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Fabrication of Nanopattern by Nanoimprint Lithography for the Application to Protein Chip

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Abstract

Nanoimprint lithography is an efficient and versatile method for fabricating nanosize patterned devices on account of its superior resolution, reliability and process speed compared to conventional lithography. Using this technique, microscale to nanoscale structures, called nanopore structures, were fabricated over a large area on a gold (Au) surface under mild conditions, such as room temperature and low pressure. The fabricated nanopatterns were characterized by field emission scanning electron microscopy. A protein array was fabricated on the pore shaped patterns and characterized by fluorescence microscopy. The proposed patterning process of the protein on Au substrates can be applied as potentially usable elements in the development of biosensors and other bioelectronic devices.

Keywords: Nanoimprint lithography, Nanopore shaped pattern, Protein array, Prostate specific antigen

Introduction

The ability to fabricate patterns on the micro and nanoscale is very important in micro and nanotechnology for many applications, such as magnetic data storage, optoelectronic devices, and biochip[1-4]. One-dimensional (1D), Two-dimensional (2D), and Three-dimensional (3D) nanostructures have attracted considerable attention from the scientific and engineering community over the past decade. Uniform patterns on the micro to nanoscale can be formed using a variety of lithographic techniques, such as ion beam sputtering, focused ion beam, and electron beam lithography[5-7]. However, these techniques have some drawbacks, such as their high cost and large area patterning. There is a need for simple and flexible processes because they are the key step in realizing the next generation of nanostructured biodevices.

Nanoimprint lithography (NIL) is one of the most promising technologies for high-throughput nanoscale patterning. NIL is a low cost, high throughput, and fast method for fabricating patterns with a micro to nano scale resolution[8-10]. There are two types of NIL, thermal nanoimprint lithography and ultraviolet-nanoimprint lithography (UV-NIL). Among these NIL methods, UV-NIL is a more promising technology compared to thermal nanoimprint lithography in view of its cost effectiveness and broad range of emerging applications. In UV-NIL, a transparent stamp with a nano and microscale pattern is pressed onto a thin resin layer or resin droplets at room temperature under normal pressure. The resin is then cured by exposing it to UV light above the stamp. In nine out of ten cases, the stamp can be separated easily from the patterned layer on the substrate during the releasing step because the stamp is coated with a self-assembled monolayer. Normally uniform patterns with a large area are transferred to the underlying substrate by reactive ion etching (RIE), followed by metal deposition and a lift-off method of the remaining resin. This lithography technique has several advantages compared to other conventional lithography techniques, such as low cost, large area recording, defect-free and speed.

A protein chip is defined as a collection of miniaturized spots consisting of proteins arranged on a solid substrate that allows many tests to be performed simultaneously in order to achieve higher throughput screenings[11-13]. The fabrication of two-dimensional patterns of protein on solid surfaces is an essential technology in the development of a protein chip. Protein chips have potential for greater sensitivity in diagnostic tests and may allowed for the discovery of currently undetectable disease markers. Many patterning techniques, including dip-pen nanolithography and nanocontact printing, have been used to create two-dimensional arrays of biomolecules such as DNA,

