Nanogap Field-Effect Transistor Biosensors for Electrical Detection of Avian Influenza**

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Field-effect transistor (FET)-type biosensors have shown potential for use in label-free electrical detection with high sensitivity.[1–5] An ion-selective FET (ISFET) utilizes a reference electrode in the form of an aqueous solution. Its conductance is dependent on charged species on the surface of the ISFET.[6–8] The sensitivity of nanowire FETs (NWFETs) is high enough to enable single-molecule detection due to the high surface-to-volume ratio of the nanowires, although the operational principle of NWFETs is similar to that of ISFETs.[9–12] However, previous FET-type biosensors lack compatibility with the standard complementary metal oxide semiconductor (CMOS) process and monolithic integration with readout circuits and signal processing systems.[13–17]

In this Communication, we report the development of a nanogap FET fabricated by the standard CMOS process. After fabrication of a conventional FET with a top gate, which consists of a chromium layer and polycrystalline silicon on top of it, the chromium layer of the gate was selectively removed by wet-etching techniques to form a 20-nm-thick nanogap between the gate and the gate oxide on top of the channel. This approach enables researchers to realize on-chip integration of biosensors with readout circuits and signal processing systems through the same process steps, which are compatible with the conventional CMOS process.

The self-assembled monolayer (SAM) method has been widely used for immobilizing biomolecular probes on a solid surface.[18,19] In order to form SAMs on the surface of the semiconductor FET, silane modification has been performed using 3-aminopropyltriethoxysilane (APTES)[1,3,20] and aldehyde propyltrimethoxysilane (APTMS)[18,21] to derivatize the amine group and aldehyde group, respectively. These methods, however, are not applicable to all types of biomolecules, and additional pre- and post-treatment is necessary for the enhancement of the surface-modification efficiency; such treatments include oxygen plasma treatment to introduce an –OH group onto the silicon surface, and a heating process to create cross linking of the SAMs.

Herein, we report an approach that uses silica-binding proteins (SBP), which bind the SiO2 surface strongly with one end and fuse the bioreceptor molecule, avian influenza (AI) antigen, with the other end by recombinant DNA technology. Hence, we demonstrate the efficient immobilization of the AI antigen (AIA) molecule on the SiO2 surface without any surface modification, a clear improvement over the results from SAMs. Nanogap FETs using the immobilization of SBP fused with AIA (SBP–AIA) are used to detect a specific antibody against AI (anti-AI). Because various SBP-fusion proteins, not only SBP–AIA, can be produced by simply cultivating recombinant microorganisms, this system should be useful in various biosensor applications by allowing effective and convenient biomolecular detection with the label-free electrical method.

The nanogap FET device, which has a nanogap beneath its gate, was fabricated using the conventional CMOS processes as shown in Figure 1A and B. A silicon dioxide (SiO2) layer of 20 nm in height was grown on the active area of a p-type silicon wafer to form the gate oxide after source/drain formation and channel implantation. A suspended chromium gate of 40 nm in height was deposited and patterned. Polycrystalline silicon, which is popularly used in conventional CMOS processes, was deposited to 500 nm in height and patterned. The nanogap FET fabricated by the standard CMOS processes supported by the Korean Ministry of Education, Science and Technology (MEST) (Y.-K.C.), the World Class University (WCU) program through the MEST (R32–2008–000–10142–0) (T.J.P. and S.Y.L.), and by the IT Leading R&D Support Project from the Ministry of Knowledge Economy through KEIT (T.I.P. and S.Y.L.), X.J.H. was supported by the Brain Korea 21 project, the School of Information Technology, and KAIST in 2007.

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This nanogap FET was used to detect a highly pathogenic AI virus of influenza type A. The AIa was first designed by multiple sequence alignment of the antigenic neuraminidases (NA) using the information present in the Influenza Virus Resource, followed by prediction of the secondary structures of the NAs. From these simulations, a highly conserved and immunogenic region for the specific AIa was designed. The AIa peptide was synthesized and used for the preparation of its specific antibody. Several protein immobilization methods have been developed based on the interaction of the protein amine groups with aldehyde, epoxide, and carboxylic acid groups on the solid surface. A similar strategy was developed by employing mixed self-assembled monolayers of nitrilotriacetic acid and alkane thiols on gold. These methods, however, require modification of the gate oxide surface. Recently, a potential silica-binding polypeptide (L2) was identified during the search for polypeptides that can bind onto an inorganic surface with high affinity. In order to find the SBP, multiple alignment was performed using the sequence of L2. An RplB protein from Mannheimia succiniciproducens MBEI55E showing 93.2% amino acid homology to L2 was identified. Sixty amino acids from the N-terminal (RplB1) and seventy amino acids from the C-terminal (RplB2) of RplB were fused and used as fusion partners to immobilize the AIa. The SBP–AIa was directly immobilized on the silica surface for the detection of the anti-AI (see Figure 1D). The binding of anti-AI to SBP–AIa immobilized on the SiO2 surface was examined via atomic force microscopy (AFM; see Supporting Information, Figure S2).

