CHITOSAN AT THE INTERFACE OF MICROFABRICATION AND BIOTECHNOLOGY

Li-Qun Wu1,2, Rohan Fernandes3,4, Hyunmin Yi1,3, David A. Small2,5, Gary W. Rubloff4,5, Reza Ghodsi6,7, William E. Bentley8,9, and Gregory F. Payne2,10

1Center for BioSystems Research, University of Maryland Biotechnology Institute
5115 Plant Sciences Building, College Park, MD 20742, USA
2Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County
1000 Hilltop Circle, Baltimore, MD 21250, USA
3Department of Chemical Engineering, University of Maryland at College Park
College Park, MD 20742, USA
4Department of Materials and Nuclear Engineering, University of Maryland at College Park
College Park, MD 20742, USA
5The Institute for Systems Research, University of Maryland at College Park
College Park, MD 20742, USA
6Department of Electrical and Computer Engineering, University of Maryland at College Park
College Park, MD 20742, USA
7Corresponding author Gregory F. Payne E-mail: payne@umd.edu

ABSTRACT

Bioensors provide exciting new opportunities to diagnose disease, detect pathogens, and discover drugs. Ideally, biosensors should effectively integrate the strengths of microfabrication with the exquisite molecular recognition capabilities of biological molecules (e.g., nucleic acids and proteins). The challenge is to adapt fabrication methods to accommodate the labile nature of the biological sensing molecules (especially proteins). Chitosan has unique properties that enable it to effectively interface between the microfabricated surfaces with labile bio-macromolecules. Specifically, chitosan has pH-dependent electrostatic properties that permit it to be "templated" onto micropatterned surfaces in response to an applied voltage, chitosan has nucleophilic amines that allow standard coupling chemistries to be used to tether biological molecules to deposited chitosan films, and chitosan has gel-forming abilities that may provide unique opportunities for creating microfluidic channels. We review our recent studies to exploit these unique properties of chitosan.

Keywords: Biosensor, Chitosan, Green Fluorescent Protein, Microfabrication, Nucleic Acid Hybridization

INTRODUCTION

There are several applications that would benefit from improvements in biosensor. A physician could more quickly diagnose disease if s/he had a disposable biosensor that could be used in the office. A food manufacturer could better insure food safety if s/he had a robust biosensor to routinely test for the presence of pathogens. Pharmaceutical companies could more effectively discover drugs if they had biosensing capabilities that allowed high throughput screening. In principle, it should be possible to meet these diverse goals if the strengths of the biological sensing component could be effectively integrated into microfabricated devices. However, this integration has not always been successful because the biological sensing molecules (proteins and nucleic acids) are labile and incompatible with standard microfabrication operations that employ high temperatures, low pressures, and gas/solvent environments. We believe that chitosan has unique properties that allow it to serve as a versatile interface between the microfabricated device and the labile biological sensing molecules.

Chitosan has pH-dependent electrostatic properties

As illustrated by the reaction (right), when chitosan's amines are protonated, the polysaccharide is a water-soluble, cationic polyelectrolyte. At higher pH, the amines are deprotonated and chitosan becomes neutral and insoluble.

\[
\text{Soluble} \xrightarrow{\text{H}^+} \text{Insoluble} + 2n \text{H}^+
\]
When it behaves as a cationic polyelectrolyte, chitosan can be attracted to the surface of a negative electrode (i.e., the cathode) (Wu et al., 2002). Interestingly, protons can also be consumed at the cathode and a pH gradient can be established near the cathode surface. Figure 1 shows that the localized pH near the cathode surface can exceed chitosan's solubility limit (Fernandes et al., 2003). Thus, chitosan can be attracted to, and deposited onto the cathode surface.

Importantly, standard microfabrication operations allow electrodes to be patterned onto silicon wafers with high spatial selectivity. To test the spatial selectivity of chitosan deposition, we micro-patterned two electrically independent sets of gold electrodes onto a silicon wafer which had previously been treated to have a 1 µm SiO2 layer. As shown in Figure 2, the wafer was immersed in a solution containing fluorescently labeled chitosan (0.8 w/w %, pH 5.6) and the top surface was polarized to serve as a cathode (2 A/m² for 2 minutes). As illustrated in Figure 2, we expect the fluorescently labeled chitosan to be selectively deposited only on the micro-patterned gold cathode. Figure 3 shows experimental results that demonstrate that chitosan deposits onto the cathode with high spatial selectivity while no deposition occurs on the un-polarized gold surface or the SiO2/Si substrate surface (Wu et al., 2003a).

