Towards *in vivo* biosensors for low-cost protein sensing

A. Ramesh, F. Ren, P.R. Berger, P. Casal, A. Theiss, S. Gupta and S.C. Lee

In vivo biosensing requires stable transistor operation in high-salt concentration bodily fluids while exhibiting impermeability to mobile alkali ions that would otherwise render the metal-oxide-semiconductor (MOS) threshold voltage to drift. Metal oxide semiconductor capacitor structures using Al_2O_3 as the gate dielectric were soaked in a sterile physiological buffer solution (PBS) up to 24 hours and for thicknesses from 100 to 10 nm. The triangular voltage sweep technique characterised alkali ion penetration, and measured no detectable alkali ions for the Al_2O_3 capacitors. By contrast, the dose of alkali ions in silicon dioxide MOS capacitors steadily increased with increasing soak times in the PBS solution.

Introduction: In the past two decades, research and development of biosensors and their applications has undergone remarkable growth [1]. Electrical biosensors have been applied to different target applications, including glucose, pH and protein, detection and measurement.

Protein biosensors are of particular interest and importance in modern medicine for their role in the early detection and diagnosis of disease, for instance cancer. Protein biosensors based on different semiconductor materials have been explored, such as Si [2], AlGaN/GaN [3], and carbon nanotubes [4]. Si-based protein biosensors are low-cost and easily integratable onto a small chip atop a diagnostic needle complete with readout circuitry. However, Si-based protein biosensors suffer from long-term electrical drift and instability due to the diffusion of ions from high osmolarity biological buffers into the gate oxides.

Long-term stable and low-cost Si-based *in vivo* protein biosensors are needed but their translation to *in vivo* sensing applications introduces big challenges. The typical *in vivo* physiological environment contains Na^+ and K^+ ions that can be incorporated into the dielectric oxide of the field effect transistor and contribute to mobile charge [5]. These mobile ions shift within the active device depending upon voltage, causing a variable drift in the transistor threshold voltage, resulting in inaccurate protein sensing. Hence, impermeability to mobile alkali ions is key for stable transistor operation. Alternate dielectric oxides with impermeability to alkali ions, or reduced ion mobility, can help mitigate this issue. In this Letter, alkali ion penetration is cited as a critical factor for threshold voltage instability in biosensors using SiO_2 as a gate dielectric, and impermeability of ions through Al_2O_3 in high ion concentration (0.15M) physiological buffer solution is demonstrated. This allows the future realisation of low-cost Si-based *in vivo* biosensors.

Experiments: A representative field effect transistor (FET) protein sensor is illustrated in Fig. 1. For use as biosensors, the gate metal is replaced by a functionalised oxide surface with analyte-specific affinity reagents (receptors), leaving the gate 'floating' in direct contact with the solution being tested. Binding of charged analytes (protein to be detected) to these surface receptors results in a change in the charge induced in the channel, which manifests as a change in the drain current. Since a gate metal is absent, a voltage is applied to the electrolyte through a reference electrode (RE) to shift the baseline transistor bias condition and maximise transistor gain.

A simple MOS capacitor can be effectively used to determine the presence of mobile ions, such as sodium (Na^+) ions, in the oxide. The typical structure of a MOS capacitor is as shown in the inset of Fig. 1. In this study, thermally grown silicon oxide (SiO_2) is replaced by aluminium oxide (Al_2O_3) deposited by the atomic layer deposition (ALD) process. The Al_2O_3 deposition was carried out with trimethylaluminum (TMA) and water as precursors at 300°C using a Picosun Sunale^{TM} reactor. The substrates used are moderately doped ($\sim 10^{16}$ cm^{-3}) p-type silicon (100) wafers. The ALD pulsing sequence for one cycle was 0.1 s TMA pulse, 4 s N_2 purge, 0.1 s H_2O pulse, and 4 s N_2 purge. Typical ALD deposition rates of the 0.8 Å/cycle were obtained. The samples were then annealed at 700°C in nitrogen ambient. Aluminium metal was deposited on the topside and patterned by photolithography and lift-off to obtain square electrodes with various areas of 275 × 275, 550 × 550, 1100 × 1100, 1650 × 1650 and 2200 × 2200 μm^2. The square electrodes were alternatively designed, some with holes and slots to permit various levels of ion permeation. Control electrodes were also included with continuous metal and no metal. Finally, aluminium metal was deposited on the backside of the wafer to complete the capacitor fabrication, followed by a post-metallisation anneal at 450°C for 10 min in nitrogen ambient. The ALD Al_2O_3 dielectric constant was calculated to be 8.65 from *C-V* measurements with a thickness of 103 nm.