The electrical current flow between the source and the drain is controlled by the gate voltage, which induces an inversion layer beneath the gate oxide when the gate voltage exceeds $V_T$, and modulated by the gate capacitance change. By employing the constant-current method to define $V_T$, $V_T$ was defined as a gate voltage that determines a specific level of drain current—that is, 10 nA. In the n-channel nanogap FET (Figure 1B), $V_T$ is principally decided by the 20-nm height of the air nanogap region, showing a lower gate capacitance than the non-nanogap region. When biomolecules are immobilized in the nanogap, the dielectric constant of the nanogap increases and the gate capacitance also increases. Consequently, $V_T$ decreases as the gate capacitance increases. This result was verified via recovery and control experiments. Therefore, it is possible to detect specific interactions of biomolecules in the nanogap by monitoring changes in $V_T$.

To verify this concept, the drain-to-source current ($I_{DS}$) versus gate-to-source voltage ($V_{GS}$) characteristics of the nanogap FET were measured (Figure 2A). $V_T$ decreased as SBP–AIa was bound to the SiO2 gate dielectric, and further decreased with the subsequent binding of anti-AI to SBP–AIa. Initially, the device characteristics were measured with a drain-to-source voltage ($V_{DS}$) of 0.05 V after forming an air nanogap underneath the suspended gate. After SBP–AIa immobilization in the nanogap, a $V_T$ shift of $-0.23$ V was observed from the increase of the dielectric constant from unity, which

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**Figure 1.** The schematics of the nanogap FET, SBP–Ala, and anti-Al. a) Three-dimensional structure showing the silicon substrate, the source/drain, the gate oxide, and the polycrystalline silicon gate. SBP–Ala was immobilized in the vertical nanogap. b) A cross section (a–a' direction in (a)) of the device shows the nanogap filled by SBP–Ala. c) Transmission electron microscopy image of the nanogap FET with a nanogap of 20 nm in height. Platinum was deposited as a background material. d) Scheme of SBP–Ala and anti-Al on the SiO2 surface.

**Figure 2.** Electrical characteristics of the nanogap FET (from 6 devices) before and after SBP–Ala and anti-Al were immobilized in the nanogap. A) $I_{DS}$–$V_{DS}$ characteristics at $V_{DS} = 0.05$ V. B) Statistical distribution of extracted $V_T$ change after immobilization of SBP–Ala, binding of anti-Al, and recovery by detaching SBP–Ala and anti-Al.
corresponds to the air dielectric constant in the nanogap. The reason for this relatively small \( V_T \) shift is that the size of SBP–Ala is approximately 3 nm in height, thus filling only a small part of the 20-nm nanogap. After the specific binding of anti-AI to SBP–Ala, a \( V_T \) shift of \(-0.55\) V was observed, corresponding to a 10\(^3\)-factor change in \( I_{DS} \), thus assuring a high signal-to-noise ratio. This relatively large \( V_T \) shift is consistent with the fact that the height of anti-AI is approximately 12 nm, which is larger than that of SBP–Ala. Furthermore, \( I_{DS} \) increases after SBP–Ala immobilization and the specific binding of anti-AI to SBP–Ala (see Supporting Information, Figure S3). This increment of \( I_{DS} \) demonstrates an increment of the gate capacitance of the nanogap FET, meaning that the net dielectric constant is increased by the biomolecules. From the explicit quantitative modeling of the dielectric constant of the nanogap FET with the measured \( V_T \) decrements, we also confirm that the net dielectric constant increases to 1.59, 1.67, and 1.90, corresponding to air/oxide, air/SBP–Ala/oxide, and air/anti-AI/SBP–Ala/oxide, respectively (see Supporting Information, explicit quantitative modeling of the dielectric constant of nanogap FETs).