Thus, chitosan's pH-dependent electrostatic properties allow it to be deposited onto a micro-patterned surface with high spatial selectivity.

**Chitosan's amines are nucleophilic**

Chitosan has amine groups at each of its glucosamine residues. Amines, in the neutral state, are nucleophilic and can undergo a variety of reactions under mild conditions. As a result, surface amines are highly desirable when one wants to couple a bio-molecule to a surface. Figure 4 shows that we exploited standard glutaraldehyde chemistries to couple amine-terminated nucleic acids (i.e., amine-terminated single-stranded DNA) and proteins to chitosan (Yi et al., 2003a).

To demonstrate that ssDNA can be assembled onto a patterned surface, we fabricated a “chip” containing two patterned gold surfaces. The left photograph in Figure 5 shows that this nucleic acid hybridization chip fits into a 2 ml centrifuge tube. This chip was immersed in a chitosan solution (pH 3.7) and the left electrode was...
polarized to serve as a cathode while the right electrode was not polarized during the deposition step. After deposition, the chip was immersed in a glutaraldehyde solution (0.1% glutaraldehyde for 30 minutes) to activate the chitosan surface. Finally, the chip was immersed in a solution containing an amine-terminated (5’-end) and fluorescently labeled (3’-end) oligonucleotide containing 20 bases that are complementary to the dnaK gene of E. coli (20 mg/ml ssDNA). The center photograph in Figure 5 shows a bright field image of this silicon chip. The right photograph in Figure 5 was taken using 480 nm excitation light and shows that the fluorescently labeled oligonucleotide is selectively assembled onto the gold surface with the deposited chitosan. Further studies demonstrate that oligonucleotides that have been assembled onto such a chip can hybridize reversibly with target oligonucleotides that have complementary sequences - including mRNA targets (Yi et al., 2003b).

Glutaraldehyde activation can also be used for coupling proteins to chitosan. For this study we used a microfabricated surface that had a series of gold patterns similar to the pattern in Figure 2. The surface was immersed in a chitosan solution (0.8% chitosan, pH 5.6) and one set of gold surfaces was polarized to serve as cathodes for chitosan deposition. After deposition, the chitosan surface was activated by glutaraldehyde, and the surface was then contacted with a solution containing the green fluorescent protein (GFP; 0.4 mg/ml for 30 minutes). Figure 6 shows GFP is effectively assembled onto the deposited chitosan and assembly occurs with high spatial selectivity. Further, the fact that the assembled GFP is fluorescent provides evidence that the protein’s three-dimensional structure remains intact (Wu et al., 2003b; Kastantin et al., 2003).

The above results indicate that the reactivity of chitosan’s amine groups allow standard coupling chemistries (e.g., glutaraldehyde coupling) to be exploited to assemble labile biological molecules to microfabricated surfaces. This assembly occurs under sufficiently mild conditions to retain the biomolecule’s structure (and presumably functional properties).

Chitosan can form a hydrogel

Chitosan has well-known hydrogel-forming abilities when the pH is raised above about 6.3. When patterned gold surfaces are polarized to serve as cathodes and high current densities are imposed (above about 50 A/m²), then the proton consumption rate at the cathode increases substantially. The increased proton consumption rate is expected to lead to an increase in the localized pH near the cathode surface. Under such high current-density conditions, chitosan deposits from solution onto the cathode surface as a diffuse hydrogel layer. Figure 7 shows that when the hydrogel is deposited onto a patterned surface, fluidic channels can be created. These channels can be temporary in the sense that they can be dissolved away by a simple acid treatment (Fernandes et al., 2003).

CONCLUSIONS

Chitosan possess pH-dependent electrostatic properties, has nucleophilic amines and forms hydrogels. These properties provide unique opportunities for exploiting chitosan for biosensors and microsystems (or microelectromechanical systems, MEMS). We illustrate chitosan’s potential using examples from our interdisciplinary research.
ACKNOWLEDGEMENTS

Financial support for this research was provided by the United States Department of Agriculture (2001-35504-10667), the Department of Energy (DE-FG02-01ER63109), and the National Science Foundation (grant BES-0114790).

REFERENCES


