Macromolecular Research, Vol. 17, No. 3, pp 192-196 (2009)

Fabrication of Multicomponent Protein Microarrays with Microfluidic Devices of Poly(dimethylsiloxane)

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Received August 21, 2008; Revised September 24, 2008; Accepted October 17, 2008

Abstract: Recently, the multi-screening of target materials has been made possible by the development of the surface plasmon resonance (SPR) imaging method. To adapt this method to biochemical analysis, the multi-patterning technology of protein microarrays is required. Among the different methods of fabricating protein microarrays, the microfluidic platform was selected due to its various advantages over other techniques. Microfluidic devices were designed and fabricated with polydimethylsiloxane (PDMS) by the replica molding method. These devices were designed to operate using only capillary force, without the need for additional flow control equipment. With these devices, multiple protein-patterned sensor surfaces were made, to support the two-dimensional detection of various protein-protein interactions with SPR. The fabrication technique of protein microarrays can be applied not only to SPR imaging, but also to other biochemical analyses.

Keywords: protein microarray, microfluidic device, polydimethylsiloxane.

Introduction

For the diagnosis and prognosis of cancer and neurodegenerative diseases, many studies have been conducted by various methods using biomarkers such as protein and antibody microarrays as versatile screening tools.[1] Among them, the surface plasmon resonance (SPR) analysis of microarrays is widely used, because of its advantages in the *in situ* biomolecular recognition by surface analysis without labeling.[2] SPR analysis is a method of measuring biomolecular interactions by detecting the quantity of an antibody or antigen present in the protein microarrays.[3-6] Previously, SPR analysis only allowed the detection of molecular interactions one at a time. However, the multi-screening of target materials has recently been realized by the development of the SPR imaging method. To adapt this method to biochemical analysis, however, the multi-patterning technology of protein microarrays is required.

Recently, microfluidic platforms have been selected as tools for medical and biochemical analysis,[7,8] including the patterning of protein microarrays, the manipulation and analysis of biological cells,[9] DNA analysis, high-throughput screening,[10] fluidics and sample handling,[11] and separation and detection with labs-on-a-chip for clinical diagnostics,[12] because of the many advantages afforded by their miniaturization, such as the reduced requirement of expensive solvents, reagents and cells, etc..[13,14] Microfluidic devices have been fabricated using silicon (Si) or glass as substrates by the commercial semiconductor fabrication method. However, they also have several disadvantages, such as their high production costs, lack of gas permeability, and lack of optical transparency in the case of Si[15,16] and, consequently, polymeric materials including PDMS, polymethylmethacrylate (PMMA), cycloolefin copolymer (COC), polycarbonate (PC), and polyimide[17-20] have been highlighted as alternative materials. Among them, PDMS is well known as a suitable material for biomedical and clinical applications, due to its numerous advantages, such as its nontoxicity, flexibility, optical transparency, high inertness, and gas permeability.[15,16,21] Also, its fabrication method (replica molding method) is easier, cheaper, and rapider than the fabrication method of PMMA (hot-embossing method).[18]

Protein microarray technology was developed from DNA chip technology and is now applied to the identification, quantification, and functional analysis of proteins. Using these miniaturized assay systems, multiplex and parallel analysis can be achieved with the surface analysis methods mentioned above. In order to pattern protein antigens and to adsorb them from solution onto modified Au substrates, a number of methods are currently being used, including

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automated microspotting techniques and the microcontact printing (μCP) method.[22] Among these methods, the μCP method is widely used, because it is easy to transfer patterns with the polydimethylsiloxane (PDMS) stamp. However, with μCP, it is difficult to control the amount of materials, and the amount of materials transferred is very small. Also, changes in protein function can occur, because it operates by pushing the PDMS stamp.[15,16] Therefore, microfluidic devices have been highlighted as a fabrication tool for protein microarrays.

Unlike DNA chips, the fabrication technique of protein microarrays with microfluidic devices is not commercialized widely yet. However, several studies have been performed, in order to construct multiplex assay systems by means of microfluidic devices. A. R. Wheeler, *et al.*[23] and V. Kanda, *et al.*[24] fabricated microfluidic devices with PDMS, and made biotinylated bovine serum albumin (B-BSA), streptavidin, biotinylated protein A, and human/goat immunoglobulins G (IgG) patterns on the Au surface by hydrodynamic injection with a syringe pump or by a vacuum applied through a pipette tip. In these studies, the microfluidic devices needed additional equipment such as flow controllers, pumps, pressure generators, etc. Also, these devices needed large amounts of expensive reagents to make the protein microarrays. In this work, to avoid using these additional equipments and to reduce the amount of solution required, the microfluidic devices were designed to operate using only capillary force. The devices were fabricated with PDMS by the replica molding method with commercial semiconductor fabrication processes. To fabricate the protein microarrays, various solutions are introduced over the substrate in the spatially defined channels of the microfluidic devices. The constructed protein microarrays were installed on an SPR apparatus, and an SPR analysis was performed in order to observe the specific interaction between the SOD1 proteins and other biomolecules.