To prove that the \( V_T \) change was caused by the specific binding of SBP–Ala and anti-AI, a recovery experiment to remove the immobilized molecules from the nanogap was performed. For the bound proteins to be detached from the surface, the device with bound SBP–Ala and anti-AI was immersed in distilled deionized water (DDW) at 70 °C (Reference [30]) for ten minutes, and flushed with DDW several times. \( V_T \) shifted back to its initial value of close to the \( V_T \) for the fresh-air nanogap by removing the bound biomolecules (Figure 2B). From these results, it can be concluded that the negative \( V_T \) shift is attributable to the specific binding of SBP–Ala and anti-AI.

The sensing window, represented by the range of the \( V_T \) shift, was investigated by varying the concentrations (12.5, 50, and 200 \( \mu \)g/mL) of SBP–Ala. A greater shift in \( V_T \) was observed with increasing concentrations of immobilized SBP–Ala (Figure 3A). Therefore, the quantitative molecular-binding characteristics are not changed even in the small 20-nm nanogap channel. These results suggest that the nanogap implemented on the nanogap FET can be an effective structure for biomolecular binding to prevent evaporation and enlarge the binding area.

As shown in Figure 3B, the normalized sensitivity is defined as the ratio of the \( V_T \) difference between anti-AI and SBP–Ala to the \( V_T \) difference between SBP–Ala and the no-air gap. The primary purpose of this normalization is to compensate the nonuniform \( V_T \) arising from statistical fluctuations of the initial nanogap FETs. The concentration of SBP–Ala was fixed at 25 \( \mu \)g mL\(^{-1}\). When the concentration of anti-AI decreased, the normalized sensitivity decreased accordingly.

A distinctive feature of the nanogap FET is its bridge-like suspended gate, which allows biomolecules to move freely in and out of the nanogap channel beneath it. The effect of the nanogap width (\( W_{\text{gap}} \) in Figure 1A), which corresponds to the length of the trace of biomolecules passing through the nanogap channel, was analyzed for a fixed SBP–Ala concentration of 50 \( \mu \)g mL\(^{-1}\). As shown in Figure 3C, the \( V_T \) shift increased with the increase in the nanogap width, and gradually saturated. After binding of the anti-AI, the \( V_T \) shift was significant when the width was 2 \( \mu \)m, but not significant when the width was 5 \( \mu \)m (Figure 3D). This reveals that the unbound SBP–Ala within the nanogap of 5-\( \mu \)m width was over-shifted due to the slow diffusion, and bound with anti-AI. As a result, a narrower nanogap is more desirable for the detection of anti-AI with a large sensing window. This trend is attractive for the down-scaling of a sensor device.

To verify that the significant \( V_T \) shift was truly caused by the specific binding of SBP–Ala and anti-AI, control experiments using phosphate-buffered saline (PBS), which contains alkaline metal ions known to affect \( V_T \) in the metal oxide semiconductor FETs (MOSFETs), were performed. Nanogap FETs were immersed in PBS for one hour without immobilization of SBP–Ala and anti-AI. No significant \( V_T \) shift was observed (Figure 4A). Similarly, the SBP–Ala–immobilized nanogap FETs were immersed in the PBS. No additional \( V_T \) shift due to PBS was observed. Furthermore, only the specific

![Figure 3. Molecular bindings inside the nanogap with different SBP–Ala and anti-AI concentrations as well as different nanogap widths.](image-url)
anti-AI, but not the antirabbit immunoglobulin G (IgG), was selectively bound to SBP–Ala immobilized in the nanogap (Figure 4B).

In conclusion, a nanogap FET with a vertical nanogap of 20 nm, which was intentionally adjusted to the target biomolecule size, was developed as an efficient label-free electrical biosensor and used to detect AI using a specifically designed protein, SBP–Ala, which has silica-binding specificity. The structure is fully compatible with current CMOS fabrication, enabling it to be integrated into readout and control circuitry for lab-on-a-chip and point-of-care systems. The binding of SBP–Ala and anti-AI was detected by simply measuring the $V_T$ shift. As demonstrated by successful AI detection, the system developed in this study not only provides specific immobilization of biomolecules on the silica surface, but also allows simple, yet accurate, bioassays in FETs. Furthermore, because various SBP-fusion proteins for desired biosensors can be produced by simply cultivating recombinant microorganisms, this system should be useful in various biosensors by allowing effective and convenient biomolecular detections in transistors.