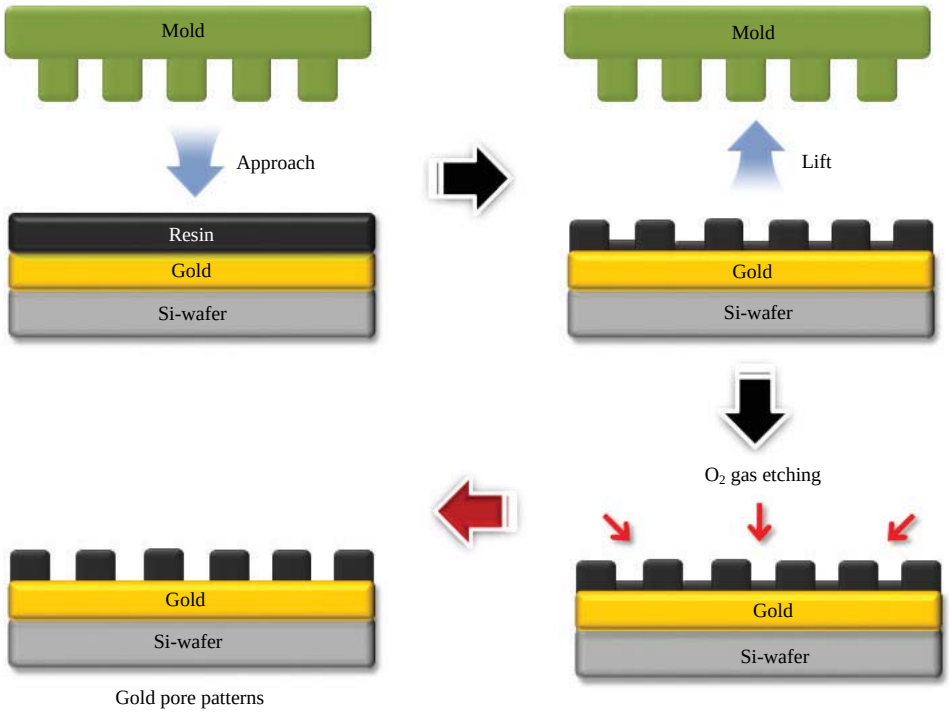
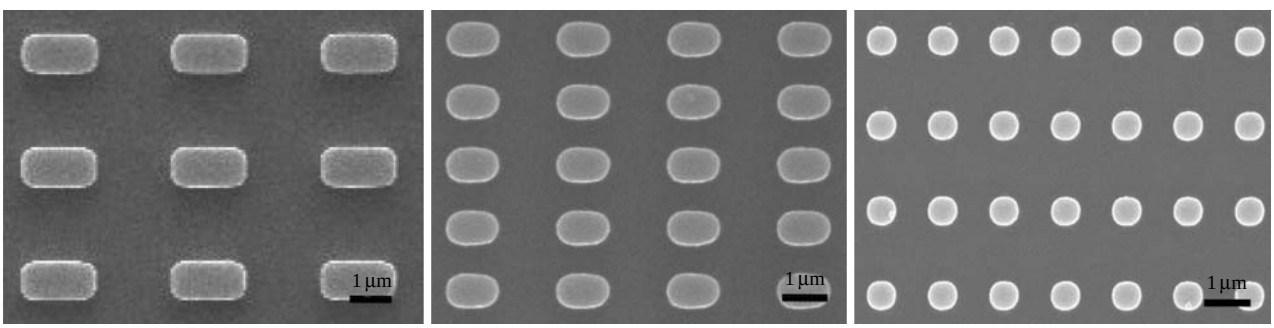


Figure 1. Schematic diagram of fabricating nanopore patterns on Au deposited Si substrate by UV-NIL.

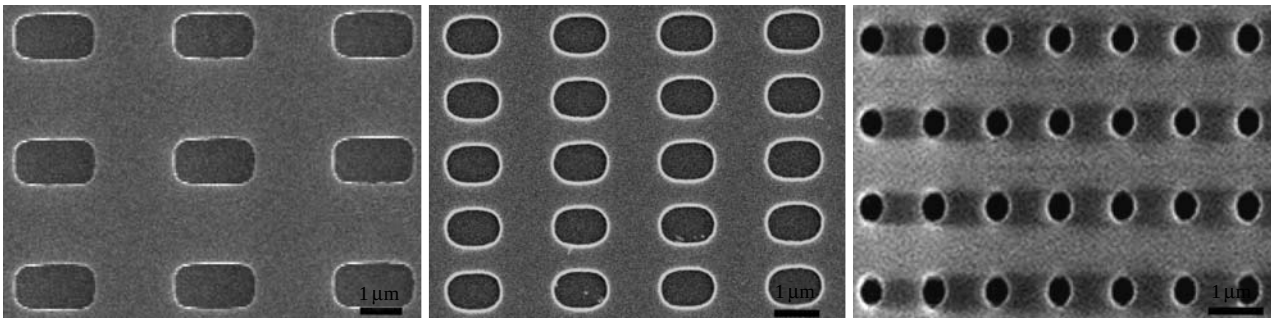


(a) (b) (c)

Figure 2. FE-SEM micrographs of (a) 2 µm dot pattern, (b) 1 µm dot pattern, (c) 500 nm dot pattern on a quartz stamp.

