VOLTAGE-PROGRAMMABLE BIOFUNCTIONALITY IN MEMS ENVIRONMENTS USING ELECTRODEPOSITION OF A REACTIVE POLYSACCHARIDE

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ABSTRACT

The amino-polysaccharide chitosan has distinct properties that make it an attractive interface material for the assembly of biomolecules onto microfabricated surfaces. Chitosan has pH-responsive electrostatic and solubility properties that allow it to be deposited and retained on cathode surfaces. Deposition is shown to be spatially controllable at \(\mu\)m levels by “templating” the chitosan onto micropatterned gold cathodes. Temporal control of deposition can be achieved depending on when the micropatterned electrodes are polarized. Chitosan also has nucleophilic amine groups that can be easily reacted using standard, amine-specific chemistries. Studies show that the sequence of chitosan deposition and chemical modification is repeatable. Finally, standard chemistries can be exploited to couple biomolecules onto chitosan films that have been “templated” onto the micropatterned gold cathodes. Specifically, we used glutaraldehyde activation to assemble the model protein, green fluorescent protein (GFP) onto a chitosan deposit. These studies demonstrate that chitosan has unique properties that allow it to serve as an interface material for the assembly of biomolecules onto microfabricated surfaces.

INTRODUCTION

The effective integration of biotechnology and microfabrication will provide robust biosensors to better diagnose disease, rapidly detect chemical/biological agents, and efficiently discover drugs. The biological sensing elements (e.g., nucleic acid probes and protein antibodies) confer the sensitivity/selectivity to these sensors while microfabrication permits miniaturization to them. Therefore, small samples can be rapidly analyzed in parallel processing format. The challenge is to effectively couple the labile bio-molecule to the microfabricated surface in a way that retains biological sensing activity.

We believe the amino-polysaccharide, chitosan is a unique material that can serve as an effective interface for the assembly of biomolecules onto microfabricated surfaces.

EXPERIMENTAL

Fabrication of gold patterned surface. The patterned surfaces were fabricated by depositing 150 Å thick chromium and then 2000 Å thick gold films on 4-inch diameter silicon wafers, which had previously been coated with 1-μm thick thermal oxide film. Patterning was achieved using photolithography in which a primer and then photoresist (Microposit Photoresist S1813) were spin-coated onto the gold surface. After soft-baking the coated wafer at 100 °C for 1 minute, a specially-designed mask was placed over the surface and the wafer was exposed to UV light (total dosage ~ 190 mJ/cm²). After 30 seconds of development, the wafer was then hard-baked at 120 °C for 10 minutes. The exposed areas were then etched away by gold and chromium etchants (TFA for gold and TFD for chromium, Transene Co), and the photoresist was removed using acetone.

Electrodeposition. The preparation of chitosan (Sigma-Aldrich Chemicals) solutions, NHS-fluorescein (5-(and 6-)carboxyfluorescein succinimidyl ester, Molecular Probes) solutions, and Fluorescently-labeled chitosan solutions were described elsewhere [1]. 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 un-patterned 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 two minutes by applying a voltage to achieve a current density of 1–2 A/m². After deposition, the wafers were removed from the solutions, rinsed for one minute with deionized water, disconnected from the power supply, and dried at room temperature. After drying, the wafers were immersed in 1M NaOH for 10 minutes to neutralize the chitosan. After neutralization, the wafers were rinsed with distilled water and dried at room temperature overnight.
Chemical reaction of chitosan deposit. NHS-fluorescin was reacted with chitosan films that had previously been deposited onto the micropatterned gold surfaces. For this study, chitosan was first deposited as described above and the dried wafer was placed in a 140 mm diameter petri dish with 35 ml PBS buffer (pH=7.4). The reaction was initiated by adding 20 µl of the dimethylformamide/ethanol (1/4) solution containing NHS-fluorescin (2.5 mg/ml). After allowing the reaction to proceed for five minutes, the wafer was removed from the solution, rinsed with distilled water and dried at room temperature overnight.

Production of Green Fluorescent Protein (GFP). GFP was expressed in E.coli BL21 (Invitrogen) using a pTrcHisB (Invitrogen) expression vector. Cells were grown under standard fermentation conditions and the fusion protein was purified using immobilized metal affinity chromatography as described elsewhere [2,3].

Assembly of model protein onto chitosan deposit. Glutaraldehyde was used to anchor the model protein (green fluorescent protein, GFP) onto the deposited chitosan surface. After chitosan was deposited onto the micropatterned gold electrode, the wafer was immersed in glutaraldehyde solution (0.05%) for 30 minutes. After glutaraldehyde activation, the wafer was extensively washed with 0.1 M PBS (Dulbecco’s Phosphate Buffered Saline, Sigma-Aldrich Chemicals) buffer and then immersed in a GFP solution (≈ 0.4 µg/ml) for 30 minutes. Two control experiments were performed at the same time. One control was a wafer with micropatterned gold lines that lacked chitosan. The second control was a wafer in which chitosan was deposited onto micropatterned gold lines but the deposited chitosan film was not activated with glutaraldehyde. Both controls were immersed in GFP solution for 30 minutes. All samples were extensively washed with PBS buffer before examination.

