Formation of Micro and Nanoscale Patterns of Monolayer Templates for Position Selective Immobilization of Oligonucleotide Using Ultraviolet and Electron Beam Lithography

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Formation of organosilane monolayer templates using ultraviolet and electron-beam (EB) lithography was investigated. The oligonucleotides were covalently immobilized with high selectivity only to the amino-monolayer modified regions locally formed on the template surfaces at micro and nanometer scale. By using EB lithography, patterned immobilization in nanometer scale, as small as 20 nm, was achieved.

Position selective immobilization of biomolecules attracts much interests in recent years for performing biological recognition and fabricating miniaturized array-based assays.[1–3] The immobilization sites of biomolecules precisely formed in micro/nanoscopic scale can be applied for high accurate biomolecule analysis such as genotyping of single-nucleotide polymorphisms, recognition of a single molecule, and so on. In order to form such sites, we attempt to develop fabrication process of patterns of the self-assembled monolayer (SAM) templates by using UV and EB lithography. These lithography processes enable to perform a template formation at wafer-scale with high throughput compared with scanning probe-based processes such as dip-pen nanolithography[4] and nanomanipulation.[5] Moreover, such templates with which the bioactive regions are locally formed are applicable to a base-template for highly integrated arrays such as light-generated DNA array[6] and for single molecule observation.

N-type Si(100) wafers covered with thermally grown SiO_2 (20-nm thick) were used in this study. Octadecyltrimethoxysilane (ODMS), (heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane (FAS), and 3-aminopropyltriethoxysilane (APS), were used as precursors. The wafers were placed into a vial with a cup filled with organosilane (ODMS or FAS) liquid, and then heated for 8 h at 110 °C.[7,8] In the case of the APS, the wafers were immersed in dehydrated toluene solvent containing 1 vol % of APS liquid at 60 °C for 7 min[9] since the formation of the APS monolayer was found to proceed easily in the liquid phase, rather than the gas phase. It has been reported that the complete monolayers using these modification procedures.[7–9] The thickness of each organosilane monolayer was estimated to be 20 Å (ODMS), 13 Å (FAS), and 6 Å (APS), respectively. These values correspond to previous reports.[7–9] Also, we have checked these modified surfaces by using atomic force microscope (AFM) and lateral force microscope (LFM). From the AFM observation, the RMS and Ra values for each modified surface are indicated to be similar to those of the bare silicon oxide surface. Also, it was confirmed that the LFM images for the monolayer surfaces were flat and homogenous, without detecting any local deviation in the friction force even in nanometer scale. Therefore, it is suggested that the modified surfaces are flat and uniformly formed at the monolayer level.

Figure 1 illustrates the process step for fabrication of the monolayer templates. For the microscale patterning, the ODMS or FAS monolayer modified substrate (**1**) was covered with conventional UV-resist and then patterning was carried out with a 350 nm UV light irradiation (**2a**). The patterned substrate was exposed with O_2 plasma at the condition of the input power of 200 W and the O_2 flow rate of 80 sccm for 1 min in order to form the patterns onto the monolayer (**2a**′), using the resist pattern as the mask for the plasma irradiation. For nanoscale patterning, EB lithography process was applied. A scanning electron microscope equipped with a thermally assisted field-emission electron gun (TFESEM) was used to expose the samples. EB was irradiated at designed area of the ODMS or FAS monolayer modified surface (**2b**). The pattering was carried out under the condition at 25 kV and 50 pA. The optimum doses for eliminating the monolayer were 400 $\mu C\,cm^{-2}$ (raster) and 2.5 $fC\,dot^{-1}$ (shot), respectively. The patterned surfaces were observed using LFM. After each patterning process, the APS monolayer was formed on the exposed clean oxide surface of the patterned region (**3**).

Oligonucleotides to be immobilized to the surface were 5′-thiol modifier-T_{20}-3′-TRITC and 5′-amino modifier-A_{20}-3′-Cy5. The TRITC(tetramethylrhodamine isothiocyanate) and Cy5 incorporated into each oligonucleotide act as fluorescent dye. The template surface was covered with the solution of crosslinker sulfosuccinimidyl 6-[3′(2-pyridyldithio)propionamide]hexanoate (sulfo-LC-SPDP) (1 mg in phosphate-buffered solution [PBS]) for 1 h, then the surface was reacted with thiol-modified oligonucleotide (10 μM in PBS) for 1 h. In the case of the bonding of amino-modified oligonucleotides, the surface was immersed in 0.25% aqueous glutaraldehyde (GA) solution for 1 h. After rinsed with deionized water, the surface was reacted with amino-modified oligonucleotide (0.1 μM in PBS) for 1 h.