Experimental

Fabrication of Microfluidic Devices. To make various protein microarrays with different types of biomolecules for SPR, a number of microfluidic devices were designed and fabricated with PDMS. These devices were designed to operate using only capillary force, without the need for additional flow control equipment, as mentioned above. With this conception, 2-D drawings of the microfluidic devices were done by AutoCAD 2002. Figure 1 shows the outline of the microfluidic devices for the SPR cell. This pattern contains 8 holes as fluid reservoirs and 4 microchannels for patterning biomolecules on the Au surface. The microchannels each have a width of 350 μm and are separated by a distance of 500 μm. The size of the devices was set to the size of the SPR glass substrate (1.75 cm×1.75 cm) to prevent fluid leakage from the boundary of the device. Between the reservoir and the microchannel, gradual expansion and contraction of the channel is applied to make the flow spontaneous.

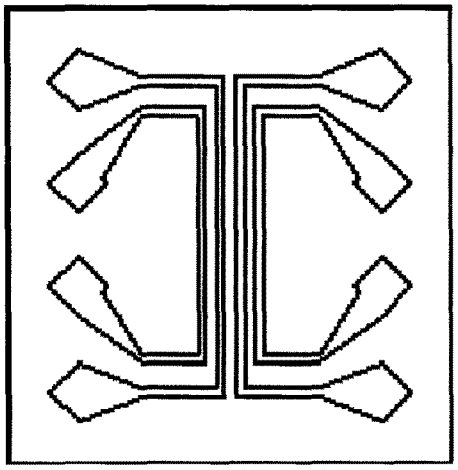

Figure 1. Selected design of microfluidic devices.

The PDMS-based microfluidic devices were designed and fabricated by the replica molding method.[9,10,25] At first, a two-dimensional pattern was drawn with a commercial CAD program. For the photolithography process, the CAD file was printed to the film mask by the Sejinsmark Company. This film mask was made with polyester and its thickness is 175 μm. The patterned region of the mask is transparent and the other region consists of black emulsion. To make the masters of the PDMS replicas, a p-type, 4-inch thick Si wafer was coated with SU-8 (an epoxy based negative resist) with a thickness of 100 μm. The pattern in the film mask was transferred to the SU-8 by photolithography, followed by different post-baking and developing steps in the clean-room facilities. This pattern transfer was done by Korea Bio-IT Foundry in Seoul. The SU-8-patterned wafer was used as the mold (master) of the PDMS replicas. The PDMS prepolymer (Dow Corning Corporation) and curing agent (Sylgard 184 kit) were mixed at a weight ratio of 10:1 by hand. The pure PDMS prepolymer has the viscosity of 5,500 mPa·s at 23 °C and the specific gravity of 1.03. After mixing, bubbles were removed by heating in a vacuum drying oven at 30 °C for 30 min. After degassing, PDMS was poured over this master and cured in the drying oven at 100 °C for 1.5 h. This cured PDMS slab was peeled off from the master and cut to the appropriate size. After cutting, reservoir holes were punched by a hole-puncher, and the surface was modified with suitable functionalities.

SPR Experiments. SPR imaging analysis for the prepared protein microarrays was carried out by a two-dimensional intensity interrogation-based SPR imaging system (SPRi LAB, KMAC, Korea), as shown in Figure 2. A p-polarized light beam with 760-nm light emitting diode was directed toward a prism/sensor chip assembly at a fixed angle. Then, the reflected beam was focused on a 2D charge coupled device (CCD) camera, the light intensity across the sensor chip

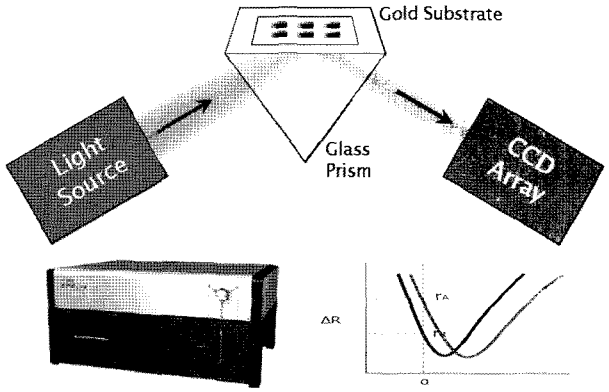

Figure 2. SPR imaging apparatus.

recorded by a computer and the SPR image results analyzed using a built-in software program. The gold sensor chip was composed of a 2-nm thick chromium and 50-nm thick gold thin-film layers on the 18-mm × 18-mm BK7 glass slide, and the space between the sensor chip and prism was filled up with a refractive index-matching oil for optical continuity.

