Capacitive immunoaffinity biosensor based on vertically paired ring-electrodes

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ARTICLE INFO

ABSTRACT

A capacitive biosensor was developed by using a vertically paired ring-electrode for the non-labeled immunoassay. The vertically paired ring-electrode was prepared by sequential deposition and etching processes. Two electrodes were layered on glass substrate by sputtering of gold layers with thicknesses of 50 nm and 100 nm, and a parylene-C film with a thickness of 550 nm was positioned between the electrode layers as a dielectric material by thermal deposition. The top layer was made by spin coating of SU-8. And then, the ring-electrodes were exposed at the wall of the layered structure by sequential etching processes. The fabricated electrodes were characterized by cyclic voltammetry of a well-known redox couple of 3,3',5,5'-tetramethylbenzidine. The non-labeled detection of antigen–antibody interaction was demonstrated by using anti-horseradish peroxidase (HRP) antibodies and C-reactive protein (CRP) as model analytes. When the model analytes were bound to the vertically paired ring-electrode, the impedance change was measured during the immunoassay steps, and the measured impedance was analyzed by using a model circuit of the ring-electrode, and the capacitance was estimated to be dependent on the adsorption of analytes between the ring-electrodes.

1. Introduction

Immunoaffinity biosensors have been developed by using the highly sensitive antigen–antibody interaction for the detection of a target analyte in complex mixtures. Usually, the non-labeled detection of immunoaffinity biosensors could be achieved by using various kinds of transducers, such as electrochemical (Mirsky et al., 1997), surface plasmon resosnance (Horan et al., 1999; Lahiri et al., 1999; Ostuni et al., 1999; Shin et al., 2010), mass sensitive (Pyun et al., 1998), and so on. Among the transducers, capacitive biosensors have been interested for non-labeled detection of antigen–antibody interactions because of the high sensitivity and the relatively simple instrumentation and so on (Berggren et al., 1998, 1999, 2001; Dijksma et al., 2001; Bard and Faulkner, 2001; Bart et al., 2005; Carrara et al., 2009a,b; Kang et al., 2010). The capacitance is determined by the equation: $C = \varepsilon \cdot A/d$, where C, ε, A, d represent capacitance, permittivity constant, area of electrode, distance between electrodes, respectively. The idea of this work is to fabricate the capacitive biosensor by using vertically positioned electrodes. In order to increase the sensitivity the distance between electrodes was minimized by using a thin parylene film as a dielectric layer. For the capacitive biosensors, many approaches have been taken in the design of interdigitated electrodes (IDEs). The IDE-type electrodes consist of comb-shaped electrodes facing each other, which are electrically separated with a micro- or nano-gap structure. Nano-gap IDEs enable to measure label-free biomolecular interactions such as DNA hybridization and antigen–antibody reactions (Yi et al., 2005; Kim et al., 2009; Zou et al., 2007). Usually, such IDE-type electrodes with the distance between electrodes of less than 1 μm have been fabricated by using costly and time-consuming processes such as E-beam lithography (Zou et al., 2007), focused ion-beam fabrication (Santschi et al., 2006), and a mass-producible submicron-gap IDE via a stepper photolithography (Ahn et al., 2011). In this work, a new type of vertically paired ring-electrode was fabricateed for the non-labeled immunoassay by using the parylene-C film with a thickness of less than 1 μm as a dielectric layer. In order to demonstrate the non-labeled immunoassay, two kinds of model analytes of anti-horseradish peroxidase (HRP) antibodies and C-reactive protein (CRP) were used with the vertically paired ring-electrodes. When the model analytes were bound to the vertically paired ring-electrode, the impedance change was measured during the immunoassay steps, and the measured impedance was analyzed by using a model circuit of the ring-electrode. From the model circuit analysis, the impedance between the ring-electrodes was calculated to be largely dependent on the capacitance change of the protein layer positioned at the current pathway. Therefore, the non-labeled detection of the target

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analyte bound to the antibodies immobilized on the wall of the ring-electrode could be achieved.

Here, the fabrication of ring-electrode was presented by controlling the thickness of parylene-C film with quartz crystal microbalance (QCM), and the fabricated electrode was characterized by using a well-known redox couple of 3,3',5,5'-tetramethylbenzidine. The non-labeled detection of antigen–antibody interaction was demonstrated by using horseradish peroxidase (HRP) and C-reactive protein (CRP) as model analytes. The impedance was analyzed by using a model circuit of the ring-electrode, and the non-labeled detection of antigen–antibody interaction was demonstrated by estimation of capacitance change during the immunoassay steps.

2. Materials and methods

2.1. Materials

C-reactive protein (CRP), anti-CRP antibodies were purchased from AbCam (Cambridge, UK). Horseradish peroxidase (HRP), anti-HRP antibody, 3,3',5,5'-tetramethylbenzidine (TMB) and other chemicals of analytical grade were purchased from Sigma-Aldrich Korea (Seoul, Korea).

