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Characteristics of Protein G-modified BioFET

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Abstract

Label-free detection of biomolecular interactions was performed using BioFET(Biologically sensitive Field-Effect Transistor) and SPR(Surface Plasmon Resonance). Qualitative information on the immobilization of an anti-IgG and antibody-antigen interaction was gained using the SPR analysis system. The BioFET was used to explore the pI value of the protein and to monitor biomolecular interactions which caused an effective charge change at the gate surface resulting in a drain current change. The results show that the BioFET can be a useful monitoring tool for biomolecular interactions and is complimentary to the SPR system.

Keywords : BioFET, Protein G, SPR

1. INTRODUCTION

Research in the field of biosensors has become popular since their applications were extended to medical diagnosis, environment, biotechnology, food safety, drug development, defense and security[1-4]. Various types of biosensors utilize optical, electrochemical, mechanical, or thermal transduction mechanisms[4]. Among these, field-effect transistor(FET)-based biosensors(BioFETs) are one of the most attractive candidates for direct and label-free electrochemical detection of biological molecules. BioFETs consist of two basic parts: the Ion-sensitive FET(ISFET) signal transduction part and the bio-recognition element("receptor") on the gate as the sensing part. The binding of a charged analyte or target molecule to the receptor produces a change in the effective charge in proximity to the surface of the gate. The analyte's electrical field influences the conductance of the FET channel. These Si-based sensors have a lot of beneficial characteristics such as on-chip integration of signal process, standardization, portability result from their small size and weight, and low-cost mass production with fast response and high sensitivity. Nowadays surface plasmon resonance(SPR) techniques have received increasing attention as powerful tools to study interaction kinetics between two biomolecules in real-time without labeling. SPR sensors detect slight changes in refractive index adjacent to the metallic sensor surface by the immobilization of biomolecules[5, 6]. In this letter, we have utilized a BioFET sensor as an analytical tool for exploring the isoelectric point(pI) of proteins and looking at biomolecular interactions by measuring the drain current variation. Qualitative information on biomolecular interactions was acquired using the SPR analysis system. In both cases, the Au surfaces of the BioFET and SPR chips were modified by a cysteine-tagged protein G for oriented immobilization of the antibody of immunoglobulin G(IgG) which is the most abundant circulating antibody and is active against foreign particles[7-9].

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2. EXPERIMENTAL

2.1 Fabrication

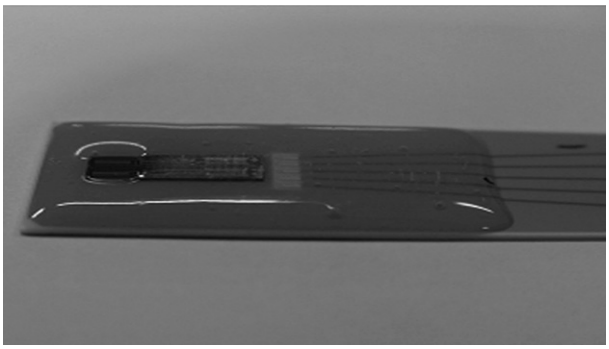

Fig. 1. Fully fabricated and encapsulated BioFET.

The basic structure of the BioFET is an ordinary n-channel metal-oxide-semiconductor FET(MOSFET) fabricated by standard CMOS process techniques. Initial cleaning of a 4 inch p-type (100) silicon wafer with resistivity of 15 Ω · cm ~ 25 Ω · cm was followed by a 5000 Å thick oxide growth by wet oxidation. To form source/drain regions, pre-deposition was performed using P2O5 after removing the deposited oxide on both regions. Next, the resulting phospho-silicate glass(PSG) was removed and a drive-in step was performed. A gate window was defined and opened. High-quality gate oxide(400 Å) was deposited by dry oxidation. Contact regions were patterned for metallization. Au/NiCr(50 nm/10 nm) were deposited by a thermal evaporator and finally defined. The gate area was 50 ㎛(length) × 2,500 ㎛(width). The fabricated FET was then attached to an alumina substrate. Polymer adhesives were used to coat the chip for encapsulation except in the gate region. Fig. 1 shows the fully fabricated FET used for our experiment-sparticles[7-9].

2.2 Measurements

Molecule binding events were monitored by a SPR analysis system(SPR LAB, KMAC, Daejeon, Korea). The system was set-up based on the Kretschmann configuration which is the most widely used set-up. Polarized light from a semiconductor laser(635 nm, 2.5 mW) irradiated the 50 nm thick Au film through a block prism(n = 1.56). The intensity of the reflected light was detected by a Si-photodiode. The incident angle was varied from 30°Δ to 80°Δ. The electrical characteristics of the BioFET were measured by a semiconductor analyzer(HP4156). To establish the solution potential an Ag/AgCl reference electrode was utilized. All measurements were carried out in PBS(phosphate buffer silane, pH 7.4) at room temperature after cleaning the modified surface by PBS. For both optical and electrical detections, the Au film was modified by sequence reactions involving the protein G, anti-IgG, BSA, and IgG.

3. RESULTS AND DISCUSSIONS

Qualitative information on the formation of protein G, the immobilization of anti-IgG and their affinity for binding of IgG could be monitored since any change in thickness or refractive index of the materials adsorbed on the Au surface resulted in a resonance angle shift. Cysteine-tagged protein G has been utilized to immobilize anti-IgG since the engineered protein G enhances better oriented antibody immobilization[7]. Initially, the protein G was formed on the Au SPR chip. Fig. 2 shows surface plasmon resonance angle shifts after a sequence injection of anti-IgG and IgG solutions onto the protein G formed Au surface. After the injection of anti-IgG solution, the resonance angle shifted about 0.2°. After the immobilization of the anti-IgG, an IgG solution was exposed to the anti-IgG immobilized Au surface, resulting in a 0.1° resonance angle shift. Here, the concentrations of the protein G, anti-IgG and IgG were 100㎍/ml, 10㎍/ml and 10 ㎍/ml, respectively. The result qualitatively shows that the anti-IgG was immobilized on the protein G, and then the IgG bound to the anti-IgG with specificity and affinity.

