

A Wireless Remote Biosensor for the Detection of Biological Agents

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Abstract:

A biosensor capable of wireless data transfer has been designed, fabricated, and tested. The sensor uses aptamers immobilized to a pair of interdigitated electrodes to detect *Staphylococcus enterotoxin B* (SEB). We have designed a solvent-free method to fabricate sensor chips comprised of interdigitated gold electrodes (150 nm thickness) evaporated onto a silicon substrate. The electrodes are functionalized with thiol-modified aptamers that were chosen based upon their bioaffinity for the target analyte. Upon contact with SEB, discriminative binding between SEB and electrode-bound aptamers occurs thereby producing an observable change in the electrical properties of the electrodes. A minimum two-fold increase in capacitance resulted from sensor exposure to SEB in comparison to exposure to non-specific proteins, thereby demonstrating device sensitivity and selectivity for the target analyte. Ultimately, we envision that this device may be used for remote detection of biological agents, thereby limiting the exposure of humans to potential biological threats.

Introduction:

A device with the ability to sensitively and selectively detect target molecules is a technology of interest because of its applications in military and public health sectors. Electronic biosensors may be manufactured at small dimensions with small features while necessitating very low power. They may also be connected to a system consisting of a data acquisition and a base station module that wirelessly transmits data from the sensor to a computer for analysis. Though electronic sensors have shown sensitivity, selectivity has been a persistent issue and has forced current biosensors to rely on optical methods such as surface plasmon resonance and molecular flow cytometry which require on-site illumination and power sources. An electronic biosensor with wireless capabilities will introduce a new generation of nanoscale biosensors.

Experimental Procedure:

Sensor Fabrication. 2 μm of Parylene C were vapor-deposited onto a 100 mm diameter silicon wafer. Two coats of photoresist were spun and soft-baked. Sensor electrodes were UV-patterned onto the wafer before post-exposure baking and development. Oxygen plasma reactive ion etching (RIE) was then employed as a parylene etchant. Electron gun

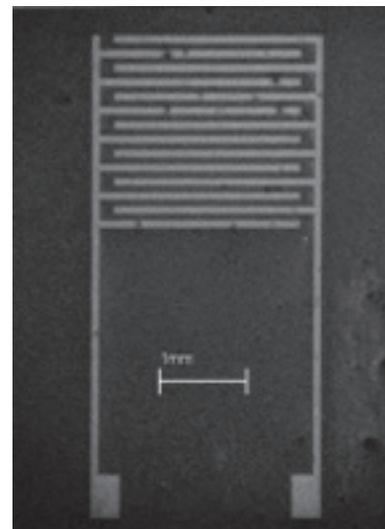


Figure 1: SEM of gold interdigitated electrodes with 100 μm interdigital spacing.

evaporation was used to deposit 10 nm titanium and 150 nm gold on top of the parylene in unexposed areas and on top of Si in exposed areas, the titanium layer facilitating adhesion at the gold-silicon interface. Residual parylene was peeled from the wafer using tweezers. Wafer dicing yielded individual chips that were wire-bonded to copper breadboard circuits. In sensor functionalization, thiol-modified nucleic acid probes with an SEB bioaffinity (“spiegelmers”) were used because of their resistivity to natural enzymatic degradation. 20 μL of 50 mM spiegelmer solution were pipetted onto the sensors and incubated for 8 hours at ambient temperatures to ensure spiegelmer immobilization onto the electrodal surface via thiol chemistry (Figure 2).

Electrical Characterization. A wire-bonded sensor chip was connected to a capacitance meter before a baseline reading was taken with phosphate buffer. After 10 minutes of equilibration, the chip was exposed to SEB. Subsequently, the chip was rinsed three times with buffer. A post-wash reading was taken while the sensor was again exposed to buffer. Residual SEB-spiegelmer binding was expected to be reflected in capacitance. The chip was incubated with 20 mM sodium hydroxide (NaOH) for 5 minutes and washed three times with buffer before a final buffer reading was taken. If

the altered capacitance was due to SEB-spiegelmer binding, the post-NaOH reading should return to baseline. The sensor was kept in the dark during characterization because previous results indicated that the sensors are light-sensitive due to photoelectric effects.

Results and Conclusions:

Preliminary electrical characterization showed sensor selectivity towards SEB, reporting nearly a two-fold increase in capacitance upon SEB addition to the sensor relative to the capacitance upon sensor exposure to bovine serum albumin (BSA). Water showed a (262 ± 15) nF capacitance increase from baseline and a (109 ± 15) nF disparity from the BSA control whereas phosphate buffer showed a (298 ± 28) nF capacitance increase and a (188 ± 48) nF disparity. Furthermore, a slightly-elevated capacitance was recorded after an initial wash which dropped back to baseline post-NaOH incubation (Figure 3). This re-occurring capacitance pattern is indicative of SEB-spiegelmer binding. These results were seen in both de-ionized water and $10\text{-}\mu\text{M}$ phosphate buffer, although significant salt-shielding effects were detected as buffer concentration increased possibly because of interactions between salts and nucleic acids at higher concentrations.

