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Conductive AFM Patterning on Oligo(ethylene glycol)-Terminated Alkyl Monolayers on Silicon Substrates: Proposed Mechanism and Fabrication of Avidin Patterns

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Abstract

Micro- and nanopatterns of biomolecules on inert, ultrathin platforms on nonoxidized silicon are ideal interfaces between silicon-based microelectronics and biological systems. We report here the local oxidation nanolithography with conductive atomic force microscopy (cAFM) on highly protein-resistant, oligo(ethylene glycol) (OEG)-terminated alkyl monolayers on nonoxidized silicon substrates. We propose a mechanism for this process, suggesting that it is possible to oxidize only the top ethylene glycol units to generate carboxylic acid and aldehyde groups on the film surface. We show that avidin molecules can be attached selectively to the oxidized pattern and the density can be varied by altering the bias voltage during cAFM patterning. Biotinylated molecules and nanoparticles are selectively immobilized on the resultant avidin patterns. Since one of the most established methods for immobilization of biomolecules is based on avidin biotin binding and a wide variety of biotinylated biomolecules are available, this approach represents a versatile means for prototyping any nanostructures presenting these biomolecules on silicon substrates.

INTRODUCTION

Micro- and nanopatterning of biomolecules on a variety of substrates has attracted widespread interest relevant to biosensing applications and fundamental studies in molecular biology and cell biology.^{1–7} Using silicon as the substrate allows for integration of microelectronic components with micro- and nanopatterns of biomolecules on the surface for biosensing^{6,8} and for guiding the attachment, growth, and interactions of neurons and axons.^{3,9} For such applications, controlling the silicon-biological interface is crucial. Ideally, this interface should be stable and free of nonspecific binding of proteins under physiological conditions to ensure that the targets only interact with the patterns presenting the ligands. Organosiloxane films presenting oligo- or poly-(ethylene glycol) (OEG or PEG) on the oxide surface of the silicon substrates were used to block nonspecific binding of proteins.¹⁰ However, siloxane films are prone to detachment through cleavage of the Si–O bonds especially in electrolyte solutions.¹¹ We and others recently used surface hydrosilylation¹² to prepare oligo(ethylene glycol) (OEG)-terminated alkyl monolayers that were directly bound on silicon substrate surfaces via Si–C bonds.^{13,14} These monolayers

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were thin (~4 nm), stable, and highly resistant to nonspecific adsorption of proteins for weeks under physiological conditions.¹⁵

Scanning probe lithography (SPL)¹⁶ techniques including nanografting and nanoshaving,^{5,17} dip pen nanolithography,⁴ and scanning probe oxidation (SPO)^{2,7,18–22} are arguably the most flexible means with the highest resolution for prototyping of any predefined nanopatterns on a variety of substrates under ambient conditions. Although SPL is a serial process and not practical for patterning over a large area, recent developments have begun to address this shortcoming, e.g., by using large conductive templates for local probe oxidation.^{23–25} Among the scanning probe nanolithography (SPN) methods, nanoshaving has been demonstrated as a unique tool for patterning on alkyl-silicon substrate surfaces.²⁶ This method allows for controlling the depth of the patterned regions by varying the applied load. The subsequent chemical functionalization of the patterned regions has yet to be demonstrated. Alternatively, scanning probe oxidation with conductive AFM (cAFM)^{18,19} is applicable to our system. During the cAFM patterning in ambient conditions, a bias voltage is applied between the conductive substrate and the AFM tip. The resultant intensive electric field across the tip–substrate junction leads to electrochemical oxidation of the film surface. Recently, Sagiv and co-workers demonstrated that the local oxidation could be controlled to convert only the methyl groups on organosiloxane monolayers on SiO₂/Si substrate surfaces to carboxylic acid groups.^{18,25} This technique has been used for patterning on a variety of organic and polymer thin films on conductive substrates^{7,21} followed by derivatization to generate patterns of nanoparticles,²⁷ alkane siloxanes,¹⁸ and dendrimers.²⁸ However, few studies have reported on using cAFM to generate nanopatterns for immobilization of biomolecules.²⁹

We recently demonstrated that cAFM could generate arrays of oxidized nanometric “holes” on the OEG monolayers which served as templates for site-specific attachment of proteins.³⁰ Later, we improved the feature size from ~90 nm to sub-10 nm, and demonstrated that degradation of the OEG layer and oxidation of the silicon interface could be minimized.³¹ However, it was unclear about the chemical nature of the patterned area, whether biomolecules could be attached on it, and whether this controlled local oxidation could be performed over a mesoscopic area.

