AN INTEGRATED MICROFLUIDIC SYSTEM FOR RAPID HBA1C AND GLUCOSE MEASUREMENT

*Tzung-Han Lee[1], Chih-Lin Lin[1], Ching-Chu Wu[1] and Gwo-Bin Lee[1,2,3]**
[1]Department of Power Mechanical Engineering, [2]Institute of Biomedical Engineering, [3]Institute of NanoEngineering and Microsystems, National Tsing Hua University, Hsinchu, Taiwan

ABSTRACT

Diet management is crucial for diabetes to avoid a variety of complications. Due to the large fluctuations in glucose levels that naturally occur over the course of a day, it has been concluded that the biomarker for long-term glycaemic control is whole-blood glycated hemoglobin since its level responds to the long-term progression of diabetes without the short-term fluctuations such as glucose. In this study, an integrated microfluidic chip was developed for measurement of glucose and HbA1c. The whole blood was first separated into plasma and red blood cells (RBC) and then respectively transported into corresponding reaction biosensors with enzyme immobilization. Eventually, both blood glucose and HbA1c levels for short-term and long-term monitoring were measured. The automatic detection system can provide quick monitoring information for the diabetes patients.

KEYWORDS

HbA1c, glucose, microfluidics, blood separation, biosensor

INTRODUCTION

Hyperglycemia may result in a variety of symptoms and long-term complications for diabetes patients. It is well known that adequate metabolic control of the blood glucose level in diabetes patients can delay and even avoid the occurrence of long-term complications. Adequate glycemic control also results in perceived improvements in overall quality of life. Therefore, early diagnosis and regular assessment of treatment effectiveness are very important for the prevention of these serious complications. Self-monitoring of blood glucose is a routine work now for self-management strategy to achieve target glycemic levels.

Electrochemical biosensors for glucose (glucose meters) play a leading role for this purpose. For the purpose of measuring daily glucose levels to control food intake and insulin usage, however, these glucose meters work although some difficulties exist. For example, blood glucose level measurements are recommended three to four times per day due to the large fluctuations in glucose levels that naturally occur over the course of a day. These problems are even more prominent for the diagnosis of diabetes and determining the link between lifestyle and medication once a patient has been diagnosed with this disease.

In addition to glucose monitoring, it has recently been concluded that the best marker for long-term glycaemic control is whole-blood glycated hemoglobin (especially, glycated hemoglobin A1c, HbA1c) since its level responds to the long-term progression of diabetes without the short-term fluctuations of glucose.

Traditionally, clinical laboratory assays for HbA1c are time-consuming, require well-trained personnel and expensive equipment and have limited availability in many areas of the world. Commercially available enzyme assay systems have offered an attractive alternative for conventional clinical tests for glycated proteins. These tests, based on the enzyme fructosyl amino acid oxidase (FAOD), are relatively rapid and have been shown to be reproducible. FAOD is expected to become a major component of glycated protein sensing and eventually be applied in simple, convenient, and economical detection systems for point-of-care treatment and self-monitoring applications [1].

One of the critical processes required for HbA1c detection is separation of RBCs from the human blood samples. It has been demonstrated that dielectrophoretic (DEP) techniques can be used for cell separation. DEP forces have been widely employed for the manipulation and separation of various dielectric particles and cells [2-3]. Differences in dielectric properties of cells and particles allows them to be manipulated by the DEP force, which has become one of the most widely used separation techniques in microfluidic devices.

Therefore, DEP system that can separate RBCs and plasma from the human blood samples are adventurous for glycemic detection. It may reduce the interference for blood glucose measurement and therefore increase the HbA1c sensitivity.

In this study, a new integrated microfluidic system to detect the concentration of HbA1c and blood glucose at the same time has been demonstrated. The system can perform sample pre-treatment and detection in a shorter period of time by incorporating DEP and electrochemical sensing techniques. In addition, micropumps and normally-closed microvalves were used to facilitate the transport of the samples and reagents automatically to reduce human operation error. The development of this integrated microfluidic system may be promising for clinical monitoring of diabetes patients.

