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A label-free immunosensor for diagnosis of dengue infection with simple electrical measurements

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ABSTRACT

The interdigitated electrodes and electrical measurements for the diagnosis of dengue infection using antigen–antibody conjugation method are reported. As a proof of concept, pre-inactivated dengue virus was firstly immobilized indirectly onto the immunosensor surface, pre-coated with sol–gel derived barium strontium titanate (BST) thin film and modified with organic self-assembled monolayer (SAM) formed by 3-aminopropyltriethoxysilane (APTS) and a cross-linker glutaraldehyde over the interdigitated electrodes. The modified sensor surface served as selective sensing probe to capture/conjugate the dengue antibody molecules present in patient's serum. Our immunosensor is based on non-faradaic process, using only de-ionized water as electrolyte during the simple electrical measurements. Both ac impedance spectroscopy and dc *I–V* measurements between the electrodes gave a clearly discernable and repeatable signal to positively identify the presence of dengue antibody in the serum. Direct correlation was obtained between the signal outputs with respect to antibody concentrations. The measured signal changes in impedance/current without/with the presence of dengue antibody were attributed to the surface conductivity change upon biomolecules immobilization and the dipole-induced interfacial polarization potential at the SAM film/biomolecules interface. By monitoring the impedance or current change, the antibody molecules in the patient's serum could be positively detected.

1. Introduction

Currently, the most sensitive and specific assay diagnosis for infectious disease is the laboratory-based polymerase chain reaction (PCR) (Mackay, 2004) or Enzyme-Linked ImmunoSorbent Assay (ELISA) methods. However, these methods are both time consuming and costly, hence not suitable for clinical screening or point-of-care molecular diagnosis in the field. The immunosensor based on antigen/antibody interaction has the potential of simple, rapid, label-free and low-cost molecular diagnosis and has attracted great interest in research and development (Daniels and Pourmand, 2007; Luppa et al., 2001). A small, disposable immunosensor device for dengue infection diagnosis, with simple interdigitated electrode design and suitable for point-of-use by unskilled users in the field is highly desirable.

The conventional electrochemical impedance spectroscopy is mainly based on the faradaic process where charge is transferred across the interface. Redox-couple, most often $[Fe(CN)_6]^{4-/3-}$, is added to the electrolyte solution. However, it was observed by some researchers that the long term presence of the redox-couple ($[Fe(CN)_6]^{4-/3-}$) reduces the activity of the protein layer (Rickert et al., 1996). Immunosensor based on non-faradaic process with no addition of reagent has also been studied and shown that this scheme was somewhat more amenable to point-of-care applications (Berggren et al., 2001; Rickert et al., 1996).

Barium strontium titanate (BST) thin films have long been receiving intensive research attentions for various applications. It has been widely used in electronic devices, such as tunable microwave filters and radio-frequency microelectromechanical capacitive switches (Morales-Cruz et al., 2005), due to their high permittivity, low loss and electric tunable feature. They are also used as transducers of various microsensors, i.e. bolometers (Hashimoto et al., 2001; Noda, 2005), gas sensor (Deng et al., 2004; Tan et al., 1999, 2003; Zhu et al., 2000), humidity sensors (Agarwal and Sharma, 2002; Agarwal et al., 2001). These microsensors employ BST thin films as the middle dielectric layers in sandwich structure capacitors, with a measurable capacitance change when the films adsorb radiant heat, gas, or water vapor. The sol–gel derived BST thin film was exploited in the area of biosensors by Tan and co-workers (Fang et al., 2006; Tan et al., 2005, 2006–2007). They demonstrated the successful immobilization of biomolecules (i.e. bovine serum albumin (BSA)) on BST thin film that deposited on Pt coated Si substrate. Three-electrode system was applied for all the measurements with an Ag/AgCl reference

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electrode, a platinum coated wafer as a counter electrode and the biomolecules immobilized BST film as a working electrode. Electrochemical impedance spectroscopy and cyclic voltammetry were applied to investigate the immune reaction between BSA and anti-BSA in the presence of a redox-couple.

In this paper, a novel immunosensor has been developed based on interdigitated electrodes, using a sol–gel derived BST thin film as sensing media. Silanization method was applied for surface modification. Thermally inactivated dengue virus was immobilized on the 3-aminopropyltrimethoxysilane (APTS)/glutaraldehyde modified surface as detection probe for the dengue antibody in human serum. The interdigitated microelectrode structure was applied in the immunosensor, which gives the advantages of ease in electrical measurements, repeatability in device fabrication process using standard microfabrication process and increment in effective sensing area. With co-planar symmetric structure, both fingers from each electrode serve as working electrode, which effectively double the sensing area. The use of BST thin film as an effective sensing media on top of the electrode provides for better coupling for the electrical field on the sensing surface rather than penetrating deep inside the electrolyte. The biomolecules that have been immobilized on the surface act as dipoles and provide an induced depletion layer on BST film surface. This in terms results in the increase in the interfacial capacitance and decrease in the interfacial resistance which are directly related to the amount of dipoles on the surface and hence the concentration of the biomolecules. Moreover, compare to conventional electrochemical immunosensors, our immunosensor is able to eliminate the use of the toxic redox-couple, i.e. $[[Fe(CN)_6]^{3-/4-}$ as liquid medium. It is based on non-faradaic process, using only DI water as the electrolyte during the measurements. Impedance spectroscopy and dc *I–V* measurements were used to semi-quantify the dengue antibody concentration in serum solution which are pre-diluted by phosphate buffered saline (PBS). Good linear relationship between electrical impedance change/current change and antibody concentration in logarithm scale was obtained for a wide range of antibody concentrations. An equivalent circuit has been setup to simulate the impedimetric measurement results and explain the sensing mechanism. Our immunosensor also has the potential to integrate with external electrical measurement circuit and microfluidic channel for the delivery of analytes. Hence, it could be developed into disposable devices which are cheap and yet robust.