In this article, we report the cAFM lithography from micro- to nanoscale on OEG-alkyl-silicon platforms. The resultant patterns were selectively derivatized with polyamidoamine (PAMAM) dendrimers, gold nanoparticles, and protein molecules (avidin). We also demonstrated that the density of avidin on the patterns could be varied by altering the bias voltage. The avidin nano-structures were capable of binding biotinylated molecules and nanoparticles, and thus could serve as a nanotemplate to present a wide variety of biotinylated biomolecules^{32,33} on inert, silicon-based platforms. Finally, we propose a general mechanism for the scanning probe oxidation on organic thin films by cAFM.

EXPERIMENTAL METHODS

OEG Monolayers

Thin films of OEG were prepared by photo-induced hydrosilylation of α -hepta(ethylene glycol) methyl ω -undecenyl ether (EG₇) on H–Si(111) surfaces (resistivity of 5–25 Ω cm) as described previously.¹⁴ Briefly, a Si(111) wafer was cleaned with NH₄OH/H₂O₂/H₂O (v/v 1:1:4) at 80 °C for 20 min, thoroughly washed with Milli-Q water (18 M Ω deionized water generated by a Milli-Q Water Purification System from Millipore), etched in 10% buffer-HF for 10 min and then in 40% NH₄F (Riedel de Haen) for 10 min under N₂ purge, and dried immediately with a flow of N₂. The H–Si (111) substrate was then placed inside a clean, dry quartz cell, and tilted with the polished silicon surface facing a droplet (~1 mg) of α -

hepta(ethylene glycol) methyl ω -undecenyl ether (**EG7**) that was placed on the inner wall of the quartz cell. After the cell was degassed at ~ 0.1 mTorr for 10 min, the wafer was allowed to fall down onto the droplets, sandwiching a thin and homogeneous layer of **EG7** between the substrate and the quartz wall. The substrate was then illuminated with a handheld 254 nm UV lamp (model UVLS-28, UVP) for 30 and 120 min, followed by washing sequentially with petroleum ether, ethanol, and CH_2Cl_2 , and finally drying with a stream of N_2 .

Scanning Probe Oxidation

Lithography and imaging were performed with a Multimode Nanoscope IIIa AFM (Digital Instruments) in contact mode under ambient conditions at a temperature range of 20–25 °C and with a relative humidity of 37–49%. The setup was described previously.³⁰ Briefly, the OEG-functionalized silicon (111) wafer was mounted on a steel disk via a double-sided carbon conductive tape in contact with a thin Pt wire (25 μm in diameter). Another Pt wire was connected to an AFM cantilever through the copper spring in the cantilever holder. The wafer on the steel disk was then mounted on the AFM scanner and insulated from each other with a parafilm. The two Pt wires were connected via two miniclamps to a BNC cable from the voltage source. The BNC cable was fixed in proximity to the scanner. During cAFM, a positive bias voltage was applied to the Si wafer while the Si cantilever was grounded. Two voltage sources were used: a digital pulse generator (Stanford Research System, Model DG535, combined with a voltage amplifier) and a Kethley voltage source. For patterning at a nanoscale, the pulse generator was used to control the pattern. To ensure that the voltage pulses are applied only when the raster scanning tip is moving in the forward direction but not in both forward and backward directions resulting in double line patterns, the square voltage pulses were programmed and triggered by the triangle input voltage driving the scanner. To generate larger patterned areas (stripes), the width of the stripes was controlled by a manual on/off switch of the voltage source. Silicon cantilevers with a force constant similar to 0.3 N/m (MikroMasch) were used for both lithography and imaging. The scanning loading force was set to about 1 nN during image and 3 nN during lithography. Two tip velocities were tested: 9 and 85 $\mu\text{m/s}$. Both topography and AFM images were simultaneously acquired at a scanning angle of 90°.

Direct Binding of Avidin on the Oxidized Patterns

The patterned films were incubated in a 0.02% avidin solution in phosphate buffered saline (PBS) buffer (pH 7.4, 0.01 M phosphate and 0.14 M NaCl, Sigma) for 5 min. The sample was washed with Milli-Q water and dried with a stream of N_2 .

