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Application of AFM Anodic Oxidation to Patterning of Biomolecules on Si

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The micropatterning of biomolecules on solid surfaces is becoming more and more important for the development of miniaturized and integrated biosensors. In this study, atomic force microscopy (AFM) was applied to the micropatterning of biomolecules on a Si surface. In this method, oxide patterns are drawn on the surface with the AFM probe by the anodic oxidation technique, and then, those oxide patterns are used as templates for the immobilization of biomolecules such as protein and DNA with the help of linker molecules. Protocols for both negative and positive patterning processes were developed. A line pattern of protein molecules as narrow as 50 nm was successfully fabricated. It was also possible to obtain "DNA wires" that connect oxide dots on the surface.

1. Introduction

Immobilization of biomolecules on semiconductor surfaces is an essential technology for the development of semiconductor-based biosensors and other bioelectronic devices. For the development of discrete biosensors such as enzyme FET (EnFET)[1] and immuno-FET, a high spatial resolution is not necessary for the immobilization of biomolecules. However, for the miniaturization and integration of such devices, and also for the development of microfluidic devices and DNA / protein chips, patterning of biomolecules at a high spatial resolution is becoming more and more important.

Various approaches have already been proposed for the patterning of biomolecules on solid surfaces.[2–14] For example, the ink–jet technique, photolithography,[5,12,14] microcontact

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printing (μCP)[11] and soft lithography[8] are expected to be high-throughput methods with spatial resolution on the micrometer scale.

Even higher resolution has been realized by techniques based on atomic force microscopy (AFM). Submicron patterning of protein has been achieved by AFM lithography[10] and dip-pen nanolithography (DPN).[9,13] The AFM-based fabrication techniques have two common advantages, namely, an extremely high spatial resolution on the nanometer scale and the possibility of precise alignment of fabricated structures to existing structures such as transistors and microelectrodes. On the other hand, the throughput of the AFM-based techniques is relatively low at present, since the patterns are not printed at one time but are drawn one by one. Development of the high-speed AFM system and the use of multiprobe arrays may be the answers to this problem.

In this study, the AFM anodic oxidation technique[6,15–26] is applied to the micropatterning of biomolecules such as protein[6,26] and DNA. In this method, oxide patterns are drawn on the Si surface by applying a dc voltage between a conductive AFM probe and the Si substrate. The dimensions of the oxide patterns can be controlled by adjusting the anodizing voltage and the anodizing time (or the velocity of the motion of the probe). The oxide patterns are then used as templates for immobilization of biomolecules with the help of linker molecules. In the following sections, details of the patterning protocols and obtained patterns are presented, and the prospects of further development and application will be discussed.

2. Materials and Methods

The Si substrates used in this experiment are n-type Si(111) with a conductivity in the range of 0.24 to 12 Ωcm. A 7×7 mm^2 sample was treated in the SC1 solution of $NH_4OH : H_2O_2 : H_2O = 1 : 1 : 10$ at 80°C for 15 min. For the covalent binding of biomolecules to the oxide surface, γ-aminopropyltriethoxysilane (γ-APTES) and glutaraldehyde were used. Octadecyltrichlorosilane (OTS) was used to prevent adhesion of biomolecules to the surface.[24,27] Protein and DNA molecules employed in this experiment are ferritin (4.6×10^5 Da, spherical, $\phi = 12$ nm),[28] and λ-DNA (48.5 kbp, L = 16 μm), respectively.

