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Short communication

Development of FET-type albumin sensor for diagnosing nephritis

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ABSTRACT

An albumin biosensor based on a potentiometric measurement using Biofield-effect-transistor (BioFET) has been designed and fabricated, and its characteristics were investigated. The BioFET was fabricated using semiconductor integrated circuit (IC) technology. The gate surface of the BioFET was chemically modified by newly developed self-assembled monolayer (SAM) synthesized by a thiazole benzo crown ether ethylamine (TBCEA)–thioctic acid to immobilize anti-albumin. SAM formation, antibody immobilization, and antigen–antibody interaction were verified using surface plasmon resonance (SPR). The output voltage changes of the BioFET with respect to various albumin concentrations were obtained. Quasi-reference electrode (QRE) and reference FET (ReFET) has been integrated with the BioFET, and its output characteristic was investigated. The results demonstrate the feasibility of the BioFET as the albumin sensor for diagnosing nephritis.

1. Introduction

The ability to detect biomolecular interactions is crucial to biotechnological research and development including clinical, environmental and food applications (Coté et al., 2003; D'Orazio, 2003; Estrela et al., 2005; Luppa et al., 2001; Patel, 2002; Rogers, 2006). Among the biomolecular interactions, the high specificity of the molecular recognition can be best typified by an antibody–antigen interaction which is the fundamental basis of all immunosensors (Luppa et al., 2001; Schöning and Poghossian, 2002). In recent decades, there are many detection modes developed for the immunosensors (Christodoulides et al., 2005; Estrela et al., 2005; Schöning and Poghossian, 2002; Shankaran et al., 2007; Wee et al., 2005). In general, optical sensors are based on the measurement of fluorescence, luminescence and reflectance. These sensors usually use signal-generating labels such as fluorophores, radioisotopes and enzymes which allows more sensitive and versatile detection mode. However, these methods involve time-consuming, multi-stage processes which are expensive and difficult to implement in portable instrumentation (D'Orazio, 2003; Luppa et al., 2001; Park et al., 2005; Shankaran et al., 2007). In the arena of portable microanalysis systems for the homecare medical instrument, electrochemical silicon-based sensors gain wide attentions (Chen et al., 2003). Among various silicon-based sensors the silicon FET-based biosensors have numerous potential advantages including small size, fast response, high reliability, low output impedance, the possible automatic packaging at wafer level, and on-chip integration of a signal processing scheme with future prospect of low-cost mass production (Bergveld, 2003; Kim et al., 2006; Park et al., 2005; Schöning and Poghossian, 2002; Sohn and Kim, 1996). The FET-based sensor has been proved to be very sensitive for any kind of electrical interactions at or nearby the gate interface. Thus, FET-based biosensor is able to detect the change in the electrical field associated with affinity binding of biomolecules (Bergveld, 2003; Schöning and Poghossian, 2002).

Nephritis is inflammation of a kidney commonly caused by infection or an autoimmune process. It has the effect of damaging and closing up the microscopic filters in kidney, which means the inflamed kidney filters out important proteins (larger molecules) from the blood. Therefore the characteristic symptom of the nephritis is proteinuria (http://en.wikipedia.org/wiki/Nephritis). Albumin

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in urine is a candidate biomarker to diagnose nephritis (Voss et al., 2005).

In this paper, we have fabricated the BioFET albumin sensor whose gate surface was chemically modified by newly developed SAM. The SAM was synthesized by the TBCEA–thioctic acid which was designed to entrap an anti-albumin selectively (Kim et al., 2000). Since the albumin is electrically charged molecule in phosphate buffer solution (PBS, pH 7.4 at 25 °C: Sigma, MO, USA), it is expected that antigen–antibody interaction on the gate surface of the BioFET would lead to a detectable change in the charge distribution. Based on this concept, we investigated the surface potential changes that were caused by binding of antigen–antibody on the gate surface. SPR was utilized to verify formation of the SAM, immobilization of antibody, and interactions of antigen–antibody. The drain current was observed after the formation of each molecular layer including BSA blocking to prevent non-specific binding. The responses of the BioFET with respect to various albumin concentrations were obtained, and the output characteristic of the BioFET/QRE/ReFET sensor system was also investigated.

2. Experiments

Complementary metal oxide semiconductor (CMOS) process technology has been applied to fabricate an n-channel MOSFET which is the key structure of the BioFET albumin sensor. The 5-in. p-type (1 0 0) single crystal silicon wafer with resistivity of 15–25 Ω cm was used as a substrate. Silicon dioxide (SiO_2) layer of thickness 450 Å was deposited. Boron ions with a dose of 1×10^{15} ions/cm^2 and energy of 80 keV were implanted on the patterned wafer to form p+ region. This step was undertaken for prohibiting formation of unwanted channels in the chip. Implantation of phosphorus ions (dose of 5×10^{15} ions/cm^2 and energy of 80 keV) was followed to form Source and Drain regions of the BioFET. Tetra-ethyl-*ortho*-silicate (TEOS, $Si(OC_2H_5)_4$) layer was deposited using plasma enhanced chemical vapor deposition (PECVD) to avoid the diffusion of the implanted impurities. After removing the oxide layer on the gate region, a good quality of SiO_2 layer with thickness of 450 Å was grown using dry oxidation. Following the removal of the oxide layer on contact regions, 50 Å-thick NiCr layer and 450 Å-thick Au layer were deposited by thermal evaporator. Photolithography (Appendix A) and etching steps were followed to define the metal pattern. To fabricate QRE, 100 Å-thick Ti layer and 1000 Å-thick Pt layer were deposited by sputtering. Lift-off process was utilized to define the QRE. The chip size was 1500 μm × 3000 μm, and the channel region was 1000 μm (width) × 20 μm (length). In this chip, both the FETs are with common drain. Fig. 1(a) shows layout of the BioFET. One of the FETs is used as BioFET for sensing target biomolecules, and the other as reference FET (ReFET) for measuring background signals. The fabricated BioFET sensor was attached to a metallized alumina substrate. For encapsulation, silicone rubber and epoxy were coated after wire bonding. Finally, Au layer on the gate region of the BioFET was opened to the outside world.