Binding of Avidin on the Oxidized Patterns in the Presence of EDC/NHS

The patterned films were incubated in a solution containing 0.1% EDC, 0.04% NHS, and 0.01% avidin in PBS (1.0 mL) at room temperature for 1 h. This was subsequently washed with Milli-Q water and imaged with tapping mode in PBS buffer using a NSC36/No Al cantilever tip. To demonstrate the attachment of biotinylated reagents to the avidin pattern, the pattern was treated with a solution of biotinylated quantum dots (1 nM from Quantum Dot Co.) at room temperature for 30 min. The sample was then washed with Milli-Q water. AFM imaging of the modified patterns was performed in tapping mode in Milli-Q water using a NSC36/No Al cantilever tip.

Fluorescent Imaging

Fluorescence images of the freshly prepared samples were obtained with an Olympus BX 41 upright fluorescence microscope with a 40 $\times$ objective and a 520 nm filter for FITC dyes.

RESULTS AND DISCUSSION

Chemical Derivatization of the Oxidized Area

We hypothesized that the cAFM process electrochemically oxidized the OEG surface to generate carboxylic acid and aldehyde as will be discussed later. The main difficulty of directly probing the products generated by cAFM is that the patterned area is too small to be characterized by conventional surface analytical techniques such as surface IR and XPS. To circumvent this difficulty, we used chemical reactions specific to a functional group to introduce handles for attachment of nanoparticles. The presence or absence of the nanoparticles on the patterned area can be easily determined by AFM. It provides an indirect probe of these functional groups. Thus, to probe the presence of COOH groups on the surface, we treated the patterned surface with NH₂-terminated PAMAM dendrimers in the presence of the activating agents *N*-hydroxysuccinimide (NHS) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in sodium acetate/acetic acid buffer (pH 5.5). After incubation of the sample in an aqueous solution of gold nanoparticles with a nominal diameter of 10 nm provided by the manufacturer (Nanopartz Inc.), AFM images revealed that the heights of the lines increased by about 10–20 nm (Figure 1c vs a). In a control experiment, Au nanoparticles did not bind to the patterned area in the absence of the dendrimer. Thus, the PAMAM dendrimers and then the gold nanoparticles were indeed bound to the pattern that was generated with 6–10 V biased voltage applied to the substrate. The lines generated with 5 V bias were visible in the friction image (Figure 1b), but few Au nanoparticles were found on the patterned area after treatment with PAMAM followed by Au nanoparticles. This result indicates that only a low density of CHO and COOH groups were generated by the local oxidation with a low bias voltage (<5 V), to which the dendrimers and then the Au nanoparticles were weakly bound and easily removed by the AFM tip. The topography image in Figure 1a shows the protrusion of the patterns, indicating that the undesirable oxidation of silicon interface occurred even with a low bias voltage of 5 V. Note that in this case the tip velocity was low, corresponding to a long duration of the electrochemical oxidation under the tip. Specifically, at the tip velocity of 9 $\mu\text{m/s}$, the duration of the tip in a spot of 10 nm (about the tip size) is ~ 1.1 ms. The results described below and our previous report³¹ indicate that a short oxidation time (hundreds of nanoseconds) could minimize the damage of the film and the oxidation of the interface.

The proposed mechanism that will be presented later suggests that aldehydes and carboxylic acids are generated during the cAFM patterning on the OEG surfaces. Amines can also react with the aldehyde groups during the reaction with the carboxylic acids. Although the resultant individual C=N—bonds are prone to hydrolysis at a low pH, the PAMAM dendrimers bound via many C=N—bonds are stable in neutral aqueous solution.

Fabrication of Avidin Nanopatterns

We found that a relatively high tip velocity allows for oxidation selectively on the surface of the OEG monolayer without apparent degradation of the OEG layer or formation of silicon oxide protrusions as suggested by the AFM topographic images. For example, stripes ca. 2 μm wide were patterned on the OEG film with a high scan rate of 85 $\mu\text{m/s}$ and bias voltages of 13–15 V. The AFM image in Figure 2a shows that the topography of the film was nearly unchanged upon cAFM patterning indicating no substantial oxidation of the silicon interface. The presence of polar groups on the patterned area is evidenced by the high friction contrast of the patterned area (Figure 2b). Treatment of the patterned surface with an avidin solution led to selective binding of a submonolayer of avidin with a height of 3–6 nm to the patterned area (Figure 2c and d). The binding occurred in the absence of activating agents for carboxylic acids, indicating that the binding is through electrostatic interactions

and/or formation of Schiff base with the aldehyde groups on the patterned surface. Remarkably, we demonstrated that the avidin density on the patterns could be varied by altering the bias voltages. As shown by Figure 2, the friction contrast (Figure 2b) of the three stripes generated with bias voltages of 15, 14, and 13 V decreased in this order, so were the apparent densities of avidin bounded on the same patterns (Figure 2c,d).