Results and Discussion

Before patterning expensive proteins to the Au surface on the glass substrate, simple tests were done to find out whether this device works well or not. Tests were done with hydrophilic red ink to provide a condition similar to that of biocompatible platforms such as the antigen-antibody interactions in the hydrophilic solution. Actually, before the modification of the surface, the physical properties of the device are hydrophobic. Therefore, although the reservoir is filled with a hydrophilic solution, the fluid does not flow through the microchannel. Without additional flow controllers or pumping equipment, natural fluid flow was not generated, as shown in Figure 3(a). To make the hydrophilic solution flow in the hydrophobic microchannels, a piece of PDMS was used as a cover on the reservoir and was strongly pushed, as shown in Figure 3(b). With this procedure, the hydrophilic solution could be made to stay within the hydrophobic microchannels, as shown in Figure 3(c). To simplify the operation procedure of the device and to bring about spontaneous fluid flow in the microchannels, surface modification by a simple chemical reaction was done. It is

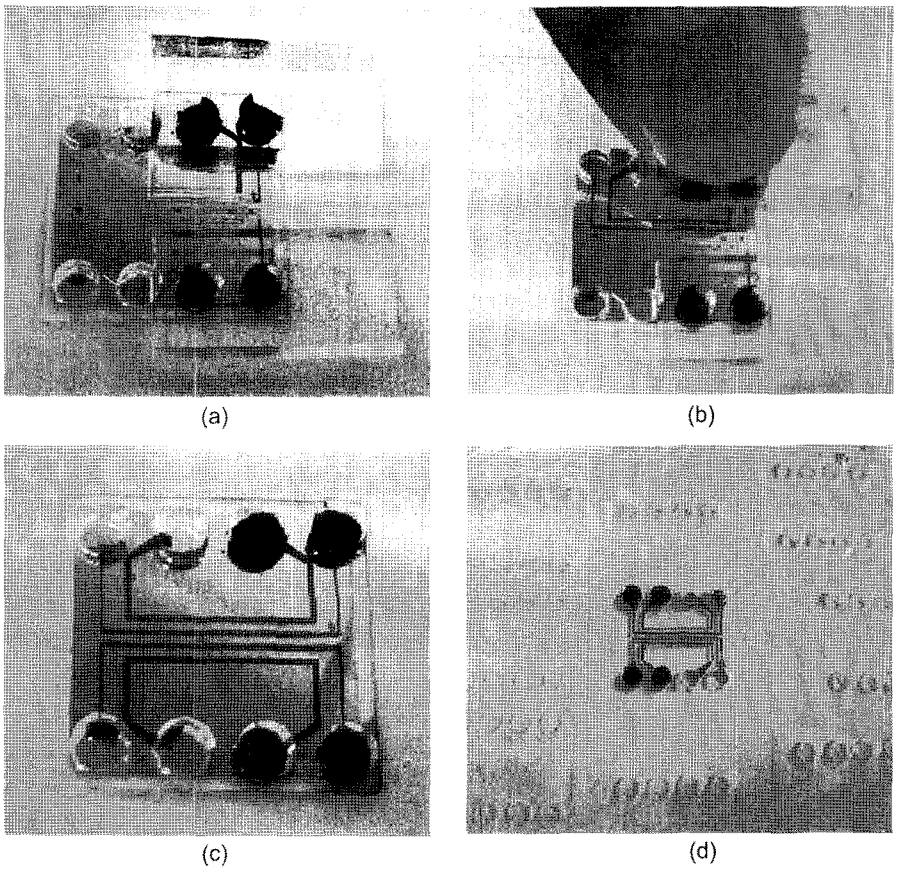

Figure 3. Test of microfluidic devices. (a) No natural flow of hydrophilic solution in the microchannels, (b) Pushing PDMS covers to accumulate solution in the microchannels, (c) Capillary flow after pumping and removing PDMS covers, and (d) Spontaneous fluid flow by surface modification of PDMS.

useful to change the physical properties from hydrophobic to hydrophilic, because the various fluids including protein solutions, antibody solutions, and buffers which were used in this work are almost all hydrophilic solutions. Firstly, in order to make the surface more active, the cured PDMS was dipped into acetone and sonicated for 5 min. After rinsing with distilled water, the PDMS slab was immersed in hydrogen peroxide (H_2O_2, 30%) solution for 1 h to change the covalent bonding of Si-O-Si to Si-OH.[26] Before using the microfluidic device, the surface of the PDMS was cleaned with distilled water and dried in the oven. With this simple surface modification, the flow in the microchannels became spontaneous, as shown in Figure 3(d). Also, without additional pushing, fluid could be accumulated within the microchannels.

