5A).

In conclusion, the formation of antibody pattern in a micrometer scale on the substrate was performed in this study by using the μCP technique, and the formation was confirmed by applying it to the antibody probe for detection of *E. coli* O157:H7.

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