

Short communication

Surface engineering for enhancement of sensitivity in an underlap-FET biosensor by control of wettability

Jee-Yeon Kim^a, Kyungyong Choi^a, Dong-Il Moon^a, Jae-Hyuk Ahn^a, Tae Jung Park^b, Sang Yup Lee^c, Yang-Kyu Choi^{a,*}^a Department of Electrical Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea^b Department of Chemistry, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul 156-756, Republic of Korea^c Department of Chemical & Biomolecular Engineering, Department of Biological Sciences, Department of Bio & Brain Engineering, Bioinformatics Research Center, Center for Systems & Synthetic Biotechnology, and Institute for the BioCentury, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

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ABSTRACT

The present work aims to improve the sensitivity of an electrical biosensor by simply changing a surface property of the passivation layer, which covers the background region except for the sensing site for electrical isolation among adjacent interconnection lines. The hydrophobic passivation layer dramatically enhances the sensitivity of the biosensor when compared with a hydrophilic passivation layer. A revamped metal oxide semiconductor field-effect transistor (MOSFET), which has a designed underlap region between a gate and a drain, is used as the electrical biosensor. A thin film of CYTOPTM and silicon nitride is used as the hydrophobic and hydrophilic passivation layers, respectively. The surface antigen and its specific antibody of the avian influenza virus were employed as the probe and target biomolecule, respectively, to confirm the enhanced sensitivity of the proposed biosensor. By using hydrophobic passivation, the limit of detection of the biosensor was improved up to 100-fold compared with that resulting from hydrophilic passivation. Therefore, a simple surface engineering to control surface wettability can notably improve the sensitivity of a biosensor without additional efforts, such as modifying the sensor structure, optimizing the bio-treatment protocol, or increasing the binding yield between a probe and its target, among other efforts.

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1. Introduction

The semiconductor-based biosensor, configured as a field-effect transistor (FET), is emerging as a powerful platform for the direct detection of biological and chemical species without a laborious labeling process. In particular, silicon nanowires (Cui et al., 2001; Stern et al., 2007; Ahn et al., 2010) and planar silicon devices (Bergveld, 2003; Im et al., 2007) have been demonstrated to be successful label-free biomolecule detection platforms. These examples have been highlighted due to their high sensitivity (Cui et al., 2001; Ahn et al., 2010), scalability, reliability, reproducibility, or possibility of on-chip integration (Stern et al., 2007; Gu et al., 2009; Lee et al., 2010). One of the important features of a biosensor is its sensitivity, the ability to discern a minimum concentration of target biomolecules. For this reason, many researchers have studied various methods, materials, and structures for optimizing this quality. However, we have found that there is little attention paid to the property of surface wettability in designs seeking to improve sensitivity. In an electrical biosensor, the interconnection lines, which connect the electrodes of a

device and the outer corresponding probing pads, are indispensable. Thus, passivation layers covering the buried interconnection lines are used for electrical isolation from adjacent interconnection lines and analytic solutions (Elfström et al., 2008; Stern et al., 2007). The most popularly used materials for passivation are silicon nitride (Si₃N₄) (Patolsky et al., 2006) and polymer (Elfström et al., 2008) because they have been shown to seal the biosensors from ambient solution or moisture satisfactorily. However, it does not appear that these studies have taken the hydrophobicity of the passivation layer into account; this is important because this property can significantly affect a sensor's performance. Because a hydrophobic surface repels water molecules, these molecules have a tendency to move from the hydrophobic to the hydrophilic surface. Consequently, the target biomolecules in the analytic solution can be confined preferentially to a hydrophilic surface enclosing a sensing region on the biosensor. Thus, it is expected that the sensitivity can be significantly increased by confining the target biomolecules to the sensing site.

