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Development of an Immunosensor to Detect Rat IgG Using Impedance Analyser

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Abstract: Antibody based biosensors are very selective and ultra-sensitive. Antigen-antibody reactions have been used in immunoassays. In this research, a biosensor which uses antigen-antibody reaction was developed to measure and detect rat IgG. Because the antigen-antibody reaction is a physical bounding between antigen and antibody, there are several ways to measure an antigen-antibody reaction. Among the methods, impedance analysis has short measuring time and possibilities of analyzing various properties of the reaction using frequency analysis. Rat IgG could be detected with developed biosensor and impedance analyzer. The biosensor showed good repeatability and availability of detecting concentration changes of rat IgG.

Keywords: Immunosensor, IgG, Sensor, Impedance

Introduction

Antigen-antibody reaction is suitable for biosensors because it has rapid reaction rate and high selectivity inherent from self defense mechanism of life. Several researches have been conducted to make diagnosis or analysis kit using antibody-antigen reaction and some products have been commercialized. Precision of sensors using antibody-antigen reaction largely depends on a transducer which detects the reaction signal.

Optical transducer consists of light source and optical sensor on which antigen is immobilized. It measures changes of light amount resulted from antibody binding on the surface of the sensor. Mass-sensitive sensors such as quartz crystal microbalance (QCM) measure frequency changes induced from antibody-antigen bindings on the sensor surface.

Recent development in electronic and communication technology brought rapid improvement of electronic parts and measurement techniques for these electronic parts. Also, technologies for production, inspection, and measurement of precision electronic parts for high frequency communication have been developed. Improvement on these technologies made possible to develop more precise and sophisticated biosensors.

In this research, an impedance type antibody biosensor that measures rat IgG was developed. Sensors were fabricated with semi-conductor fabrication process and sensing signals were analyzed with an impedance meter.

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Materials and Method

1. Sample and reagents

Anti-rat IgG and rat IgG were acquired from ELISA kit (Bethyl, USA), and other chemicals including protein G were purchased from SIGMA. For the experiment, rat IgG was sequentially diluted by 10-fold with pH 7.4 PBS solution.

2. Theory of impedance biosensor

Impedance is a measure of the difficult of passage of electric current through electric circuit or electronic components. Its unit is Ω. The impedance consists of real and imaginary component. It is represented as Z= R+jX. Real component is called resistance, R, and imaginary one is called reactance, X. Fig. 1 shows impedance in the vector space.

The impedance meter (Agilent 4294A, USA) used in this research has frequency response of 40 Hz~110 MHz and detection range of 3 mΩ~500 MΩ. As shown in Fig. 2, when antigen bound to antibody on the sensor surface, impedance is changed as electrical characteristics between two electrodes were altered. Concentration of the antigen is estimated from the measured impedance. The impedance change is depended on reactants, concentration of the reactants, and distance between the electrodes.

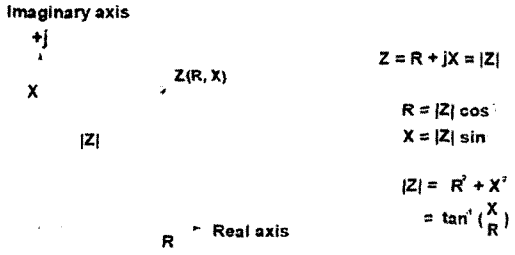
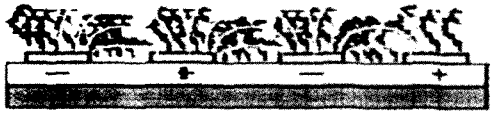


Fig. 1 Impedance (Z) consists of real part (R) and imaginary part (X).

(a) without affinity binding



(b) with affinity binding

Fig. 2 Concept of an immunosensor.