The gate surface of the fabricated BioFET was exposed to TBCEA–thioctic acid in a mixed solvent (ethanol (CH_3CH_2OH) (60%):acetonitrile (CH_3CN) (40%)) which has surface terminal groups to immobilize antibody and head groups (thiol, –SH) to link the Au gate surface with alkyl and biphenyl spacer chains. The surface consisting of SAM layer was immersed in anti-albumin solution to immobilize antibody. When the albumin solution was injected, antigen–antibody specific binding occurred. All proteins were dissolved in PBS, and all measurements were taken at room temperature in PBS after the completion of the reactions. At the end of each step, PBS was used to clean the device. The SAM formation, antibody immobilization and antigen–antibody interaction was verified using SPR system. The drain currents of the BioFET were measured after each reaction including BSA blocking. We observed potential variation at the surface of the gate using a null balance circuit. Differential amplifier method was employed to eliminate noise in the BioFET/QRE/ReFET sensor system.

3. Results and discussion

Fig. 1(b) shows a typical graph of drain voltages (V_d) versus drain currents (I_d) of BioFET after SAM formation, anti-albumin immo-

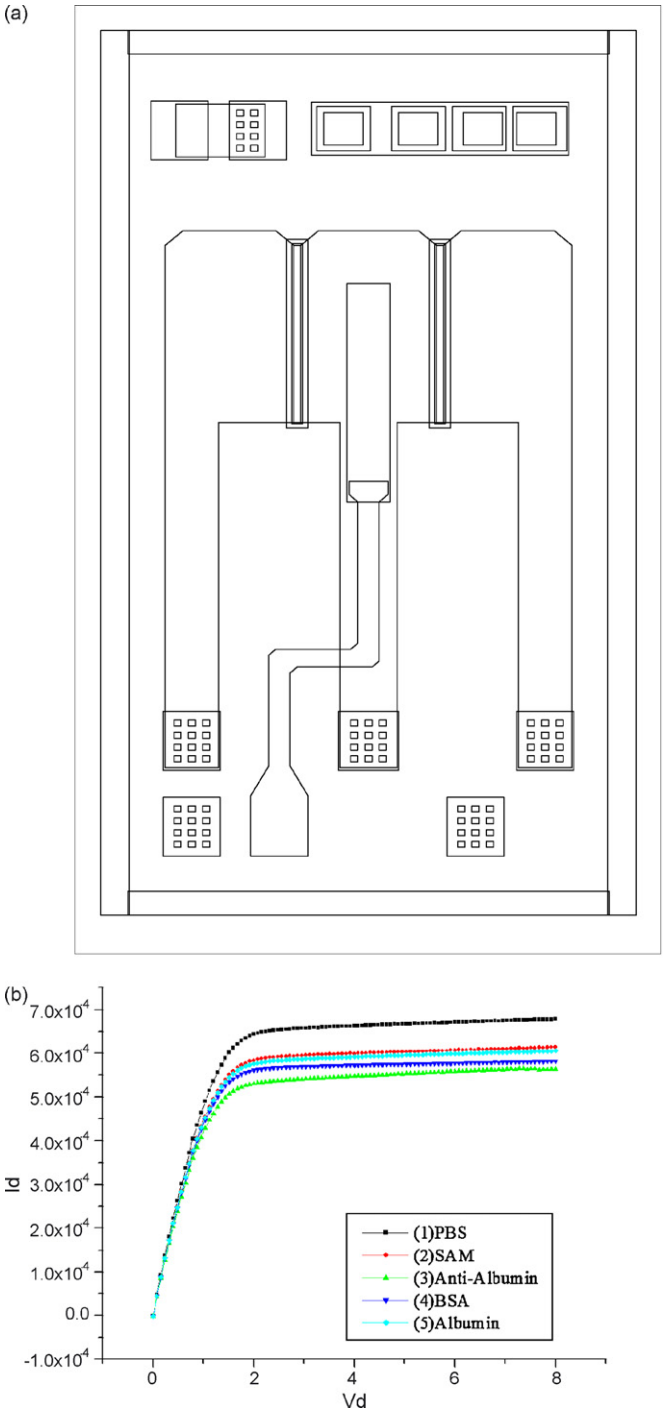

Fig. 1. (a) Layout of the BioFET and (b) I_d–V_d characteristics of the BioFET albumin sensor.