Spatially Selective Deposition of a Reactive Polysaccharide Layer onto a Patterned Template

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Received September 5, 2002. In Final Form: November 4, 2002

The amine-containing polysaccharide chitosan was selectively deposited onto patterned gold surfaces in response to an applied voltage. Standard microfabrication techniques were used to pattern gold onto silicon wafers, and these gold patterns served as templates for the electric field directed deposition of chitosan. Experiments conducted with a fluorescently labeled chitosan derivative demonstrated the spatially selective deposition of chitosan onto gold surfaces that were polarized to serve as negative electrodes. Studies with unlabeled chitosan demonstrated that a "templated" chitosan, deposited by voltage programming of electrodes, can subsequently react with standard amine-selective functional groups. This indicates that common coupling chemistries can be exploited to assemble a variety of compounds onto the deposited chitosan pattern. Thus, chitosan appears to be a unique interface material that can be "templated" onto patterned inorganic surfaces and is reactive for the subsequent assembly of organic and biological molecules.

Introduction

Microfabrication techniques are routinely applied to create patterned inorganic surfaces with nanometer to micrometer scale resolution.[1] Two approaches are emerging to extend microfabrication techniques for the creation of patterned surfaces with organic and biological materials. Extensions of photolithography commonly begin with self-assembled monolayers[2,3] that are selectively irradiated to create a pattern of freshly exposed surface. This freshly exposed surface is then reacted with a second reagent that is bifunctional—one function for attachment to the freshly exposed patterned surface and the second function for subsequent coupling of the molecules of interest. Although variations exist, lithography creates the spatial template upon which subsequent coupling occurs.[4−8] An alternative approach for creating patterned surfaces with organic and biological materials is microcontact printing (μCP). In μCP, a soft stamp (typically made of poly(dimethylsiloxane)) is created with the appropriate pattern. After "inking" the stamp with a solution containing the material to be deposited, the stamp is pressed onto the surface to transfer the pattern.[9−19]

We are examining an alternative approach for creating organic and biomolecular patterns on surfaces. This approach employs electric fields that are known to direct the assembly of particles and macromolecules to surfaces.[20,21] For instance, electrophoretic deposition has been

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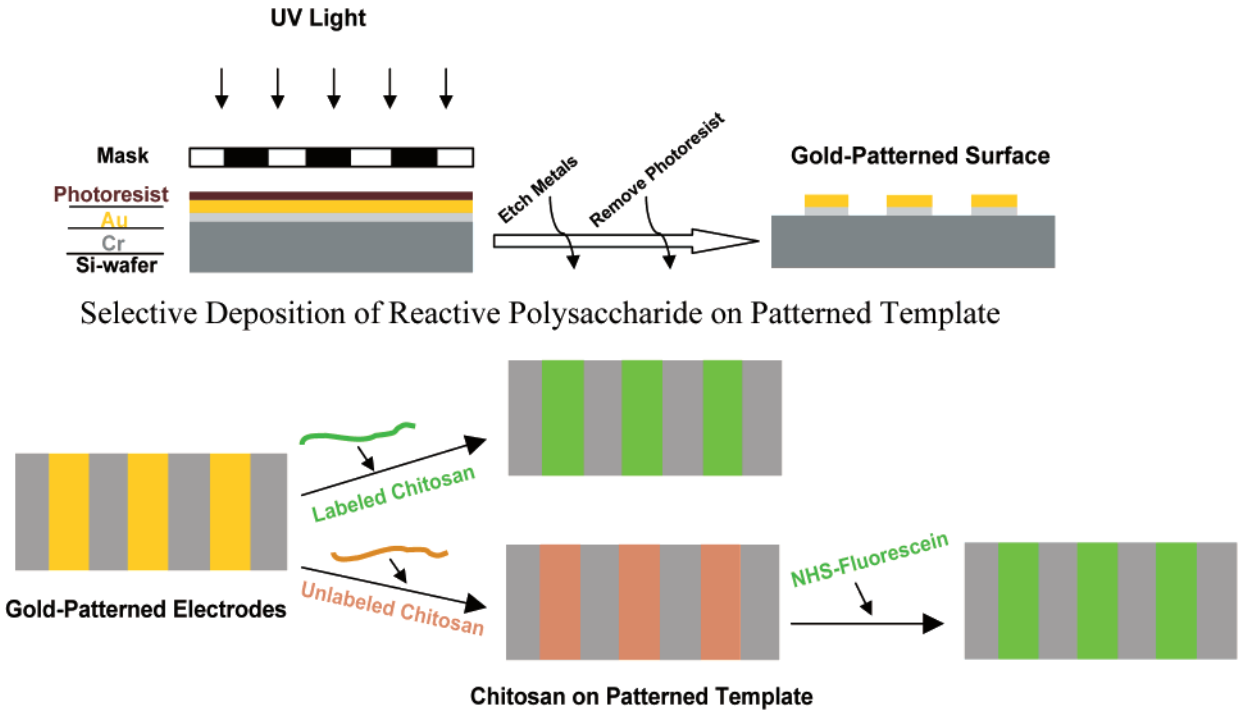
10.1021/la026518t CCC: $25.00
Published on Web 01/04/2003