Since FETs are very sensitive to surface charge at or nearby the gate, the BioFET can be applied as a tool for the measurement of pI in unknown proteins. Here, the pI of protein G was measured. The concentration of protein G 10 ㎍/ml, in this case. The applied voltage on the reference electrode was 3 V. Drain current responses of protein G to different pH solutions were observed as shown in Fig. 3. Firstly, we have the measured drain current characteristic of the BioFET in PBS without immobilization of protein G. The current was 0.986 mA at a drain voltage of 5V. After the adsorption of protein G on the Au gate surface, the drain current was measured at the same drain voltage but in different pH solutions, pH 4, pH 7, and pH 10. The drain currents were found to be 1.02 mA, 0.948 mA and 0.845 mA, respectively. If we ignore the thickness of protein G, the pI of protein G can be inferred from these results to be between 4 and 7. It has been reported that the pI of protein G ≈ 4.5[10].

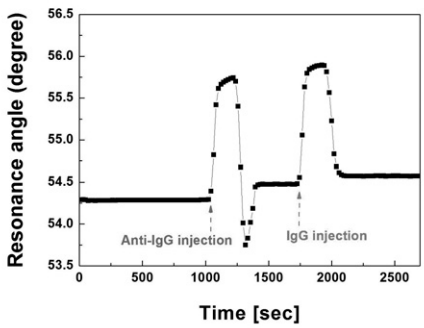

Fig. 2. Surface plasmon resonance angle shifts after sequence injection of anti-IgG and IgG solutions into the protein G immobilized Au surface.

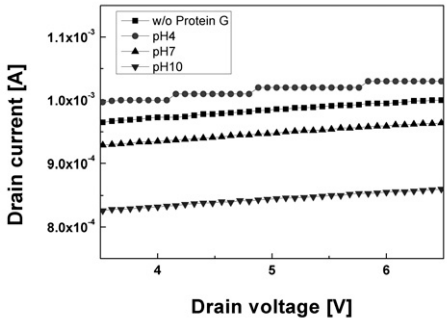

Fig. 3. Drain current response of protein G to different pH solutions.

Adsorption of protein G, immobilization of anti-IgG, prevention of non-specific binding by BSA(bovine serum albumin) and anti-IgG and IgG interaction were monitored using the BioFET. The structure of the BioFET with biomolecular interactions on the gate surface is illustrated in Fig. 4. The concentrations of protein G, anti-IgG, BSA and IgG were 200μg/ml, 100μg/ml, 10μg/ml and 10 μg/ml, respectively. The output response of the BioFET to each protein interaction is shown in Fig. 5. As expected from previous results, adsorption of protein G and the measurement of drain current in PBS were equivalent to applying negative voltage to gate electrode since the pI of protein G is less than the pH of PBS. Thus, the drain current decreased after adsorption of protein G. The drain current variation ratio was 2.4 %. After anti-IgG immobilization, BSA injection and antibody-antigen interaction, the drain current variation was 4.1 %, 1.3 % and 6.9 %, respectively.

Thus the BioFET can be utilized as an analytical tool to monitor biomolecular interactions and the results can be compared to those given by SPR analysis systems. If the BioFET is coupled with SPR, the hybrid system would be a powerful tool to analyze biomolecular interactions.

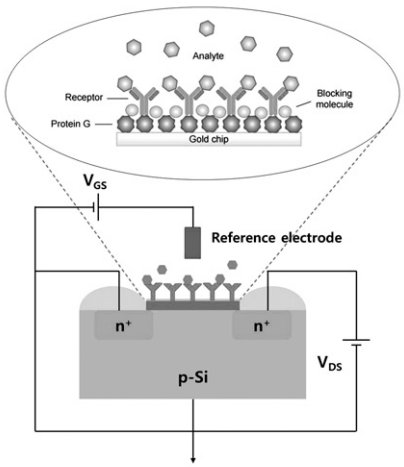

Fig. 4. BioFET structure with biomolecular interactions on gate surface.

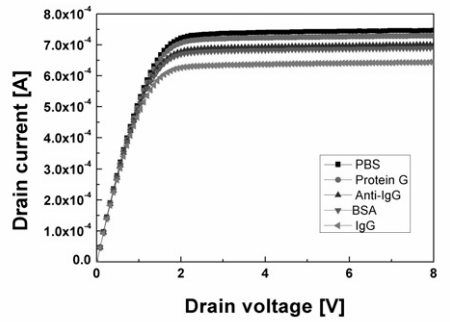

Fig. 5. Drain current characteristics of BioFET during various biomolecular interactions.

4. CONCLUSIONS

Label-free detection of biomolecular interactions can be assessed using a BioFET fabricated by conventional CMOS fabrication techniques. Qualitative information on biomolecular interactions such as immobilization of anti-IgG on protein G and interaction between anti-IgG and IgG was acquired by a SPR analysis system. The BioFET can be used to explore pI values of protein. Biomolecular interactions can be monitored using the BioFET by measuring drain current. The results show that the BioFET can be used as a monitoring tool for biomolecular interactions.

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