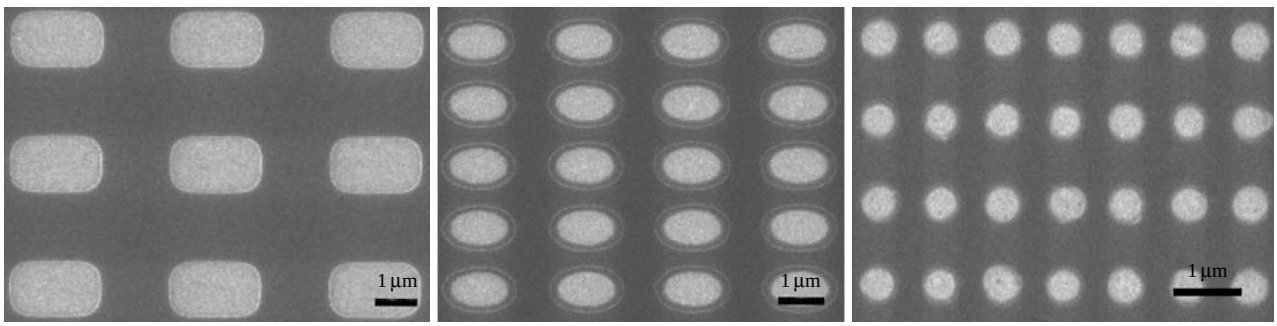
peptides and proteins immobilized on solid surfaces[14,15]. Despite the utility of these approaches, the development of protein micro/nanoarrays over a large area is still difficult due to the need to fabricate regularly spaced protein platforms at low cost and high speed. Therefore, other techniques are still needed to fabricate stable and uniform protein patterns although constant technological advances have been made with the aim of broadening the understanding of a variety of surface-mediated biological recognition events[16,17].

In this study, microscale to nanoscale structures, called nanopore structures, with a large area were fabricated on gold (Au) surfaces under mild conditions, such as room temperature and low pressure, using UV-NIL (Figure 1). Field emission scanning electron microscopy (FE-SEM) was used to characterize the surface structure and three dimensional appearance. The fabricated substrates were used to develop a protein chip, where a monoclonal antibody to prostate specific antigen (PSA) was immobilized on a gold surface with zinc ions and mecaptohexadecanoic acid (16-MHA) as a chemical linker[18]. The fabrication of the protein array was examined by fluorescence microscopy. Overall, the proposed patterning process of the protein can be a useful method for fabricating nanoscale biochips.



(a) (b) (c)

Figure 3. FE-SEM micrographs of the imprinted (a) 2 μm dot pattern, (b) 1 μm dot pattern, (c) 500 nm dot pattern on Au deposited Si substrate.



(a) (b) (c)

Figure 4. FE-SEM micrographs of the fabricated (a) 2 μm pore pattern, (b) 1 μm pore pattern, (c) 500 nm pore pattern on Au deposited Si substrate.

Results and Discussion

Fabrication of Nanopore Shaped Patterns using Ultraviolet-nanoimprint Lithography

Figure 2 shows FE-SEM images of the quartz stamp with a 2 μm, 1 μm and 500 nm dot patterns carved by e-beam lithography, respectively. The size of the entire quartz stamp was 2 cm × 2 cm. The size of an Au deposited Si substrate was the same as that of the quartz stamp in order to prevent breakage of the stamp and substrate during imprinting. Figure 3 shows FE-SEM images of imprinted dot patterns on the Au substrate. The diameters of imprinted dot patterns were 2 μm, 1 μm and 500 nm, respectively. The patterns of the quartz stamp were transferred to the Au substrate successfully and uniformly. The size and shape of the imprinted patterns was an exact negative of the stamp patterns. Any defects and air cracks were not observed.

The vacuum step was maintained in order to eliminate air bubbles between the stamp and substrate. The UV exposure time was 70 seconds. The cured resin pattern can burn if the resin between the quartz stamp and substrate is exposed to UV for too long. The imprinting pressure was 15 bars and the holding time was 30 minutes. Many residual layers can be generated on the pattern if the imprinting pressure is too low. On the other hand, if the imprinting pressure is too high, the stamp and substrate can be broken during imprinting. Therefore, a proper imprinting pressure is very important for high quality pattern transfer as well as economics. The amount of dropping resin used was 0.5 μL. Although a lot of resin was dropped on the substrate by a micropipette, the excess resin could be squeezed out during the holding time.

Imprinted patterns on the Au deposited Si substrate generally contain residual layers. Therefore, an O_2 plasma etching is needed to remove the residual layers. The O_2 plasma etching process is very important for fabricating the pore shaped patterns. Proteins cannot be immobilized on pore patterns if an etching process is not carried out successfully. Figure 4 shows FE-SEM images of the fabricated pore shaped pat-