Scheme 1



used to assemble colloidal particles[22−27] and proteins[28−30] onto electrode surfaces. Recently, Gao et al.[31] extended this approach and exploited an electric field to direct the spatially selective deposition of CdTe nanocrystals. In this method, they first fabricated a surface with patterned electrodes and then used a combination of an applied voltage and layer-by-layer assembly to generate a multilayer with spatial resolution in the lateral directions.

We report the spatially selective deposition of the biopolymer chitosan onto patterned electrode surfaces. Chitosan is an amino-polysaccharide that under moderately acidic conditions (pH < 6) is a soluble, cationic polyelectrolyte. Previously, this polyelectrolyte was observed to deposit onto an un-patterned negative electrode in response to an applied voltage.[32] The thickness of the deposit varied from tens of nanometers to micrometers depending on the deposition time, applied voltage, and chitosan concentration. At higher pH, chitosan's amino groups are deprotonated and the polymer becomes insoluble. We observed that after the chitosan deposit was "neutralized" with base, it could be retained on the electrode surface in the absence of an applied voltage (i.e., the deposit is stable under neutral conditions). If desired, the deposits can be removed from the surface by treatment with acid to dissolve away the chitosan.

To deposit chitosan with spatial selectivity in the lateral direction, Scheme 1 shows that we first used standard photolithography methods to pattern silicon wafers with gold lines with widths as small as 20 μm. Then, electric field directed deposition was initiated on the gold surfaces by polarizing them to serve as negative electrodes. As indicated in Scheme 1, we performed two types of deposition studies. In our first study, we created a fluorescently labeled chitosan and deposited this labeled chitosan from solution. In our second study, Scheme 1 shows a two-step approach in which chitosan was first deposited onto the negative electrode and then reacted with a fluorescein derivative activated with NHS to be reactive toward chitosan's amino groups.

Materials and Methods

Chitosan from crab shells (85% deacetylation) and phosphate-buffered saline (PBS) tablets were purchased from Sigma-Aldrich Chemicals. 5-(and 6-)-Carboxyfluorescein succinimidyl ester (NHS-fluorescein, excitation maximum 495 nm and emission maximum 519 nm) was purchased from Molecular Probes and stored desiccated at −20 °C in a dark container until use. Silicon wafers with 1 μm thick thermal oxide film (four inch diameter) were obtained from MEMC Electronic Materials. The gold and chromium used for sputtering onto the wafer were purchased from Kurt J. Lesker Co. The primer was hexamethyldisilazane (HMDS, Microelectronic Materials). The photoresist (Microposit Photoresist S1813) and developer (Microposit Developer 352) were purchased from Shipley Co. The etchants (TFA for gold and TFD for chromium) were obtained from Transene Co.

