FABRICATION OF SILICON NANOPLATE AND NANOWIRE BIOSENSOR ARRAYS WITH HIGH SPECIFICITY AND SUB-PICOMOLAR LIMITS OF DETECTION

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ABSTRACT

Diagnostic platforms based upon microfluidics coupled to ion-selective field effect transistors (ISFET's) offer great potential to address some world health goals since they can be made portable, low-cost, miniaturized, and sensitive. We have fabricated arrays of accumulation mode, fully depleted silicon nanowires (50 nm width) and nanoplates (2um width) of 30nm thickness on SOI substrates, all of which are individually addressable. The devices respond to changes in charge density well and have excellent time stability in fluid. By coupling probe proteins to the ISFET surface, we are able to detect analytes down to pg/mL levels.

KEYWORDS: nanowire, biosensor, biointerfacing, monolayer

INTRODUCTION

Silicon based biosensors have proven to be extremely useful tools for a variety of bio-analytical applications. After the first introduction of the adaption of a standard Metal-Oxide-Semiconductor Field Effect Transistor (MOSFET) for physiological measurements in 1970 by Bergveld [1] and the initial demonstration of the detection of pH [2], ISFETs have been used for a variety of other applications, such as the detection of various proteins [3-8], the indication of immunological reactions [9], and the monitoring of cell activity [10-11].

Silicon field effect devices with dimensions ranging from 2um width (nanoplate) to 50nm width (nanowire) were fabricated in house using a top-down fabrication approach and a silicon dioxide gate dielectric. The surface charge sensitivity of both nanowires and nanoplates versus pH was calculated by extraction of the threshold voltage and nanoplates were observed to have lower sensitivity than nanowires. The devices also exhibited good time stability in fluid with varying pH changes, and exhibited the maximum sensitivity to charge change in the subthreshold regime, as expected for a field effect device. Using the monofunctional silane protocol developed for protein conjugation, these devices were functionalized with antibodies acted against Mouse immunoglobulin G (IgG). By monitoring the change in the threshold voltage of the devices, we were able to sense protein concentrations of Mouse IgG down to 10fM, with subpicomolar sensitivities being readily achieved. Control experiments using PEG 100,000 and Rabbit IgG yielded no change, within error of the measurements, indicating the devices can perform with high selectivity toward the analyte in question. By decreasing the gate dielectric thickness, and thus increasing the capacitance, we were able to increase the percent change of the threshold voltage to the same analyte concentrations, increasing the sensitivity from picomolar to femtomolar.

THEORY

The charge density in the silicon channel of an ISFET is related to surface potential via:

$$\Delta\sigma_{Silicon} = C_D \Delta\psi_0 \approx \frac{\varepsilon_D \varepsilon_0}{t_D} \Delta\psi_0 \quad (1)$$

where C_D is the dielectric capacitance, $\Delta\psi_0$ is the change in surface potential at the oxide/fluid interface, ε_D is the dielectric constant of the gate dielectric (3.9 for SiO_2), and t_D is the thickness of the dielectric. The coupling of changes in potential at the surface to changes in charge in the silicon, given by the dielectric capacitance, is a critical factor that ultimately determines device sensitivity. Moreover the changes in surface potential are not always Nernstian in behavior and can be related through the following equation:

$$\Delta\Psi_o = -2.3\alpha \frac{RT}{F} \Delta pH_{bulk} \quad (2)$$

where α is a sensitivity factor which accounts for this non-ideal coupling. Proteins in solution are usually charged, with their charge depending on the isoelectric point of the protein. Thus, when they bind to the surface they alter the surface potential in a similar manner as a change in pH, changing the charge density in the channel, and thus changing the conductance in the channel and the inherent threshold voltage of the device. This principle allows for silicon nanowires and ISFET's in general to operate as biosensors.

