



Reusable urine glucose sensor based on functionalized graphene oxide conjugated Au electrode with protective layers



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ABSTRACT

An electrochemical based system with multiple layers coated on a functionalized graphene oxide Au electrode was developed to measure glucose concentration in urine in a more stable way. Two types of gold printed circuit boards were fabricated and graphene oxide was immobilized on their surface by chemical adsorption. Multiple layers, composed of a couple of polymers, were uniformly coated on the surface electrode. This device exhibited higher electrochemical responses against glucose, a greater resistivity in the presence of interferential substances in urine, and durable stabilities for longer periods of time than conventional units. The efficiency in current level according to the order and ratio of solution was evaluated during the immobilization of the layer. The fabricated electrodes were then also evaluated using hyperglycemic clinical samples and compared with the patterns of blood glucose measured with commercially available glucose meters. Our findings show that not only was their pattern similar but this similarity is well correlated.

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1. Introduction

The International Diabetes Foundation (IDF) reported that approximately 382 million people worldwide have diabetes in 2013 and this figure is predicted to rise to 592 million by 2035. Although electrochemical based methods, combined with enzymes [17,10] and nanomaterials [11,12,14], have been predominantly used for measuring glucose levels, there is still a need for the development of a reusable, resistant to interferential substances device capable of non-invasive monitoring. Especially, for invasive approaches to glucose sensing, while a large amount of research has been undertaken and technological advances achieved, still more optimizations, such as improvements to person dependent calibration, need for low cost production, and long term stability, remain to be achieved to make a device that can be commercialization [1,3]. Electrochemical measurement of glucose in urine is, at some point, an appropriate candidate for a non-invasive approach. It is easy to fabrication, has a rapid assay time, and most importantly has low-cost production. However, one critical issue to be considered is the stability of the device during

measurements in urine. Therefore, efficiently block inferential substances in urine and enhance electron transfer is the most important issue when developing urine glucose meters. Recently, a urine glucose meter has been developed and commercialized, this device has exhibited stable and quantifiable results in the presence of inferential substances by focusing on blocking them [6,7,9]. For the facilitation of electron transfer, conducting nanomaterials were introduced and systematically placed on the surface of electrodes and characterized [5,15,2,18,16]. It has been well described in previous studies that electron transfer efficiencies or amperometric responses to glucose concentration is significantly increased as nanoparticles are applied on the electrodes when compared those without nanoparticles [8,4]. Our previous studies have demonstrated the direct attachment of nanoparticles containing graphene oxide to the electrode and their electrochemical characteristics are used to determine the level of glucose. We fabricated metalloidal polymer hybrids (MPHs), a nanomaterial composed of polyethylene glycol (PEG) and silver-silica material, and functionalized this on the surface of graphene oxide nano-sheets to form a functionalized graphene oxide (FGO). In previous study, FGO on gold electrodes (Au-FGO) exhibited a durable affinity at the electrode and showed good amperometric responses with different concentrations of glucose in the TES buffer and in urine collected from patients with hyperglycemia [13].

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In this study, we developed a new enzyme hybrid material composed of multiple layers on an Au-FGO electrode. Multiple layers, composed of the enzyme hybrid and polymers, were spin coated layer by layer on to the Au-FGO. Our findings show that the multiple layers with Au-FGO exhibit a more stable response to glucose in the presence of interference substances in urine. In addition, measurements of urine samples of patients with hyperglycemia ($n = 30$) show good correlation with blood glucose of same patients measured by commercially available glucose meters.

2. Materials and methods

This prospective study was approved by the institutional review board at our institute and informed consent was obtained from all subjects. Urine and serum sample collection was performed between December 2012 and May 2013 from 30 subjects that met our inclusion criteria and consented to participate. The number recruited was based on sample size estimation, and the inclusion criterion was that patients be scheduled for orthopedic surgery at our hospital.

2.1. Chemicals

Silver nitrate (AgNO_3), tetraethoxysilane (TEOS), sodium borohydride (NaBH_4), ammonium hydroxide (NH_4OH), poly(ethylene glycol) (PEG) ($M_n = 10,000$ g/mol), and 3-aminopropyltriethoxysilane (3-APTES) were purchased from Sigma–Aldrich. Glucose oxidase (GOx) (from *Aspergillus niger*, >100 U/mg), anhydrous ethanol ($\text{C}_2\text{H}_6\text{O}$), albumin from bovine serum (BSA), glutaraldehyde, nafion[®] perfluorinated resin, 1H,1H,2H,2H-perfluorodecyl acrylate, 1,3-bis(trifluoromethyl) benzene,

D-(+)-glucose (reagent grade), and *N*-[Tris(hydroxymethyl) methyl]-2-aminoethanesulfonic acid sodium salt (TES) buffer were also obtained from Sigma–Aldrich.