Chitosan solutions were prepared by adding chitosan flakes to water and incrementally adding small amounts of HCl to the solution to maintain the pH near 3. After being mixed overnight, the chitosan solutions were filtered to remove undissolved material, and the pH of solution was adjusted using NaOH (1 M). NHS-fluorescein solution was prepared by first dissolving 2.5 mg of NHS-fluorescein in 200 μL of dry dimethylformamide (DMF) and then adding 800 μL of ethanol.

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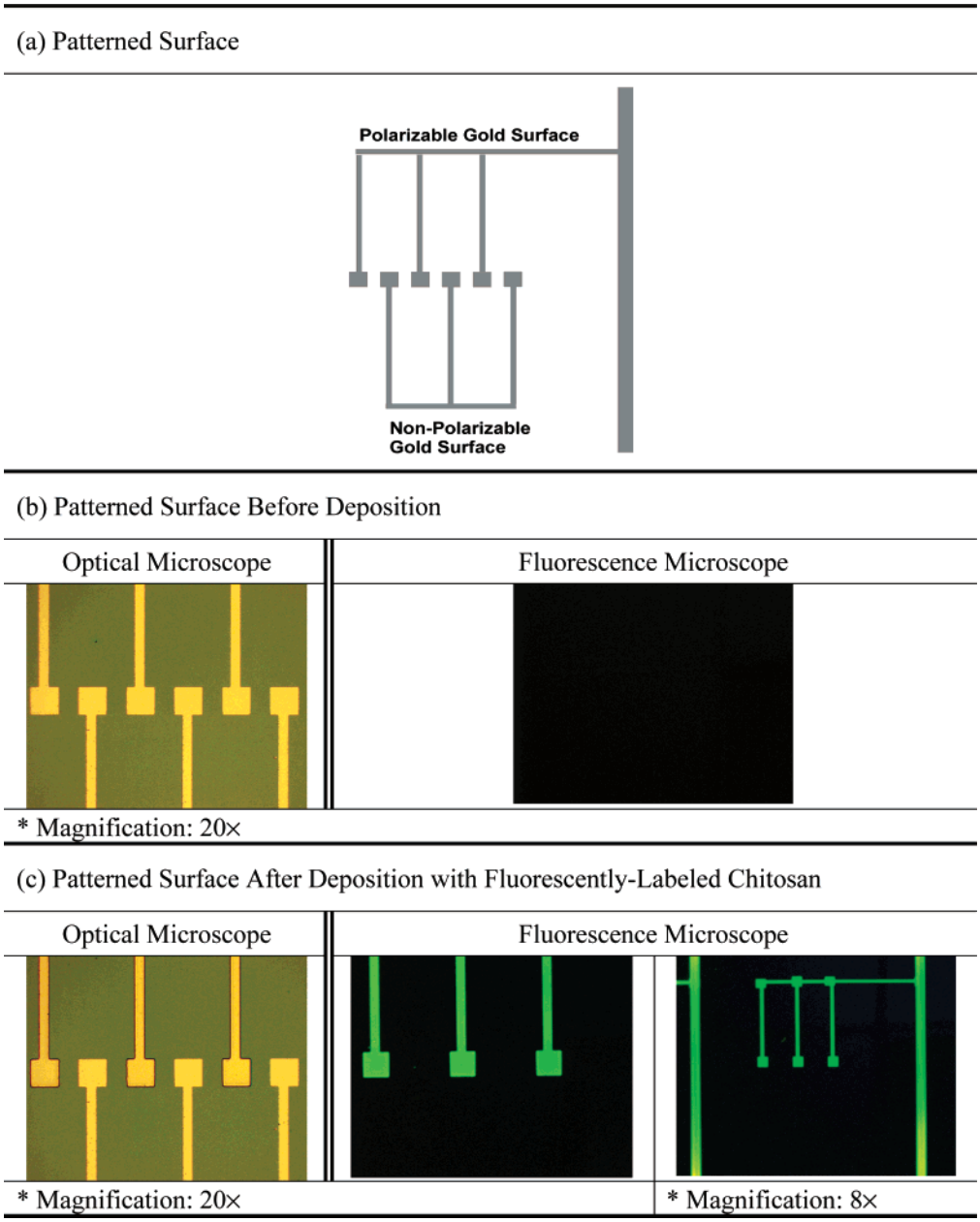