To confirm the selective attachment of avidin to the patterns and that the attached proteins are functional, a series of experiments were performed on the $10 \times 10 \mu\text{m}^2$ squares generated by local oxidation followed by attachment of fluorescent avidin (FITC-avidin). The results are summarized in Figure 3. To enhance the binding of avidin to the pattern, we used EDC as the activating agent to promote formation of amide bonds between avidin containing a large number of amino groups on the periphery and the patterned surface containing carboxylic acids and aldehydes. FITC-avidin molecules were selectively bound to the cAFM patterned area as shown by the fluorescent image in Figure 3a.

To confirm that the bound avidin is functional, that is, capable of binding biotinylated molecules, we first treated the sample with free avidin (no fluorescent label, Figure 3b) followed by biotinylated BSA. The presence of biotin groups on the resultant pattern was supported by the fluorescent image in Figure 3b obtained after treatment of the pattern of biotinylated BSA with FITC-avidin. As a control, upon treatment of the avidin pattern with native BSA without biotin modification, only a small amount of FITC-avidin nonspecifically bound to the pattern as shown by the fluorescent image in Figure 3c. Lastly, FITC-labeled biotin selectively bound to the avidin pattern as shown by Figure 3d.

Finally, we demonstrated the high resolution patterning of avidin nanostructures with sub-30 nm feature size (Figure 4) with a tip velocity of $85 \mu\text{m/s}$ on the OEG-alkyl films. As shown by the AFM topography image in Figure 4a and the line profile in Figure 4d, the apparent depth of the oxidized lines generated with 10 V bias voltage was $\sim 0.4 \text{ nm}$, which corresponds to the length of one methoxyl ethylene glycol unit. Note that the actual depth should be smaller due to the artifact generated by the higher friction at the oxidized spots.^{34,35} Substantial oxidation of the silicon interface leading to protrusion of silicon oxide was not observed in the AFM image. A protrusion was generated on the nanopattern after treatment with avidin in the presence of EDC, as shown by Figure 4b and g. The height of the protrusion was measured to be $\sim 1\text{--}1.5 \text{ nm}$ above the OEG film surface (Figure 4e). Considering the $\sim 0.4 \text{ nm}$ depth of the patterned area, the AFM measured height of the avidin immobilized on the patterns was still much lower than the expected height ($\sim 4.3 \text{ nm}$) from the crystal structure of avidin. The reason remained unclear. Nonetheless, the resultant avidin nanostructures could serve as a nanotemplate for selective attachment of biomolecules or nanoparticles with a biotin tag.

Thus, we incubated the avidin nanopattern in a 1 nM solution of Qdot biotin conjugates (Quantum Dot Co.). The AFM topography images (Figure 4c and h) showed the binding of the nanoparticles to the avidin lines, increasing the height by $\sim 2 \text{ nm}$ (Figure 4f). The AFM images also show a high selectivity for binding of avidin to the cAFM-generated patterns on OEG and the biotinylated nanoparticles to the avidin patterns. Few particles on the nonpatterned area were present. Some might be due to the very low level of nonspecific adsorption, and some were brought by the AFM tip swiping from the pattern.

Proposed Mechanism of Scanning Probe Oxidation on OEG Film Surfaces

Although many reports on local probe oxidation of organic thin films have been published,^{7,18,19,21,27,28} discussion on the mechanism of the process even on simple alkyl monolayers has been limited.^{18,22,35–37} Here, we propose a mechanism for the process in general and the possible products generated on OEG-alkyl monolayers on silicon.

