Optimizing the Delivery of Toxins and Glycans to Photonic Biosensor Surfaces

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Abstract and Introduction:

Photonic biosensors allow for precise, real-time, and label-free detection of biomolecules by using micro-ring resonators, which are ring-shaped waveguides that permit different frequencies of light to resonate, as a function of the concentration of target molecules near the biosensor surface [1, 2]. The localization of target molecules to the surface occurs through a biochemical bond; in this case, that bond was between target toxin proteins and carbohydrate glycans, which were attached to the surface using a glutaraldeyde-based surface treatment [3].

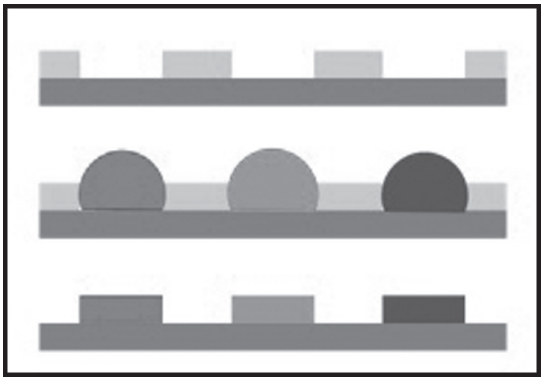
The focus of this project was to optimize the delivery of glycans and toxins to the surface of the biosensor with high resolution and high throughput, while fabricating the biosensor to be reusable and have the ability to multiplex, or detect multiple toxins at the same time. In order to fabricate a multiplexing biosensor, different glycans needed to be dispensed on the various micro-ring resonators on the biosensor surface. Therefore, a system of microfluidic channels was printed, allowing users to dispense solutions with high throughput. In order to increase resolution and reduce droplet size, an intermediate layer of 15-micron parylene was deposited above the microfluidic channels for delivering reagents, in a process known as "print-and-peel" [4]. It was shown that a system of channels to deliver glycans could be patterned on top of this parylene, and could be peeled off mechanically along with the parylene, making the biosensors reusable.

Finally, contact angle measurement revealed the need for a pumping mechanism, so a cladding layer of poly-dimethylsiloxane (PDMS) was activated with ultraviolet ozone treatment, bonded to the microfluidic channels, and tested for leaks. Successful demonstrations of these surface components have paved the way for the fabrication of a complete biosensor that can be effectively used to detect toxins.

Experimental Procedure:

In order create a system of microfluidic channels, 15-micron series 2000 SU-8 photoresist was spin-coated onto a test silicon wafer. The photoresist was then exposed at a dosage of 140 mJ/cm^2. While this was sufficient to create channels in photoresist, in order to test the print-and-peel method, a 15-micron layer of parylene was deposited, using the Specialty Coating Systems Parylene Labcoater, on top of the photoresist. In some samples, a release agent (2% micro-clean) was first lightly swabbed to facilitate the eventual parylene peeling.

The parylene was then coated with another layer of the same SU-8, which, however, needed to be exposed at 210 mJ/cm^2. This created successive layers of SU-8, separated by parylene, which could be mechanically peeled off, allowing reusability and even higher resolution (see Figure 1).



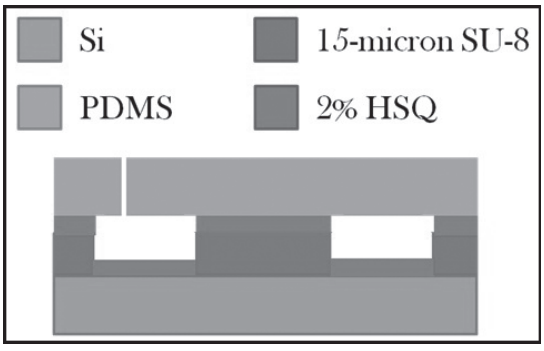
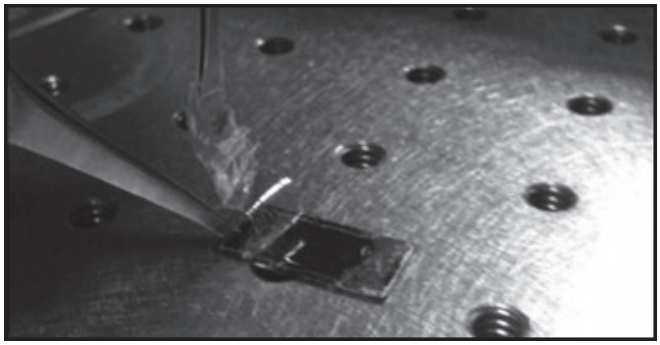


