Nanoscopic Surface Architecture Based on Scanning Probe Electrochemistry and Molecular Self-Assembly

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Abstract: Coplanar nanostructures consisting of two different types of organosilane monolayers have been fabricated using scanning probe microscopes (SPMs) and served as templates for the area-selective immobilization of various materials. The first organosilane monolayer, which had been uniformly prepared on a substrate, was locally degraded through electrochemistry of adsorbed water at the junction of the monolayer and the SPM probe. This probe-scanned region chemisorbed molecules of the second organosilane, resulting in the creation of a self-assembled monolayer (SAM) confined to the SPM-defined pattern. Latex nanoparticles or proteins selectively assembled onto this patterned SAM.

Organic molecules such as proteins are the basis of various functional processes of living cells. These processes are made possible not by organic molecules alone but also require organized molecular assemblies, i.e., so-called supramolecular systems. In order to artificially construct such supramolecular systems and apply those to both electronic and chemical devices[1,2] and to fundamental scientific research,[3] there has been an increasing demand for technology by which desired molecules could be arranged onto predetermined locations. Positioning atoms and molecules by the use of scanning probe microscopy (SPM) is one possibility.[4] Although this method has an ultimate spatial resolution down to the atomic or the molecular scale, it is limited by its very low processing speed.

In this paper, we report a method by which a number of molecules can be arranged at one time onto positions spatially defined in the near-molecular scale. Our approach combines scanning probe lithography (SPL) with the self-assembling of organosilane molecules. A trimethylsilyl ([$(CH_3)_3$Si-], TMS) monolayer on a silicon substrate was locally removed by the SPL process.[5,6] The region where the monolayer had been removed was then selectively modified with a self-assembled second monolayer of organosilane molecules. This self-assembled monolayer (SAM), confined onto the patterns predefined by SPL, served as a template onto which a large number of desired molecules could be immobilized. Such patterned organosilane SAMs, terminated with chemically reactive functional groups, have been used for the fabrication of microstructures composed of a variety of materials, including proteins, fluorescent molecules, metals, and biological cells.[7−11] However, the minimum dimensions of these structures have remained in the micrometer scale due to the limitations of photolithography. This resolution limit can be overcome by lithographically patterning SAMs using charged particle beams, such as electron beams.[12,13] The area-selective deposition of monolayers and minerals has been demonstrated using SAMs patterned by electron and ion beams.[14,15] However, the reported results did not achieve resolution beyond that of photolithography. SPM is also a powerful means of fabricating minute-scale structures on organosilane SAMs.[5,6,16−18] Marrian *et al.* have demonstrated the local deactivation of an amino-terminated organosilane SAM through electron irradiation from an STM tip in vacuum.[16] Müller et al. have converted azide groups of an SAM to amino groups using the chemical reaction catalyzed by a platinum-covered probe of an atomic force microscope (AFM).[18] These two reports are very interesting, demonstrating the fabrication of minute patterns with chemical properties different from those of a surrounding SAM surface. However, their methods are based on particular chemical reactions and, therefore, lack versatility. On the contrary, the approach

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(1) Birge, R. R., Ed. *Molecular Electronics and Bioelectronics;* American Chemical Society: Washington, DC, 1994.

(2) Bard, A. J. *Integrated Chemical Systems;* John Wiley & Sons, Inc.: New York, 1994.

(3) Masuhara, H. In *Microchemistry: Spectroscopy and Chemistry in Small Domains;* Masuhara, H., De Schryver, F. C., Kitamura, N., Tamai, N., Eds.; North-Holland: Amsterdam, The Netherlands, 1994; pp 3−20.

(4) Avouris, P. *Acc. Chem. Res.* **1995**, *28*, 95.

(5) Sugimura, H.; Nakagiri, N. *Langmuir* **1995**, *11*, 3623.

(6) Sugimura, H.; Nakagiri, N. *J. Vac. Sci. Technol.* **1996**, *A14*, 1223.

(7) Dulcey, C. S.; Georger, J. H., Jr.; Krauthamer, V.; Stenger, D. A.; Fare, T. L.; Calvert, J. M. *Science* **1991**, *252*, 551.

(8) Ichinose, N.; Sugimura, H.; Uchida, T.; Shimo, N.; Masuhara, H. *Chem. Lett.* **1993**, 1961.

