Deposition of DNA Rafts on Cationic SAMs on Silicon [100]

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Received June 2, 2006. In Final Form: August 14, 2006

We demonstrate a guided self-assembly approach to the fabrication of DNA nanostructures on silicon substrates. DNA oligonucleotides self-assemble into "rafts" 8 × 37 × 2 nm in size. The rafts bind to cationic SAMs on silicon wafers. Electron-beam lithography of a thin poly(methyl methacrylate) (PMMA) resist layer was used to define trenches, and (3-aminopropyl)triethoxysilane (APTES), a cationic SAM precursor, was deposited from aqueous solution onto the exposed silicon dioxide at the trench bottoms. The remaining PMMA can be cleanly stripped off with dichloromethane, leaving APTES layers 0.7−1.2 nm in thickness and 110 nm in width. DNA rafts bind selectively to the resulting APTES stripes. The coverage of DNA rafts on adjacent areas of silicon dioxide is 20 times lower than on the APTES stripes. The topographic features of the rafts, measured by AFM, are identical to those of rafts deposited on wide-area SAMs. Binding to the APTES stripes appears to be very strong as indicated by "jamming" of the rafts at a saturation coverage of 42% and the stability to repeated AFM scanning in air.

Introduction

DNA has great potential as a material for the construction of nanostructures. Duplex DNA is relatively rigid, with a persistence length of over 50 nm, and the base-pairing interaction between complementary DNA strands is strong and selective. Duplex DNA has been used as a "glue" to hold nanoparticles together[1−4] or to direct them to micrometer-scale features.[5,6] However, the potential of DNA as a structural template is much greater than its use as a glue. Branching motifs, such as hairpin and crossover sequences, can be incorporated to create two- and three-dimensional branching patterns. DNA has been used to create a variety of self-assembling two-dimensional periodic arrays,[7] three-dimensional structures,[8,9] and even mechanical devices.[10−12] Winfree and Seeman demonstrated the self-assembly of two-dimensional lattices consisting of tens of thousands of double-crossover tiles, which self-assemble in solution and are sturdy enough to be deposited onto mica substrates for AFM imaging.[13] The success of early efforts to "decorate" these periodic lattices with DNA hairpins, metal nanoparticles, and biomolecules[14] points to a possible role for DNA arrays as self-assembling templates for nanocircuitry. Such a role would be greatly facilitated if the DNA arrays could be deposited in a controlled manner on substrates compatible with standard silicon CMOS technology. The DNA arrays could then act as a bridge between the nanometer-scale world of molecules and nanoparticles and semiconductor technologies.

The stable and specific attachment of DNA to solid substrates is a prerequisite for "DNA chip" technologies, and several methods have been developed to bind single- and double-stranded DNA in desired spatial locations. A simple method is to functionalize the DNA with a thiol-derivatized nucleotide[15,16] and place a microdot of the solution onto a desired location on a gold substrate. Other covalent bond-forming reactions, such as amide formation, can also be employed to attach a chemically functionalized DNA strand to a functionalized self-assembled monolayer on gold, silicon oxide, or H-terminated silicon.[17,18] Lithographic methods have been used in conjunction with covalent

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10.1021/la0615948 CCC: $33.50
Published on Web 11/15/2006