EXPERIMENTAL

Top down fabrication was utilized in this work to create the silicon nanoFET devices. The fabrication flow began with bonded Silicon on Insulator (SOI) wafers. Electron beam lithography and optical lithography were used to define the nanowires and nanoplate. The source and drain regions were doped with boron (simulated doping ~10^{19}/cm3), the the SiO2 gate dielectric was formed via dry oxidation to a thickness of ~150 Å. A 4000 Å thick passivation layer of PECVD

nitride was deposited over the entire wafer, and a dry CF4 RIE etch was then used to etch the passivation layer directly over the device active area. During this step, the passivation layer over a platinum electrode close to the devices was also etched to expose the on-chip fluid gate.

The chips were placed into ceramic packages (Global Chip Materials 28 pin lead sized brazed package) and microfluidic channels were aligned to the chip using a mask aligner. Individual devices were contacted using wire bonding to the package. Teflon tubing was inserted into the ends of the channel, and the entire setup was covered with slow drying epoxy to insulate the devices and to mitigate fluid leakage issues. Fluid was exchanged using the tubing and syringe pumps with syringes containing the various different solutions for pH and protein sensing. Electrical current measurements and applied biases were controlled by a semiconductor parameter analyzer (Keithley 4200).

RESULTS

We have fabricated arrays of accumulation mode, fully depleted silicon nanowires (50 nm width) and nanoplates (2um width) of 30nm thickness on SOI substrates, all of which are individually addressable. The top down images of the sensors are shown in Figure 1A(left) and 1B (left), while Figure 1A (right) and 1B (right) show corresponding SEM x-sections of the released devices. We also developed a vapor based silanization method which uses monofunctional alkoxysilanes to form high density, subnanometer monolayers for biointerfacing.[12] After conjugation to a polyethylene glycol (PEG) linker, we were able to achieve a high density conjugation, denoted by a large shift in the device threshold voltage. **Figure 1C** shows the drain current vs. gate voltage (Id-Vg) curve of a nanoplate sensor before and after conjugation of the Texas Red goat anti-mouse IgG antibody in 1mM NaHCO3, pH 8.4. Fluorescent micrographs taken before and after attachment of the antibody verify the high density conjugation. We tested the device sensitivity using immunoglobulins (IgG's) because their properties are similar to other membrane bound target proteins, such as receptor tyrosine kinases.

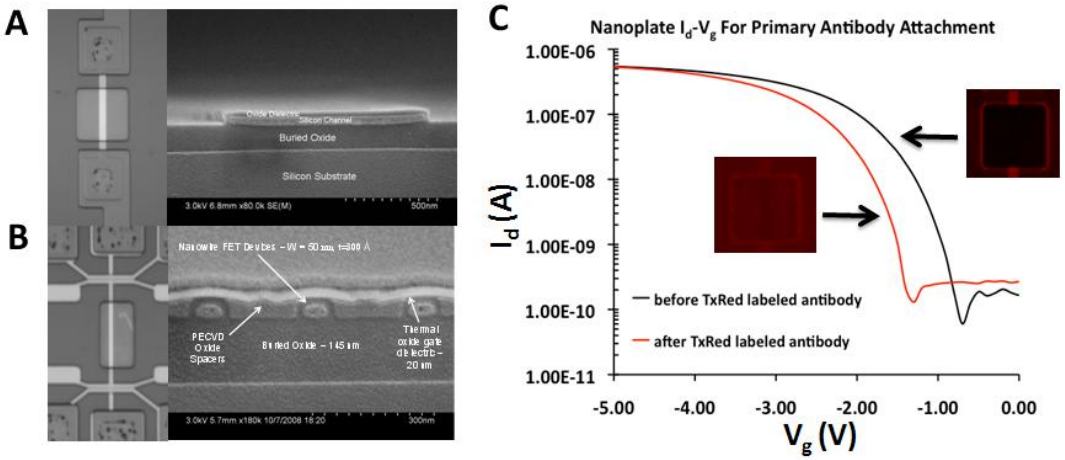

Figure 1. Top down micrograph of nanoplate (A, left) and nanowire (B,left) sensor inside the release window, and SEM x-sections of the nanoplate (A, right) and nanowire (B,right) sensors. Id-Vg curves of the primary antibody attachment are in (C) along fluorescent micrographs from before and after attachment.

