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Developing Electrochemical Impedance Immunosensor for the Detection of Myoglobin

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Abstract:
In the present work, electrochemical impedance immunosensor is developed for detection of myoglobin. The procedure involves the immobilization of myoglobin antibodies on the interdigitated gold electrodes (IDE) using alkanethiol self-assembled monolayer (SAM) for binding myoglobin antigens available in the blood serum/aqueous solution. Then the sensor system is characterised using alternating current (AC) electrochemical impedance spectroscopy (EIS), in which the change in impedance spectra is observed across the frequency range of 0.1 Hz to 10 MHz. This work mainly focuses on understanding and improving the impedance immunosensor for better sensitivity readings (~1 ng/mL) by adjusting the interdigitated electrode configuration, incubation times, reagent sample volumes and immobilisation protocols. Myoglobin is one of the premature indentifying cardiac protein markers to monitor minor heart attacks. Hence, the developed immunosensor has potential of using as a point-of-care diagnostic device. The protocol developed in this work can be useful for detecting other cardiac markers like Troponin and CK-MB by using respective antibodies.

Key words: electrochemical, impedance spectroscopy, immunosensor, myoglobin, self-assembled monolayer, fluorescent detection

Introduction

In recent years, impedance based direct immunosensors have made huge progress for the rapid detection of biomolecules. Electrochemical impedance spectroscopy is emerging as a reliable method for the analysis of interfacial property changes of modified electrode surfaces, which eventually used as bio-recognition event [1]. Impedance technique analyzes both the resistive and capacitive properties of the medium containing biomolecules and electrolytes. Impedance-based biosensors have been widely applied because of their high sensitivity and label-free operation. Motivated by the remarkable advantages, researchers are engaged to produce a variety of impedimetric biosensors to monitor various biochemical reactions at the surface of electrodes by immobilizing biomolecules such as enzymes, proteins, nucleic acids, etc. [2-8].

In this work, we fabricated gold interdigitated electrodes (IDEs) with various dimensions. IDEs were modified by alkanethiol self-assembled monolayers (SAMs) for the antibody immobilizations. Finally, alternating current (AC) impedance spectroscopic studies were performed after immobilization of antibody and the sensitivity of the immunosensors, at various IDEs dimensions were tested.

Materials and Methods

Materials

11-mercapto-1-undecanoic acid (MUA), 11-mercaptoundecanoal (MU), N-hydroxysuccinimide (NHS) and 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich Chemicals, Ontario, Canada. Capture and detection myoglobin antibodies (antimyoglobin 7C3 and 4E2) were obtained from Hytest Ltd, Turku, Finland. Other chemicals (phosphate buffer saline (PBS), Sodium Chloride (NaCl), Ethanol, etc.) were of analytical grade and used without further purification.

Apparatus

Impedance measurements were performed using VersaSTAT Impedance Analyzer (VersaSTAT 4, Princeton Applied Research,

Oak Ridge, TN, USA) with VersaStudio software.

Fabrication of Microfluidic Chip with Integrated IDE's

First, IDE's were patterned on the glass substrate using standard photolithography process. Four different types of electrode configurations were used in the present work (Table 1). IDE is made-up of gold/chromium layer with 300nm thickness. PDMS microchannels (10mm long, 2 mm width and 100 micron depth) with two reservoirs (inlet and outlet) were fabricated using soft-lithography process. Next, PDMS microchannel was aligned and bonded to IDE using oxygen-plasma treatment. Microfluidic chip with integrated IDE is illustrated in Fig.1.

Immobilization of Antibodies on Gold Electrode

Step1: Cleaning of Gold surface

Microfluidic chip with integrated IDE was cleaned in ethanol, isopropyl alcohol and deionized water and dried with nitrogen gas.

Step2: Preparation of alkanethiol self-assembled monolayer (SAM)

4mM of MUA and 1mM of MU were prepared in ethanol and stored at room temperature. Then, IDE were immersed in a mixture of 4mM MUA / 1mM MU (1:1; v/v) for overnight to obtain SAM. Then SAM prepared samples were washed in ethanol to remove unbound thiols and dried with nitrogen gas.