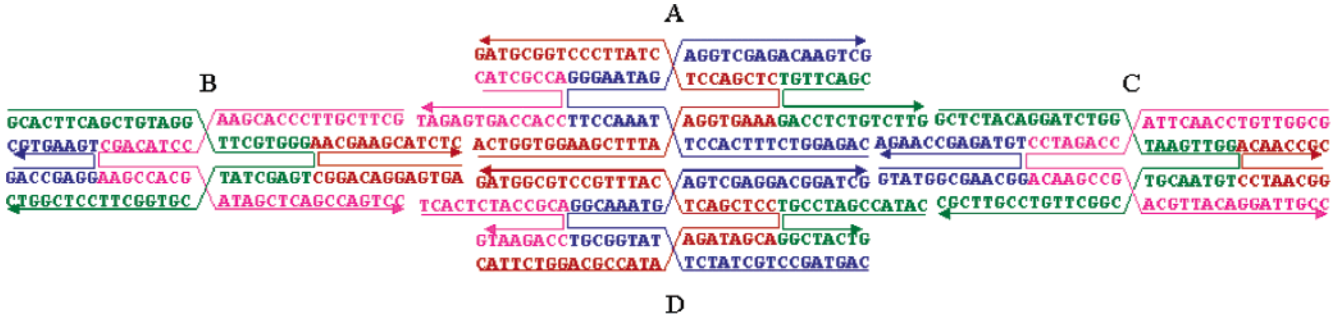

Figure 1. Schematic structure and DNA sequences for A, B, C, and D tiles of the four-tile raft. The four strands in each tile are represented by the red, pink, blue, and green lines; arrowheads indicate the 3′ end of each strand.

attachment to pattern the deposition of single-stranded and duplex DNA on various surfaces,[19−21] and dip-pen nanolithography[22,23] and nanografting[24,25] have been used to "write" lines and dots of thiol-functionalized DNA onto substrates with resolution down to about 50 nm and write times of 1−100 μm/s. Covalent attachment of DNA to substrates gives very strong binding so the DNA will not wash away in subsequent steps. However, it requires chemical modification of the DNA, binding is kinetically irreversible, and only one nucleotide is covalently attached, so the orientation of the DNA strand on the surface is not well controlled. These features may hinder tile−tile alignment and are undesirable for attaching DNA tiles to surfaces.

Since the water meniscus in contact-mode AFM imaging can exert more than 1.0 nN of force on adsorbed molecules,[26] DNA must be strongly adhered to the surface at multiple points for reproducible imaging. DNA bases can adsorb directly to gold, but the interaction is not very strong and also requires that the bases be free to interact with the surface, which is not possible with duplex DNA.[27,28] Electrostatic interactions yield very strong multipoint adhesion between duplex DNA and cationic surfaces. Freshly cleaved mica adsorbs enough Mg^{2+} to adhere DNA to the surface for contact-mode AFM imaging under liquid and in air.[29] Anionic surfaces such as mica or silicon oxide can also be treated with poly(lysine) to form a very thin (<5 Å) polycation film to which DNA binds.[30] Silicon oxide films can also be functionalized with a self-assembled monolayer of (3-aminopropyl)triethoxysilane (APTES), which at neutral pH is cationic and binds DNA.[31]

Although the characterization of two-dimensional DNA lattices relies extensively on AFM imaging of lattice fragments on mica,[32] there have been no reports of patterning of DNA tiles or lattices by control of the underlying surface chemistry. Here, we report that electron-beam lithography (EBL) can control the deposition on silicon surfaces of DNA "rafts" which self-assemble from 4 DNA tiles.

Results

Design of DNA Nanostructures. DNA oligonucleotides were programmed to self-assemble into four-tile rafts that are ∼37 nm long, 8 nm wide, and 2 nm high (the height of duplex DNA is ∼2 nm).[33] The schematic structure of the four-tile raft and the individual tile sequences are shown in Figure 1. The physical structure of the rafts was verified by biochemical assays and non-contact-mode AFM imaging on mica.

Attachment of DNA Rafts to Silicon Surfaces. The native silicon oxide film on silicon is negatively charged in neutral or basic aqueous solutions[34] and repels the sugar−phosphate backbone of DNA. As expected, the four-tile rafts failed to adhere to native oxide films on Si(100) (Figure 2b). The native oxides were treated with an aqueous APTES solution (Figure 2c) to deposit a cationic anchoring layer. The triethoxysilane functionality slowly hydrolyzes in the presence of water to form large aggregates unsuitable for thin-film deposition. However, when dry APTES is freshly mixed with water and the solution is applied immediately to the silicon oxide surfaces for 5 min, a 0.7−1.2 nm film of APTES can be reproducibly formed. The film is not a perfect monolayer, but the observed film thickness is close to the theoretical thickness of ∼0.7 nm for an APTES monolayer,[35] and the RMS roughness over 1 μm^2 is only 0.15 nm. Using aqueous APTES solutions that are not freshly prepared produces unacceptably thick and rough multilayers of APTES.