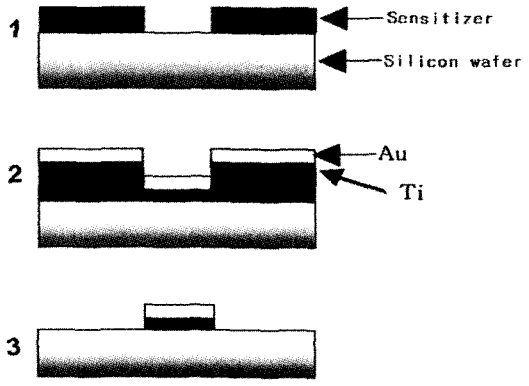


Fig. 3 Process of silicon sensor (1) Photo-lithography (2) Metal evaporation (3) Lift off.

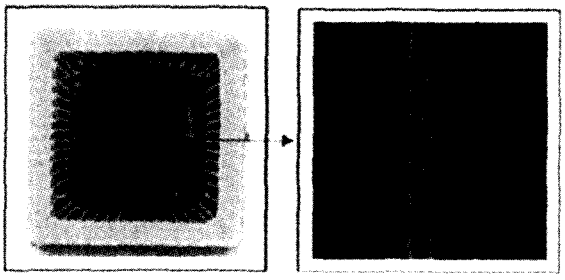


Fig. 4 shows a microscopic image of a developed sensor.

3. Sensor fabrication

Both silicon wafer and glass could be used as supporting layer of biosensor. However, silicon wafer is more durable and more integratable than glass layer. In this research, silicon wafer was used as the supporting layer. Also, gold (Au) was selected as an electrode because it less affects biological materials and is easy to bind with antibody. Since, gold is difficult to bind with silicon wafer, titanium (Ti) was used as an adhesion layer. To adhere titanium and gold on the sensor surface, thermal evaporator was used to.

Sensor pattern was printed on the surface with photo resist and gold was deposited on the pattern. And then the surface was lifted off with acetone. Fig. 3 shows the procedures of sensor fabrication.

Sensors were designed to have 2 μm of distance between electrodes and 10 μm of electrode width. Fig. 4 shows a microscopic image of a developed sensor.

In order to measure impedance accurately, the surface of the sensor was cleaned by immersing in the 60℃ of pirahna solution (30 vol.% H_2O_2 and 70 vol.% H_2SO_4, v/v) for 5 minutes and washing it with ethanol and deionized water. After clean the sensor surface, protein G Layer was formed on the surface by immersing the sensor in the protein-G solution (10 mg/l protein-G in 10mM phosphate buffer (pH 7.4) containing 0.14 mol/l NaCl and 0.02% (w/v) thimerosal (PBS)) for 2 hours at room temperature. Sensor was treated with 0.1% tween 20 solution for 20~30 minutes and washed with PBS. Next, anti-rat IgG was put on the sensor surface and incubated in the environmental control chamber with settings of 5℃ of temperature and 90% of relative humidity. Before rat IgG measurement, sensor was cleaned again with PBS.

4. Measurement

Background signal before reacting with antibody was measured to compensate variation of impedance characteristics of sensors resulted from nonuniform binding of antigen between each sensor. And then, antibody was applied and impedance was measured. Detailed measurement procedure is as follows:

Background impedance measurement: impedance was measured with impedance meter 10 minutes after applying 150 ul of PBS onto the sensor surface. For each sensor, 10 measurements were made and averaged for analysis. Frequency settings of 100 Hz, 1 KHz, and 10 KHz were used for the measurements.

Antibody measurement: Salmonella antibody was applied

on the sensor and the sensor was put into an incubator set to 37℃ and 90% RH for 30 minutes. Unbound antibody was washed away with PBS and impedance was measured.

Results and Discussions

Fig. 5~Fig. 8 shows impedance changes according to concentrations of the rat IgG. In each figure, lower graph shows background impedance. To find the relationship between impedance and antibody concentration, difference between without and with rat IgG application was used.

Frequency range for impedance measurement was from 40 Hz to 110 MHz. Among the frequencies, 100 Hz was the best to detect antibody concentration changes as shown in Table 1.

In that frequency, impedance difference according to the changes of antibody concentration was most apparent. Below the 100 Hz, influence of signal noise became predominant. In this research an impedance biosensor was developed to detect rat IgG. The sensor was designed to have 2 μm of distance between electrodes and 10 μm of sensor width.