In the present study, we demonstrate the effect of a hydrophobic passivation surface, which enhances the sensitivity in terms of the change in the current of the FET-based biosensor caused by the biomolecules and the least detectable concentration. An underlap-FET biosensor, which is a planar FET structure

* Corresponding author. Tel.: +82 42 350 3477; fax: +82 42 350 8565.
E-mail address: ykchoi@ee.kaist.ac.kr (Y.-K. Choi).

having poly-crystalline silicon (poly-Si) gate electrode and containing an intentional offset (underlap) between the gate and the drain (Lee et al., 2010), was proposed to improve sensor performance. The offset region is a sensing site for which the channel potential is subtly influenced by the presence of biomolecules. Since poly-Si gate electrode controls the channel potential, reference electrode usually used in an ISFET is not necessary for the underlap-FET. Two types of passivation layers, Si_3N_4 for the hydrophilicity and CYTOP™ (Asahi glass company, Japan) for the hydrophobicity, were used for a controlled comparison of the sensitivity because sensitivity is influenced by the surface wettability of the passivation layer. Therefore, all other subsequent steps and sensor structures in the present study follow previous works, except for the passivation layer.

2. Experimental methodology

Throughout the experiment, the underlap-FET biosensor was employed to characterize the biosensor performances. The underlap region between the gate and the drain served as a sensing region. When negatively charged biomolecules are immobilized on the underlap region, the electron paths from the source to drain become pinched and, consequently, the drain current decreases, as a result of an increased barrier height of channel potential. For positively charged biomolecules, this trend is reversed. Therefore, the target biomolecules can be detected by detecting a change in the drain current before and after the introduction of the biomolecules. As previously described, every interconnection line that connects to the gate, the source, and the drain should be electrically isolated from the analytic solution. Therefore, a passivation layer (Kim et al., 2012; Choi et al., 2012) is necessary, and its surface wettability may result in an enhancement of sensitivity.

2.1. Fabrication

The underlap-FET biosensor was fabricated on a p-type bulk silicon wafer. To suppress the device-to-device leakage current, the local oxidation of silicon (LOCOS) was utilized. Afterwards, the electrical channel region was defined by photoresist to accommodate the underlap region, and the source and drain (S/D) electrode was formed by applying an arsenic ion-implantation with an energy of 30 keV and a dose of $5 \times 10^{15} \text{ cm}^{-2}$. It should be noted that the S/D electrode is no longer self-aligned against the gate in this process flow. As a gate dielectric layer, a thermally grown silicon oxide (SiO_2) with a thickness of 20 nm was used. Subsequently, n^+ *in-situ* heavily doped poly-Si with a thickness of 200 nm was deposited by low pressure chemical vapor deposition (LPCVD) and patterned as a gate electrode. Due to the intentional offset between the gate and the drain, some fraction of the channel is uncovered by the gate, i.e., the underlap region is left near a drain side. Then, an inter-dielectric oxide layer was deposited in order to isolate and interconnect each device electrode by LPCVD. Then, contact holes were delineated by an additional mask, and a wet etching process using HF etchant was used to connect the subsequent interconnection lines over the contact holes and the buried electrodes for the gate, the source, and the drain (device electrodes) probing. In order to connect the interconnect electrodes between device electrodes and outer probing pads, n^+ *in-situ* heavily doped poly-Si was deposited by LPCVD, and the contact holes were filled. To avoid an electrical short between the interconnection electrodes and the analytic solution, passivation layers of SiO_2 (thickness, 50 nm) and Si_3N_4 (thickness, 150 nm) were sequentially deposited by LPCVD. The passivation layers covering the background layers were opened by a dry etching process, with

the exceptions of the probing pad areas and the sensing region. Afterwards, the underlying inter-dielectric oxide layer on the underlap-FET was selectively etched with a diluted HF solution, and additional thermal oxidation on the underlap region was carried out to create a thin SiO_2 layer to stabilize the sensing region. Finally, forming gas annealing was applied to cure possible etching damages and to reduce interface traps. To introduce the hydrophobic layer, CYTOP™ (Asahi glass company, Japan) with a thickness of 40 nm was selectively coated on the passivation region by a lift-off process. It should be noted that the top-most layer in the present design is the newly covered CYTOP™ (hydrophobic) layer in the experimental group. In contrast, the CYTOP™ coating was not employed in the control group, i.e., the top-most layer is the pre-existing Si_3N_4 (hydrophilic).