Experimental Section

Fabrication process: A p-type silicon wafer was used as a starting material, and, onto it, a 70-nm-thick buffer oxide was grown by thermal oxidation, 170 nm of silicon nitride was deposited, and a 10-nm-thick mask oxide was grown by thermal oxidation. For the definition of an active channel region, optical lithography using light of 436-nm wavelength with 6612K positive photore sist was used. After channel-stop implantation (boron, 10 keV energy and $5 \times 10^{15} \, \text{cm}^{-2}$ dose), local oxidation of silicon (LOCOS) to improve isolation among adjacent devices was performed to make silicon dioxide of 1-μm thickness through wet oxidation. The remaining silicon nitride on the active channel region was used as an implantation stopper for subsequent source and drain formation. After the nitride removal followed by source/drain implantation (phosphorous, 20 keV energy and $2 \times 10^{15} \, \text{cm}^{-2}$ dose), channel implantation (boron, 10 keV energy, $5 \times 10^{12} \, \text{cm}^{-2}$ dose) was performed prior to a gate oxidation to adjust $V_g$. All ion impurities introduced by the implantation were activated by rapid temperature annealing (RTA) for 30 s at 1000 °C.

A 20-nm gate oxide was grown by thermal dry oxidation, and the 40-nm chromium layer was deposited using an electron-beam evaporator. The base pressure of the vacuum chamber was lower than $1 \times 10^{-6} \, \text{Torr}$ and the deposition rate of Cr was about $0.3 \, \text{Å} \, \text{s}^{-1}$. The Cr layer was delineated by optical lithography and etched by CR-7 (Cyantek Corp.). For gate-electrode formation, in situ n-type-doped polycrystalline silicon of 500 nm was deposited by the low-pressure chemical vapor deposition (LPCVD) method. The top side of the 20-nm chromium layer was consumed by formation of a chromium silicide layer between the chromium and the polycrystalline silicon due to its deposition temperature of 580 °C. The polycrystalline silicon was patterned by optical lithography with AZ6612K photoresist. The polycrystalline silicon was patterned by a well-known etchant ($\text{HNO}_3:\text{H}_2\text{O}:\text{HF} = 100:40:3$). A forming-gas annealing was carried out for 1 h at 400 °C under a flow of 95% $\text{N}_2$ and 5% $\text{H}_2$ to improve the interface quality of the gate oxide and the channel silicon. The native oxide residing on the sacrificial chromium layer was etched by buffered oxide etchant (BOE). Finally, the remaining chromium of 20 nm for the sacrificial layer was laterally etched by CR-7 for 5 min in order to form the nanogap channel underneath the suspended gate, with various nanogap widths from 2 to 5 μm.

Procedures of protein preparation and binding: Polymerase chain reaction (PCR) experiments were performed with a PCR Thermal Cycler T1 (BioMetra) using the high-fidelity PCR System (Boehringer Mannheim). Restriction and DNA-modifying enzymes were purchased from New England Biolabs. All DNA manipulations including restriction digestion, ligation, and agarose gel electrophoresis were carried out following the standard procedures [31]. The DNA sequences of all clones were confirmed by sequencing with an automatic DNA sequencer (ABI Prism model 377, Perkin Elmer).

The restriction enzyme sites are indicated by the underlined sections. For the construction of the plasmid expressing the SBP and antiregion of Al, the DNA fragment encoding the N-terminal region of RplB was first amplified by PCR with the primers P1 (5'-GGAATTCATATGCTATGTTAATGTAAGCGG-3') and P2 (5'-AGTACGTCACTTTGTATAGCAACCACCTGAT-3') using the genomic DNA of Mannheimia succiniciproducens MBEL55E as a template. The DNA fragment encoding the C-terminal region of RplB and the Ala fusion protein was amplified by PCR with the primers P3 (5'-TATCGTCCAGTACTGGAATGGGATGGAAGTGCCCC-3') and P4 (5'-GTAGCTCGAGATCAGGATGTTCGCTTCGGTCAGCTATCAGCGAACATTTGCCCAGCACAGCTGAATC-3') using the genomic DNA of Mannheimia succiniciproducens MBEL55E as a template. The amplified product obtained by PCR was digested with Ndel and SalI, and SalI and Xhol, respectively. All fragments were ligated into the Ndel and Xhol restriction sites of PET-22b(+)-(Novagen) to construct pET–rplB12–Ala. The sequence of pET–rplB12–Ala contains 18 nucleotides encoding a six-histidine tag at the C-terminus of RplB–Ala for easy purification by metal-affinity chromatography.
For the production of RplB12–Ala fusion protein, an *Escherichia coli* BL1(DE3) strain harboring pET–rplB12–Ala was cultivated in 100 mL of Luria–Bertani (LB) medium (10 g L\(^{-1}\) tryptone, 5 g L\(^{-1}\) yeast extract, and 5 g L\(^{-1}\) NaCl) supplemented with ampicillin (Ap, 100 \(\mu\)g mL\(^{-1}\)) in a shaking incubator at 37 °C and 200 rpm. Cell growth was monitored by measuring the absorbance at 600 nm (\(OD_{600}\)).