MATERIALS AND METHODS

Experimental procedures

Figure 1(a) shows a photograph of the integrated microfluidic chip integrated with electrochemical detection devices. Figure 1(b) schematically shows the components in the detection device. Figure 1(c) shows an exploded view of the chip. After loading the blood sample, it will be absorbed into the PDMS microchannel due to its hydrophilic property. When applying an AC voltage on the DEP electrodes, red blood cells will be attracted by DEP force and plasma will keep moving forward. The whole

blood will be separated into two components, red blood cells and plasma, with this approach. Then, micropumps and microvalves of the glucose electrodes were used to transport the plasma sample into the detection area. Note that the red blood cells will be attracted by DEP force during the plasma transport process. When the glucose detection was finished, the DI water for cell lysis [4] was injected into the microchannel and then the DEP force was turned off. The valve of HbA1c was then opened and the red blood cells were transported into the HbA1c detection area afterwards.

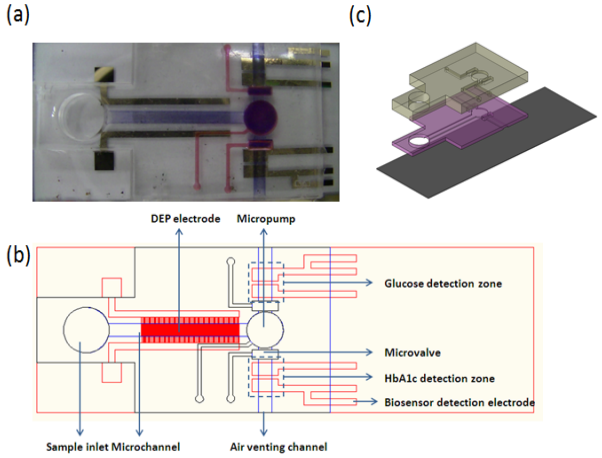

Figure 1: (a) A photograph of the integrated microfluidic chip integrated with DEP electrodes and electrochemical biosensors. Blue color indicated the fluid channel layer and red color indicated the air chanel layer. (b)A schematic diagram and (c) an exploded view of the microfluidic chip. There were three layers in the microfluidic chip. The layer 1 (air layer) was for valves and pumps. The layer 2 (liquid layer) was for sample/reagent flow. The layer 3 (glass) was for detection and DEP electrodes, which was deposited with Au electrodes on the glass substrate.

Chip fabrication

The fabrication process could be described as follows. First of all, a glass substrate was spin-coated with AZ photoresist (Figs.1 (a)-(b)). In order to pattern the Au electrodes on the glass, standard lithograph process was then performed (Figs. 1(c)-(d)). A thin layer of 150-Å chromium and another layer of 1000-Å gold were deposited, followed by a standard lift-off process using the ultrasonic wet bench with acetone (Figs. 1(e)-(f)). The electrodes were then immersed into the cysteamine solution to form the thio group of cysteamine on the Au surfaces and the amino group was extended out of the surface (Figs. 1(g)) [5]. The modified electrodes were immersed into the solution with glutaraldehyde and enzyme again. Note the glutaraldehyde will crosslink the amino group of cysteamine and amino group of enzymes to immobile enzyme covalently on the Au electrode (Fig. 1(h)) [6]. Finally, the hydrophilic PDMS layers were bonded on the glass equipped with the enzyme-based electrodes by oxygen plasma treatment (Fig. 1(i)).