2.2. Reagents and bio-experiment procedures

To characterize the sensor performance, a surface antigen (Ala) and its specific antibody (anti-AI) from the avian influenza (AI) virus were used as the probe and target biomolecule, respectively. In this experiment, silica binding protein (SBP) (Gu et al., 2009) was used because it helps the Ala to strongly immobilize onto the Si oxide surface without additional complicated surface modification steps. This complex, SBP-Ala, is a recombinant protein in which Ala is initially fused with SBP. The underlap-FET biosensor with the passivation layer was immersed into phosphate-buffered saline (PBS) solution (pH 7.4) containing SBP-Ala of 25 $\mu\text{g}/\text{ml}$ for 1 h and then rinsed with deionized water to immobilize the Ala onto the underlap-FET. After the SBP-Ala immobilization, the drain current of the underlap-FET biosensor was measured using a semiconductor parameter analyzer (Agilent 4156C). The drain current of the biosensor was measured at a gate voltage (V_G) of 0.7 V, which is the subthreshold voltage of the underlap-FET, and a drain voltage (V_D) of 0.05 V. A subthreshold voltage indicates that V_G is smaller than the threshold voltage (V_T), which determines the on-state or the off-state of the biosensor. During the drain current measurement, another PBS droplet including anti-AI was introduced, and the drain current was continually traced. Because the anti-AI has a pI value of 6.02, it shows negatively charged characteristic at pH 7.4. Therefore, anti-AI bound to the underlap region raises the barrier height of the channel potential in the underlap region and results in a reduction of the drain current flowing through the underlap-FET.

In addition, FITC (fluorescein isothiocyanate)-conjugated anti-rabbit IgG and gold-conjugated anti-rabbit IgG (Sigma-Aldrich) were used to demonstrate the anti-AI immobilization on the active area. For this measurement, SiO_2 substrate passivated with nitride or nitride/CYTOP™ layer was exposed to the SBP-Ala solution of 25 $\mu\text{g}/\text{ml}$ for 1 h followed by anti-AI solution of 100 ng/ml for 2 min for receptor–target molecule immobilization. Then, the substrate was exposed to FITC-conjugated anti-rabbit IgG or gold-conjugated anti-rabbit IgG solution of 100 $\mu\text{g}/\text{ml}$ for 2 h in order to label the anti-AI immobilized on the SiO_2 surface for visualization.

3. Results and discussion

A schematic of the underlap-FET biosensor is shown in Fig. 1(a). The source, drain, and gate electrodes are connected with the outer contact pads via the interconnection lines, and those interconnection lines are encapsulated by the passivation layers. Fig. 1(b) shows cross-sectional scanning electron microscopy (SEM) images of the passivation layers (Si_3N_4 and CYTOP™ film). Fig. 1(c) and (d) are microphotographs of a water droplet, which show the surface wettabilities on the Si_3N_4 (hydrophilic)

and CYTOPTM (hydrophobic) layers, respectively. As shown in the figures, the Si₃N₄ surface exhibits hydrophilic characteristics with a contact angle of 19°, and the CYTOPTM surface exhibits hydrophobic characteristics with a contact angle of 111°. This hydrophobicity of the CYTOPTM surface is expected to increase the

sensitivity of the biosensor. The hydrophobic surface is extremely difficult to wet, implying that the water molecules are repelled from the hydrophobic surface. Thus, the repelled water molecules help to assemble the target biomolecules in the analytic solution on the designed hydrophilic surface, i.e., the sensing region of the biosensor (Fig. 1(f)). Therefore, even with the same concentration of the target biomolecule, the sensitivity of the hydrophobic-passivated biosensor would be greater than that of the hydrophilic-passivated biosensor.