The system during cAFM in air on an OEG-alkyl monolayer is illustrated in Figure 5. It consists of (1) a silicon tip with a ~1–2 nm thick oxide layer, (2) a monolayer of OEG-alkyl chains (calculated length for the OEG chain: ~25 Å; for the alkyl chain: ~15 Å; in agreement with the measured ellipsometric thickness of the film: 32–38 Å) bound to the Si(111) surface through Si–C bonds, (3) a water meniscus rapidly formed between the tip and the hydrophilic OEG surface especially when the bias voltage is turned on,³⁸ and (4) O₂ diffusing into the water meniscus. The lithography process was performed with the positive voltage applied to the silicon substrate (as the anode) and the silicon tip being grounded (as the cathode).

Scanning probe oxidation of silicon substrates without an organic film has been extensively studied.³⁹ During this process, oxidation occurs at the silicon interface (anode) via combination of holes with hydroxyl anions (HO[−]) to form protruding silicon oxides. For silicon substrates covered with an organic thin film, the anodic oxidation at the silicon interface is hampered by diffusion of hydroxyl anion across the film, to an extent depending on the composition, thickness, and density of the film, the bias voltage, and the duration.

In general, the local probe oxidation of organic thin films on silicon was proposed to involve hydroxyl radicals generated by electrochemical reaction within the water meniscus, albeit without further discussion on the details.^{18,22,35,37} Here, we propose that, in addition to hydroxyl radicals, the local probe oxidation may also be initiated by hydrogen radicals generated in the system. There are five routes to generate hydroxyl radicals and hydrogen radicals (eqs (1)–(5), Figure 5). Among them, four routes take place at the AFM tip (cathode, eqs (1)–(4)). Equation (1) depicts the sequence of steps and the corresponding standard reduction potentials (E^0) for reducing O₂ to hydroxyl anions through OH radicals as a transient intermediate.⁴⁰ Equations (2) and (3) represent the generation of hydrogen radicals by injection of an electron to the silanol or water molecules on the silicon tip. In the above processes, the electrons need to tunnel from the AFM tip through the 1–2 nm thick oxide layer. While eq (1) is a favorable process, probably very few OH radicals are available to react with the organic film before being rapidly reduced to HO[−] ($E^0 = +1.77$ V). On the other hand, hydrogen radicals can undergo hydrogen abstraction on the organic film to form H₂ and carbon radicals. This process is pseudo-first order to hydrogen radicals generated by eqs (2) and (3) with $E^0 < 0$, and thus can compete with the combination of the hydrogen radicals to form H₂, which is a second-order process. Although the hydrogen abstraction reaction by hydrogen radicals has been described on organic thin films under ultrahigh vacuum,⁴¹ to our knowledge the process during local probe oxidation has not been reported. The resultant carbon radicals react rapidly with O₂ to generate peroxy radicals that are degraded to produce carboxylic acid, aldehyde, and alcohol groups on the film surface. The specific case for oxidation of OEG surfaces will be discussed later.

It is worth noting that the processes of eqs (1)–(3) is self-limited due to the accumulation of hydroxyl anions that inhibit the electron tunneling from the tip. On the other hand, we note that hydroxyl radicals can also be generated by direct water discharge at a high effective potential (more than 3.0–3.5 V, eq (4)),⁴² and by anodic oxidation of hydroxyl anion with $E^0 = +1.77$ V (eq 5).⁴³ In the case for silicon substrates covered with a densely packed alkyl monolayer (without OEG top layer), the diffusion of hydroxyl anions to the anode is hampered. However, if the monolayer of the alkyl chains is sufficiently thin (chain length no more than 12–16 carbon atoms), the hydroxyl anion on the film surface may still be oxidized by one electron tunneling through the alkyl layer to the anode at a bias voltage above E^0 .⁴⁴ Since the resultant hydroxy radicals are on the monolayer surface, only the film surface is oxidized as observed by Sagiv and coworkers.¹⁸

