Micropore Immunosensors for Fast Disease Diagnostics

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Introduction:

Single molecule biosensors using etched silicon micropores provide an alternative to, and an advantage over, traditional immunoassays. By reducing the sample volume necessary for the assay and supplying an electrical readout of the measurements, the silicon micropore system reduces the cost, time, and chemical reagents involved in current standard immunoassays. These differences, as well as the portable nature of the biosensor, make it ideal for point-of-care diagnostics.

For this project, a microbead-based assay was performed. A constant voltage was applied across a silicon micropore filled with an ionic electrolyte solution. The pore acted as a resistor. The ionic electrolyte solution contained suspended 1 μm diameter silica beads. As a microbead entered and traveled through the pore, it increased the resistance of the pore, therefore causing a reduction in the measured current. This decrease in current, ΔI corresponds to the ratio of the diameter of the bead 'd', to the diameter 'D' and length, 'L' of the pore.

Equations:

$$(1)\ L = RA/\rho$$

$$(2)\ \Delta I = I_g(d^3/LD^2)$$

The detection of the translocation of the beads through the pore via resistive pulse measurements is similar to the method used for Coulter Counting. The silica beads and the micropore itself were then biofunctionalized with an anti-body specific to the antigen being tested for, in this case, Interleukin-6. In the presence of a sufficient concentration of the antigen, the functionalized beads bound inside of the pore and caused a sustained reduction in the measured current.

Device Fabrication and Characterization:

The silicon micropores were fabricated using a series of photolithography processes and a deep silicon reactive ion etcher. First, a pore with a desired diameter of 100 μm was patterned and etched into the backside of the silicon wafer.

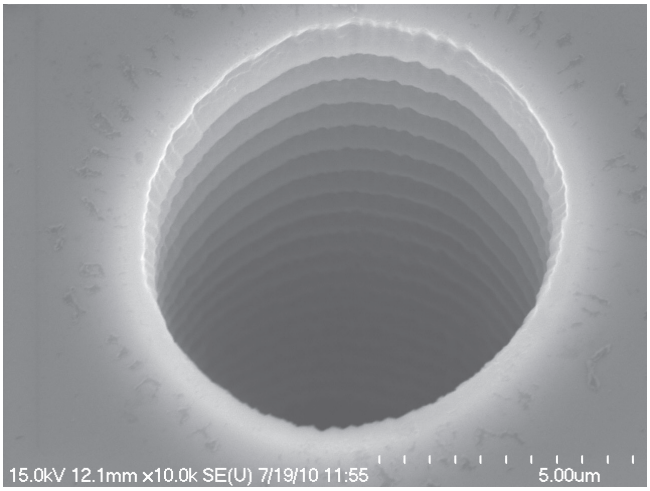

Figure 1: SEM image of frontside (8 μm) pore.

Then, a pore with a desired diameter of 5 μm was aligned, patterned, and etched on the topside of the wafer, in series with the 100 μm pore.

Due to the relationship between the measured reduction in current, bead, diameter, pore length and pore diameter, it was necessary to characterize the micropore, specifically the length and diameter of the topside pore. This was accomplished using the field-emission scanning electron microscope (FESEM) to determine the diameter, which was found to be 8 μm (Figure 1).

This value was then substituted into Equation 2, along with the measured value of electrolyte solution conductivity ρ to determine pore length, which was determined to be 32 μm. Each of these values was also substituted into Equation 1 to predict the change in current that resulted from the passing of a 1 μm bead in the fabricated micropore.

Biofunctionalization and Experimental Procedure:

The silicon micropore and the silica microbeads can be functionalized with a series of different biomolecules,

depending on the identity and specificity of the target biomarker. For this experiment, the pore and beads were functionalized with anti-Interleukin-6 (anti-IL6). This monoclonal antibody specifically binds to Interleukin-6, an antigen linked to rheumatoid arthritis and several cancers.