To assess the sensitivity of the biosensor, a sensing metric was used: the drain current ratio (δ), defined as the final drain current after the introduction of anti-AI divided by its initial current (~ 100 nA) before the introduction of anti-AI ($\delta = [\text{drain current with anti-AI}] / [\text{drain current without anti-AI}]$). Here, the concentration of the anti-AI is in the range from 10 fg/ml to 100 pg/ml, as shown in Fig. 2(a). The current ratio is changed remarkably in the CYTOPTM-passivated biosensor; however, the current ratio is not changed in the Si₃N₄-passivated biosensor. Fig. 2(b) demonstrates the sensitivity (δ^{-1}) improvement following the control of the surface wettability in the passivation layer. At an anti-AI concentration of 1 ng/ml, the values of δ^{-1} were about 4.3 and 3900 for the Si₃N₄- and the CYTOPTM-passivated biosensors, respectively. Moreover, the limit of detection of the biosensor was 0.28 pg/ml (1.9 fM) for the CYTOPTM-passivated biosensor, whereas it was 28 pg/ml (0.19 pM) for the Si₃N₄-passivated biosensor. It should be noted that there is no other change present, such as a structural modification of the underlap-FET or protocol of anti-AI and SBP-Ala treatment; only the passivation layer is controlled.

As expected, the biosensor with the hydrophobic passivation layer clearly showed an improved sensitivity for antibody detection. To confirm the proposed scenario and to explain why the hydrophobic passivation layer enhances sensitivity, we attempted to visualize the antibody binding by using fluorescence dyes and gold nano-particles (Au-NPs). In this experiment, the SBP-Ala and the anti-AI were immobilized on the samples, which are comprised of a common buried SiO₂ layer and passivated individually with a Si₃N₄ or a CYTOPTM layer. Then, the fluorescent (FITC) conjugated anti-rabbit IgG was attached to the immobilized anti-AI (Fig. 3(a)). The fluorescence images shown in Fig. 3(b) and (c) clearly confirm that the anti-AI is dominantly bound to the active region (the non-passivated area [SiO₂]), which was defined by the passivation pattern for both the CYTOPTM- and nitride-passivated sample. Moreover, to verify the aforementioned increased sensitivity, we used immunogold (10 nm)-conjugated anti-rabbit IgG to visualize the antibodies immobilized on the substrate.