2. Material and methods

2.1. Reagents

Barium acetate ($Ba(CH_3COO)_2$, 99%) and glacial acetic acid (CH_3COOH) were purchased from Merck. Strontium acetate ($Sr(CH_3COO)_2{\cdot}1/2H_2O$) was purchased from Nacalai Tesque, Japan. Titanium butoxide ($Ti(O(CH_2)_3CH_3)_4$, 97%), 3-aminopropyltriethoxylsilane (APTS), glutaraldehyde (50%), phosphate buffered saline (PBS, powder) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich. Acetylacetone ($CH_3COCH_2COCH_3$) was obtained from Fisher Scientific. 2-Methoxy-ethanol was purchased from Panreac Quimica SA. Acetone, 2-propanol and ethanol were purchased from BESTCHEM. Double adhesive Mylar-D® polyester film was purchased from Fralock Div. of Lockwood Ind. Inc., California. De-ionized water was purified using a MilliPore Simplicity unit to a resistivity $\geq$18.2 MΩ cm. Dengue virus solutions (DENV-2, thermally inactivated) and serum with dengue antibody were received from DSO National Laboratories. All reagents were used as received unless otherwise specified.

2.2. Device fabrication

Three major steps were involved in device fabrication, namely bottom interdigitated electrode fabrication, BST thin film deposition and device patterning.

The bottom interdigitated electrode (IDE) was deposited on thermal oxide coated Si substrate using photolithography and E-beam evaporation techniques. The electrode finger is 100 μm in width and 50 nm in thickness. The gap between each electrode finger is 25 μm. 10 nm of Ti was deposited prior to gold deposition and served as the adhesion layer.

The BST thin film was deposited on the IDE coated substrate by sol–gel technique. 0.15 M sol solution was prepared using barium acetate, strontium acetate and titanium butoxide as the starting precursors; acetic acid and 2-methoxy-ethanol as the solvents. Acetylacetone was used as additive to increase the stability of the solution. Spin coating method was used for the thin film deposition. Each layer was spun on at 3000 rpm for 60 s. It was dried at 150 °C and pyrolyzed at 375 °C for 5 min each. Four layers were deposited to achieve a film thickness of around 100 nm. The final film was annealed in quartz furnace at 500 °C in simulated air ambient.

The final step of device fabrication was to pattern the device and expose the bottom electrode for electrical probing. Photolithography was used with a second mask to define the contact pad at the two ends of each device. Diluted HF solution (HF:DI water = 1:50) was used to etch away the BST thin film at the contact pad opening. Fig. 1a shows the photomicrograph of the ready device. Under field emission scanning electron microscopy (FESEM), the BST thin film deposited on both SiO_2/Si substrate and gold electrode are dense and uniform, as shown from the device cross-section images in Fig. 1b. Good film continuity was also observed at the edge of gold electrode. The thickness of the gold electrode layer as well as BST thin film layer are both close to what we have expected.

2.3. Surface modification and biomolecules immobilization

BST thin film surface was modified using silanization method for biomolecules immobilization. The samples were thoroughly cleaned with acetone, 2-propanol and de-ionized water before being treated with UV/ozone dry stripper (Samco, Bench Top, UV-1) for 20 min. This commercial UV/ozone dry stripper emits UV light principally at 254 nm (intensity 10–13 mW/cm^2) and 185 nm (intensity is 10% of the 254 nm line). The ozone is generated by the silent discharge ozone generator inside the system. Under the UV light, the ozone is decomposed to oxygen molecule and oxygen atom, which is very active that can clean and activate the surface by introducing –OH to the BST thin film surface. The samples were then immersed in a freshly prepared ethanolic APTS solution (6%, *v*/*v*) for 2 h, followed by dip rinsing with ethanol for 1 min and dried with a N_2 stream. The samples were incubated at a N_2-filled chamber at room temperature for 1 h. These pretreated samples were treated with a cross-linker by incubating in glutaraldehyde PBS solution (1.25%, *v*/*v*) for 1 h, and then rinsed with PBS solution. They were then exposed to thermal inactivated dengue virus solution at 4 °C for overnight. BSA solution (5 mg/mL) was used as blocking agent afterwards to cover the excess active terminals to prevent any unspecific binding. The human serum solutions with different concentration of dengue antibody were prepared by serial dilution with PBS. The dengue virus immobilized samples were then exposed to serum solution with dengue antibody for 2 h at room temperature for antibody/antigen conjugation. The samples were rinsed thoroughly with PBS buffer to remove any loosely attached antibody. They were cleaned with DI water subsequently to remove any PBS residuals. Fig. 2a shows