Figure 2a shows LFM image of the surface of the microscale patterns of the ODMS monolayer after the plasma treatment. The bright and dark regions in the image correspond to the plasma

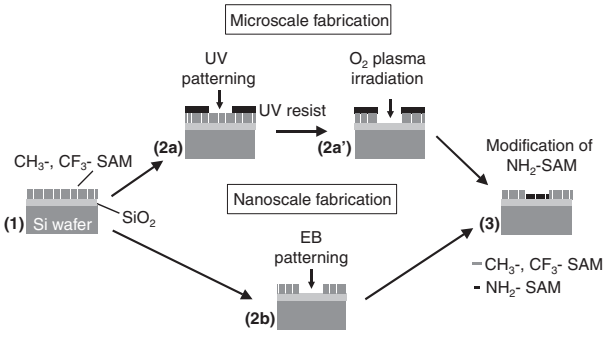

Figure 1. Process steps for fabrication of the monolayer templates by lithography.

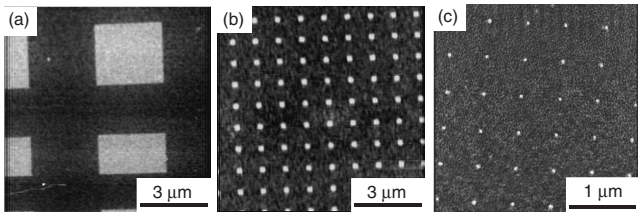

Figure 2. Representative LFM images of the patterned surfaces. (a); The specimen after the O_2 plasma irradiation and the removal of UV-resist in the microscale patterning process. (b); The specimen after the irradiation of EB at the dose of $400\,\mu C\,cm^{-2}$ (raster mode). (c); The specimen after the irradiation of EB at the dose of $2.5\,fC\,dot^{-1}$ (shot mode).

irradiated and masked areas, respectively. The plasma-irradiated areas exhibit stronger lateral force than the masked areas covered with the hydrophobic monolayer. Since the exposed area is SiO_2 with hydrophilic feature, it could chemically interact with a Si-probe surface, which is also hydrophilic because of the formation of surface oxide, resulting in a higher friction force.

Figures 2b and 2c show LFM images of the ODMS surface after the EB irradiation. The monolayer was known to be degradated by exposure of the EB, so that it has the possibility to be applied for high resolution EB resist since the thickness of the monolayer is extremely thin.[10] Bright patterns indicating the exposure of hydrophilic SiO_2 surface are also clearly observed. The feature sizes of the bright regions, which were the irradiated parts, were 200×200 nm squares (for raster mode) and 20 nm diameter dot (for shot mode), respectively.

Then, the aminosilane was deposited on the patterned SiO_2 regions. Similar LFM images are observed from the patterned surface after the deposition of APS. In addition, from the AFM investigation, the morphology of irradiated and unirradiated region was well corresponded to that of the bare surface. Therefore, the aminosilane molecules only deposited onto the exposed region.

Figures 3a and 3b show fluorescent microscope images of the micro-patterned template surface after immobilization of the fluorescent-labeled oligonucleotides. As is seen in these images, bright dot-patterns, which indicate the existence of oligonucleotides, were clearly observed. The diameter and period of the bright regions were 10 and 50 μm, respectively, which correspond to that of the APS patterned regions formed on the surface.

Next, the immobilization of the oligonucleotides was also attempted by using the nanoscale templates. The feature sizes of the bright regions were 200 nm diameters, which were almost equal to that of the patterned APS regions (Figure 3c). In contrast, when the patterned surfaces without modification of aminosilane were used, no bright region was observed from the fluorescent microscope image, indicating that the oligonucleotides were not immobilized to the surface. It was indicated that the oligonucleotides were position selectively immobilized only to the APS modified sites formed on the patterned monolayer surface at micro and nanometer scale, and ODMS modified surfaces act as a layer for preventing the adhesion of oligonucleotides and so on. Such a controlled immobilization was also achieved when the template surface crosslinked with biotin–avidin molecule

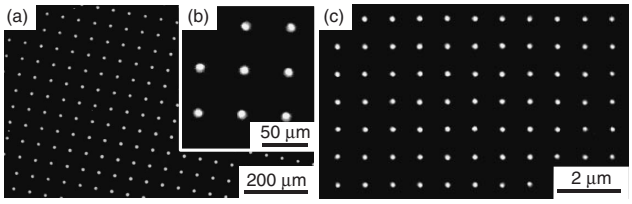

Figure 3. Fluorescent microscope images of the APS / ODMS patterned surface covalently immobilized with (a-b) TRITC-labeled 5′-thiol modified oligonucleotide via sulfo-LC-SPDP, (c) Cy5-labeled 5′-amino modified oligonucleotide via GA.

was used. In addition, similar behaviors were observed in the case of using APS/FAS patterned monolayer templates. Therefore, it was suggested that these patterned monolayer templates were applicable to the surface for immobilization of oligonucleotides with high selectivity. Especially, it is expected that the nanoscale templates are effective as those for the single molecule immobilization and formation of the molecular-assembly structure, since the monolayer pattern can be fabricated with the resolution as high as 10 nm by using the EB lithography.

In summary, we investigated the formation of patterned monolayer templates at micro and nanometer scale by using UV and EB lithography processes. When the patterned monolayer templates were applied to those for immobilization of oligonucleotide, controlled immobilization at micro and nanometer scale was achieved. These templates were expected to be effective as the immobilization of various biomolecules.

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