Figure 1. Spatially selective deposition of fluorescently labeled chitosan onto a patterned gold surface in response to an applied voltage. (a) Schematic showing the two sets of gold surfaces—the top set was polarized to be negative while the bottom set could not be polarized. (b) Photomicrographs before deposition using an optical microscope (left photo) and fluorescence microscope (right photo). (c) Photomicrographs after deposition (2 A/m^2 for 2 min) using an optical microscope (left-most photo) and fluorescence microscope (the two right photos).

Fluorescently labeled chitosan derivatives facilitate visualization,[33] and we prepared our labeled chitosan by reacting a chitosan film with NHS-fluorescein. The chitosan film was made by adding 50 mL of a 0.4% (w/v) chitosan solution (pH 3.0) to 140 mm diameter Petri dishes. The Petri dishes were oven-dried overnight at 45 °C, and then the dried films were neutralized by immersion in 1 M NaOH for 3−4 h. After neutralization, the films were washed thoroughly with distilled water and equilibrated with a 0.1 M PBS buffer. This buffer was prepared by dissolving PBS tablets in double distilled H_2O and adjusting the pH to 7.4. The labeling reaction was initiated by adding 20 μL of NHS-fluorescein solution (the DMF/ethanol solution described above) into a Petri dish containing a chitosan film in 35 mL of PBS buffer. After allowing 30 min for reaction, the yellowish-green-colored chitosan films were then rinsed with distilled water and dissolved in a dilute HCl solution (pH = 3). For purification, the fluorescein-labeled chitosan was precipitated by adjusting the pH to about 9 using NaOH. The precipitant was then collected and rinsed with distilled water. After purification the fluorescently labeled chitosan was redissolved in a dilute HCl solution and the pH was adjusted to 5.6. To determine the polymer concentration, aliquots of known mass were oven-dried, and the residue was weighed.

The patterned surfaces were fabricated by depositing 150 Å thick chromium and then 2000 Å thick gold films on 4-in. diameter silicon wafers, which had previously been coated with 1 μm thick thermal oxide film. As indicated in Scheme 1, patterning was achieved using photolithography in which a primer and then photoresist were spin-coated onto the gold surface. After soft-backing the coated wafer at 100 °C for 1 min, a specially designed mask was placed over the surface and the wafer was exposed to UV light (total dosage ~190 mJ/cm^2). After 30 s of development, the wafer was then hard-baked at 120 °C for 10 min. The exposed areas were then etched away by gold and chromium etchants, and the photoresist was removed using acetone.

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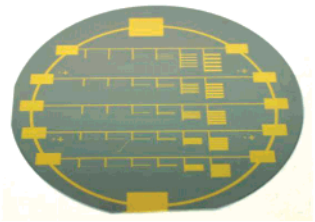

Line (μm)	20	50	100	500	1000
Space (μm)	500	500	500	1000	1000
	100	100	200	500	500
	20	50	100	200	300
	10	30	50	50	100
	5	10	10	10	50

Figure 2. Photograph of a patterned surface with gold lines of varying widths and varying spaces. The table next to the photograph lists the widths of the gold lines and the spaces.

For deposition, the patterned wafers were immersed in solutions (pH = 5.6, 0.8% (w/w) polymer) containing either fluorescently labeled chitosan or unlabeled chitosan, and the patterned gold surfaces were polarized to serve as negative electrodes. The positive electrode in these experiments was an unpatterned gold-coated silicon wafer. The two electrodes were connected to a dc power supply (model 6614C, Agilent Technologies) using alligator clips. Deposition was performed for 2 min by applying a voltage to achieve current densities of 1−2 A/m^2. After deposition, the wafers were removed from the solutions, rinsed for 1 min with deionized water, disconnected from the power supply, and dried at room temperature. After drying, the wafers were immersed in 1 M NaOH for 30 min to neutralize the chitosan. After neutralization, the wafers were rinsed with distilled water and dried at room temperature overnight.