The analytes were flown onto the sensor with the Id-Vg curves taken using the fluid gate, and showed increasing threshold voltage shifts for increasing mouse IgG concentrations (**Figure 2A**). A buffer rinse showed a small recovery, as expected. The devices showed little response (<10mV) to 1pM concentrations of the nonspecific analytes (**Figure 2B**), while exhibiting a ~100 mV shift for the equivalent mouse IgG concentration. By thinning the top gate oxide to ~80Å we were able to achieve 8fM analyte concentrations by monitoring the threshold voltage (**Figure3A**). In comparison to a 150Å gate oxide, the threshold shifts are larger (**Figure3B**) and may lead to even higher sensitivity for clinical applications.

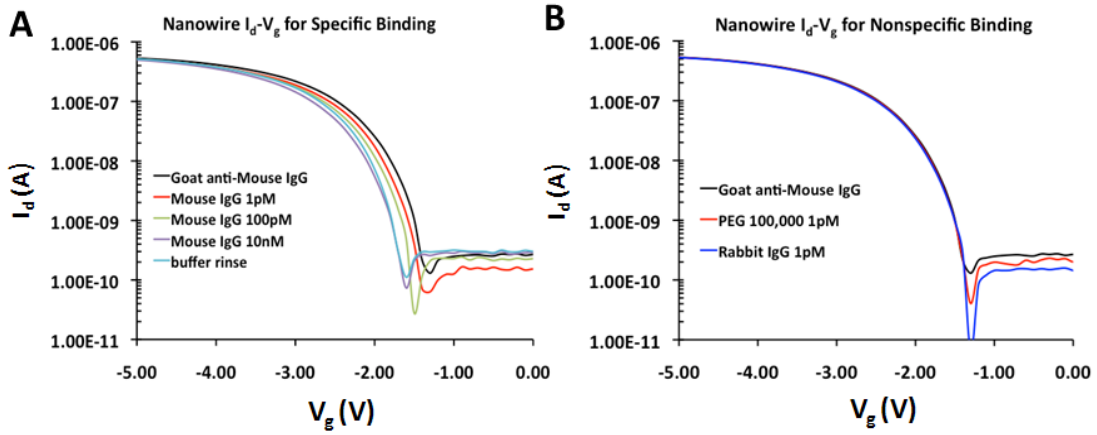

Figure 2. Id-Vg curves of a nanowire for various mouse IgG concentrations and the buffer rinse are shown in (A). The threshold voltage shifts to left with increasing protein concentration. Id-Vg curves for 1pM concentrations of nonspecific binding analytes are in (B), showing a lack of threshold response.