The details of the AFM anodic oxidation technique are described elsewhere.[22,23,25] Figure 1 shows the schematic of the AFM anodic oxidation system. The Si surface is locally oxidized by applying a dc voltage between the gold-coated probe and the substrate, while the distance between the probe and the surface is controlled by the contact-mode operation of AFM. Arbitrary patterns can be drawn by controlling the motion of the probe using a computer program. Figures 2(a) and 2(b) show examples of oxide lines drawn by AFM anodic oxidation of (a) bare and (b) SC1-treated Si surfaces, respectively. The anodizing voltage was 16 V and the AFM probe was moved at the constant velocity of 2.3 μm/s. The hydrophilic nature of the SC1-treated Si surface, which is covered with a thin oxide,[29] results in enhanced oxidation in comparison with oxidation of a bare Si surface.[25] From the viewpoint of spatial resolution, oxidation of the bare Si surface is more advantageous for drawing narrow lines. As for the reproducibility, however, oxidation of the SC1-treated surface was more stable. Oxidation of the bare Si surface was occasionally interrupted, probably due to inhomogeneous distribution of adsorbed water on the surface.

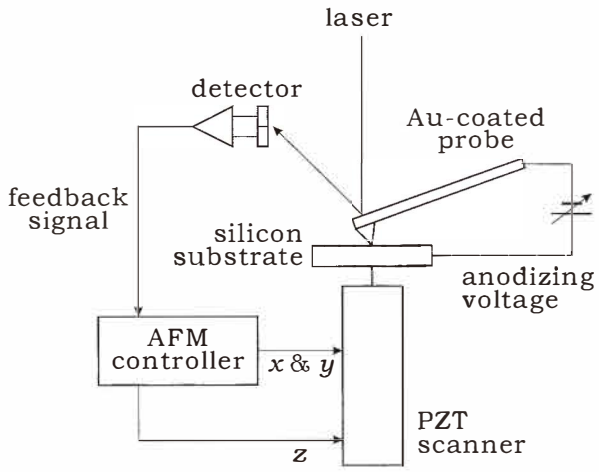

Fig. 1. Configuration of AFM anodic oxidation system.

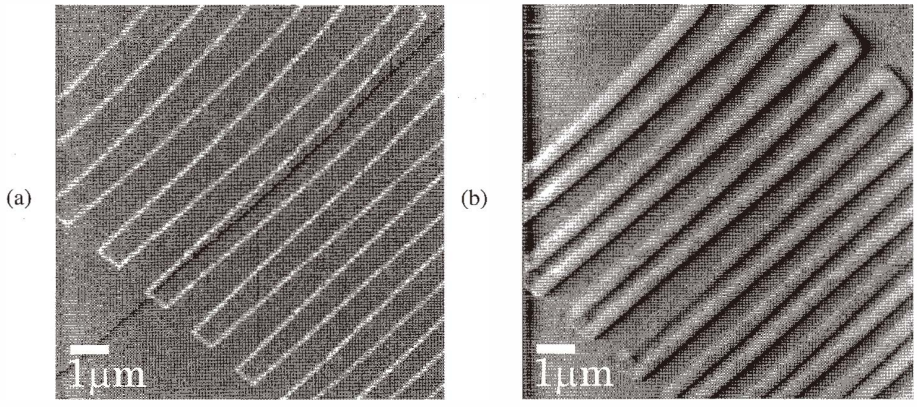

Fig. 2. Oxide lines drawn by AFM anodic oxidation of (a) bare and (b) SC1-treated Si surfaces.

Two kinds of patterning processes are possible, namely, negative and positive patterning processes.[7] In a negative patterning process, biomolecules are immobilized on the surface region that is not oxidized. In a positive patterning process, on the other hand, biomolecules are immobilized only on the oxidized surface. In certain applications, a positive patterning process would be more useful, particularly when various kinds of biomolecules are to be successively immobilized on the same surface.