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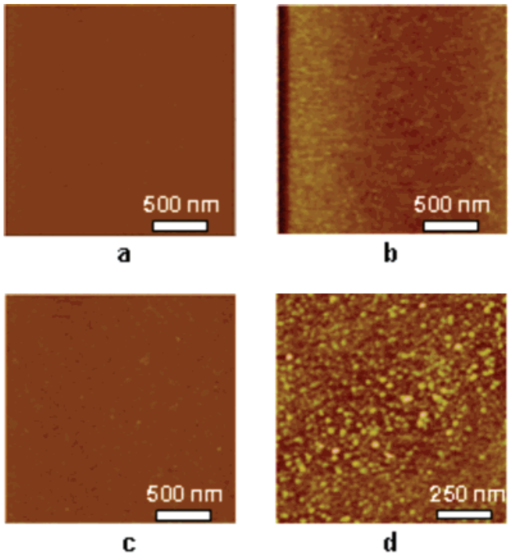

Figure 2. Attachment of DNA rafts to chemically treated silicon surfaces: tapping-mode AFM images in air: (a) bare Si(100) substrate with a thin layer of oxide (0.06 nm RMS); (b) bare substrate after exposure to DNA rafts; (c) substrate treated with aqueous APTES (0.15 nm RMS); (d) APTES treated substrate after exposure to DNA rafts for 4 h.

The use of aqueous solution was driven by compatibility with the electron-beam resist poly(methyl methacrylate) (PMMA). APTES films are normally deposited from various dry organic solvents, from the vapor phase, or from acidic solution (1% HOAc). However, the PMMA resist is soluble or swellable in most organic solvents, so deposition from organic solvents is not practical. Exposure of the developed PMMA to the low pH (3.5) of the 1% HOAc solution made the PMMA trenches swell and collapse, and exposure to APTES vapor (from a drop of pure APTES heated to 80° C) did not yield discernible lines of APTES after lift-off.

At pH 8.0, the APTES amines are expected to be partially protonated,[36] which should give the silicon surface a positive charge. DNA rafts (Figure 2d) adhere to the APTES monolayer with coverage of about 120 rafts/μm^2 (about 4%) after 4 h of exposure to a solution of the preformed rafts in buffer solution. The height of the rafts measured by tapping-mode AFM was 2.0 $\pm$ 0.2 nm on the APTES surfaces. The lateral dimensions of the rafts were measured as $54 \pm 1 \times 32 \pm 1$ nm; the measured lateral dimensions do not match the theoretical ones (37×8 nm) due to tip convolution. In addition to rafts, smaller particles can be seen on the surface, presumably individual tiles and partial rafts.

Electron-Beam Lithography and DNA Patterning. Electron-beam lithography is an important patterning technology for the semiconductor industry because of its high resolution and ability to fabricate arbitrary patterns. In one of the basic pattern-transfer methods, lift-off, an electron beam is scanned over a resist material such as PMMA to define the patterned area, the exposed material is removed with a development solvent, metal is deposited, and the remaining resist is stripped off with another solvent. This method is usually used to form metal wires which are tens to hundreds of nanometers thick and 20−200 nm wide. We have shown that a lift-off-like process can be applied to molecules that bind to silicon dioxide.[37] Molecular lift-off requires a very flat substrate (e.g., native oxide rather than thermal oxide) and care during the development and stripping steps to minimize the amount of PMMA debris and achieve clean patterning at the molecular scale.[38] The usual lift-off solvent for PMMA, acetone, leaves behind numerous 2−10 nm particles of PMMA debris.

The lift-off process used for deposition of DNA rafts is shown in Figure 3. The Si(100) substrates were first patterned with a large locator pattern using optical lithography to facilitate electron-beam lithography and repeated AFM imaging of the same area of the sample. Then they were cleaned, etched, and chemically oxidized to form a smooth native oxide. A 30 keV electron beam with a diameter of 2 nm was used to expose 60−90 nm PMMA resist atop the native oxide film. These exposures were conducted on a converted Hitatchi S-4500 scanning electron microscope. The pattern was developed to expose 100−120 nm wide trenches down to the silicon oxide surface (Figure 4a,a″), and then the sample was treated with an aqueous solution of APTES to form an APTES film in the trench bottoms. The remaining PMMA was cleanly removed by methylene chloride to yield 100−120 nm wide stripes of APTES on the silicon oxide, Figure 4b. These APTES stripes have a topographic height of 0.7 nm, and their RMS roughness is 0.15 nm, about the same as the roughness of a wide-area film of APTES.