Furthermore, upon addition of a protein cocktail containing BSA, green fluorescent protein (GFP), and SEB in water, there was an initial capacitance increase of (288.5 ± 0.5) nF. Upon system stabilization, the capacitance settled at 237-nF whereas the control cocktail (BSA+GFP) showed a 61-nF increase from baseline, a 176-nF disparity (Figure 4). The capacitance returned to baseline post-NaOH incubation, demonstrating SEB selectivity during exposure to protein arrays and proving viability for practical sensing applications.

Voltaic characterization using a wireless system was performed as well; however, the data acquisition module was flawed in design. Nevertheless, the sensors were capable of successful wireless data transfer and preliminary voltaic results showed efficient SEB detection with the characteristically-elevated voltage readings indicative of SEB-spiegelmer binding.

Future Work:

The next steps involve acquiring consistent electrical characterization results with phosphate buffers that have environmentally-relevant salt concentrations and conducting voltaic experiments using the wireless biodetection system upon re-designing of the data acquisition module.

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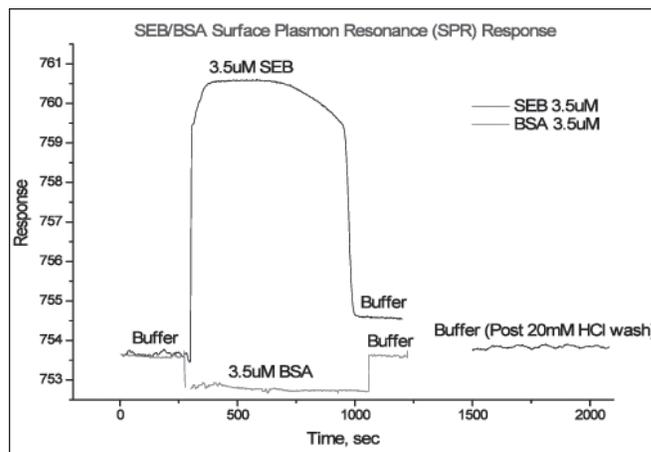


Figure 2: SPR confirmed effective functionalization of electrodes by spiegelmers and spiegelmer functionality.

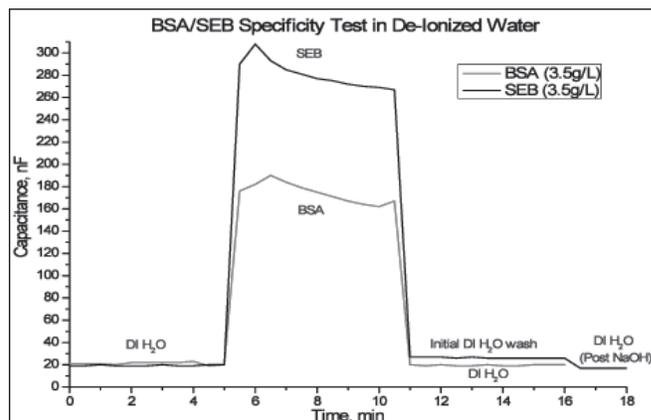


Figure 3: Specificity assays. There is a two-fold capacitance increase upon sensor exposure to SEB with elevated post-wash capacitance.

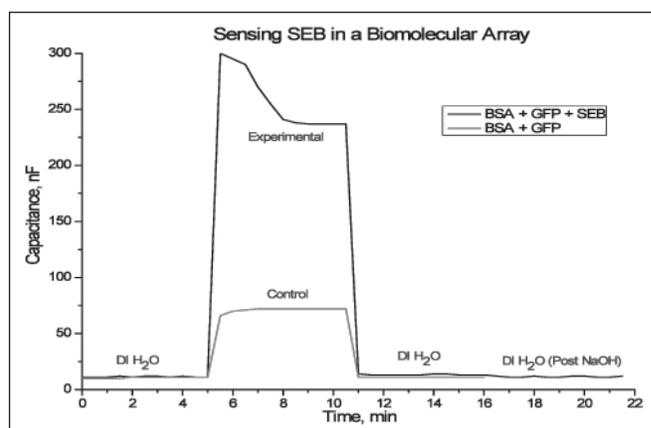


Figure 4: Selectivity assay. The BSA+GFP+SEB capacitance response was four times greater than the BSA+GFP capacitance response, demonstrating sensor selectivity for SEB.