Figure 3 shows the protocol for the negative patterning of biomolecules. The SC1-treated surface is modified with 2% γ-APTES in ethanol at RT for 40 min to introduce

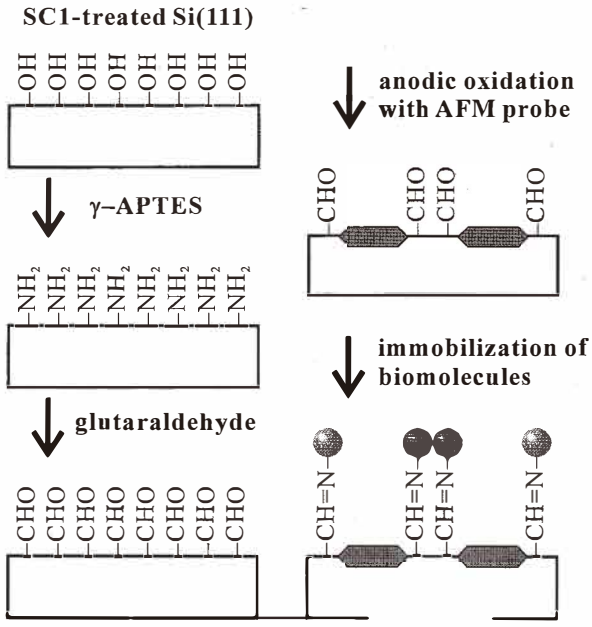

Fig. 3. Protocol for negative patterning of biomolecules on Si.

amino groups on the surface. The surface is further modified with 25% glutaraldehyde at 4°C for 90 min to introduce aldehyde groups. Then, the sample is mounted on the AFM stage and oxide patterns are drawn by anodic oxidation. Biomolecules are covalently bound to the aldehyde groups, which remain only outside the oxide pattern. The immobilization of biomolecules can be carried out by immersing the sample in a solution containing biomolecules. When the volume of the solution is limited, a drop of the solution may be put on the surface. Finally, the surface is rinsed with deionized water to remove the biomolecules from the oxide surface.

Figure 4 shows the protocol for the positive patterning of biomolecules. At first, the Si surface is treated with 1 mM OTS in n-hexadecane : tetrachlorocarbon : chloroform = 10 : 1 : 1.5, followed by rinsing in chloroform and ethanol.[24,27] The OTS layer protects the surface from adhesion of biomolecules. After drawing oxide patterns, amino and aldehyde groups are successively introduced on the oxide surface, on which biomolecules are immobilized.

3. Results and Discussion

3.1 *Negative patterning of protein molecules*

Figure 5 shows examples of AFM images, in which negative patterns of ferritin molecules are formed on the Si surface. In Fig. 5(a), ferritin molecules are immobilized outside the arc-shaped oxide band with a width of approximately 200 nm. The apparent

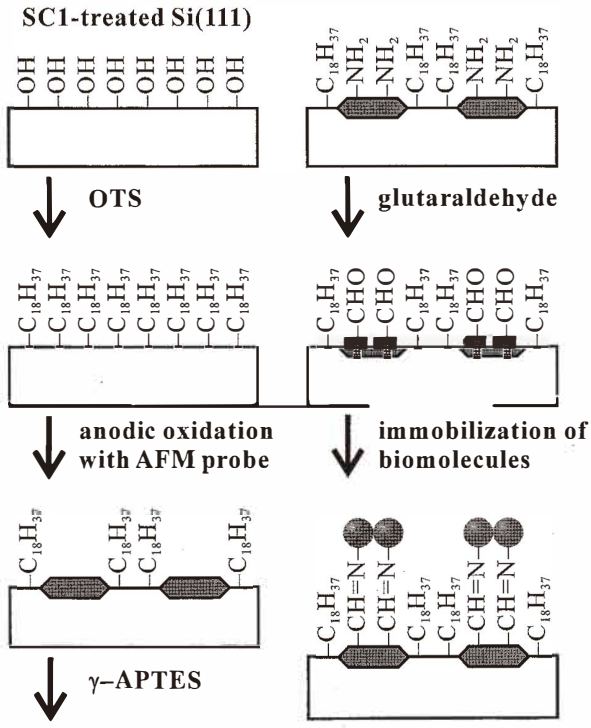

Fig.4. Protocol for positive patterning of biomolecules on Si.

