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Ratio of HbA_{1c} to hemoglobin on ring-shaped interdigital electrode arrays based on impedance measurement

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ABSTRACT

It is essential to monitor the long-term glucose concentration in the blood of diabetic patients, and glycated hemoglobin (HbA_{1c}) has become one of the most prominent markers of glycemic control in diabetes. This study presents an on-chip biosensor for detecting HbA_{1c} as a ratio to total hemoglobin (Hb) based on impedance measurement, which allows for a label-free low-volume sample. The ring-shaped interdigital electrodes were coated with the self-assembled monolayer (SAM) to immobilize the proteins and measure the impedance deviations. The roughness of the glass substrates was further improved by buffer oxide etchant (BOE), while distribution uniformity of the proteins was also improved and verified by fluorescent images. Various concentrations of Hb and HbA_{1c} were measured via before–after impedance deviations. After the HbA_{1c} separation process, the ratio of HbA_{1c} to total Hb was measured by the differential capacitance (ΔC) of the proteins calculated from the equivalent circuit model. ΔC rises with the volume percent of HbA_{1c} from 1% to 15% in 200 ng/μL Hb and 200 ng/μL HbA_{1c}. The proposed detection method is very close to actual point-of-care diagnostics for diabetic patients, and features the advantages of low-cost and easy fabrication.

1. Introduction

It is clearly acknowledged that diabetes has become a major public health problem. The American Diabetes Association (ADA) has estimated the total cost of diagnosed diabetes have rise from $174 billion in 2007 to $245 billion in 2012, which translates to a 41% increase over a 5-year period [1]. Therefore, the main objective of this research was to provide a low-cost and simple device to detect this long-term disease.

Although many other proteins are also glycated in the diabetic and non-diabetic states, HbA_{1c} is now considered the most dependable for long-term markers of glycemic control. HbA_{1c} occurs over the entire 120-day life span of a red blood cell, so it has been thought to represent the average blood glucose level over the past 2–3 months [2]. Due to its stable characteristic, it is unaffected by the influence of daily meals and water consumption, both of which impact glucose level measuring. However, most importantly, with the help of HbA_{1c} detection, one does not have to bear the pain of injections for daily glucose level monitoring. With the amino-terminal valine of hemoglobin's beta chain, HbA_{1c} is formed by a non-enzymatic reaction of glucose [3]. The HbA_{1c} marker is frequently interpreted as a ratio (HbA_{1c}/total Hb). In general, diabetes is considered to be under good, fair or poor control at values of <7%, 7–8%, and >8% respectively, which means that more than 7% could constitute diabetes [4].

Many methods which have rapid and lower concentration detection of HbA_{1c} have been proposed such as electrophoresis [5], cation-exchange chromatography [6], Raman spectrometry [7], boronate-affinity chromatography [8,9], the immunoturbidimetric method [10], electrochemical [11,12], grapheme nanotube [13,14], and ion-sensitive field-effect transistors [15]. Due to their composite operation procedures or dedicated instruments, however, most of the above methods are not portable, and so do not meet clinical requirements [16]. In addition to the above methods, electrical biosensors have the high sensitivity together with their compatibility, portability, low cost, and disposability by modern microfabrication technologies [17,18]. Besides, most of previous studied mentioned above used multilayer films or other complex

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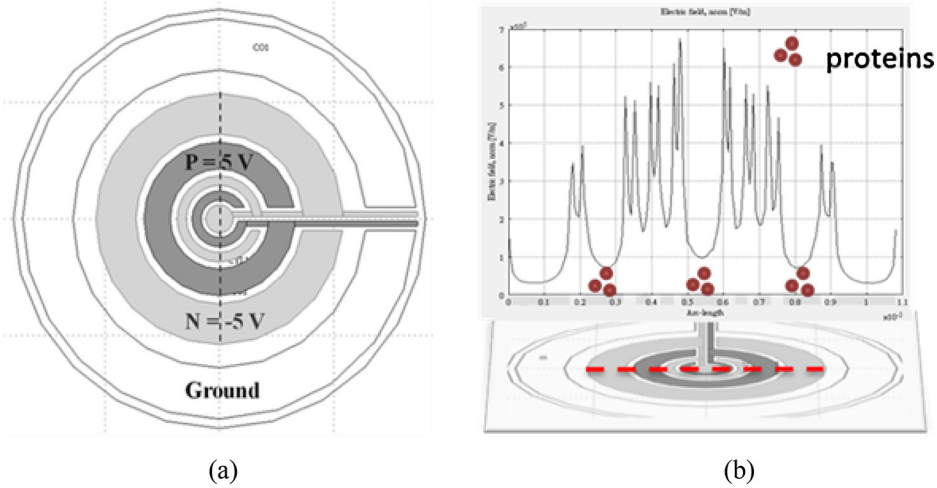

Fig. 1. (a) Schematic diagram of the electrode, which combines a ring structure and capability of concentrating proteins. (b) COMSOL simulation result of the electric field on the electrode surface. The background is phosphate buffered saline (PBS) with a conductivity (σ) of 1.6 S/m and a relative permittivity (ε_r) of 80. The electric field can create a downward force that drives proteins to be immobilized on the capturing electrodes [12].

structures and made it difficult to achieve electrical signal transduction for proteins [19]. Together, these advantages comprise the reason why electrical biosensors have been rapidly developed over the last few years.