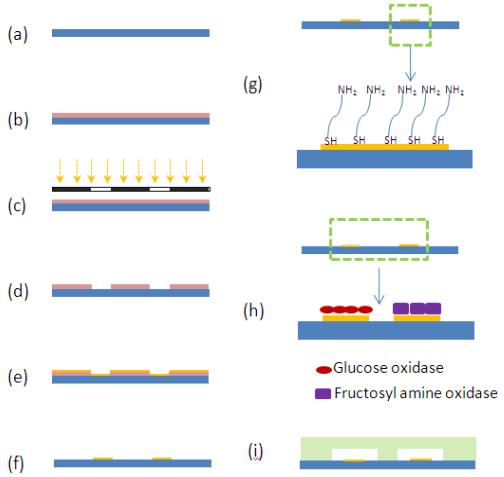

Fig. 2: Microfabrication process of Au electrodes for sensing glucose and HbA1c; (a) clean a glass substrate, (b) spin-coat AZ photoresist on the substrate, (c)expose the substrate by UV light under the patterned photomask, (d) pattern the substrate layer after wash process, (e) deposit chrome and gold by e-beam evaporation, (f) lift off the PR to leave metal patterns on the glass surface, (g) immerse the electrodes into the cysteamine solution to assemble the amine group on the Au electrodes, (h)immerse the electrodes with the amino group into the solution with glutaraldehyde and enzymes (glucose oxidase and fructosylamine oxidase) to immobile the enzyme on the Au electrodes, and (i) assemble the PDMS layers with the glass.

DEP platforms

A scanning electron microscope (SEM) image of the microfabricated planar electrode arrays is shown in Figure 3. This electrode array had a feature periodicity of 50 µm and an electrode gap of 50 µm. This castellated electrode design was chosen because of the features such as simple fabrication, ease to distribute DEP force over a large portion of microchannels, ease to expose samples to electrodes. Furthermore, it also allows flow-through mode operation, high flow rate applications. It was reported that it only required low magnitude AC signals and may expose particles to strong electric fields [7].

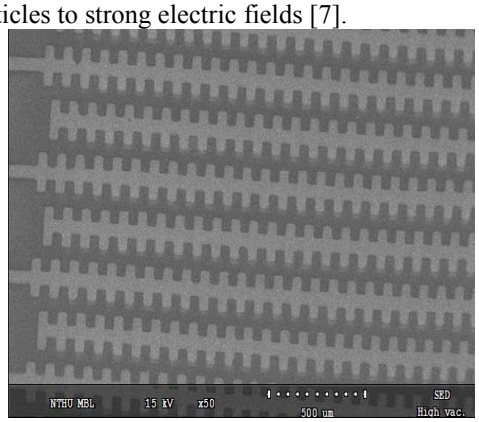

Figure 3: SEM photograph of the castellated DEP electrodes.

RESULTS AND DISCUSSION

Characterization of capillary micropump

The driving force for liquid transport is the capillary force to form a suction force. The advantage of capillary pump is that it can be performed without any external device. It only needs hydrophilic property for the capillary pump to work; however, PDMS has an inherent hydrophobic property. Long-lasting hydrophilic surface property could be formed through APTES surface modification [8]. It demonstrated the hydrophilic surface can maintain for 4 weeks. The modification process and result was shown as Figure 4. The contact angle for water was measured to be 15 °.

As expected, the solution could not fill the microchannel due to the inherent hydrophobic surface before surface treatment. After surface modification, the solution could fill the microchannel quickly. The result was shown as Figure 5.

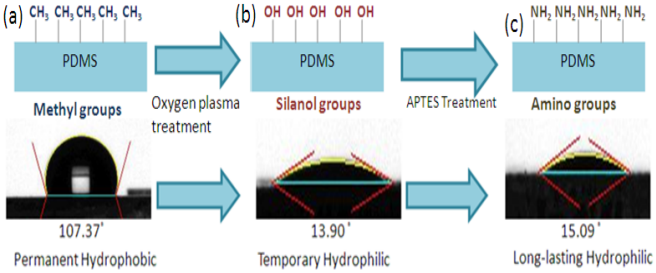

Figure 4: Representative images of water contact angle on PDMS surfaces after surface modification. (a) For unmodified PDMS, the WCA was 107 °; (b) After oxygen plasma treatment, the WCA was 14 °; (c) after APTES treatment, the WCA was15 °.