In our system, however, the hydroxyl anions on top of the OEG-alkyl layer (Figure 5) are unlikely to be directly oxidized via one electron tunneling through the ~ 40 Å thick organic layer to the anode. Rather, the electric field facilitates the diffusion of the anion into the OEG layer toward the anode. The closer the hydroxyl anion gets to the anode, the more likely it will be oxidized to a hydroxy radical through the electron tunneling process, which can take place before it gets to the 10-carbon-thick alkyl layer, as illustrated in Figure 5. The resultant highly reactive hydroxy radicals will then rapidly abstract a hydride from the adjacent OEG chains **1**, generating the carbon radicals **2** inside the film. Unlike alkyl radicals in which the unpaired electron occupies a nonbonding orbital, the unpaired electron orbital in the $R^1-O-\dot{C}H-R^2$ radicals has a higher energy due to the two-orbital three-electron interaction with the lone electron pair orbital of the O-atom.⁴⁵ Therefore, these α -oxy radicals are easily oxidized by single electron transfer,⁴⁵ leading to the carbocations **3** that are quenched by the hydroxyl anion to form the hemiacetals **4**. Further oxidation of **4** in a similar way may generate esters. If the cAFM patterning process stops at this stage, treatment of the pattern in aqueous solution should lead to hydrolysis of the hemiacetals and esters, generating a hole presenting aldehydes, alcohols, and carboxylic acids in the film A. This proposed mechanism explains our previous observation that the patterns generated with a high voltage were “etched” upon incubation in aqueous solutions.^{30,31} With slow scanning on a low-density film, the oxidation will continue eventually to form the unwanted silicon oxide protrusion as illustrated in the film A (Figure 5), which were found in Figure 1 and many reports by others.^{7,18,19,21,27,28,35,36,46}

The above oxidation process occurring inside the film requires the diffusion of hydroxyl anion toward the anode. This process may be blocked when a densely packed film is used, especially at a short duration of bias voltage. Under such conditions, it is possible to control the oxidation on the film surface (Figure 5). We propose that this process is initiated by hydrogen radicals (atomic hydrogen) and/or hydroxy radicals generated at the AFM tip as the cathode through eqs (1)–(4) (Figure 5).

Reactive oxy radicals are involved in many lithographic methods for patterning of OEG/PEG films.⁴⁷ The autoxidation of PEG initiated by $HO\cdot$ was shown to generate formate esters, alcohols, and small amounts of esters.⁴⁸ The general autoxidation process is initiated by hydrogen abstraction from PEG with $HO\cdot$. This initiation step generates a carbon radical that can undergo chain scission and oxygen addition leading to oxidation products. Since hydrogen abstraction with $H\cdot$ and $HO\cdot$ radicals is diffusion-controlled, it should only take place at the surface of the OEG monolayer, as illustrated by **5** in Figure 5, leading to carbon radicals **6**. The radicals could react with oxygen to form the peroxy radicals **7** or undergo chain scissoring. Studies on PEG radicals have established that reaction with oxygen dominates chain scissoring for the PEG radicals.⁴⁸ The resultant peroxy radicals on the film can rapidly receive an electron from the Si tip to generate peroxy anions that are protonated by water to form the hydroperoxides **8** that can undergo thermal decomposition. Our previous model study suggested that the thermal degradation of the hydroperoxides **8** does not involve radicals, and generates mostly alcohols, ether, and esters.¹⁵ The “naked” hydroxyl anions in our system can rapidly hydrolyze the ester groups to carboxylic acids as shown in B. The hydroperoxides **8** can also be electrolyzed to the oxy radicals **9** that rapidly undergo β -cleavage to generate the carbon radicals **10**, formaldehyde, and formate. The carbon radicals **10** are reduced by electrons from the anode to the OEG derivatives **5**. The same process from **5** to **10** is repeated until the electric field is turned off. Note that each cycle results in removal of one ethylene glycol unit. Once the electrical potential is turned off, the unstable oxy radicals **9** rapidly undergo β -cleavage to generate the carbon radicals **10**. The reactive carbon radicals in **6** and **10** are quenched by O_2 to generate the relatively stable peroxy radicals that slowly propagate through the autoxidation process, involving hydride abstraction at the adjacent OEG chain followed by combining with O_2 to regenerate

the peroxy radicals. The peroxy radicals can be terminated by reaction with alcohols accumulated on the surface to form an aldehyde in **B** and an OOH radical.^{48,49} Our previous model study of the autoxidation on OEG film has suggested that the autoxidation process generates mostly alcohols and aldehydes on the surface.¹⁵ The proposed mechanism above suggests that the oxidation is confined within the water meniscus between the tip and the film surface. Hence, the pattern resolution would be determined by the size of the water meniscus, which increases with the applied voltage,³⁸ duration, and the relative humidity.

