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Signal Enhancement of Human IL5 Immunoassay by Enzyme Catalyzed Ag Reduction Beyond Limit of Debye Screening Length on Ion-Sensitive Field Effect Transistors

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1. Introduction

There is increasing interest in applying ion-sensitive field effect transistors (ISFETs) as biosensors converting a biological signal into an electrical one.[1] A major driving force for this progressive reserach is the process compatibility with the state-of-the-art complementary metal-oxide semiconductor (CMOS) technologies providing various advantages, such as: easy integration, high reliability, possibility of automatic packaging at wafer level, mass production, and precise process control.

A concept of direct immunosensing by an ISFET was introduced by Schenck in 1978.[2] It was expected that the formation of an antibody–antigen complex on the gate of an ISFET would lead to a detectable change in the charge distribution and thus, directly modulate the drain current of the ISFET. However, despite intensive studies on immunoFETs, the previous results were unsatisfactory due to its fundamental limitations, debye screen length (small response signal of a 10 mV with a wide concentration range of 10^{-7} ~10^{-10} M under an ideal condition) compared to any other biosensor application such as emzymeFETs or DNAFETs.

In this study, to break through traditionally exposed problems of ImmunoFETs, we proposed a new immuno-signal detectable method with highly amplified signal. The major concept is not direct immunosensing of antibody-antigen complex but indirectly sensing using enzyme catalyst reaction. Here, the immuno-signal was excessively amplified up to 0.437 V by enzyme catalyzed Ag reduction from mili responsive voltage shift by origin antibody-antigen complex. Therefore, this immunoassay is very promising alternative to overcome limit of Debye screening length on ImmunoFETs.

2. Experiment

p-type (100) bulk silicon films with a resistivity of 10-20 Ω cm was used as a starting material. Then, a 100 nm-thick phosphorus-doped poly-silicon layer was deposited at the source/drain area using low-pressure chemical vapor deposition. Subsequently, the SiO_2 layer was grown by thermal oxidation with a thickness of 28 nm as a sensing membrane. After deposition of a 150 nm-thick Al layer for the source/drain contacts by e-beam evaporator. Meanwhile, the major procedures shown in Figure 1 to realize high performance ImmunoFETs were as following various steps; O_2 plasma → 1% 3-aminopropyltrimethoxy silane (APTMS) → 1 M succinic anhydride → 0.1X IL5 antibody → 10% Bovine Serum Albumin (BSA) → 100 ng/ml IL5 in 1mg/ml BSA → 10 μg/ml biotin Anti-IL5 + 10 ug/ml Streptavidin-alkaline phosphatase (SAv-ALP) in 1 mg/ml BSA → 5 mM ascorbic acid-phosphate + 10 mM $AgNO_3$. For last process, Ag precipitators by ALP induce highly amplifed signal on surface potential.

3. Result and Discussion

The SiO_2 sensing membrane revealed a generally reported sensitivity value of 34.6 mV/pH with a linearity of 99.46% presented in figure 2. It implys the membrane of a device senses well the variation of surface potential by biomolecule. Also, to ensure stability of membrane, drift characteristic of SiO_2 membrane (1.47 mV/h shown in Figure 3) was measured in pH 7 solutions for 12 hours. The figure 4 showed the immune-sensing properties of human IL5 by enzyme catalyzed Ag reduction. For direct immunosensing properties, the small amount of responsive voltage (77.9~78.05 mV variation with IL5 concentration range of 10^{-6} ~10^{-9} M) was shifted by antibody-antigen complex despite low concentration of 0.001X PBS solution. This is because it can be seen that only potential changes which occur within the order of the Debye length can be detected. The dimensions of macromolecules, like antibodies, are much longer than those of the double layer. However, immuno-sensing characteristics were largely amplified

by enzyme catalyzed Ag reduction reaction shown as figure 1(d) with outstanding responsive voltage variation of 0.437 V which is five time larger value than origin was observed. On the other hand, small voltage shifts were occurred at control sample (without IL5) even though same process was applied. This newly proposed immunoassay using ISFETs will create various opportunities in realization of a high quality health care system.

4.Conclusion

Immuno-sensing signal of human IL5 immunoassay on a ion-sensitive field effect transistors (ISFETs) was sucessfully amflied to as high as 0.437 V from a few mili voltage by enzyme catalyted Ag reduction reaction. This new dectection method is a promising alternative to overcome a chronic problem in debye screening length on the ImmunoFETs.