Step3: Preparation of EDC/NHS for antibody activation

400mM of EDC and 100mM of NHS were prepared in freshly filtered Millipore deionized water, aliquoted and stored separately at -20^{o}C. Then IDE were immersed in a mixture of 400mM EDC / 100mM NHS (1:1; v/v) for 10 minutes. This step helps to activate the sample surface to immobilize the antibodies.

Step4: Immobilization of Myoglobin antibodies

Capture myoglobin anitbodies were prepared in PBS at concentration of 100 µg/mL. 2 µl solution of myoglobin antibodies were injected into the PDMS channel and kept for 10 min incubation time. This step was used to immobilize the antibodies on IDE.

Step5: Testing of Antibody Immobilization

Antibody coated IDE was cleaned several times by injecting PBS buffer into the channels to test the performance of immobilization.

Impedance measurements

Figure 1 shows the experimental set-up arrangement for the impedance measurements. Electrochemical impedance measurements were performed using 1M NaCl solution as an electrolyte without any redox species for different IDE sensors. For the impedance measurements, a sinuous modulated AC potential of 10mV at frequency range of 0.1 Hz to 1MHz was applied across IDE. Bode plot was plotted for the magnitude (Ohms) and phase angle (deg) of impedance.

Tab. 1: Details of different IDEs used in this work

Type of electrode configuration	Width of electrode (µm)	Gap between the electrodes (µm)
IDE1	25	25
IDE2	25	50
IDE3	70	30
IDE4	75	25

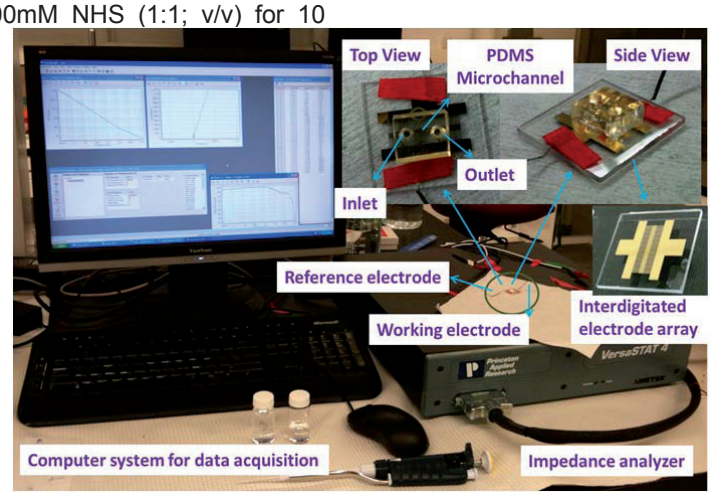

Figure 1.Experimental set-up for the impedance measurement with microfluidic chip with integrated IDE.

Results and Discussion

The development of sensor protocol is characterized after each modification on the gold surface with ellipsometer, Fourier – Transform Infrared Spectroscopy (FTIR), atomic force microscopy (AFM), and contact angle measurement system. This characterization identified the respective linked molecules produced after each step. Thickness of alkanethiol SAM is measured as ~0.84 nm in ellipsometer. Since the main focus of the paper is to show the impedance spectra of IDEs at different steps, we have not presented the characterization results here.