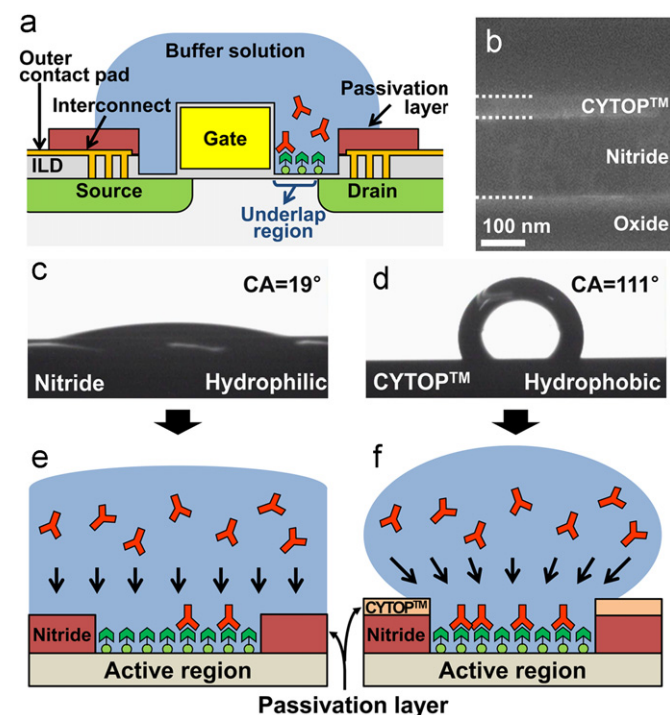


Fig. 1. Schematic of the underlap-FET biosensor and cross-sectional photographs of a water droplet to show the wettability, with corresponding schematics to convey the enhanced sensitivity: (a) underlap-FET biosensor with a passivation layer and interconnection lines between the source, drain, gate electrode, and outer contact pad, (b) cross-sectional SEM image of the passivation layer. A 40 nm-thick layer of CYTOPTM and a 150 nm-thick layer of nitride were deposited on the oxide (inter-layer dielectric, ILD) layer, (c) photograph displaying a water droplet on the nitride surface. Its contact angle is 19°, which is clearly a hydrophilic surface, (d) photograph displaying a water droplet on the CYTOPTM surface. Its contact angle is 111°, which indicates a hydrophobic surface, (e) for the hydrophilic passivation layer, the biomolecules will have same probability to diffuse to the surface, and (f) hydrophobic surface repels the water molecules, so the biomolecules inside the solution will also be repelled from the hydrophobic surface. Therefore, for the hydrophobic passivation layer, the biomolecules will be concentrated in the hydrophilic area after moving away from the hydrophobic region.

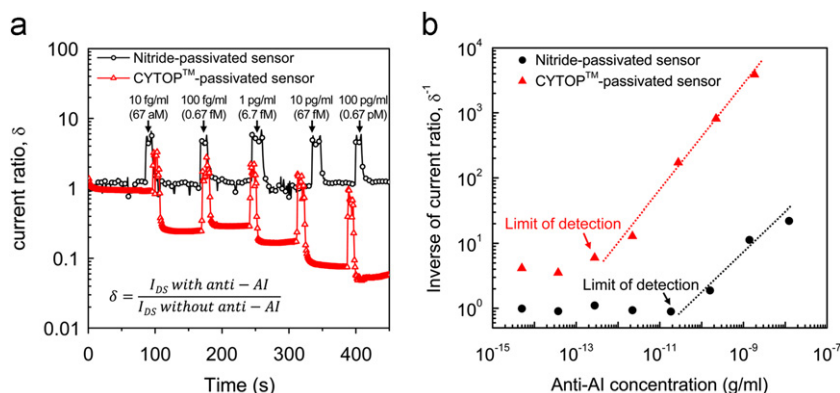


Fig. 2. Label-free and direct sensing of the AI antibody: (a) current responses with increasing concentrations of anti-AI (from 10 fg/ml to 100 pg/ml). The current ratio, δ , is defined as an I_{DS} value normalized to the I_{DS} value before anti-AI immobilization. The CYTOPTM-passivated sensor responds to a lower level of anti-AI than the nitride-passivated sensor and (b) sensitivities (inverse of current ratio) for various concentrations of anti-AI as obtained from nitride- and CYTOPTM-passivated sensors. The CYTOPTM-passivated sensor shows a 100-fold-lower limit of detection (1.9 fM) than the nitride-passivated sensor.