Acknowledgment

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Reference

[1] P. Bergveld, IEEE Trans. Biomed. Eng., 17, 70-71, 1970.
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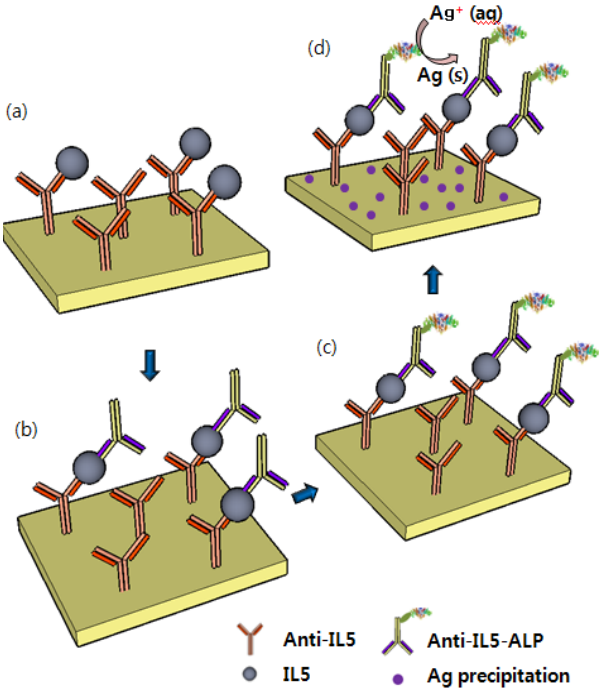

Fig. 1. Schematic of the sequence of events occurring for the sandwich assay of IL5 antigen.

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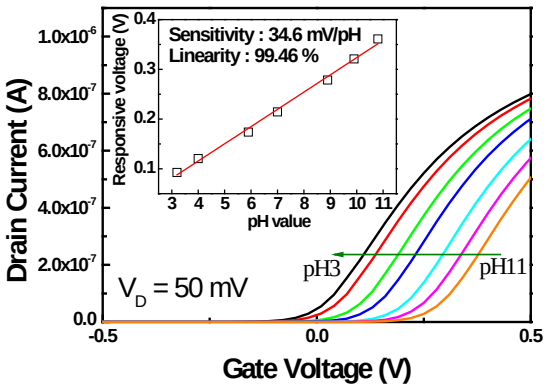

Fig. 2. Transfer curves of a SiO_2 MOSFET in different *p*H buffer solutions. The drain bias is set at 50 mV. The responsive voltage of the sensor for each *p*H buffer solution shown in the insets of Fig. 2. It was defined as the corresponding gate voltages to the drain current (Reference current) of 100 nA

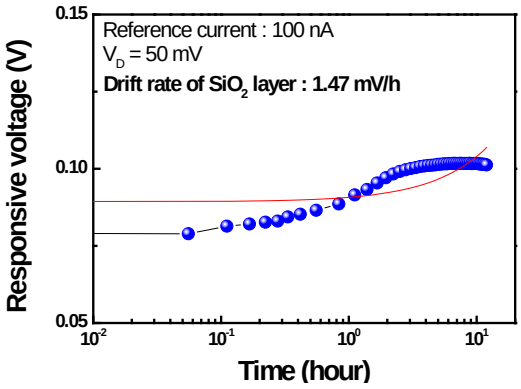

Fig. 3. Drift characteristic of the SiO_2 membrane in pH 7 buffer solution for 12 hours.

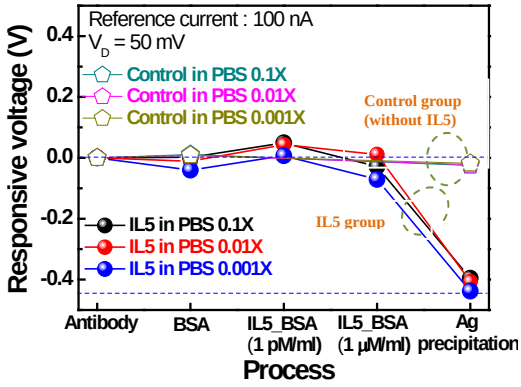

Fig. 4. Responsive voltage tendency of ISFETs with control (without anti-IL5) or anti-IL5 for each process. The IL5 detection signal enhancement by Ag precipitation was not observed in control group but anti-IL5 group