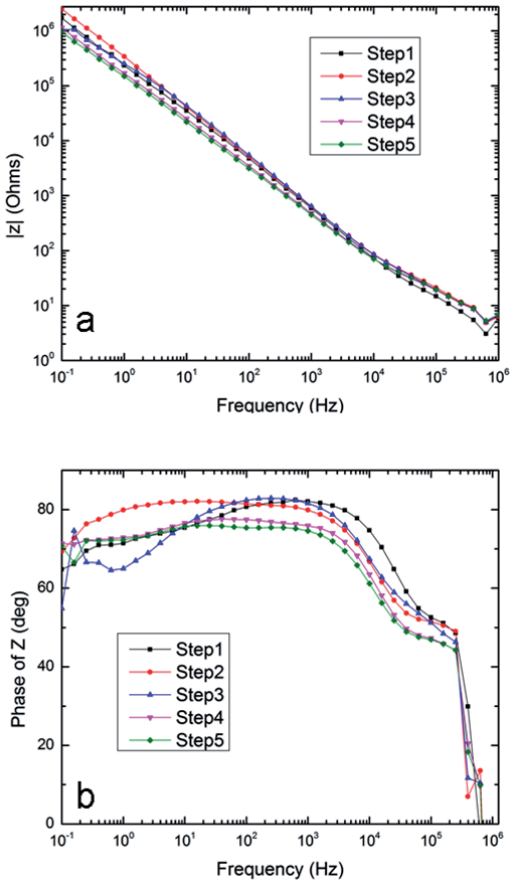

Fig. 2. Illustrations of Bode plot of impedance spectra for IDE3 after each step; (a) |Z| vs frequency; (b) the phase angle of Z vs frequency.

We measured the impedance after each processes mentioned above. The whole frequency spectra showed a difference in both impedance and phase angle, possibly due to the change of double layer capacitance (C_{dl}) and charge transfer resistance (R_{ct}). Bode plot can be useful to understand the characteristics frequency range of the sensors. Two representative impedance spectra are shown in Fig. 2 and Fig. 3. Figure 2 shows the effect of each process on the impedance spectra and Fig. 3 shows the effect of electrode dimensions on the impedance spectra. The results shown in Figs. 2 and 3 are matches with the existing literature results on impedimetric sensors.

In the present work, we used 1M NaCl solution as electrolyte without any redox species, whereas most other reports considered $[Fe(CN)_6]^{-3/-4}$ as redox species. Redox species increases Faradaic reaction, which will increase the insulation of surface. This can be easily detected by change in resistance (R_{ct}). In non-Faradaic case (the present study), impedance spectra is dominated by equivalent capacitance change in the set-up due to double layer formation at the electrode surfaces.

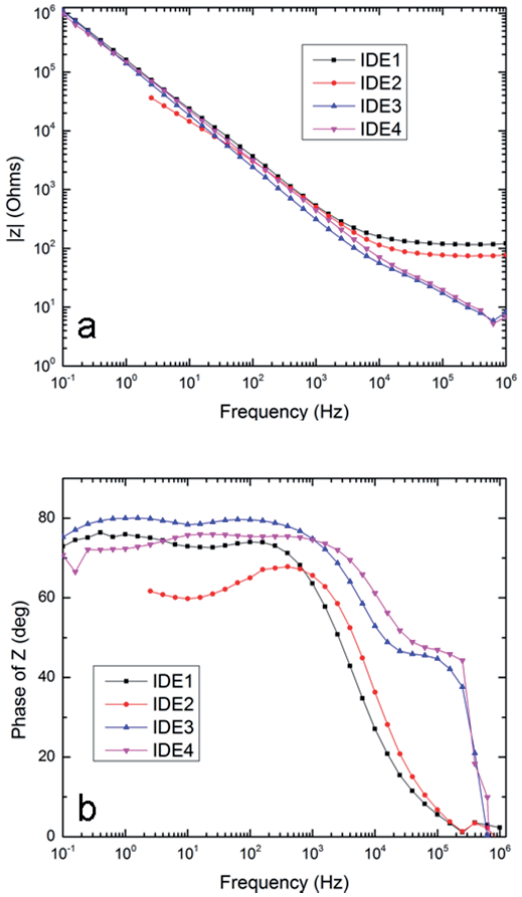

Fig. 3. Illustrations of Bode plot of impedance spectra for various IDEs after step 5; (a) |Z| vs frequency; (b) the phase angle of Z vs frequency.

These Bode plots are used to decide the working frequency range to study the performance of sensor. At higher frequencies, the increase in electrode area is the dominant

parameter for change in impedance as observed in Fig. 3a. From the results (Figs. 2a and 3a), it is observed that 10 Hz to 10KHz is the frequency range where one can observe a large change in impedance.

Conclusion

The present work discussed the methodology used to develop electrochemical impedance immunosensor for myoglobin detection. The reagent volume and incubation times were reduced by using microchannels. In future, we will study the effects of electrode size, gap size, and thickness of electrode.

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