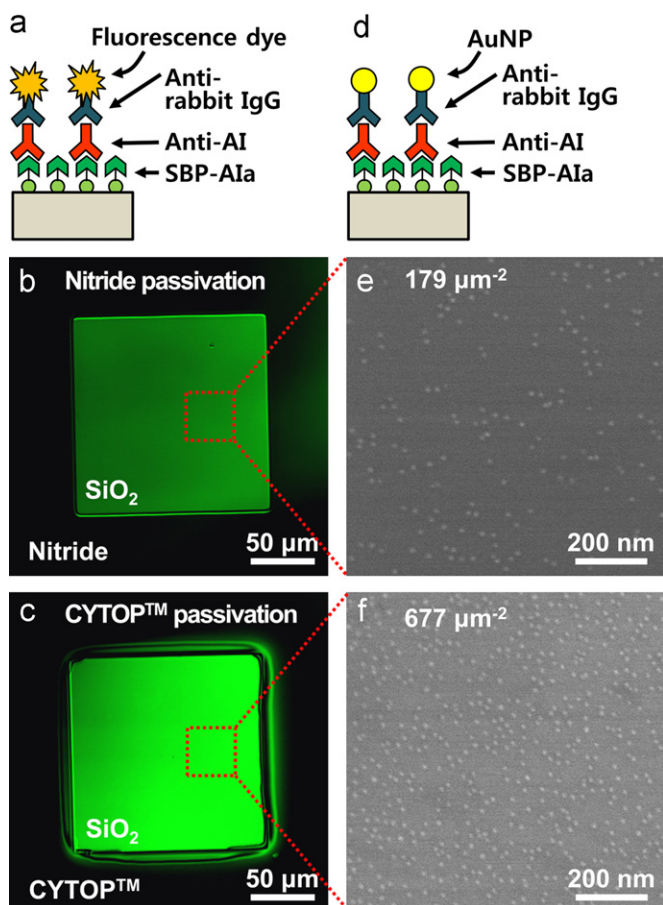


Fig. 3. The binding probability test: (a) sandwich assay by using FITC-conjugated anti-rabbit IgG, (b) fluorescence image of the nitride-passivated sample, (c) fluorescence image of the CYTOP™-passivated sample. For both (b) and (c), the anti-AI was predominantly immobilized in the active region (square pattern) as opposed to the passivated region, (d) sandwich assay by using immunogolds (10 nm)-conjugated anti-rabbit IgG, (e) SEM images of the active area for the nitride-passivated sample and (f) SEM images of the active region for the CYTOP™-passivated sample. The mean numbers of the attached immunogolds are $179 \mu\text{m}^{-2}$ (standard deviation, $\sigma=46 \mu\text{m}^{-2}$) and $677 \mu\text{m}^{-2}$ ($\sigma=39 \mu\text{m}^{-2}$) for the nitride and the CYTOP™ passivation layers, respectively.

The results of the sandwich immunoassay will clarify that larger number of biomolecules will be immobilized in case of hydrophobic passivation, which is expected to repel the biomolecules and confine them to the active region, compared to hydrophilic passivation. The surface of samples with CYTOP™ passivation and Si_3N_4 passivation were imaged by scanning electron microscopy (SEM), and the images are shown in Fig. 3(e) and (f). The attached immunogolds were counted in each sample. It is noteworthy that the binding yield is different between the hydrophilic Si_3N_4 and the hydrophobic CYTOP™ samples. The density of immunogolds attached to the active region was approximately 3.8 times larger in the CYTOP™-passivated sample than in the Si_3N_4 -passivated sample. It is straightforward that larger number of immobilized antibody on the substrate will enlarge the change of electrical signal. Consequently, these data support the hypothesis that the hydrophobic passivation layer contributes to enhancing the

sensitivity of the biosensor through amplification of the number of biomolecules immobilized on the sensing region. Although further studies are required to understand these phenomena quantitatively, it is believed that the amplified number of immobilized antibody for hydrophobic passivation is crucial to enhance the sensitivity of the biosensor.

4. Conclusions

An underlap-FET biosensor design utilizing a hydrophobic passivation surface was proposed, and its sensitivity was dramatically enhanced compared to a biosensor covered with a hydrophilic passivation surface. Its biosensing ability was characterized by using a specific antigen and antibody of the avian influenza virus as the probe and the target biomolecules, respectively. The sensitivity of the biosensor was enhanced more than 100-fold in the hydrophobic CYTOP™-passivated biosensor. In addition, it was found that the biomolecules were immobilized at a higher density in the CYTOP™-passivated sample when compared to the Si_3N_4 -passivated sample. The hydrophobic passivation surface increased the binding probability of biomolecules by confining the biomolecules inside the designed hydrophilic sensing region. Therefore, a control experiment confirmed that the hydrophobic passivation surface greatly improved the sensitivity. Surface engineering to control the wettability of a surface can be directly applied to various structured biosensors and provides a breakthrough for improving the sensitivity, requiring neither the sacrifice of biosensor characteristics nor the use of any extra design efforts, such as modification of biosensor structure, optimization of the bio-treatment protocol, or other efforts to increase the binding yield between the probe and target biomolecules.

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