Characterization. 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 (band 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

Electrochemical Deposition. Chitosan has pH-dependent electrostatic properties that allow it to be deposited onto microfabricated surfaces. Figure 1 shows that at low pHs (below about 6) chitosan’s amine groups are protonated and chitosan behaves as a water-soluble polyelectrolyte. At higher pHs (above about 6.5) chitosan’s amine groups become deprotonated and chitosan becomes neutral and insoluble. Chitosan’s pH-responsive properties can be exploited as illustrated in Figure 2 where the chitosan chains are shown to travel through an acidic solution to a cathode surface. Once chitosan arrives at the cathode surface, it can be deposited by an increase in pH – either an electrochemically-generated localized increase in pH or by removing the electrode and immersing it in a basic solution. After neutralization, the deposited chitosan is insoluble and can be retained on the cathode surface even in the absence of an applied voltage. In previous studies we observed that the thickness of the deposited chitosan film could be varied from 20 nm to 2 µm depending on the deposition conditions [4,5].

![Figure 1. Structure and acid-base reaction of chitosan.](image1)

![Figure 2. Deposition of chitosan on cathode surface in response to an applied voltage.](image2)
The strengths of microfabrication can also be exploited for the deposition of chitosan. Specifically, gold electrodes can be micro-patterned onto a surface and these micropatterned electrodes can serve as templates for the selective deposition of chitosan. Figure 3a shows a schematic of a surface where gold lines were patterned onto a silicon wafer with a 1 µm thermal oxide layer. This wafer was immersed into a slightly acidic solution of fluorescently-labeled chitosan (pH 5.6) and the upper gold lines were polarized to serve as cathodes while the lower lines were not polarized. As illustrated in Figure 3a, we expect the fluorescently-labeled chitosan to selectively deposit onto the gold “template” that is polarized to serve as the cathode. The fluorescence micrograph of Figure 3b demonstrates that the labeled-chitosan is selectively deposited onto the cathode surface while no deposition was observed on the un-polarized gold electrodes. The lines in Figure 3a are 125 µm wide and thus Figure 3b demonstrates reasonably high resolution for the selective deposition of chitosan [1]. (Resolution was examined in a separate study to be discussed later.)

In summary, chitosan has pH-dependent electrostatic properties (including pH-dependent solubility) that allow it to be deposited from solution onto a cathode surface and, when neutralized, this polysaccharide remains where it is deposited even in the absence of an applied voltage (i.e. you can “put” a chitosan film on the cathode surface and “keep” it there). Microfabrication allows deposition to be controlled spatially and temporally based on where the electrodes are patterned and when they are polarized.

Chemical Modification of Chitosan. Another desirable feature of chitosan is that this polysaccharide has amine groups at nearly every repeating sugar residue. Amines are convenient because they can undergo various chemical reactions under facile conditions. The reactivity of amines is commonly exploited for chemically-modifying surfaces [6-8] and a range of amine-specific coupling chemistries have been developed. In previous studies, we deposited chitosan from solution onto micropatterned gold electrodes and then subjected the deposited chitosan to reaction with a fluorescein derivative that is activated to react with amine groups (NHS-fluorescein). Results from that study demonstrated that chitosan deposition and its subsequent reaction occurred with high resolution. Specifically, deposited and reacted chitosan could be visualized on gold lines as thin as 20 µm [1] (near the resolution limit for the photolithography step used to pattern gold onto the wafer surface).

We used the device in Figure 4a to examine the repeatability of the two steps - chitosan deposition and its subsequent chemical modification. Specifically, we patterned a wafer to have two sets of gold patterns that were electrically distinct. In our experiments, we polarized the top set of electrodes to serve as cathodes while the bottom set was un-polarized. This wafer was immersed in a slightly acidic chitosan solution (pH 5.6) for 3 minutes. After washing with distilled water, it was stored in PBS buffer and then NHS-fluorescein solution was added and allowed to react for 20 minutes. After reaction, the wafer was visualized using UV illumination. Figure 4b shows that the two steps - deposition and modification – are quite repeatable.

Bio-Functionalization of Chitosan. Amine-specific coupling chemistries are commonly exploited to crosslink biomolecules, or to couple biomolecules to surfaces. Figure 5 shows that glutaraldehyde can be used to activate chitosan surfaces for subsequent biomolecule coupling. The upper path in Figure 5 shows that amine-terminated oligonucleotide probes can be coupled to the glutaraldehyde-activated chitosan surface. This approach has been used to create biosensors based on nucleic acid hybridization [9]. The lower path in Figure 5 shows that glutaraldehyde activation can be used to anchor proteins to surfaces. Because proteins are considerably more labile than nucleic acids, more care is required to couple the protein to prevent destruction of its structure and biological activity.
To examine protein coupling, we used the common model protein green fluorescent protein (GFP). After depositing chitosan onto the patterned gold surface, the chitosan film was activated with glutaraldehyde, extensively washed with buffer, and then contacted with a GFP-containing solution. Figure 6 shows that GFP is selectively coupled to the chitosan film that had been templated onto the patterned gold cathodes. As described in the “Experimental” section, two control experiments were performed – one in which chitosan was not deposited and a second in which chitosan was deposited but not activated with glutaraldehyde. No fluorescence was observed in either control.

**Figure 6.** Glutaraldehyde-coupling of model protein (green fluorescent protein, GFP) to selectively deposited chitosan.

**CONCLUSIONS AND FUTURE WORK**

Chitosan has pH-dependent electrostatic and solubility properties that allow it to be deposited and retained in response to an applied voltage. Additionally, chitosan’s amines can be reacted under mild conditions using standard and specific coupling chemistries. We have exploited these unique properties to deposit chitosan films onto micropatterned electrode “templates” and then to assemble the model protein, GFP, onto the deposited film. Deposition and protein assembly occurred with high spatial resolution and repeatability. Currently, we are integrating these two-dimensional biomolecule assemblies into microfluidic devices.

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**Reference**