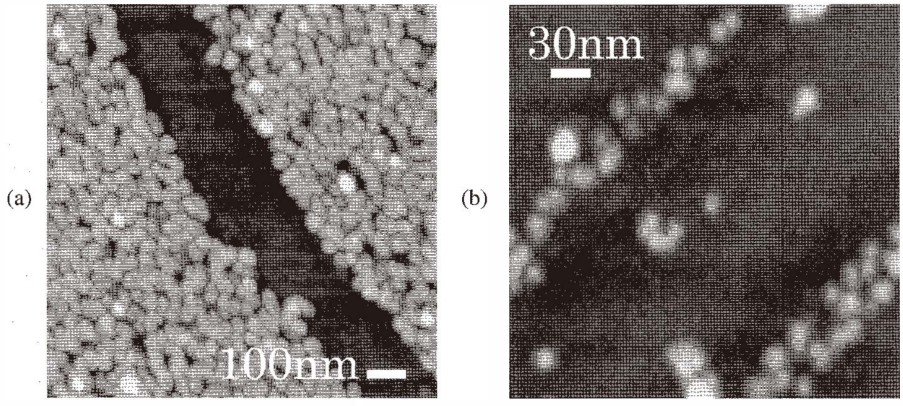

Fig. 5. Negative patterns of protein molecules. (a) Ferritin molecules are immobilized outside a 200-nm-wide line of oxide. (b) Ferritin molecules are confined within a width of approximately 50 nm.

height of the molecule above the oxide surface is 12–18 nm. Because of the probe artifact due to the high aspect ratio of the ferritin molecules, the apparent diameter of the molecule is much larger than the actual value of 12 nm. The ferritin molecules are, therefore, not as densely immobilized on the surface as they are observed to be in the AFM image. Figure 5(b) shows another example of negative patterning, in which the ferritin molecules are confined within lines as narrow as 50 nm or approximately four times the diameter of the molecule.

3.2 *Positive patterning of protein molecules*

As mentioned earlier, positive patterning is expected to be more advantageous for certain applications than negative patterning. In the case of positive patterning, even after drawing of an oxide pattern and subsequent immobilization of biomolecules onto the pattern, another oxide pattern can be additionally drawn as a template for immobilization of different biomolecules. Such a process may be useful in cases where various kinds of biomolecules are to be integrated on the same surface.

Figure 6 shows examples of positive patterns of protein molecules immobilized on (a) lines and (b) dots of oxide drawn by the AFM anodic oxidation technique. The height of the oxide pattern is 6–8 nm. As can be seen in the AFM images, the density of ferritin molecules immobilized on the oxide pattern in Fig. 6 is much lower than the density of ferritin molecules immobilized outside the oxide pattern in Fig. 5, which may be due to the difference in the density of linker molecules bound on the anodic oxide and on the SC1 oxide. Furthermore, in the case of positive patterning, a few ferritin molecules were observed also outside the oxide pattern, which may be due to the imperfection of the OTS passivation.

3.3 *"DNA wire" structures*

The present method is expected to be applicable not only to the immobilization of protein molecules but also to the immobilization of DNA molecules, which is required for the development of DNA chips and sensors. Unlike the protein molecules, whose dimensions are on the nanometer scale, the DNA molecules can have length at the micrometer level. In the following examples, therefore, DNA molecules are immobilized on the surface in such a manner that they connect oxide dots. In addition to sensor applications, such "DNA wire" structures may be useful, for example, for studying the mechanical and electrical properties of DNA molecules. The position-controlled immobilization of DNA molecules on the surface may also be useful for the construction of nanostructures using DNA molecules as building blocks.[30,31]

Figure 7 shows AFM images of λ-phage DNA molecules immobilized on arrays of oxide dots with different densities. The height of the oxide dots is 6 nm. In the case of Fig. 7(a), where the density of oxide dots is low and the gap between neighboring dots is approximately 1 μm, the immobilized DNA molecules were almost straight. For a higher density of oxide dots with the gaps smaller than 200 nm, the DNA molecules became zigzaged, as shown in Fig. 7(b). This result suggests the possibility of arranging DNA molecules along an arbitrary path on the Si surface, by placing oxide dots as "stepping stones" along the path.