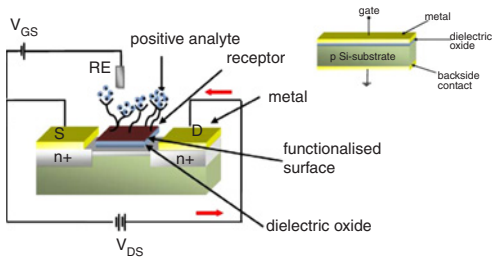

Fig. 1 *Field effect transistor protein biosensor*

A sensing channel connects the source (S) and drain (D) with a reference electrode (RE). When a target protein binds to the receptor, it induces charges in the substrate, causing a change in the current flow between the source and drain
Inset: Typical structure of a MOS capacitor used in this study

Thermally grown silicon oxide (SiO_2) was used as the control sample. Dry silicon oxide was grown in an atmospheric tube furnace at 1050°C with an oxygen ambient followed by a 20 minute nitrogen anneal at the same temperature. The SiO_2 oxide thickness was measured to be 116 nm with a calculated dielectric constant of 3.8.

The *in vivo* physiological environment can be simulated by conducting experiments in physiological buffer solutions (pH 7.4, 0.15 M Na^+, K^+). Natural *in vivo* protein environments contain comparable concentrations of alkali ions at a similar pH. Hence, immunity of transistor electrical response to these environments serves as a viable proof of the applicability of Si-based FET sensors for *in vivo* measurements.

Permeation of mobile charges into the oxide can be quantified using the triangular voltage sweep (TVS) method. The MOS sample is heated to a temperature ($\sim$250°C) to increase ion mobility. The MOS capacitor is stressed for 5 minutes with a 1 MV/cm electric field across the oxide, moving all the mobile ions to one side of the capacitor plate. A triangular voltage ramp is subsequently applied to the gate of the capacitor. A quasi-static capacitance-voltage *C-V* measurement is performed. As the polarity of voltage changes, a displacement current due to mobile ion movement, results in a peak in the measured capacitance. Next, a high frequency *C-V* measurement is performed, where the ions do not have sufficient time to respond, and no significant peak due to mobile ions is observed. Using this as the baseline, the area between these two curves (high frequency and low frequency) is determined by integration to obtain the mobile ion charge density within the oxide. Finally, MOS capacitors with ALD Al_2O_3 and thermal SiO_2 gate dielectrics were soaked in a PBS solution for varying amounts of time and subsequently measured by the TVS technique.

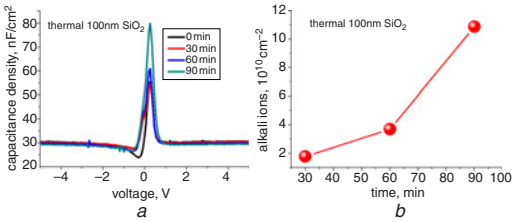

Fig. 2 *Triangular voltage sweep measurements of typical thermal SiO_2 MOS capacitor at 250°C (Fig. 2a). Relationship between increases in alkali ions concentration with increasing soak times in PBS for control SiO_2 based capacitor (Fig. 2b); line is joins of measured data*