DNA rafts attach specifically to the APTES stripes forming a partial monolayer. In the images shown in Figure 4c,c″,c‴, several rafts can be seen lying across each 110 nm wide APTES stripe. The height of the rafts is 2.7 nm in cross section, but that includes the 0.7 nm thick APTES adhesion layer, so the height of the DNA rafts matches that of duplex DNA. Repeated imaging of the same area does not appear to damage the DNA rafts or move them around on the surface. This result demonstrates that EBL with molecular lift-off is a promising approach to guide the deposition of DNA rafts on silicon.

Efforts to use poly(lysine) as the adhesion layer for this lift-off process were unsuccessful. Several experiments confirmed that the poly(lysine) is not uniformly deposited into 60 nm trenches even after 2 h of soaking; this may be due to the fact that the hydrodynamic radius of the 750 kDa poly(lysine) is about 35 nm, so it has trouble diffusing into the trench bottoms. In addition, poly(lysine) does not tolerate the stripping solvent (methylene chloride) used to remove the PMMA resist, and 1−10 nm tall blobs and lines, composed of denatured protein or PMMA debris, are observed. APTES remains the best choice for the adhesion layer because its molecular precursor can access the trench bottoms freely, and the APTES film is chemically stable to the solvent used to strip away the PMMA resist.

Discussion

The question that we are trying to address in this study is whether DNA nanostructures deposited on silicon surfaces could potentially act as a bridge between top-down lithography and bottom-up self-assembly. We have shown here that standard silicon wafers modified with a cationic SAM are a suitable substrate for binding of DNA rafts and also that electron-beam lithography can be applied to pattern the surface deposition of self-limiting structures made from DNA, and several groups[39] have shown that non-DNA components such as metal nano-particles can be attached to specific locations on large DNA tile

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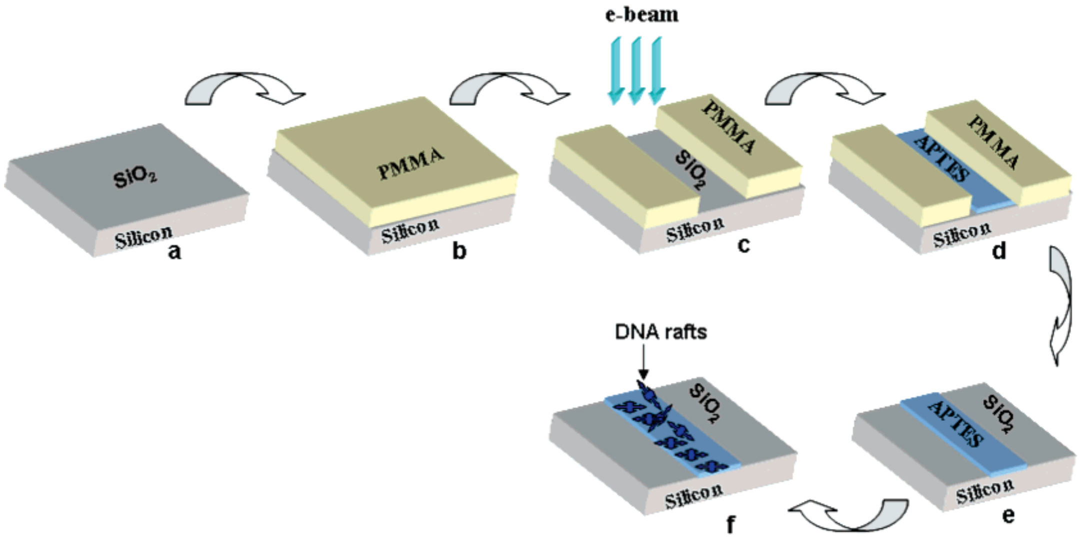

Figure 3. Schematic diagram of the molecular lift-off process: (a) silicon substrate with a thin layer of oxide, (b) ~100 nm thick PMMA spin coated onto the substrate, (c) PMMA trenches defined by EBL and development, (d) sample soaked in 0.05% APTES solution for 5 min, (e) lift-off of PMMA with CH_2Cl_2, (f) DNA raft solution 4 h, rinse.

