Extended Abstracts of the 2014 International Conference on Solid State Devices and Materials, Tsukuba, 2014, pp592-593

Signal Amplification of Immune-Field-Effect Transistors Using Enzyme Catalyzed Ag Reduction to Overcome Debye Screening Length

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1. Introduction

Abnormal accumulation of Amyloid-beta peptides in the brains induces Alzheimer disease which is very fatal to the human beings.[1] Enzyme-linked immunosorbent assay (ELISA) is one of the most typical methods to quantify Amyloid-beta peptides with an enzyme conjugated secondary antibody. Recently, as an advanced and simplified alternative to the immunoassays, field-effect transistors (FETs)-based immunesensors have received a great attention, owing to the fast and inexpensive analyses, and possibility to miniaturize the immuneassay system. However, the application embeds a crucial impediment induced by the Debye screening length.

In this study, we amplified an immune signal of human interleukin 5 (IL5) by projecting sandwich immuneassay on the classic ISFET. The core of this assay is to trigger the amplification of electric signals through the enzyme catalyst reaction. Here, the type of a labeled enzyme is very crucial to obtain a larger sensing margin, which means we need to choose an appropriate protocol to be suitable for the ISFET platform. We applied two types of enzyme for the detection for human IL5 in the same protocol: alkaline phosphatase (ALP) and horseradish peroxidase (HRP). When we applied ALP as a label, the immuno-signal was largely amplified up to 0.395 V, due to sliver precipitation precipitated by ALP. On the other hand, the signal amplification of HRP-labeled sample is somewhat trivial to quantify concentration of molecule. Proposed assay can be alternative reading method of ELISA with electric signals, instead of large-scale optical reading system.

2. Experiment

After O_2 plasma treatment, the gate surface was immersed into an ethanol solution containing 1% (v/v) APTMS for 1 h to functionalize the silicon oxide surface with amine groups and were baked on a hot plate at 130◦C for 30 min. Sequentially, 1 M succinic anhydride was introduced to form carboxyl group. The surface was activated with 0.1 M 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 0.025 M n-hydrosuccinimide (NHS) in distilled water for 15 min. To immobilize the antibodies, the chips were incubated in phosphate-buffered saline (PBS: pH 7.4) containing 100 ng/ml of anti-human IL5 for 1 h. Non-specific binding was prevented by using ethanolamine and bovine serum albumin (BSA). The chips were reacted within a PBS solution that contained 1 mg/ml BSA and different concentrations of IL 5 for 1 h, and 10 μg/ml biotinylated anti-human IL5. The chip was incubated for 30 min. Here, the protocol was divergent. Streptavidin-ALP (SAv-ALP) conjugate and streptavidin-HRP (SAv-HRP) conjugate (each 10 μg /ml in PBS) was added for 30 min, respectively. Finally, in the ALP labeled sample, 5 mM ascorbic acid 2-phosphate (AA-P) and 10 mM $AgNO_3$ in 0.1M Tris-HNO_3 pH 9.8 buffer solution was added. The other sample was immersed with 5 mM 4-chloro-1-naphtol (CN) and 10 mM H_2O_2 in PBS buffer solution. The brief procedure is described in Figure 1.

3. Result and Discussion

Figure 2 (a) shows the transfer curves of ALP-labeled sample responding to each step of ELISA process. Initially, we put 1 pg/mL and 100 ng/mL human IL5 diluted in BSA into the chamber in succession at the immobilized anti-IL5 layer described in figure 1 (a). The threshold voltage shift caused by reaction from antibody-antigen complexes is trivial, due to the Debye screening limit. After binding the detection anti-IL5 conjugated to ALP as shown in figure 1 (c), we added $AgNO_3$ and AA-P into the sample. Interestingly, a large amount of the threshold voltage of 0.395 V was negatively shifted. On the

other hand, controlled sample which did not contain IL5 did not response to the mixture solution of AA-P and $AgNO_3$, as shown in Figure 2 (b) We repeated the reaction several times and the summary is presented in figure 3 (a). Immuno-sensing signal were largely amplified by enzyme catalyzed Ag reduction reaction with an outstanding responsive voltage variation of 0.395 V. The Ag precipitated surface is corroborated from SEM image of Figure 4 (a).