The immobilization of the recombinant protein was performed in a nanogap device. Prior to assembly, the nanogap device was cleaned thoroughly with acetone and DDW and dried in a stream of high-purity N\(_2\). The nanogap device was then placed into a SBP–Ala solution for 1 h at 25 °C, followed by washing with PBS as well as DDW, and dried in a stream of N\(_2\). After further cultivation for 6 h, cells were harvested by centrifugation at 10 000 g for 10 min at 4 °C and disrupted by sonication (Braun Ultrasonics) for 1 min at 20% output. After centrifugation at 16 000 g for 10 min at 4 °C, the supernatant containing the soluble protein fraction was obtained for the purification of fusion proteins using the Ni-NTA column (Qiagen) without further purification steps. Protein concentration was determined by Bradford’s method using bovine serum albumin (BSA, Sigma) as a standard.

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**Synthesis of polyclonal antibodies against AI surface antigen:**

Polyclonal antibody was provided by Peptron (Daedeoan, Korea). Polyclonal rabbit serum was produced by immunization with a peptide corresponding to the neuraminidase protein residues 291–302 (CRDNWKGSNRPI-NH\(_2\)) of H5N1 and H9N2-type AI-containing cysteine for conjugation. It was synthesized and conjugated to maleimide-activated keyhole limpet hemocyanin (KLH; Pierce Chemical) by a N-[4-maleimidobutyryloy]succinimide ester (GMBS) conjugation method, and conjugated to ovalbumin (OVA; molecular weight (\(M_w\) = 45 000), which serves as a nonrelevant carrier protein for enzyme-linked immunosorbent assay (ELISA). Female rabbits (age 12–22 weeks) were injected 3 times at 21-d intervals with 500 mg of peptide–KLH conjugate in Freund’s complete adjuvant (FCA; Pierce Chemical) according to the manufacturer’s protocol. Serum was screened by indirect ELISA using the peptide–KLH conjugate. Each well of the 96-microwell ELISA plates was coated with peptide–OVA conjugate (10 w/v\%) in 50 mM carbonate buffer (pH 9.0), and the plates were incubated overnight at 4 °C. Without blocking, 100 \(\mu\)L of antiserum or hybridoma supernatant was incubated for 45 min at 37 °C. Bound antibody was detected with goat antirabbit IgG-horseradish peroxidase and O-phenylenediamine dihydrochloride (Sigma). The titer of the rabbit antisera following immunization was about 1:100 000. This antibody was purified through the column and concentrated up to 1.6 mg mL\(^{-1}\).

**Measurement method and verification by simulation:**

Field-emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM) images were obtained using a Philips XL 30 AFEG SEM (Eindhoven) and FEI Tecnai F20, respectively. AFM images were collected from Nanoscope III (Digital Instruments).

The bindings of SBP–Ala and anti-Al were detected by measuring \(V_I\), shift of the suspended nanogap FETs. All electrical measurements were done with a semiconductor parameter analyzer (HP4156C). The electrical characteristics were measured after each immobilization step (i.e., measuring the data after binding of SBP–Ala and anti-Al, respectively). The gate, drain, and source currents were measured while the gate voltage was swept from 0 V to 4 V. At mean time, the source was grounded, and 50 mV was applied to the drain. All measurements were performed on a probe station. These measured data were compared to the simulation data. It was found that the normalized sensitivity showed a decreasing trend with decreasing concentration of anti-Al. This tendency was in agreement with the simulation results obtained with the semiconductor simulator (Silvaco).

**Keywords:**

avian influenza · biosensors · field-effect transistors · nanogap materials · silica-binding proteins