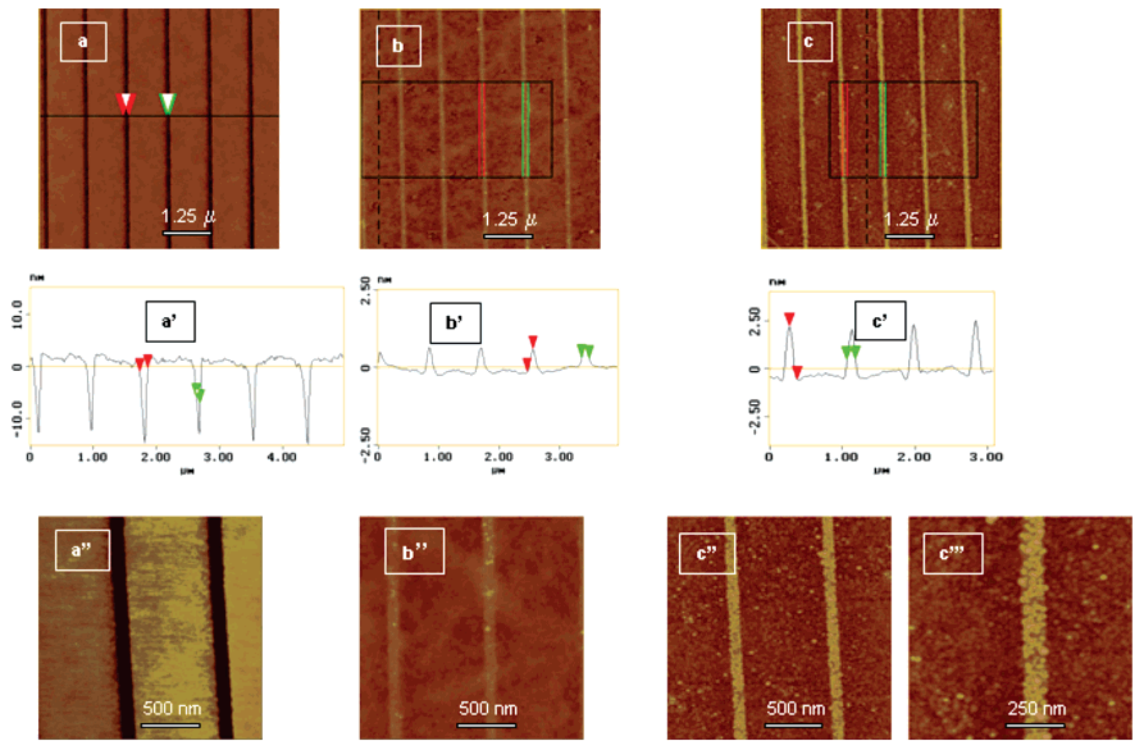

Figure 4. AFM topographic images during the lift-off process: (a, a′′) PMMA resist after development of exposed areas, (b, b′′) APTES lines after stripping of PMMA, (c, c′′, c′′′) four-tile DNA rafts bound to APTES lines at different magnifications, (a′, b′, c′) cross sections of the images.

arrays. Thus, in principle it seems possible to use the DNA nanostructures to bootstrap our way to sublithographic assembly of non-DNA components.

Among the issues which must be addressed are the thermodynamics and kinetics of assembly. The DNA nanostructures used in this study measure 8 × 37 nm, large enough to be positioned on lithographically defined features and directly observed by AFM. Recent work by Rothemund[40] has demonstrated that much larger DNA nanostructures (over 100 × 100 nm) can self-assemble with a high yield and low defect levels. The size of these structures suggests that the thermodynamics of DNA assembly is not likely to be the main “sticking point”. However, the electrostatic interaction between APTES SAMs and DNA is so strong that binding appears to be kinetically irreversible, at least under our buffer conditions. Repeated imaging of rafts on APTES/Si does not result in rafts moving about or detaching from the surface. In a mathematical study, Talbot et al.[41] showed that irreversible binding between a surface and ovals