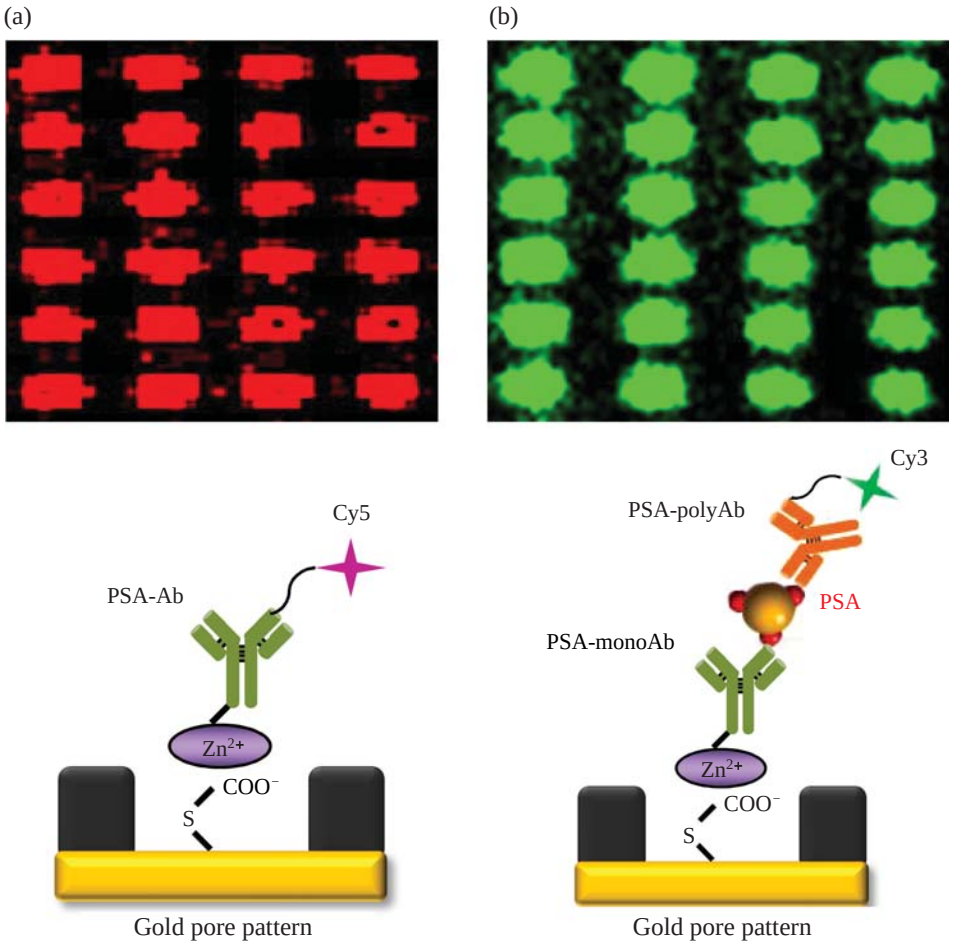


Figure 5. Fluorescence microscope images of the immobilized protein; (a) Pore pattern+16-MHA+Zn ion+Monoclonal antibody to PSA labeled with Cy5. (b) Pore pattern+16-MHA+Zn ion+Monoclonal antibody to PSA +PSA+Polyclonal antibody to PSA labeled with Cy3.

terns on the Au deposited Si substrate. As shown in Figure 4(a), (b) and (c), the size of fabricated pore shaped patterns were 2 μm, 1 μm and 500 nm diameter, respectively. The bright area indicates the Au surface area. The images show that O_2 plasma etching had been carried out successfully. The dark area is the UV-curable resin. In NIL process, etching time is very important because a lengthy O_2 plasma etching time has a deleterious effect on the transfer patterns from the stamp to substrate because plasma ions etch not only the residual layers but also the imprinted resin patterns.

Fabrication of a Protein Chip by using Imprinted Pore Patterns on Au Substrate

A protein can be immobilized on Au pore patterns by using chemical linkers. However, the protein cannot be immobilized on the resin because it consists of a hydrophobic polymer material. The fabricated pore shaped patterns on Au deposited Si substrate was treated with mercaptohexadecanoic acid (16-MHA) and $Zn(NO_3)_2 \cdot 6H_2O$, in turn. 16-MHA was self-assembled on the Au surface and the carboxylic terminal groups of 16-MHA were coordinated to Zn^{II} ions. Proteins were easily immobilized on the functionalized pore shaped patterns by charge interaction between protein and Zn^{II} ions.