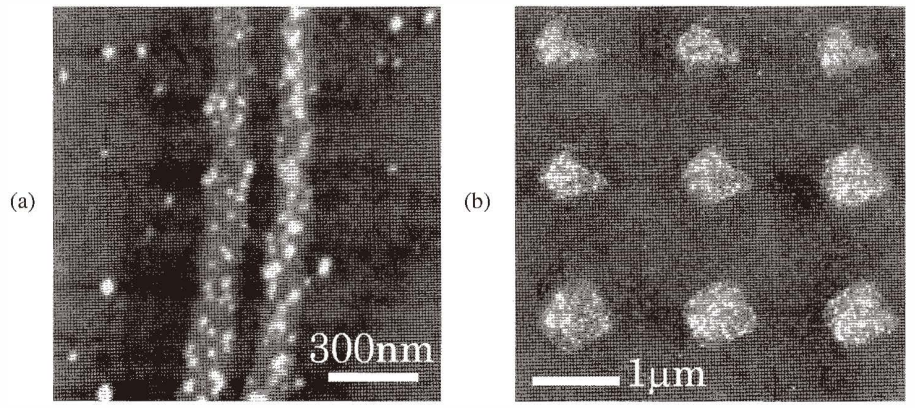

Fig. 6. Positive patterns of protein molecules. (a) Ferritin molecules are immobilized on 150-nm-wide lines of oxide. (b) Ferritin molecules are immobilized on oxide dots of 1 μm diameter.

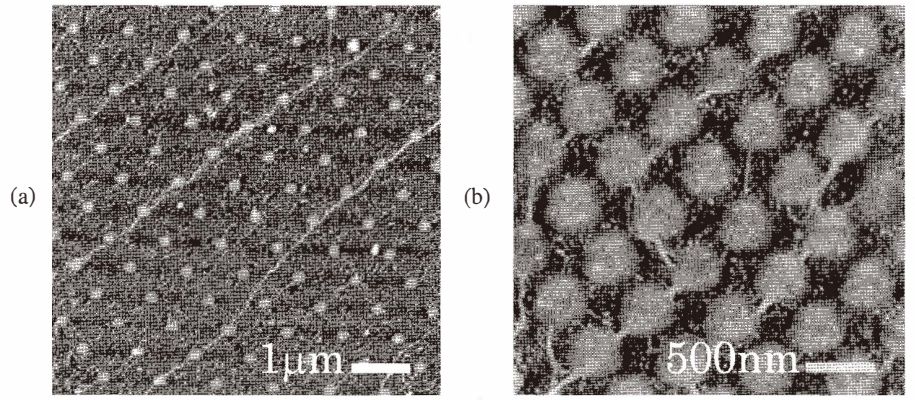

Fig. 7. λ-phage DNA molecules immobilized on arrays of oxide dots with different densities.

4. Conclusion

In this study, protocols for the negative and positive patterning processes of biomolecules on Si were developed on the basis of the AFM anodic oxidation technique. In the case of negative patterning, protein molecules were immobilized outside the oxide pattern. Protein molecules could be confined within lines as narrow as 50 nm. In the case of positive patterning, protein molecules were immobilized on the surface of the anodic oxide, but the density of immobilized molecules was lower than that in the case of negative patterning. It was also possible to obtain "DNA wire" structures, in which DNA molecules connected the oxide dots. Further development of the present method is expected to realize the patterning of biomolecules at even higher resolution, ultimately on the single-molecule scale.