2.2. Fabrication of nano-gap-ring electrode

The vertically paired ring-electrode was prepared on a glass substrate by a sequential deposition of gold electrode (100 nm), parylene-C film (550 nm), gold electrode (50 nm), SU-8 layer (1.7 μm). Two gold layers were sputtered with a thickness of 100 nm by using a metal mask with a square-shaped opening (10×10 mm^2), and the square-shaped superimposed area was designed to be 5×5 mm^2 as shown in Fig. 1. Between the gold layers, parylene-C (Femto Science Co., Korea) film with a thickness of 550 nm was thermally deposited by the following polymerization steps: (1) evaporation of parylene dimers at the temperature of 160 °C, (2) pyrolysis for the production of highly reactive p-xylene radical at the temperature of 650 °C, and (3) deposition on the gold layer at room temperature (Jeon et al., 2010, 2011). The whole coating procedure was reproducibly carried out by using a microprocessor-controlled parylene coater from Femto Science Co (Korea). After the parylene-C dimer was completely evaporated, the deposition was carried out by supplying the evaporated parylene-C monomer gas into the deposition chamber under vacuum condition of less than 5 Pa at room temperature. For the monitoring of parylene-C deposition, the QCM response was measured from the beginning of evaporation step, and the mass changings on the QCM sensor from the deposition of a parylene film were converted into the frequency shift based on the Sauerbrey equation: $\Delta f = -2\Delta m f_0^2 / [A(\mu_q \rho_q)^{1/2}]$, where ρ_q is the quartz density, μ_q is the crystal shear module, f_0 is the crystal fundamental frequency, A is the piezoelectrically active area of crystal (defined by the area of the deposited metallic film on the crystal), and Δm and Δf correspond to mass and system frequency changes, respectively (Ansorena et al., 2011; Harbecka et al., 2011). For the thickness control of parylene-C film, the QCM response was measured from the beginning of evaporation step, and the thermal deposition was finished when the QCM frequency shift reached to the value corresponding to the targeted thickness of the parylene film-C. As a top layer, the SU-8 coating (1.7 μm) was made on the gold layer as a protective layer by the spin-coating. The hole-shaped area with a diameter of 2 mm was exposed to UV light for the etching process, and then the SU-8 layer was baked at 110 °C.

The ring-electrodes were exposed by the sequential etching processes as shown in Fig. 1. The hole-shaped opening with a diameter of 2 mm was made at the superposed area of gold layers. The ring-electrodes were exposed by a series of etching process. As the first step, the SU-8 layer was etched by dipping in a commercial developer (Micro Chem, USA). The gold layer was etched by dipping in 1% ferricyanide solution with 1% NaCN for 30 s at room temperature (Utaka et al., 2007). The parylene film was etched by LF-plasma treatment with a power of 10 W for 1 h (Jeon et al., 2010, 2011). The second gold layer on the glass substrate was also etched by dipping in 1% ferricyanide solution with 1% NaCN for 1 min at room temperature.

2.3. Electrochemical measurements

The cyclic voltammetry (CV) was performed by using a potentiostat from IVIUM Technologies (Compactstat, Netherlands). For the characterization of the ring-electrodes, three-electrode system was prepared by using the ring-electrode as a working electrode. An external reference electrode of Ag/AgCl and a counter electrode made of Pt-wire were used for the analysis of a well-known redox couple of 3,3',5,5'-tetramethylbenzidien (TMB). The cyclic voltammogram (CV) of TMB sample was obtained at the potential range from −400 mV to +600 mV (versus Ag/AgCl) at the scanning rate of 30 mV/s. The TMB solution was prepared to be 0.02 mg/ml in 100 mM sodium citrate buffer at pH 5.0 with 0.2% hydrogen peroxide.

The impedance measurement was also performed by using the potentiostat from IVIUM Technologies (Compactstat, Netherlands), and two ring-electrodes were used for the impedance measurement at the frequency range from 100 Hz to 100 kHz with an applied potential of 10 mV. The measured impedance of the electrode sensor was analyzed by using an equivalent circuit model which consists of the interfacial electrode impedance consisted of the constant phase
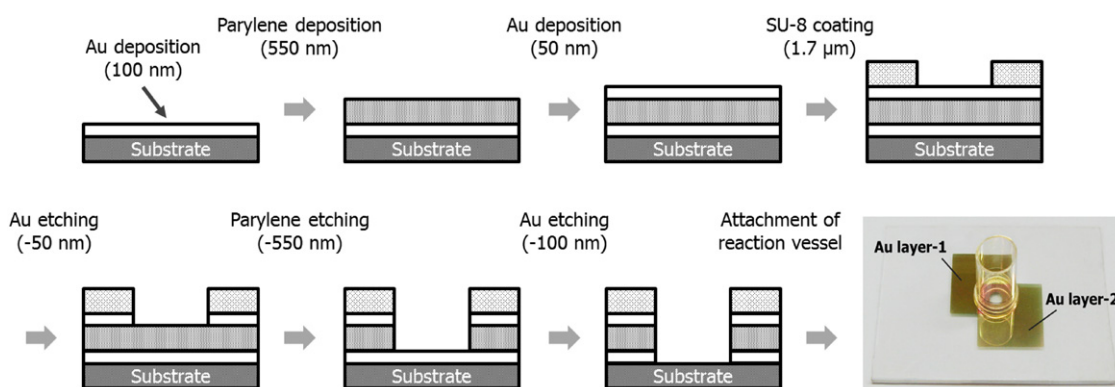

Fig. 1. Fabrication process of vertically paired ring-electrode. The electrode was prepared by a sequence of deposition processes and a series of etching processes.