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with an aspect ratio of 4 gives a final coverage of 0.55; ideal packing of the same ovals would give a coverage of 0.95. We assume an effective size of 10 × 40 nm for each raft, on the basis of the normal packing density of plasmid DNA on mica, which forms “fingerprint” structures with about 2 nm spacing between adjacent DNA helices. After 4 h, the saturation coverage of DNA rafts on the APTES lines was measured as 0.42, which suggests that the rafts are “jammed” under our deposition conditions. Bussiek et al. demonstrated that the supercoiling of DNA plasmids binding to APTES-treated mica can be controlled by salt concentration to allow the DNA to relax on the surface.[42] A similar approach applied to APTES films on silicon may allow the DNA rafts more freedom to diffuse in two dimensions and thus to pack into more ordered structures on the APTES stripes.

Conclusions

Silicon wafers that are modified with a cationic SAM can be used as a substrate for attachment of DNA nanostructures. Electron-beam lithographic patterning of the cationic SAM guides the binding of the DNA nanostructures and offers a potential pathway for nanofabrication at sublithographic length scales.

Methods

Preparation of Substrates. Silicon [100] (MEMC Electronic Materials, Inc., Malaysia) wafers were cleaned in piranha solution (1:3 30% H_2O_2/concentrated H_2SO_4) at 90° C for 30 min, rinsed with distilled water, and dried with a stream of N_2. *Caution: Piranha solution is a strong oxidant and can cause explosions when mixed with organic solvents!* The cleaned wafers were etched in a solution of 10% HF (∼1 min) until they became completely hydrophobic, indicating removal of the surface oxide. RCA cleaning procedures[43] were carried out next. The silicon wafers were first cleaned in a $NH_4OH/H_2O_2/H_2O$ (1:1:5) solution at 70° C for 10 min, rinsed with water for 5 min, then immersed in $HCl/H_2O_2/H_2O$ (1:1:5) solution at 70° C for 10 min, rinsed again with water, and finally dried with nitrogen.

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Electron-Beam Lithography. Silicon substrates for EBL were patterned with locator designs as described.[44] They were then cleaned as described above for SiO_2 substrates. A 60−90 nm thick film of 950000 g/mol PMMA was spun on, and the substrate and film were baked at 160 °C in an oven for 5 h. EBL was performed in a modified Hitachi scanning electron microscope at 30 keV with a spot size of 1−2 nm; the resolution was determined by imaging of gold nanoparticles. Exposed PMMA was developed with isopropyl alcohol/methyl isobutyl ketone (3:1) with 1.5% v/v methyl ethyl ketone at room temperature.[45] The samples were dried, immediately soaked in a 0.05% (v/v) solution in deionized water for 5 min, and dried with nitrogen. The samples were stored in a vacuum desiccator overnight to evaporate physisorbed APTES molecules from the surface. For EBL samples, the remaining PMMA was lifted off by soaking in a vial of clean CH_2Cl_2 for 3 × 1 min.

DNA Deposition. DNA rafts were prepared by annealing stoichiometric amounts of the 16-component single strands as described previously.[46] AFM samples were prepared by pipetting 20 μL of DNA raft solution (1 μM) onto the silanized surface of silicon and allowing the solution and silicon to stand for 4 h. Because of the poor spreading of DNA solution on the silanized surfaces, a clean glass coverslip was placed on top of the 20 μL drop of DNA solution. After deposition, the surface was rinsed gently with Milli-Q purified water and dried under a stream of nitrogen.

Atomic Force Microscopy. The atomic force microscope images shown were obtained using a Nanoscope IIIa Multimode instrument (Digital Instruments, California) in tapping mode. Silicon cantilevers were chosen from Nanosensors (type PPP-NCH).

Acknowledgment. We thank the Radiation Lab (University of Notre Dame and Department of Energy) for access to a Digital Instruments Multimode Nanoscope IIIa atomic force microscope. This research was supported by DARPA Grant N00014-01-1-0658, National Science Foundation Grant ECS 03-04630, and Intel.

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