As indicated by the bottom path in Scheme 1, some experiments were performed in which NHS-fluorescein was reacted with chitosan after it had been deposited onto the patterned gold surfaces of the wafers. For this study, chitosan was first deposited as described above and the dried wafer was placed in a 140 cm diameter Petri dish with 35 mL of PBS buffer (pH = 7.4). The reaction was initiated by adding 20 μL of the DMF/ethanol solution containing NHS-fluorescein. After the reaction was allowed to proceed for 5 min, the wafer was rinsed with distilled water and dried at room temperature overnight.

The patterned wafers were examined using an optical microscope (model FS70, Mitutoyo Corp.), and photographs were taken with this microscope using a digital camera (Nikon DXM 1200). The patterned surfaces were also examined using a fluorescence stereomicroscope (MZFLIII, Leica) using a fluorescence filter set (GFP Plus) with an excitation filter at 480 nm (slit width of 40 nm) and an emission barrier filter at 510 nm. Photomicrographs were prepared from the fluorescence microscope using a digital camera (Spot 32, Diagnostic Instruments).

Results and Discussion

Our first study examined the selective deposition of fluorescently labeled chitosan onto a patterned surface. For this study, a silicon wafer was patterned to have two independent sets of gold surfaces. Figure 1a shows that only the top set of gold surfaces could be polarized to serve as an electrode. Figure 1b shows photomicrographs for this patterned surface before deposition. The photomicrograph in the left of Figure 1b was obtained using an optical microscope and shows the patterns of the two sets of gold surfaces. The photomicrograph in the right of Figure 1b shows that prior to deposition, no image could be obtained from this patterned surface when a fluorescence microscope was used.

For deposition, the wafer was immersed in a solution containing the labeled chitosan and a negative voltage was applied to the polarizable gold surfaces. After 2 min of deposition, the wafer was removed from the solution, rinsed with deionized water, and then disconnected from the power supply. After neutralization and rinsing, the wafer was dried and then examined. The photograph from the optical microscope (left-most photo in Figure 1c) shows only slight differences between the polarizable and non-