CONCLUSION

In summary, we showed that local oxidation nanolithography with conductive AFM could be performed on the OEG-alkyl-silicon platform without substantial degradation of the OEG layer and oxidation of the silicon interface. The resultant nanotemplates could be selectively derivatized with nanoparticles and protein molecules (avidin). The densities of avidin molecules on the patterns could be varied by altering the bias voltage. The avidin nanostructures fabricated were able to bind biotinylated molecules and nanoparticles. Our proposed mechanism for the cAFM patterning on OEG-terminated alkyl monolayers on silicon suggests that the process generates mostly alcohols together with carboxylic acids, aldehydes and peroxides on the OEG film, and that it is possible to limit the oxidative fragmentation to the outmost ethylene glycol units.

A wide variety of biotinylated biomolecules are commercially available or can be prepared using well-established protocols for site-specific biotinylation.³² Our method for fabricating any nano- and microscale patterns of avidin provide a versatile means of prototyping of structures presenting these biomolecules on an inert background on silicon substrates. Although only cAFM patterning is described here, we expect that micropatterning using conductive stamps²⁴ can also be performed for high-throughput fabrication on the OEG platforms.

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Figure 1.

Contact mode AFM height (a, $3 \times 3 \mu\text{m}^2$, 30 nm contrast) and friction (b, 1.0 V contrast) images of the patterns (lines) generated by cAFM using the same tip on an OEG-alkyl film with an applied bias voltage of 4, 5, 6, 7, 8, 9, and 10 V (corresponding to the lines from left to right) and a tip velocity of $9 \mu\text{m/s}$. The sample was then derivatized with PAMAM dendrimers, followed by treatment with gold nanoparticles, and the topography of the same area imaged with contact mode AFM (c, $3 \times 3 \mu\text{m}^2$, 30 nm contrast). (d) and (e) are line profiles designated by the white lines in (a) and (c), respectively.

Figure 2.

(a) Topographic and (b) the corresponding friction contact mode AFM images of the lithographic patterns on OEG films generated with a bias voltage of 15, 14, and 13 V (left to right) and an identical tip velocity of $85 \mu\text{m/s}$; (c) topographic contact mode AFM images of the same regions after treatment with an avidin solution; (d) line profiles of image (a) and (c) designated by the white lines; scanning size, $12 \times 12 \mu\text{m}^2$; z range, 10 nm, 0.2 V.

Figure 3.

Fluorescence images of four $10 \times 10 \mu\text{m}^2$ squares on OEG films (ellipsometric thickness $\sim 36 \text{ \AA}$) generated by cAFM followed by treatment with EDC and fluorescent FITC-avidin (a); with EDC and nonfluorescent avidin, then biotinylated BSA, followed by FITC-avidin that binds the biotinylated BSA (b); with EDC and avidin, then BSA (without biotin label), followed by FITC-avidin with little binding due to the lack of biotin on the pattern (c); and with EDC and avidin, followed by fluorescently labeled biotin (d).

Figure 4.

Topographic AFM images obtained in contact mode of the lithographic patterns generated on OEG films with a tip velocity of $85 \mu\text{m/s}$ under bias voltage of 10 V at a relative humidity of 37% (a), and the topographic images of the same pattern obtained in tapping mode in water upon treatment with avidin in the presence of EDC (b and g), followed by binding with biotinylated Qdot (c, h) with line profiles (d, e, and f) designated by the white lines in (a), (b) and (c), respectively. Scanning size: $6 \times 6 \mu\text{m}^2$ for (a–c) and $2.5 \times 2.5 \mu\text{m}^2$ for (g) and (h); z range: 10 nm for (a–c) and 5 nm for (g) and (h).

Figure 5.

Illustration of the cAFM system and two pathways (a and b) for the oxidation of the OEG-alkyl monolayers. Pathway a: oxidation near the silicon interface (anode), initiated by anodic oxidation of the HO^- (that arrives near the alkyl layer) via hole tunneling from the interface to form $\text{HO}^\bullet$, followed by hydride abstraction and formation of hemiacetals and esters, finally upon hydrolysis generating a hole presenting alcohols, aldehydes, and carboxylic acids in A. Pathway b: oxidation on the film surface initiated by formation of $\text{HO}^\bullet$ and $\text{H}^\bullet$ at the AFM tip (cathode) through eqs (1)–(4), followed by a series of reactions forming hydroperoxides and peroxy radicals that decompose to form alcohols, aldehydes, and carboxylic acids in B without substantial degradation of the OEG film. A short duration of bias voltage and high packing density of the film can block the Pathway a and favor the Pathway b.