Figure 1, top: In the print-and-peel method, parylene (patterned) is etched, solutions (hemispheres) are dispensed, and parylene is then peeled.

Figure 2, bottom: Schematic of the final surface layers on the biosensor.



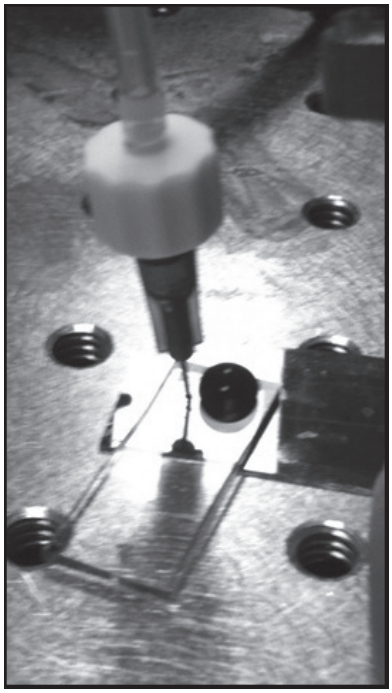


Figure 3, above: Parylene, sandwiched between two layers of SU-8, is successfully peeled off.

Figure 4, left: As solution is pumped through the microfluidic channels, some leakage occurs.

In addition, after the wafer was treated with glutaraldehyde (in order to bind glycans to the surface), the surface wettability was characterized using a contact angle goniometer. The need for a pumping mechanism was realized, and, in order to pump into the SU-8 channel, a layer of 2% hydrogen silsesquioxane (HSQ) was spin-coated on the parylene.

Then, on some samples, the HSQ surface and the layer of PDMS were activated with an ultraviolet ozone treatment for two minutes, and then, the two surfaces were mechanically bonded. The PDMS served as a cladding layer through which solutions could be pumped into the microfluidic channels (see Figure 2).

Results and Conclusions:

After the system of SU-8 microfluidic channels had been created and coated with parylene, which was itself coated with SU-8, the peeling-off of parylene was attempted. In order to facilitate this peeling-off, some SU-8-coated samples had been swabbed with 2% micro-clean. It was found that 100% of the samples swabbed with the micro-clean peeled off (see Figure 3), while only 50% of the non-swabbed samples peeled off, and in the latter case, the peeling often resulted in lower-quality channels in the lower layer of SU-8.

At the same time, it was found that the glutaraldehyde-based protocol for binding glycans increased the contact angle of water on the wafer from 52.0° to 68.1°. This increase in hydrophobicity suggested that treated microfluidic channels would be unable to carry solutions on their own; thus, a pumping mechanism was created by cladding the channels with PDMS (with a thin layer of HSQ in between to facilitate bonding), permitting a syringe to pump solutions through the microfluidic channels. Because this resulted in significant leakage, in some samples, the HSQ-coated channels and PDMS were both activated. It was empirically found that a two-minute ozone activation resulted in the strongest bond and the least leakage (see Figure 4).

Several aspects of reagent delivery have been demonstrated and optimized over the course of this project. As these components are perfected, the fabrication of a toxin-detecting photonic biosensor becomes more possible, allowing for efficient detection of glycan-binding toxins, such as ricin and the cholera toxin B, which endanger many human populations.

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