Micropatterning of Si Surface with Protein Molecules by the AFM Anodic Oxidation Method

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Micropatterns of protein molecules were fabricated by a method based on anodic oxidation of Si surface by atomic force microscope (AFM). In this method, an arbitrary pattern of oxide is drawn on the Si surface by applying a voltage between the conducting AFM probe and the Si substrate. The oxide pattern is then used as a template for immobilization of protein molecules. The obtained patterns were investigated with AFM and scanning electron microscope (SEM).

Key Words : Protein, Patterning, Silicon, Atomic Force Microscope (AFM), Anodic Oxidation, Nanofabrication

1 Introduction

Patterning of protein molecules on solid surfaces1 - 19 is an important technology for the development of biosensors, protein chips, cell culture and tissue engineering applications. Various methods of protein patterning have been developed including those based on photolithography or photochemical modification of the surface, in which the pattern is transferred onto the surface by exposure to light through a photo mask. There are also other types of methods such as ink-jet technique,2 electron beam lithography,7, 18, 19 microcontact printing (μCP) or soft lithography,8 dip-pen nanolithography (DPN),20 anodic oxidation by atomic force microscope (AFM),6, 14, 15 nanoimprint lithography,16 and protein patterning in microfluidic channels.10, 17

Among the various types of protein patterning methods, those based on AFM6, 9, 12, 14, 15 are advantageous not only for the extremely high spatial resolution, but also for the possibility of precise alignment of the protein patterns to existing structures on the surface by referring to the AFM image acquired prior to the patterning process. Sugimura and Nakagiri6 proposed a patterning method based on the AFM anodic oxidation.20 - 29 In this method, the Si surface is covered with a self-assembled monolayer (SAM) of organosilane. An oxide pattern is drawn on this surface by applying dc voltage between the substrate and the conducting AFM probe. The surface of the anodic oxide pattern is then modified with another organosilane with different affinity to protein molecules. Finally, the protein molecules are immobilized on the patterned surface. This method has been applied to patterning of latex6 and gold20 nanoparticles as well as protein molecules.

Two types of protein patterning are possible by the AFM anodic oxidation method, i.e., the negative and positive patterning, in which protein molecules are immobilized outside and inside the oxide pattern, respectively. As described later, the protocol of positive patterning is more complicated, but it might be more useful for applications where multiple proteins are immobilized.

In this paper, both negative and positive patterning of protein molecules by the AFM anodic oxidation method is demonstrated and the obtained patterns are investigated with AFM and scanning electron microscope (SEM).

2 Experimental

The Si substrates, 7 × 7 mm² in size, are cut out from n-type Si(111) wafers with resistivity of 0.24 – 12 Ω cm. As a protein molecule to be immobilized on the Si substrate, ferritin (horse spleen, Aldrich),30 a cellular iron-storage protein with a molecular weight of 450 kDa, is used. A ferritin molecule consists of 24 subunits forming a spherical shell that can store iron as hydrated iron oxide in its internal cavity. The inner and outer diameters of the shell are 6 and 12 nm, respectively.

Anodic oxidation is carried out with a commercial AFM (SPI-3700 / SPA-300, Seiko Instruments) with an additional computer for positioning of the AFM probe (Au-coated pyramidal SiN₄, Olympus) and control of anodization. The X- and Y-axes of piezo scanners are driven by the output of a 16-bit D/A converter (PCI-6052E, National Instruments) via an amplifier (M-2629B, MESS-TEK), while the Z-axis is controlled by the AFM con-
controller in the contact mode. A software program developed with LabVIEW (National Instruments) is used to move the AFM probe at a typical velocity of 2.3 μm/s and to apply an anodizing voltage of 8 – 20 V. Anodization is performed in air at a relative humidity of 45 ± 5 % and room temperature of 25 ± 3°C. The obtained patterns were investigated by tapping-mode AFM and field-emission-type high resolution SEM (S-900, Hitachi).