Table 1 Frequency v.s. Impedance (unit: g/mℓ)

Freq.	7.6×10^{-6}	7.6×10^{-7}	7.6×10^{-8}	7.6×10^{-9}
100 Hz	7.01×10^{3}	9.85×10^{3}	1.49×10^{3}	1.66
1 KHz	4.94×10^{2}	4.56×10^{2}	14×10^{2}	2.39
10 KHz	5.88	-9.99	-5.97	0.32

Experiment results showed that detection limit of the sensor was 7.6×10^{-9} g/mℓ.

Impedance type biosensor has simple preprocess and measurement procedures, and it requires relatively short measurement time. Since it has high measurement precision it can detect very little amount of analyte. For further improvement, researches on electrode design and sensor fabrication are needed.

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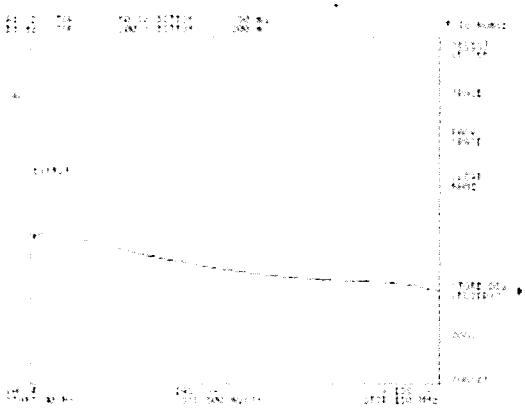

Fig. 5 Rat IgG concentration 7.6×10^{-8} g/mℓ.

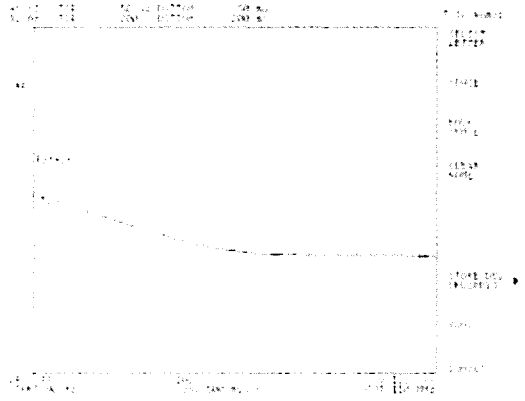

Fig. 6 Rat IgG concentration 7.6×10^{-7} g/mℓ.

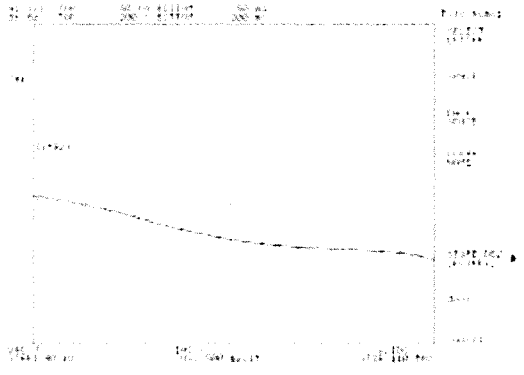

Fig. 7 Rat IgG concentration 7.6×10^{-9} g/mℓ.

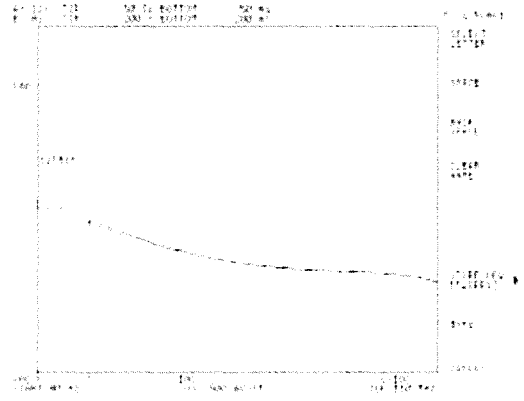

Fig. 8 Rat IgG concentration 7.6×10^{-6} g/mℓ.

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