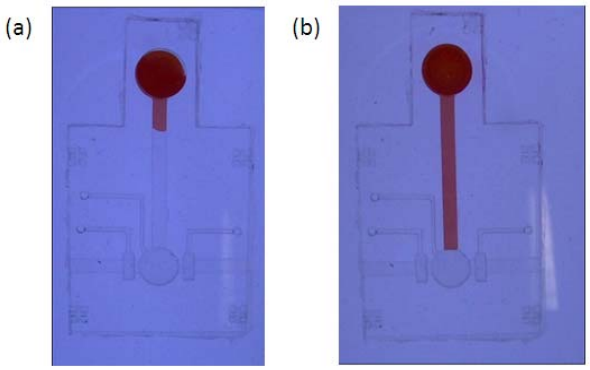

Figure 5: Effect of surface modification applied for the capillary pump; (a) For unmodified PDMS , the solution could not fill the channel completely. (b) For surface-modified PDMS with amino group, the solution could fill the channel quickly.

Detection of HbA1c and glucose by electrochemical biosensors

Glycated hemoglobin A1c (HbA1c) is a hemoglobin molecule in which the *N*-terminal valine residue of the β subunit has been modified by blood glucose. This modification is a nonenzymatic reaction of glucose with free amino groups, proceeding through a Schiff base intermediate to produce a relatively stable product [9]. This HbA1c can be digested to small glycated peptide fructosyl valine (FV) that can be further oxidized by the enzyme fructosylamine oxidase (FAO). Enzymatic assay of HbA1c is therefore based on the oxidation of FV.

The basic concept of the glucose biosensor is based on the fact that the immobilized GOx catalyzes the oxidation of β-D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide. Hydrogen peroxide is oxidized at the electrode and the electrode easily recognizes the number of electron transfers, and this electron flow is proportional to the number of glucose molecules present in blood.

The physiological level of HbA1c in the blood is between 4% and 15% of the total hemoglobin (glucose range is from 80–200 mg/ml) [10]. The concentration of commercially available HbA1c samples is 10% and was then diluted by PBS to various concentrations for detection. The cyclic voltammetry was operated with a scan range from -0.4 to 0.4 V at a scan rate of 0.1 V/s by using a potentiostat equipped with a computerized electrochemical analyzer.

The influence of the HbA1c concentration on peak current difference at 400m V is shown in Fig. 6(a). The current values for (a) 7% , (b) 6%, (c) 5%, (d) 4%, (e) 3%, (f) 2%, (g)1% and (h) 0% (PBS only) of HbA1c are 14.44, 13.36, 10.93, 10.00, 9.19, 5.39, 3.08 and 0.88 µA, respectively, indicating that the peak current difference increases with increasing HbA1c concentration.

Figure 6(b) shows voltammograms obtained at different glucose concentrations. As expected, an increase in the glucose concentration leads to a decrease in the resulting current at 40mV oxidative voltage, which was due to the increasing consumption of the mediator, ferricyanide, resulting from oxidization by peroxide that was the product of glucose oxidation process.

The influence of the glucose concentration on peak current difference at 40 mV is shown in Fig. 6(b). The current values for (a) 40 mg/dL, (b) 50 mg/dL, (c) 80 mg/dL, (d) 100 mg/dL, (e) 150 mg/dL and (f) 200 mg/dL of glucose are 21.70, 20.80, 17.80, 14.60, 12.90 and 10.80µA, respectively, indicating that the peak current difference increases with increasing glucose concentration.