Based on the good result obtained from the pre-test, protein microarrays were constructed on a 50-nm thick Au film deposited on SF10 glass by the fabricated microfluidic devices. Among the various proteins, copper-zinc superoxide dismutase 1 (Cu-Zn SOD1), which is known as the cause of the progressive neurodegenerative disease, amyotrophic lateral sclerosis (ALS), was selected as the target material. SOD1 enzyme protects cells from harmful metabolic waste such as reactive oxygens and toxic free radicals. When the mutation occurs in the genetic code of SOD1, SOD1 cannot convert reactive toxic wastes to harmless water. These mutations are linked to familial ALS (FALS) which accounts for 5-10% of ALS.[25,27-31] There are over 100 different genetic changes associated with SOD1. Among them, A4V is well known as the most common mutation in North America.[25,28,29]

Briefly, the self-assembled monolayer (SAM) of 11-mercaptoundecaionic acid (MUA) on the Au film was formed by treatment with a 1 mM MUA ethanolic solution for 18 h. For the formation of the protein microarrays on the MUA treated Au thin film by covalent bonding, the MUA treated surface was first activated by 30 min exposure to a 1:1 mixture of 0.4 M *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS) aqueous solution. The protein solutions were dissolved in separate PBS buffer solutions at a concentration of 0.1 mg/mL. The proteins were WT-holo SOD1, WT-apo SOD1, A4V-holo SOD1 and A4V-apo SOD1. For the fabrication of the multi-component protein microarrays, the different types of SOD1 proteins were then introduced into the microfluidic device for 4 h. After flushing the device with PBS, the microarrays were imaged using the SPR imaging apparatus, as shown in Figure 2.

Figure 4 presents the multi-component protein microarrays fabricated with the microfluidic device. As shown in Figure 4, 4 kinds of proteins are adsorbed onto the pretreated Au substrates. There is no significant difference in the refractive intensity between the 4 channels, however the intensity difference between the channels and the other regions shows that the proteins are patterned on the spatial

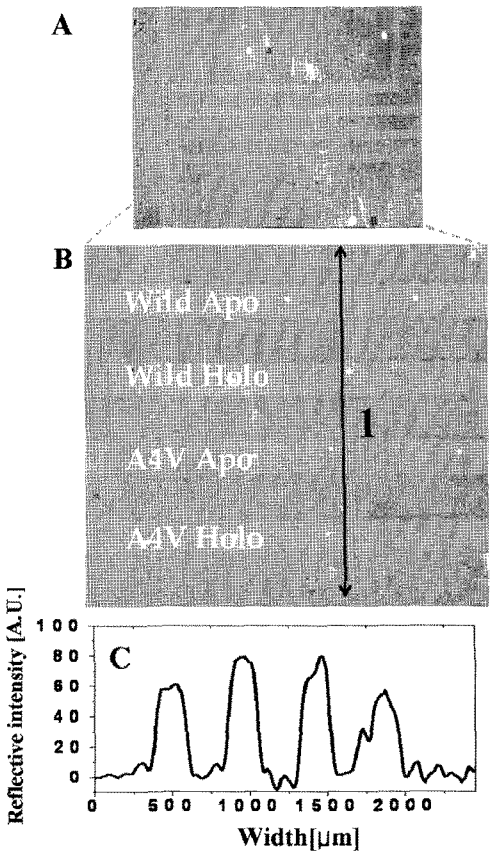

Figure 4. Fabrication of multi-component protein microarrays.

areas which are exposed to the solutions by the flow channels. Therefore, using these microfluidic devices, different proteins were adsorbed on the substrate for the surface analysis. Also, unlike CP, the amount of materials transferred can be controlled by adjusting the exposure time of the solutions. Moreover, after the first patterning of the proteins, a multiplex and parallel protein microarray which has 16 protein-patterned microspots can be constructed by the second patterning of biomolecules perpendicular to the first patterning.

Conclusions

For the patterning of protein antigens on the Au surface for use in SPR imaging experiments, microfluidic devices were designed and fabricated with PDMS by the replica molding method. To avoid using additional flow control equipment and to reduce the amount of solution required, the microfluidic devices were designed to operate using only capillary force. The flow in the microchannels driven only by capillary force was verified by simple microfluidic experiments. Multiple protein-patterned sensor surfaces on the Au substrate were constructed by these microfluidic devices. The multi-patterned protein microarrays constructed by this method can be applied not only to SPR imaging, but also to other biochemical analyses.

Acknowledgments. This research was supported by the Korea Science and Engineering Foundation (KOSEF R01-2006-000-10239-0) and by the ministry of commerce, Industry and Energy of Republic of Korea (2007-E-1D25-P-02-0-000).

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