Figure 5 shows fluorescence microscope images of the immobilized proteins on the fabricated pore shaped patterns. The red dot in Figure 5(a) indicates the immobilized monoclonal antibody to PSA labeled with Cy5 on the pore shaped pattern. The dark area is the UV-curable resin. Although several washing procedures were applied to the patterned surface, the shape of the protein pattern was maintained on the overall region. This is because the immobilized protein is surrounded by a resin area. The interaction of immobilized monoclonal antibody to PSA and PSA was examined by fluorescence microscopy. Figure 5 (b) shows a fluorescence microscope image of sandwich structure consisting of the immobilized monoclonal antibody to PSA, PSA and polyclonal antibody to PSA labeled with Cy3. From Figure 5(b), it could be confirmed that the fabricated protein arrays were

still kept activated.

Conclusions

Using NIL, microscale to nanoscale structures, called nanopore structures, were fabricated over a large area on an Au surface under mild conditions, such as room temperature and low pressure. The fabricated nanopatterns were characterized by FE-SEM. A protein array was successfully fabricated on the pore shaped patterns with 16-MHA and zinc ions as a chemical linker and characterized by fluorescence microscopy.

In conclusion, nanoimprint lithography is an efficient and versatile method for fabricating protein arrays on account of its superior resolution, reliability and process speed compared to conventional lithography. Moreover, further decreases in pore size will allow the production of smaller protein arrays. Overall, the proposed patterning process of the protein on Au substrates can be applied to nano-scale bio chips and nanoplatform techniques.

Materials and Methods

Materials

The resin for the Ultra-Violet (UV) polymerization of NIP-K28 UV curable resin was purchased from Zenphotonics co. (Korea). This resin is a colorless, clear liquid with a viscosity < 10 cPs consisting of a perfluorinated acrylate monomer mixed with various agents such as a viscosity modifier, an anti-sticking agent and a UV photo-initiator. Tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (TFOCS) was purchased from Gelest, Inc. (USA). Cy5-labeled antibody to prostate specific antigen (PSA) and Cy3-labeled polyclonal antibody to PSA were purchased from Adcam, co. (USA). Mercaptohexadecanoic acid (16-MHA), sulfuric acid, hydrogen peroxide, and all other chemicals were purchased from the Aldrich Chemical Co. (USA) and used without further purification.

Fabrication of Nanopore Shaped Patterns on Au Deposited Si Substrate by Nanoimprint Lithography

Ti (height of 10 nm) was deposited on a Si substrate. After depositing Ti, an Au film (height of 100 nm) was deposited on the Si substrate by sputtering (ALPS-C03, alpha plus, Korea). The Au deposited Si substrate was cut into 2 cm × 2 cm pieces using a dicing saw (DAD522, Disco, Japan). The Au wafers were cleaned by immersion in a piranha solution ($H_2SO_4 : H_2O_2 = 7 : 3$ (v/v)) at 70˚C for 10 minutes. The surface of the quartz stamp was treated with a hydrophobic self-assembled monolayer to help allow the easy release of the quartz stamp. The monolayer of releasing material TFOCS was formed on the surface of quartz stamp by self-assembly method. A contact angle of approximately 135˚ was measured after placing a water droplet on the surface of the quartz stamp.

Figure 1 shows a schematic diagram of the process used to fabricate the nanopore shaped patterns on Au deposited Si substrate. The imprinting process was carried out using NANOSISTM 610 (NND. co, Korea). This equipment has the advantage of a small residual layer after the imprinting process. A vacuum step was carried out for 30 minutes in the imprint chamber to squeeze the excess resin and remove the bubbles. The holding time (stamp and substrate) was more than 30 minutes to disperse the resin uniformly over the entire substrate area. After UV curing of the resin, the quartz stamp was detached from the substrate under normal pressure. The residual resin layers were removed by etching the imprinted samples by O_2 plasma (Miniplasma. Co., Korea). After O_2 plasma etching, the Au surface was exposed to immobilize the protein in the pore shaped pattern.

Immobilization of Protein Molecules on Pore Patterns on Au Substrate

The fabricated pore shaped pattern was immersed in 16-MHA solution (100 mM) for 12 hours at room temperature to form self-assembled monolayers (SAMs). After rinsing the 16-MHA immobilized pore pattern several times with absolute ethanol and deionized water, the carboxylic acid groups of 16-MHA were coordinated to Zn^{II} ions by immersing the 16-MHA immobilized pore pattern into an ethanolic solution of $Zn(NO_3)_2 \cdot 6H_2O$ (5 mM) for 6 hours. The patterns were rinsed with absolute ethanol and deionized water, and dried under a stream of N_2 gas to eliminate any residual impurities. This functionalized pore shaped pattern was immersed in a solution of 100 μg/mL Cy5-labeled antibody to PSA in PBS buffer for 12 hours at room temperature. After 12 hours, the protein immobilized pore pattern was rinsed several times[18].

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