Results: Fig. 2*a* shows the result of TVS measurements for a typical 100nm SiO_2 MOS capacitor. TVS measurements were conducted prior to dipping in PBS solution and after soaking in PBS for 30, 60, and 90 minutes. It should be noted that thermal SiO_2 shows a mobile ion peak prior to soaking in PBS due to incorporation of some alkali ion contamination from the tube furnace during thermal oxidation. Additionally, as the soak time in the PBS solution is increased, a clear linear increase in the mobile ion peak is observed in SiO_2. This indicates

significant penetration of ions from the PBS solution into the SiO_2 oxide. The area between consecutive curves quantifies the increased mobile charge (alkali ions) after each soak and is determined by numerical integration. Table 1 and Fig. 2*b* tabulate the increase in alkali ion penetration into SiO_2 MOS capacitors with increasing soak times in the PBS solution.

Table 1: Relationship between increased alkali ion concentration into thermal SiO_2 oxide (~ 100 nm) and PBS soak times

Time (min)	0	30	60	90
Δ[Alkali ions] $\times 10^{10}$ cm^{-2}	0	1.77	3.69	10.87

The experiment was then repeated with a 100 nm-thick ALD Al_2O_3 gate dielectric. The results are depicted in Fig. 3*a*. No response due to alkali ion penetration is observed up to 24 hours soak time. This was consistent amongst all four electrode configurations, floating-gate, slots, holes, and opaque. Reduction in oxide thickness provides an additional benefit of increasing capacitance, hence increased sensitivity to analyte charge. Hence, MOS capacitors with reducing ALD Al_2O_3 oxide thicknesses were also fabricated and soaked in PBS as described above. Figs. 3*b–d* depict the TVS measurement results for 50, 25, 10 nm Al_2O_3 thicknesses, respectively. Again, no mobile ion response was observed for soak times in PBS up to 24 hours for *any* of these thinner Al_2O_3 oxide thicknesses, regardless of electrode perforations.

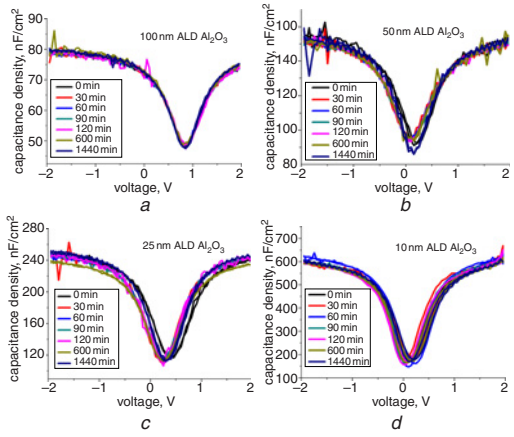

Fig. 3 *Triangular voltage sweep measurements of MOS capacitors with Al_2O_3 dielectric at 250°C for 100, 50, 25, and 10 nm oxide thicknesses (Figs. 3a–d, respectively)*

Note the absence of any time varying mobile ion peak superimposed on the baseline *C-V* waveform that instead shows a natural progression of depletion and inversion in the channel

Conclusion: Silicon *in vivo* protein biosensors suffer from long-term electrical drifting and instability due to the contamination of alkali ions from high osmolarity biological buffers. In this reported work, a Si based MOS capacitor with a high-k Al_2O_3 dielectric deposited by ALD has been fabricated. High-k dielectric layers not only prevent alkali ions diffusion from high osmolarity biological buffers into the gate oxides but also result in enhanced device sensitivity due to increased electrostatic coupling. MOS capacitors with Al_2O_3 gate dielectric show no measurable peak before and after soaking in the PBS solution, indicating no alkali ions penetration for various oxide thicknesses of 100, 50, 25, 10 nm. Thus, Si-based FETs with Al_2O_3 as their oxide dielectric are attractive candidates for the realisation of future low-cost biosensors for *in vivo* protein sensing.

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One or more of the Figures in this Letter are available in colour online.

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