First, the antibody, anti-IL6, was attached to the beads. To create a lasting binding between the inorganic silica beads and organic antibodies, the beads were coated with amine group. They were incubated overnight with glutaraldehyde, which acted as a linker between the amine group and antibody, then rinsed in TRIS buffer solution, and finally, incubated for two hours with anti-IL6. The micropore was functionalized separately, using the same glutaraldehyde and anti-IL6 incubation protocol as the beads.

The functionalized micropore was sandwiched between two custom-made Teflon® chambers with wells. Bovine serum albumin was left to incubate in the wells and micropore to coat any silicon surface not covered in anti-IL6. Varying concentrations of IL6, from 200 pM to 1 μM, were added into each well and left to incubate for two hours. The IL6 bound to the anti-IL6 antibodies present in the micropore. The functionalized beads were added into the wells. Ag/AgCl electrodes were dipped in each well and a constant voltage of 400mV was applied, causing the beads to move. As the beads migrated through the pore, the anti-IL6 on their surface interacted with the IL6 present in the micropore. This caused the beads to stick inside the micropore. The current through the pore was measured, and bead sticking and binding events were recorded.

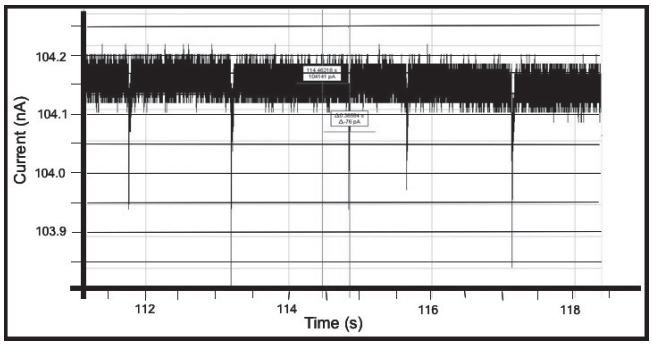

Figure 2: Translocation of amine-coated beads through an unfunctionalized pore.

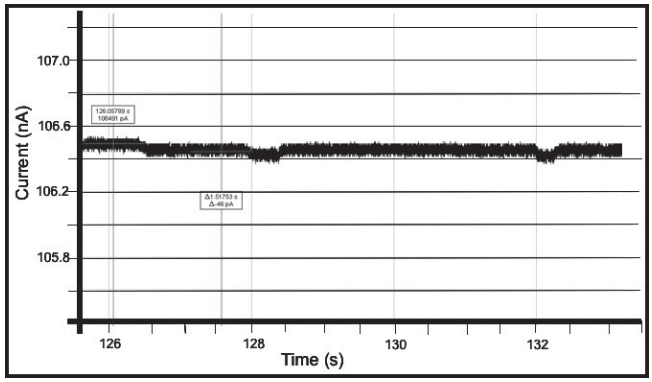

Figure 3: Bead binding and bead sticking events with functionalized beads in functionalized pore with 10 nM IL6.

Results and Conclusions:

In the experiments, we were able to observe the translocation of beads through an unfunctionalized pore (Figure 2). After biofunctionalizing the beads and pore, bead translocation events were still observed in the presence of low concentrations of IL6. Increasingly higher concentrations of IL6 resulted in more frequent and pronounced bead sticking and blocking events (Figure 3).

It was found that very high concentrations of IL6, in the micron range, caused the blocking of the pore in the absence of beads. This was due to the multiple binding sites on the antigen, which caused it to bind to itself and multiple antibodies, creating bridge-like structures and blocking the pore.

Through this series of experiments, it was found that a lower concentration of beads and antigen, and an increase in incubation time with glutaraldehyde and anti-IL6 resulted in greater consistency of data. These experiments showed that the detection of biomarkers, such as Interleukin-6, can be achieved using a silicon micropore and bead-based assay.

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References:

[1] Mathew, T.; Joshi, P.; Prasad, S.; Goryll, M.; Spanias, A.; Thornton, T.J.; "Silicon Based Pore Systems for Emerging Biosensor Applications"; ASME Congress and Exposition, 2, 263-270 (2009).