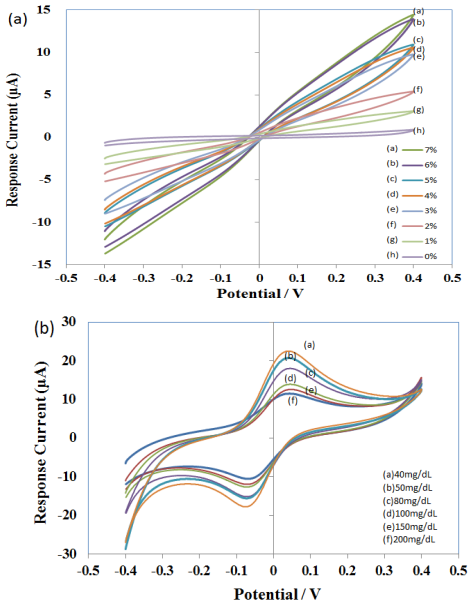

Figure 6: Electrochemical biosensing of HbA1c and glucose by using enzyme-based electrodes; (a) Cyclic

voltammograms of the assay at different HbA1c concentrations; (b) Cyclic voltammograms of the assay at different Glucose concentrations

Sensitivity and linearity of the detection

Figure 7 shows the relationship between the output signals of the electrochemical sensor and the analytic concentrations. The experimental results demonstrate that the developed sensor can perform accurate measurement of the HbA1c sample with concentrations ranging from 0% to 7% (n = 3) and glucose samples with concentrations ranging from 40 mg/dL to 200 mg/dL (n = 3).

Figure 7(a) exhibits the linear detection range at +400 mV is from 0% to 7% of total hemoglobin concentrations that are within the physiological range. Measurements on higher HbA1c concentrations are undergoing. Figure 7(b) exhibits that the linear detection range at +40mV is from 40 mg/dL to 200 mg/dL of glucose that are within the physiological range. This developed system may provide a promising platform for detecting HbA1c and glucose during routine monitoring of diabetes patients.

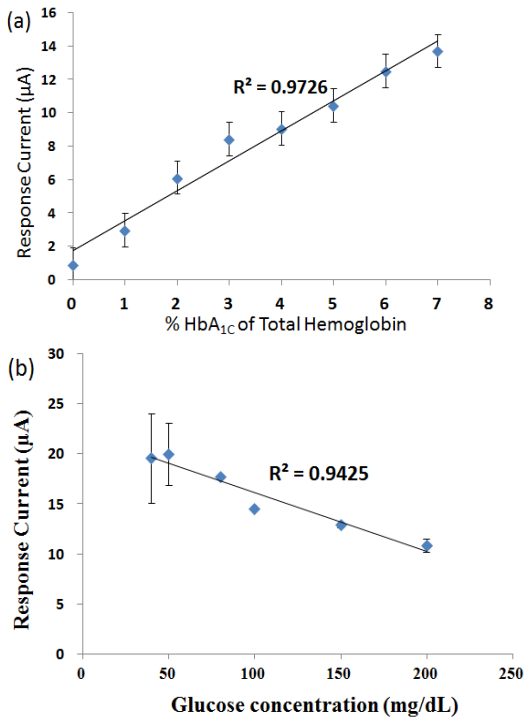

Fig7: Calibration curves from the developed assays as a function of target concentrations. (a) Signal current levels of HbA1c were measured at +400 mV from the background-subtracted voltammograms for respective analyte concentrations. (b) Signal current levels of glucose were measured at +40 mV from the background-subtracted voltammograms for corresponding analyte concentrations.

CONCLUSION

A new integrated microfludic system was developed to carry out the entire process for detection of HbA1c and glucose, including sample pre-treatment and biosensing process in an automatic microfluidic system. The sensitivity and linearity of the assays have been tested by using commercial samples. Therefore, the proposed microfluidic system is promising for diabetes patients that can provide easy and quick monitoring information.

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CONTACT

*Dr. Gwo-Bin Lee, Tel: +886-3-5715131 Ext.33765;
gwobin@pme.nthu.edu.tw