(9) Britland, S.; Perez-Arnaud, E.; Clark, P.; McGinn, B.; Connolly, P.; Moores, G. *Biotechnol. Prog.* **1992**, *8*, 155.

(10) Kleinfeld, D.; Kahler, K. H.; Hockberger, P. E. *J. Neurosci.* **1988**, *8*, 4098.

(11) Stenger, D. A.; Georger, J. H.; Dulcey, C. S.; Hickman, J. J.; Rudolph, A. S.; Nielsen, T. B.; McCort, S. M.; Calvert, J. M. *J. Am. Chem. Soc.* **1992**, *114*, 8435.

(12) Lercel, M. J.; Redinbo, G. F.; Pardo, F. D.; Rooks, M.; Tiberio, R. C.; Simpson, P.; Craighead, H. G.; Sheen, C. W.; Parikh, A. N.; Allara, D. L. *J. Vac. Sci. Technol.* **1994**, *B12*, 3663.

(13) Lercel, M. J.; Craighead, H. G.; Parikh, A. N.; Seshadri, K.; Allara, D. L. *Appl. Phys. Lett.* **1996**, *68*, 1504.

(14) Mino, N.; Ozaki, S.; Ogawa, K.; Hatada, M. *Thin Solid Films* **1994**, *243*, 374.

(15) Rieke, P. C.; Tarasevich, B. J.; Wood, L. L.; Engelhard, M. H.; Baer, D. R.; Fryxell, G. E.; John, C. M.; Laken, D. A.; Jaehnig, M. C. *Langmuir* **1994**, *10*, 619.

(16) Marrian, C. R. K.; Perkins, F. K.; Brandow, S. L.; Koloski, T. S.; Dobisz, E. A.; Calvert, J. M. *Appl. Phys. Lett.* **1994**, *64*, 390.

(17) Perkins, F. K.; Dobisz, E. A.; Brandow, S. L.; Koloski, T. S.; Calvert, J. M.; Rhee, K. W.; Kosakowski, J. E.; Marrian, C. R. K. *J. Vac. Sci. Technol.* **1994**, *B12*, 3725.

(18) Müller, W. T.; Klein, D. L.; Lee, T.; Clarke, J.; McEuen, P. L.; Schultz, P. G. *Science* **1995**, *268*, 272.

S0002-7863(97)01027-5 CCC: $14.00

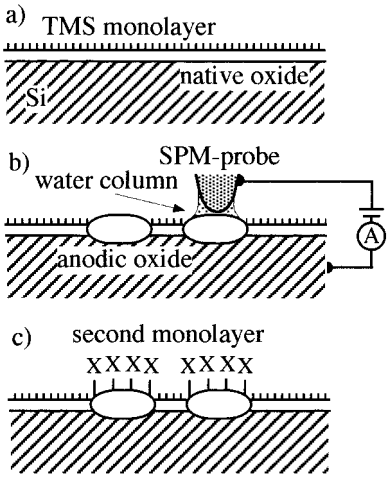

Figure 1. Schematic diagram of scanning probe lithography. (a) Formation of the TMS monolayer. (b) Patterning of the TMS monolayer by SPM through localized electrochemical reactions. (c) Second organosilane monolayer coating, area-selectively deposited onto the region where the first monolayer was removed. The average height of the protruded region is 0.5 nm.

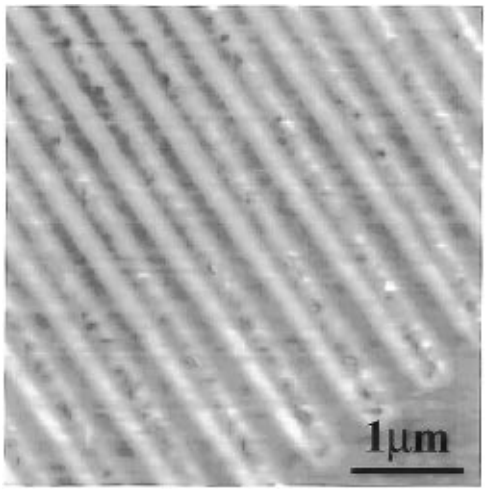

Figure 2. AFM image of the line pattern fabricated on a Si sample covered with a TMS monolayer resist. The lines were generated by STM-based scanning probe anodization with a sample bias (V_s) of +5.0 V, a reference current (i) of 0.2 nA, and a scane rate (v) of 1.2 μm/s in N_2 with a relative humidity (RH) of 20%. The image was acquired after the patterned sample was rinsed in pure water.

presented in this paper provides a more general means by which nanostructures with various chemical functionalities can be fabricated.