3 Results and Discussion

3.1 Negative patterning

Figure 1 shows the protocol for negative patterning of protein molecules on Si. In this case, protein molecules are immobilized outside the oxide pattern drawn by AFM anodic oxidation. After 10 min of sonication in acetone, the Si surface is treated with the modified SC1 process (NH₄OH : H₂O₂ : H₂O = 1 : 1 : 10 at 80°C for 15 min) to form a thin oxide layer with hydroxyl groups. After drying at 40°C for 15 min, the sample is immersed in 2% γ-aminopropyltriethoxysilane (γ-APTES) in ethanol for 40 min to introduce amino groups. The sample is then rinsed with ethanol and dried with nitrogen. In the next step, the sample is immersed in 25% glutaraldehyde (GA) at 4°C for 2 hours to introduce aldehyde groups. The sample is then rinsed with deionized water and dried with nitrogen. An oxide pattern is drawn on this surface by AFM anodic oxidation. Finally, the sample is treated in 0.5 μM colloidal suspension of ferritin at pH 6.8 for 30 min, followed by sonication in deionized water for 30 sec. The ferritin molecules are covalently bound to the aldehyde groups outside the oxide pattern and those physisorbed molecules on the oxide surface without covalent linking are removed by sonication.

Figures 2(a) and (b) show AFM and SEM images of the obtained patterns, respectively. The ferritin molecules are immobilized only outside the oxide lines. The apparent sizes of molecules in Fig. 2(a) are 20 – 30 nm, which is larger than the actual size of a ferritin molecule, 12 nm. This is an artifact due to the tip radius of the AFM probe. On the other hand, the white dots in the SEM image, Fig. 2(b) show the iron cores inside ferritin molecules, which are smaller than the outer shell. Even though the appearances of Fig. 2(a) and Fig. 2(b) are quite different, the densities of immobilized ferritin molecules are 2.6 × 10⁷ μm⁻² in both cases, which is about 32% of close-packing. The apparent height of molecules in Fig. 2(a) was 10 – 15 nm, which is consistent with the diameter of a ferritin molecule.

3.2 Positive patterning

In comparison to negative patterning, the protocol of positive patterning is more complicated as shown in Fig. 3. In this case, the protein molecules are immobilized only on the surface of the oxide pattern drawn by AFM anodic oxidation, and the rest of the Si surface must be protected from binding or adhesion of protein molecules. For this purpose, the Si surface is covered with a long chain of octadecyltrichlorosilane (OTS), which makes the Si surface inaccessible for protein molecules, whereas the surface of the oxide pattern is modified with γ-APTES for anchoring protein molecules with glutaraldehyde.

At first, the Si surface is treated with the modified SC1 process to form a thin oxide layer. The oxide surface is then treated with 1 mM OTS in hexadecane / carbon tetrachloride / chloroform for 10 min, followed by rinsing in chloroform and ethanol for 20 min each to remove the polymeric residuals. The oxide pattern is drawn on this surface by AFM anodic oxidation, in which the Si surface under the OTS layer is oxidized. Amino and aldehyde groups are successively introduced on the surface of anodic oxide by γ-APTES and glutaraldehyde, respectively. The sample is then dipped in a solution with 20 μM colloidal suspension of ferritin at pH 6.8 for 30 min for immobilization. Finally, the sample is sonicated in detergent (Contaminon N, Wako Pure Chemical Industries) for 10 min to remove protein molecules that are not covalently bound to the surface.

![Fig. 2](image_url)  (a) AFM and (b) SEM images of a negative pattern of ferritin fabricated on a Si surface. The ferritin molecules are immobilized outside the oxide pattern (a part of a circular pattern).
molecules. Even though many white dots are observed outside the anodic oxide in Fig. 4(a), the heights of these dots are well below 10 nm, and therefore, they are not ferritin molecules.

4 Conclusion

Micropatterns of ferritin molecules were fabricated on Si by negative and positive patterning protocols based on anodic oxidation by AFM. The density of ferritin molecules in the case of negative patterning was $2.6 \times 10^4$ µm$^{-2}$, which was about 32% of close-packing. The positive patterning protocol produced a pattern with less uniform immobilization of ferritin molecules. This technique is expected to be applicable to immobilization and patterning of other functional protein molecules on Si, which would be useful for the development of bioelectronic devices on the submicron scale.

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References