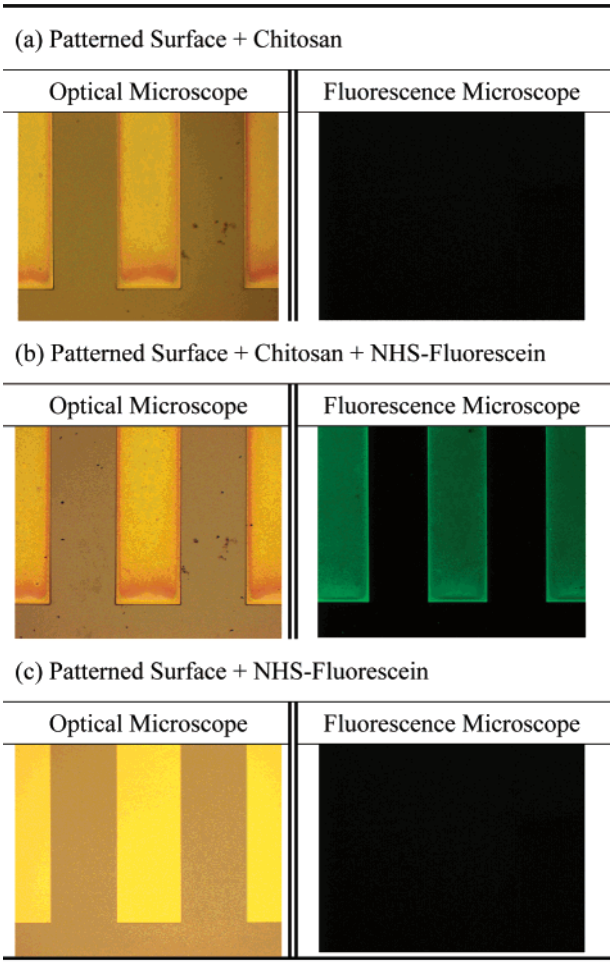

Figure 3. Spatially selective deposition of unlabeled chitosan and subsequent reactions of the "templated" chitosan deposit. (a) Photomicrographs of patterned surface after chitosan deposition (1 A/m^2 for 2 min) but before NHS-fluorescein reaction. (b) Photomicrographs of patterned surface after both chitosan deposition and NHS-fluorescein reaction. (c) Photomicrographs of a "control" in which the patterned surface was incubated with the NHS-fluorescein solution without prior deposition of chitosan. In all cases the left photographs were obtained using an optical microscope and the right photographs were obtained using a fluorescence microscope. The gold lines were 1 mm wide and separated by 1 mm spaces.

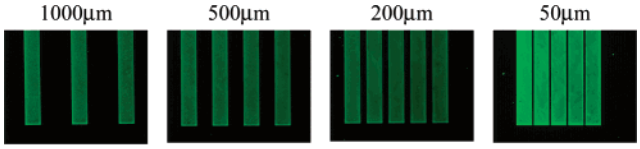

Figure 4. Resolution of spaces between 500 μm wide gold lines after chitosan deposition and subsequent reaction with NHS-fluorescein. Deposition and reaction were the same as described for Figure 3. The widths of spaces were 1000, 500, 200, and 50 μm.

polarizable sets of gold surfaces. The two photographs from the fluorescence microscope show dramatic differences with obvious images from the upper set of gold surfaces (which had been polarized to be negative), and no fluorescent images from the nonpolarized, lower set of gold surfaces. For convenience fluorescence micrographs are shown at two different magnifications in Figure 1c.

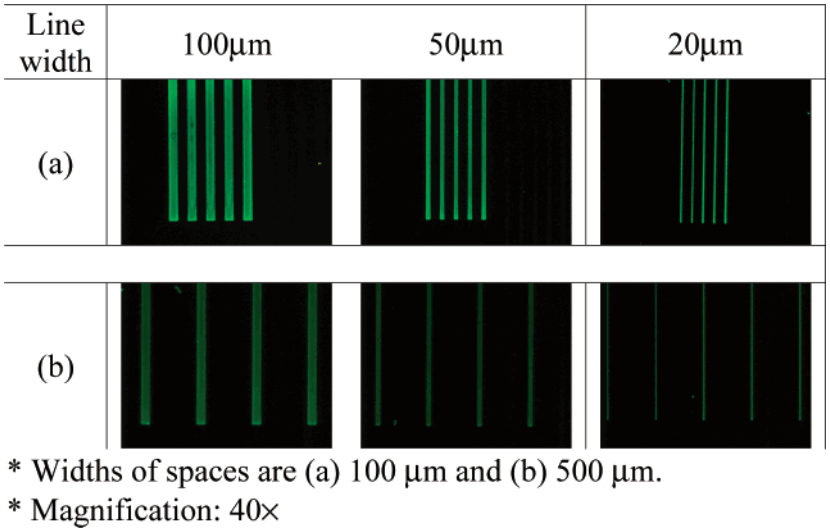

Figure 5. Resolution of chitosan deposition and subsequent reaction of NHS-fluorescein with gold lines of varying widths separated by spaces of (a) 100 μm and (b) 500 μm. Deposition and reaction were the same as described for Figure 3. The widths of gold lines were 100, 50, and 20 μm.

In summary, Figure 1 shows that the patterned gold surface serves as a template for the spatially selective deposition of the fluorescently labeled chitosan. Further, no deposition was observed on the unpolarized gold surfaces. Thus, deposition occurs only in response to an applied voltage (or current)—indicating that deposition can be controlled temporally and spatially based on when and where the voltage is applied.