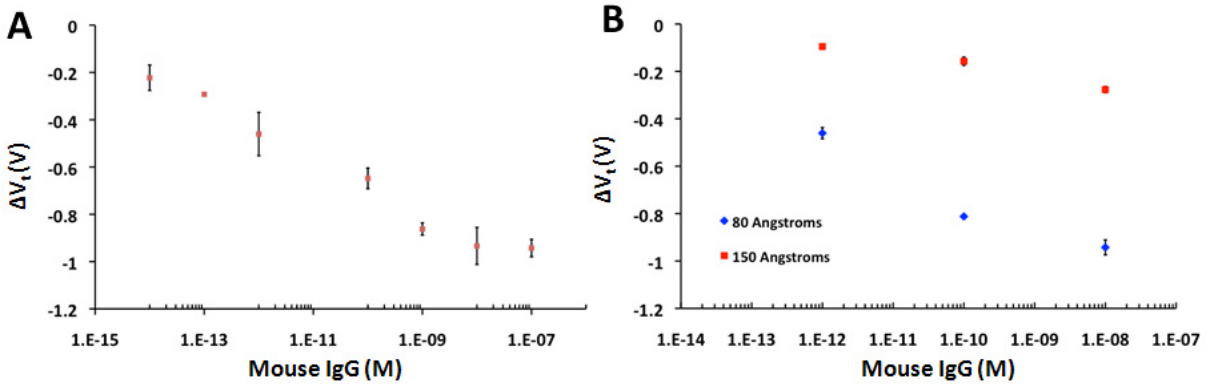

Figure 3. Change in threshold voltage of a nanowire for mouse IgG concentrations from 8fM to 80nM for an 80 angstrom thick oxide is in (A). A comparison between the threshold voltage shift for an 80 angstrom and 150 angstrom oxide thickness is shown in (B) for the same mouse IgG concentrations. The shifts for the 80 angstrom oxide are much larger than for the 150 angstrom oxide.

CONCLUSION

We have fabricated robust SOI silicon nanowires arrays using top-down fabrication techniques. The devices show sensitivity to charge via pH experiments, and good stability over time when introduced to solutions of different pH values. Using our vapor based functionalization method, we were able to conjugate the probe antibodies in high affinity to the surface, and detect the target analytes in femtomolar concentrations. Moreover, the chemistry and the nanowires were shown to have high specificity, with little response to the introduction of similar antibodies and poly(ethylene glycol)

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REFERENCES

[1] P. Bergveld, "Development of an Ion-Sensitive Solid-State Device for Neurophysiological Measurements," *Ieee Transactions on Biomedical Engineering,* vol. 17, pp. 70-71, 1970.

[2] P. Bergveld, "Development, Operation, and Application of Ion-Sensitive Field-Effect Transistor as a Tool for Electrophysiology," *Ieee Transactions on Biomedical Engineering,* vol. 19, pp. 342-351, 1972.

[3] B. H. Vanderschoot and P. Bergveld, "Isfet Based Enzyme Sensors," *Biosensors,* vol. 3, pp. 161-186, 1987.

[4] B. H. Vanderschoot and P. Bergveld, "The Ph-Static Enzyme Sensor - an Isfet-Based Enzyme Sensor, Insensitive to the Buffer Capacity of the Sample," *Analytica Chimica Acta,* vol. 199, pp. 157-160, Aug 15 1987.

[5] P. Bergveld, "A Critical-Evaluation of Direct Electrical Protein-Detection Methods," *Biosensors & Bioelectronics,* vol. 6, pp. 55-72, 1991.

[6] J. Kruise, *et al.*, "Detection of Charged Proteins by Means of Impedance Measurements," *Sensors and Actuators B-Chemical,* vol. 6, pp. 101-105, Jan 1992.

[7] J. C. Vankerkhof, *et al.*, "Development of an Isfet Based Heparin Sensor Using the Ion-Step Measuring Method," *Biosensors & Bioelectronics,* vol. 8, pp. 463-472, 1993.

[8] W. Olthuis, *et al.*, "Characterization of Proteins by Means of Their Buffer Capacity, Measured with an Isfet-Based Coulometric Sensor-Actuator System," *Biosensors & Bioelectronics,* vol. 9, pp. 743-751, 1994.

[9] R. B. M. Schasfoort, *et al.*, "Modulation of the Isfet Response by an Immunological Reaction," *Sensors and Actuators,* vol. 17, pp. 531-535, May 17 1989.

[10] W. H. Baumann, *et al.*, "Microelectronic sensor system for microphysiological application on living cells," *Sensors and Actuators B-Chemical,* vol. 55, pp. 77-89, Apr 20 1999.

[11] M. Lehmann, *et al.*, "Non-invasive measurement of cell membrane associated proton gradients by ion-sensitive field effect transistor arrays for microphysiological and bioelectronical applications," *Biosensors & Bioelectronics,* vol. 15, pp. 117-124, Jun 2000.

[12] B. Dorvel, *et al.*, "Vapor-Phase Deposition of Monofunctional Alkoxysilanes for Sub-Nanometer-Level Biointerfacing on Silicon Oxide Surfaces," *Advanced Functional Materials,* vol. 20, pp. 87-95, 2010.