Meanwhile, immune ISFET with HRP label was evaluated in figure 3 (b). The procedure performed under the same procedure with ALP-labeled sample before the step described in figure 1 (c). Similar application related to application of HRP label to the immune ISFET has already reported.[2] The catalytic activities of HRP conjugated to antibody changes pH in solution after treatment with H_2O_2. We added CN in order to evaluate the influence of other substrate, blue products, on the signal change, but the result is unsatisfactory to quantify concentration of IL5 compared to ALP-labeled sample, as shown in figure 3 (b). From the result, we needs to choose an appropriate label, in order to induce the signal enhancement in the ISFET.

4.Conclusion

Immune ISFET was realized using ALP label for detecting human IL5 immuno-signal. The Ag precipitation which was catalyzed by enzymes, dramatically enhances the sensing margin of binding events. Consequently, this assay is a promising alternative to provide reading method of ELISA with electrical signal. It is expected that the established electrical biosensor can find wide application in a diverse range of areas, including drug discovery, clinical diagnostics, and environmental monitoring.

Acknowledgment

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2013R1A1A2A10011202).

Reference

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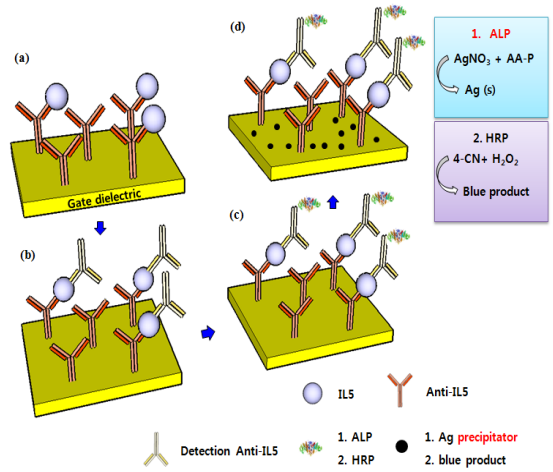

Fig. 1. Schematic of the sequence of events occurring for the sandwich assay of IL5 antigen.

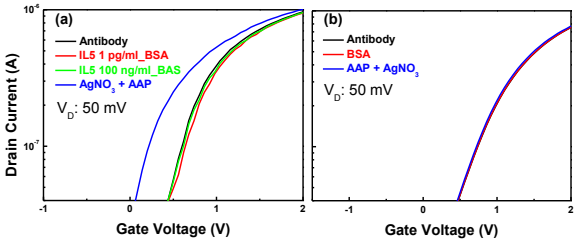

Fig. 2. Transfer curves of (a) ALP-labeled ISFETs and (b) control sample in each process. The large shift of threshold voltage can be observed from Ag precipitation.

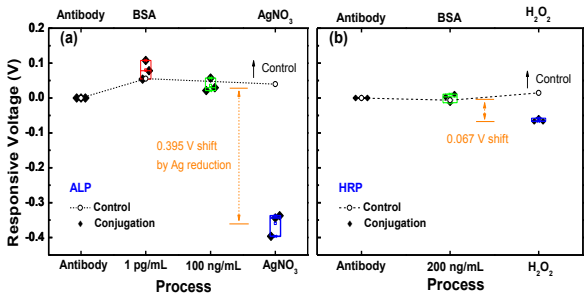

Fig. 3. Responsive voltage shift of (a) ALP-labeled ISFET and (b) HRP-labeled ISFET.

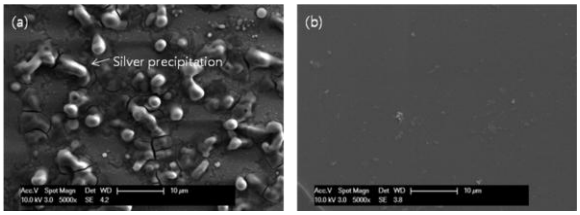

Fig. 4. Surface SEM images of (a) ALP-labeled ISFET and (b) control sample.