In our second study we deposited unlabeled chitosan onto a patterned surface and examined the spatial selectivity for subsequent coupling reactions. For this study, the wafer in Figure 2 was patterned to have a variety of gold lines with different widths and different spaces between the lines. The table next to the photograph lists the dimensions of the various lines and spaces and shows that the lines vary in width from 20 to 1000 μm. For deposition, this patterned wafer was immersed in a chitosan solution and the gold surface was polarized to be negative for 2 min. After deposition, the wafer was neutralized, rinsed, and dried as described above. Figure 3a shows photomicrographs of the region of the wafer patterned with 1 mm wide gold lines spaced 1 mm apart. The left image in Figure 3a is from the optical microscope and it shows both the lines and spaces in this region. The right image in Figure 3a shows no fluorescence was observed after chitosan deposition (i.e., unlabeled chitosan is not fluorescent).

The next step in this study was to contact the wafer with a solution containing NHS-fluorescein. This fluorescein derivative is activated to react with amine groups and should react with any chitosan that had been deposited onto the gold pattern. After the patterned wafer was allowed to react with the NHS-fluorescein solution, the wafer was rinsed with distilled water and dried. The left photograph in Figure 3b shows that the NHS-fluorescein treatment had little effect on the patterned surface when the wafer was examined with an optical microscope. In contrast, the right photograph in Figure 3b was obtained with the fluorescence microscope and shows a distinct fluorescent pattern. This photomicrograph indicates that chitosan had been deposited onto the patterned gold template, and this "templated" chitosan layer underwent reaction with the amine-reactive fluorescein derivative.

Figure 3c shows results for a control in which the patterned wafer was directly treated with NHS-fluorescein (without prior deposition of chitosan). After this treatment, the wafer was rinsed and dried. The photograph from the optical microscope shows the distinct gold pattern while no pattern was observed using the fluorescence microscope. The observations in Figure 3c are consistent with the expectation that there is no reaction between NHS-fluorescein and either the gold or silicon oxide surfaces of the wafer.

To further characterize the spatial selectivity of chitosan deposition and subsequent NHS-fluorescein coupling, we examined several regions of the patterned surface using the fluorescence microscope. Figure 4 shows photomicrographs for surfaces with 500 μm wide lines separated by spaces of different widths. These photographs show that the lines were well-resolved even when they were separated by only 50 μm. Figure 5 shows lines of varying widths (100, 50, and 20 μm) separated by either 100 or 500 μm spaces. These images demonstrate good deposition and subsequent fluorescein coupling even when the patterned gold lines were only 20 μm wide.

Conclusions

We believe there are two important conclusions from this study. First, a fluorescently labeled derivative of the aminopolysaccharide chitosan was deposited in a spatially selective manner onto patterned gold surfaces in response to an applied voltage (or current). In principle, these results are similar to those of Gao et al.[31] who used an electric field to direct the layer-by-layer assembly of CdTe-poly(diallyldimethylammonium chloride) multilayers onto a patterned surface. In both studies, standard microfabrication techniques were used to create patterned electrode surfaces that served as the template for electric field directed deposition. For the case of chitosan, or fluorescently labeled chitosan, the deposited layer is stable after neutralization because chitosan is insoluble at pH values above about 6.3. The deposited chitosan can however be removed from the surface by washing with acid.

The second, and more important, conclusion is that a "templated" chitosan deposit provides a spatially resolved surface that is prepared for subsequent coupling reactions. In particular, chitosan's primary amine groups are reactive, and various selective chemistries can be exploited to couple additional compounds onto the patterned surface. In our case, NHS-fluorescein reacted with the "templated"

chitosan deposit and we observed a lateral resolution of a few micrometers. Thus, we believe chitosan is a unique material that can be programmed to "template" onto a patterned surface and can offer the reactive amine functionality upon which other compounds (e.g., biomacromolecules) can be assembled.

Acknowledgment. Partial funding for this study was provided by the National Science Foundation (BES-0114790) and the Department of Energy (DE-FG02-01ER63109).

LA026518T