Template preparation is schematically illustrated in Figure 1. A TMS monolayer was prepared on the native oxide upon Si substrates (Figure 1a). Hexamethyldisilazane ($[(CH_3)_3Si-NH-Si(CH_3)_3]$, HMDS) was used as the precursor. The HMDS molecules react with hydroxyl groups on the surface of the substrate to form covalent siloxane (Si−O−Si) bonds and liberate NH_3 as a reaction byproduct.[19] The thickness of the monolayer was estimated to be less than 1.0 nm by both AFM and ellipsometry. This value is consistent with the reported thickness of a similar organosilane monolayer having a short alkyl chain.[20] After monolayer deposition, the surface became hydrophobic, with water contact angles of about 100°, while contact angles for the substrates were typically 5° or less after cleaning.

Each Si substrate coated with the TMS monolayer (Si-TMS) was patterned by the probe tip of an SPM, applying a bias voltage to the probe/sample junction (Figure 1b). The TMS monolayer was locally removed from the probe-scanned surface areas. These regions became hydrophilic, while the unscanned regions remained hydrophobic.[5] The probe/sample junction is connected through an adsorbed water column when the microscope is operated in the presence of atmospheric water vapor. Using such adsorbed water, we have achieved the anodization of Ti and Si surfaces.[21−23] In a manner similar to the localized anodization of metals and semiconductors, the degradation mechanism of a TMS monolayer by SPM was also concluded to be electrochemistry in the water column, on the basis of experimental results on the effect of humidity.[24] Figure 2 shows an AFM image of scanned lines on a Si-TMS sample.[25] Owing to volume expansion, which accompanied the anodization of the substrate Si, the probe-scanned regions slightly protruded from the surrounding unscanned areas. This volume expansion appeared to occur after the removal of the TMS monolayer.

As we have previously demonstrated,[5] since the regions where the TMS monolayer is degraded preferentially become wet with water, they are concluded to be hydrophilic. These regions are most likely terminated with OH groups. Thus, they can be expected to become once again reactive to organosilane molecules. It is possible to fix a different type of organosilane molecule onto these scanned regions. The unscanned regions are unreactive due to termination with the TMS monolayer. Consequently, functional groups other than alkyl groups can be attached onto the regions predefined by the SPM probe scans (Figure 1c). In order to demonstrate this hypothesis, an organosilane molecule terminated with an amino group was selected for deposition. The patterned Si-TMS samples were washed in distilled water and ethanol, in that order, and dried by N_2 blow. They were then immersed for 5 min in a fresh solution of 1 vol % of (3-aminopropyl)triethoxysilane ($[H_2N(CH_2)_3Si(OC_2H_5)_3]$, APS), 4 vol % of CH_3COOH, and 4 vol % of H_2O in methanol. Finally, the samples were rinsed twice in methanol and once in distilled water.

The APS molecules were expected to react with the surface of the regions where the TMS monolayer had been removed. Accordingly, an APS monolayer confined to the probe-scan pattern should form. An amino group is known to react with an aldehyde group to form the chemical bonding shown by eq 1:

$$-NH_2 + -CHO \rightarrow -N{=}CH- + H_2O \qquad (1)$$

The amino-covered surface will preferentially adsorb aldehyde-

(19) Hertl, W.; Hair, M. L. *J. Phys. Chem.* **1971**, *75*, 2181.
(20) Wasserman, S. R.; Tao, Y.-T.; Whitesides, G. M. *Langmuir* **1989**, *5*, 1074.
(21) Sugimura, H.; Uchida, T.; Kitamura, N.; Masuhara, H. Jpn. *J. Appl. Phys.* **1993**, *32*, L553.
(22) Sugimara, H.; Kitamura, N.; Masuhara, H. *Jpn. J. Appl. Phys.* **1994**, *33*, L143.
(23) Sugimura, H.; Uchida, T.; Kitamura, N.; Masuhara, H. *J. Phys. Chem.* **1994**, *98*, 4352.
(24) Sugimura, H.; Okiguchi, K.; Nakagiri, N. *Jpn. J. Appl. Phys.* **1996**, *35*, 3749.