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References

1 S. Caras and J. Janata: Anal. Chem. **52** (1980) 1935.
2 A. S. Blawas and W. M. Reichert: Biomaterials **19** (1998) 595.
3 S. K. Bhatia, J. L. Teixeira, M. Anderson, L. C. Shriver-Lake, J. M. Calvert, J. H. Georger, J. J. Hickman, C. S. Dulcey, P. E. Schoen and F. S. Ligler: Anal. Biochem. **208** (1993) 197.
4 M. Hengsakul and A. E. Cass: Bioconjugate Chemistry **7** (1996) 249.
5 J. F. Mooney, A. J. Hunt, J. R. McIntosh, C. A. Liberko, D. M. Walba and C. T. Rogers: Proc. Natl. Acad. Sci. USA **93** (1996) 12287.
6 H. Sugimura and N. Nakagiri: J. Am. Chem. Soc. **119** (1997) 9226.
7 D. V. Nicolau, T. Taguchi, H. Taniguchi and S. Yoshikawa: Langmuir **15** (1999) 3845.
8 R. S. Kane, S. Takayama, E. Ostuni, D. E. Ingber and G. M. Whitesides: Biomaterials **20** (1999) 2363.
9 R. D. Piner, J. Zhu, F. Xu, S. Hong and C. A. Mirkin: Science **283** (1999) 661.
10 K. Wadu-Mesthrige, S. Xu, N. A. Amro and G.-Y. Liu: Langmuir **15** (1999) 8580.
11 A. Bernard, J. P. Renault, B. Michel, H. R. Bosshard and E. Delamarche: Adv. Mater. **12** (2000) 1067.
12 A. Douvas, P. Argitis, C. D. Diakoumakos, K. Misiakos, D. Dimotikali and S. E. Kakabakos: J. Vac. Sci. Technol. B **19** (2001) 2820.
13 K.-B. Lee, S.-J. Park, C. A. Mirkin, J. C. Smith and M. Mrksich: Science **295** (2002) 1702.
14 H. Sorribas, C. Padeste and L. Tiefenauer: Biomaterials **23** (2002) 893.
15 H. C. Day and D. R. Allee: Appl. Phys. Lett. **62** (1993) 2691.
16 H. Sugimura, T. Uchida, N. Kitamura and H. Masuhara: Jpn. J. Appl. Phys. **32** (1993) L553.
17 E. S. Snow and P. M. Campbell: Appl. Phys. Lett. **64** (1994) 1932.
18 T. Hattori, Y. Ejiri, K. Saito and M. Yasutake: J. Vac. Sci. Technol. A **12** (1994) 2586.
19 E. S. Snow and P. M. Campbell: Science **270** (1995) 1639.
20 K. Matsumoto, S. Takahashi, M. Ishii, M. Hoshi, A. Kurokawa, S. Ichimura and A. Ando: Jpn. J. Appl. Phys. **34** (1995) 1387.
21 J. A. Dagata: Science **270** (1995) 1625.
22 W. C. Moon, T. Yoshinobu and H. Iwasaki: Jpn. J. Appl. Phys. **38** (1999) 483.
23 W. C. Moon, T. Yoshinobu and H. Iwasaki: Jpn. J. Appl. Phys. **38** (1999) 6952.
24 J. Zheng, Z. Zhu, H. Chen and Z. Liu: Langmuir **16** (2000) 4409.
25 W. C. Moon, T. Yoshinobu and H. Iwasaki: Jpn. J. Appl. Phys. **41** (2002) 4754.
26 T. Yoshinobu, J. Suzuki, H. Kurooka, W. C. Moon and H. Iwasaki: Electrochim. Acta **48** (2003) 3131.
27 A. N. Parikh, D. L. Allara, I. B. Azouz and F. Rondelez: J. Phys. Chem. **98** (1994) 7577.
28 I. Yamashita: Thin Solid Films **393** (2001) 12.
29 T. Hattori, K. Takase, H. Yamagishi, R. Sugino, Y. Nara and T. Ito: Jpn. J. Appl. Phys. **28** (1989) L296.
30 Y. Zhang and N. C. Seeman: J. Am. Chem. Soc. **116** (1994) 1661.
31 E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman: Nature **394** (1998) 539.