Numerous biosensors for HbA_{1c} detection have been reported [10,20]. However, despite HbA_{1c} being mixed with Hb in human red blood cells, most of these biosensors detect only concentrations of HbA_{1c} [16]. If clinical demands are to be met, detection methods should include some acting mechanism, such as HbA_{1c} separation, before practical analysis. Son et al. [21] present a disposable biochip for measuring the ratio of HbA_{1c} to hemoglobin in blood. The condition for blood lysis was determined empirically with 2% Triton X-100, a neutral detergent. Son et al. [22] detect the ratio of HbA_{1c} using electromechanical assay with magnetic particle. The results shown that the HbA_{1C} samples of different concentrations ranging from 0% to 15% could be detected with magnetic particle under the working electrode. Chen et al. [23] present a novel microfluidic immunoassay system based on three-component shell/shell/core structured magnetic nanocomposite Au/chitosan/Fe_3O_4 as affinity matrix for specific recognition and detection of HbA1c. According to measurement results, HbA_{1c} responded linearly in the concentration of 0.05–1.5 $\mu g\,mL^{-1}$, with the detection limit of 0.025 $\mu g\,mL^{-1}$. The above methods, however, require extra chemical or physical agents, which will inevitably increase the cost or require some procedure which may lead to greater device complexity. To overcome those disadvantages, a more convenient way, boronic acid modification, has been developed to act as a significant application used to separate HbA_{1c} from other non-glycated Hb, allowing for label-free biosensors. For biosensors, surface modification to bind the specific analyte at the electrodes is a crucial and useful process before measurement. Self-assembled monolayer (SAM) technology is an effective process of biomolecule immobilization, which is formed on surfaces by spontaneous adsorption on a solid surface. This simple process makes SAM manufacturable and thus attracts much attention for use in biosensors. Not only is the SAM process simple, but it also has many advantages such as ultra-thin, well ordered and stable [24]. With gold an inert and biocompatible material that is easy to acquire, SAMs are commonly adopted on gold surface with thiols bonding [25].

Previous study presents a biosensor for only HbA_{1c} detection [16]. However, to meet the needs of clinical, detection methods should include some long-term mechanism [26], such as the separation of glycated hemoglobin. Hence, this study presents a device which can detect proteins on flat glass substrates for the ratio of HbA_{1c} to total Hb. The device is composed of a microfluidic channel and Au electrode arrays for HbA_{1c} separation before importing the sensor part and measurement for the ratio of HbA_{1c} to total Hb. Further, we investigated the influence on impedance measurement and distribution of the proteins caused by surface roughness [27]. Hb and HbA_{1c} were initially immobilized on the electrodes on which the SAMs were coated. Afterward, by impedance measurement, various concentrations of Hb and HbA_{1c} were detected. At the end of the study, the ratio of HbA_{1c} to total Hb from 1% to 15% was further analyzed from the equivalent circuit model.

2. Device design and fabrication

2.1. Materials

HbA_{1c} and Hb were supplied by Exocell, thiophene-3-boronic acid (T3BA), 3-mercaptopropanoic MPA, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydrosuccinimide (NHS), anti-Hb, immunoglobulin conjugated with fluorescein isothiocyanate (IgG-FITC) and phosphate buffer saline (PBS: pH 7.4, 0.2 g KCl, 1.44 g $NaHPO_4$, 8 g NaCl and 0.24 g KH_2PO_4 in 1:l distilled water) were purchased from Sigma–Aldrich Chemical Co. The solvents, namely ethanol, acetone, isopropanol and other chemicals, were of analytical grade and acquired commercially.

2.2. Electrodes

Coplanar electrodes are commonly used as detecting electrodes in biosensor devices. Due to the electric field lines passing through the molecules, impedance of the sensor will change. By measuring the impedance change, the concentration of the analytes can be indicated. Such coplanar electrodes are often presented in interdigital form. In terms of manufacturing process difficulty, coplanar electrodes are easier to fabricate than parallel facing electrodes [8]. However, interdigital electrodes usually encounter difficult conditions such as electric field non-uniformity due to fringing corner field and edge effects. In order to overcome these problems, a spiral electrode is applied. Compared to a traditional comb structure such as an interdigital electrode, the spiral electrode offers a continuous and uniform electric field [28]. In this study, we used a ring-shaped interdigital electrode which applies alternating current (AC), electro-osmosis (ACEO) and dielectrophoresis (DEP) to focus proteins on the electrode. Fig. 1 is the schematic diagram of the electrode, which combines the ring structure and capability to