(25) We have reported in this paper only our results on template preparation by STM. However, patterning of the TMS monolayer can also be conducted with AFM, as we have previously demonstrated.[26,27] The STM and AFM used were an SPI-3600 (Seiko Instruments) and an Autoprobe CP (Park Scientific Instruments), respectively. Due to the independence of AFM's feedback control from the patterning mechanism, i.e., electric current, AFM lithography has great versatility over a wide range of patterning conditions. Using AFM, we have achieved a maximum patterning speed of 500 μm/s and a lateral resolution of 20 nm. We believe that template preparation will be possible at this patterning speed, which is much higher than the speed of normal atomic manipulation.
(26) Sugimura, H.; Okiguchi, K.; Nakagiri, N.; Miyashita, M. *J. Vac. Sci. Technol.* **1996**, *B14*, 4043.

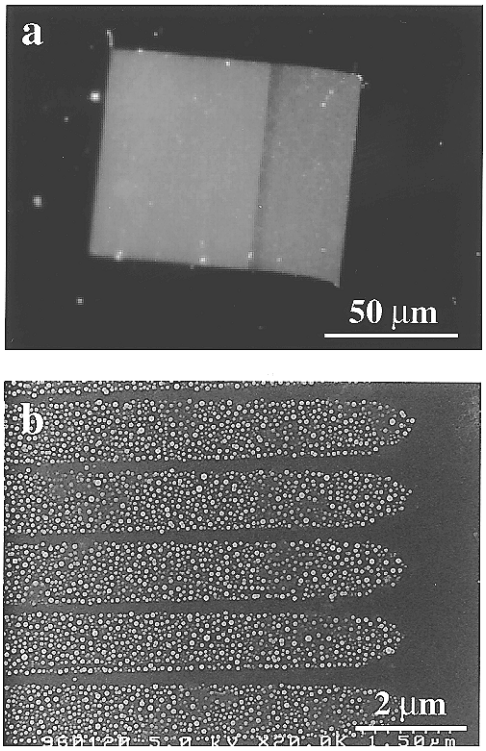

Figure 3. Fluorescence optical (a) and scanning electron (b) images of fluorescent nanoparticles immobilized onto an SPM-generated pattern. Patterning was conducted using the STM at $V_s = +5.0$ V and $v = 0.2$ μm/s in N_2 with 80% RH. After APS treatment, the patterned sample was immersed for 30 min in 50 mL of morpholinoethane sulfonic acid buffer (MES; 50 mM, adjusted to pH 6.5 by adding aqueous NH_4OH solution) with 0.2 mL of an aqueous suspension of aldehyde-modified latex particles (Molecular Probes, L-5401, 2% solids in H_2O, diameter = 29 nm ± 20.1%). These samples were again rinsed in distilled water and then blown dry.

modified materials. In order to confirm the area-selective growth of the APS monolayer, the sample was labeled with aldehyde-modified fluoresent latex nanoparticles. Figure 3a is a fluorescence optical micrograph of the nanoparticles area-selectively condensed onto the probe-scanned regions. A part of the area where the fluorescent nanoparticles were adsorbed was magnified by scanning electron microscopy, as shown in Figure 3b. A submonolayer of the nanoparticles is seen to be spatially arranged onto the probe-scanned lines. Since specific adsorption, as demonstrated by these images, was not observed on a sample untreated with the APS solution, we conclude that this adsorption is based on the chemical reaction between the amino and aldehyde groups and, furthermore, is evidence of the area-selective growth of an APS monolayer on the probe-scanned region.

Such an APS monolayer can be modified not only with nanoparticles but also with a variety of organic molecules, such as proteins, by using a suitable cross-linker between an amino group and a molecule. An example is shown in Figure 4. Protein molecules (horseradish peroxidase, HRP, Sigma) selectively assembled onto an APS-modified template by using glutaraldehyde as a cross-linker between the amino groups of the APS monolayer and those of HRP. Figure 4a shows a topographic image of the sample acquired in the intermittent contact mode, in which an AFM probe is vibrated at a frequency near its resonance and touches the sample surface once each vibration cycle.[27] As seen in Figure 4a, the brighter, probe-scanned region protrudes about 4−5 nm from the surrounding area. Since this protrusion height is larger than the sum of the substrate anodization and the thickness of the APS monolayer,[28] something has probably been adsorbed onto the region. In order to confirm that the protein had, in fact, been adsorbed, we acquired a phase contrast image (Figure 4b) simultaneously with the topographic image in Figure 4a. This image maps phase lag between the phases of the applied ac voltage to the probe vibrator and the observed cantilever vibration. It has been reported that adsorbed organic molecules showed larger phase lag than a rigid substrate due to the differences in softness and/or viscosity between them.[29] As is clearly seen in Figure 4b, the protruding region in Figure 4a shows a larger phase lag compared to the surrounding region, where there were no adsorbates. It is most likely that the protein molecules assembled area-selectively onto the APS monolayer confined to the SPL-defined patterns.

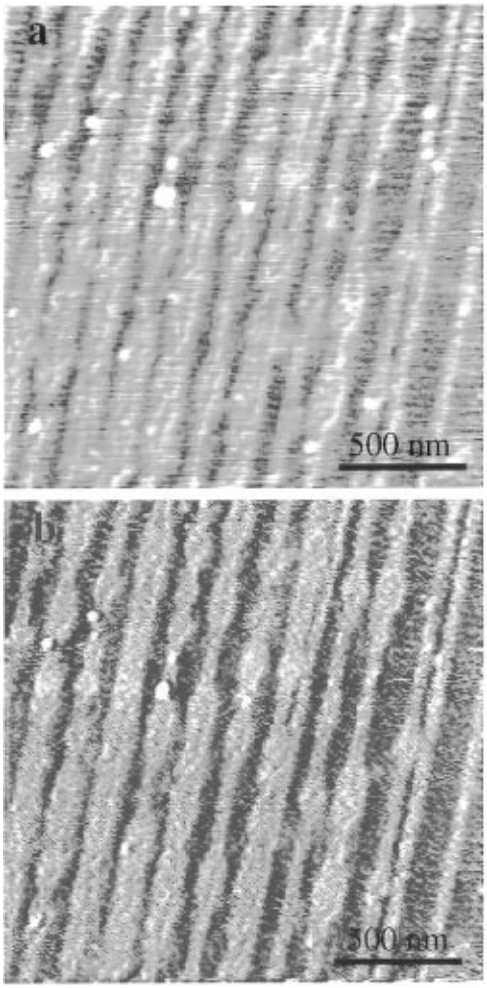

Figure 4. AFM images of an APS-treated sample modified with HRP. The APS-treated samples were immersed for 30 min in 2.5 mL of a 50% glutaraldehyde aqueous solution (Kishida Chemical) diluted with 100 mL of phosphate-buffered saline (Biowhittaker, PBS) and then rinsed with pure water. Next, the samples were soaked in a 1 mg HRP (Sigma) solution in 5 mL of PBS. The samples were then washed in distilled water and blown dry. The height of the protruding regions in image a is in the range of 4−5 nm.

The two-step surface modification presented here, i.e., local removal of an organosilane monolayer through SPL, followed

(27) A microfabricated Si probe was used (Nanosensor, G2 T3-5 L125R; mechanical resonant frequency of about 250 kHz).

(28) The height of the probe-scanned region in a Si-TMS sample scanned at a sample bias of +5 V was estimated to be less than 1 nm (ref 24). The thickness of an APS monolayer is considered to be less than 1 nm since its has an alkyl chain length of only three ethylenes.

(29) Tamayo, J.; García, R. *Langmuir* **1996**, *12*, 4430.

by the selective chemisorption of a second monolayer of organosilane molecules, provides a method for fabricating patterns with areas of various chemical reactivities and, therefore, shows promise in the preparation of templates for constructing molecular assemblies. Using such chemically reactive patterns as templates, nanostructured assemblies of molecules such as fluorescent molecules, redox reagents, catalysts, and biologically active species can be synthesized at a reasonable processing speed. This technique will contribute to the future realization of biomimetic chemical systems and novel molecular devices.

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