2.2. Fabrication of Au electrode PCB

As shown in Fig. 1 the Au electrode PCB was fabricated. Approximately 250 gold electrode chips on each 4 in glass wafer were fabricated during the process (Fig. 1(a)). Each electrode chip is composed of working, counter, and reference electrode, these are denoted as WE, CE, and RE, respectively (Fig. 1(b)). Prior to being diced into each chip, the glass wafer was spin coated with the aforementioned layers to form multilayers on top of the Au-electrode. Each electrode chip was then diced and glued to the region indicated by arrows (Fig. 1(c and d)). We fabricated two types of multilayer Au-chips, one is for in-house use as a prototype and the other is for a portable prototype (not shown in this article). Shown in Fig. 2 is a customized prototype of the read out system for the fabricated chips. As can be seen in Fig. 2(a), the layout and PCB of the read out circuit, each board has five readout channels that are able to collect amperometric data from Au PCBs implemented in each channel. In a single run, five different Au-PCB chips can be mounted and with the assistance of a lever each platform can be precisely inserted into the desired solutions kept in eppendorf tubes (Fig. 2(b)).

2.3. Formation of multiple layers on FGO-Au electrode

We fabricated an Au electrode system comprised of working, auxiliary, and reference electrodes to simplify the fabrication process. Each electrode chip was fabricated by a semiconducting processes including a photoresist coating, patterning, lift off, and

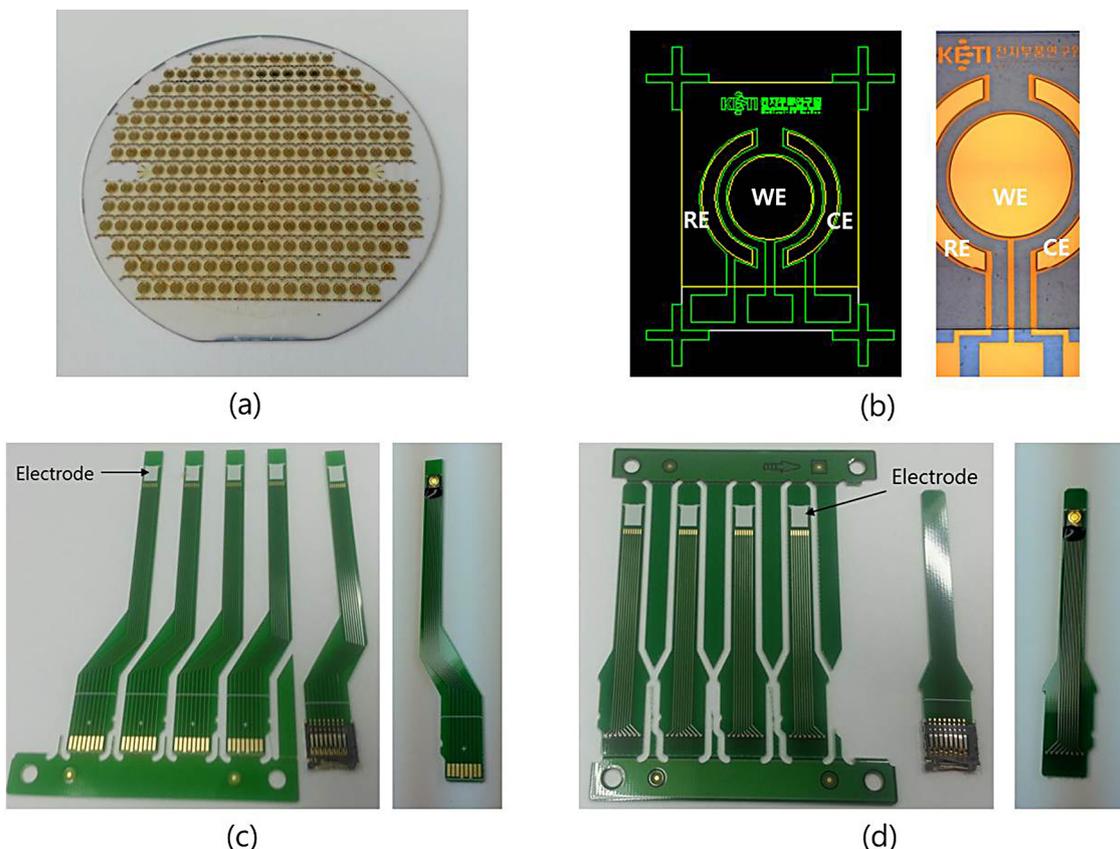


Fig. 1. (a) Fabricated gold electrodes chip on 4 in glass wafer. (b) Each chip is composed of three electrodes, working, counter, and reference, and they are all made of gold. Each chip can be mounted on the region indicated by the arrow in Fig. 1(c and d). Two types of PCB chips were fabricated for (c) stationary and (d) portable use.

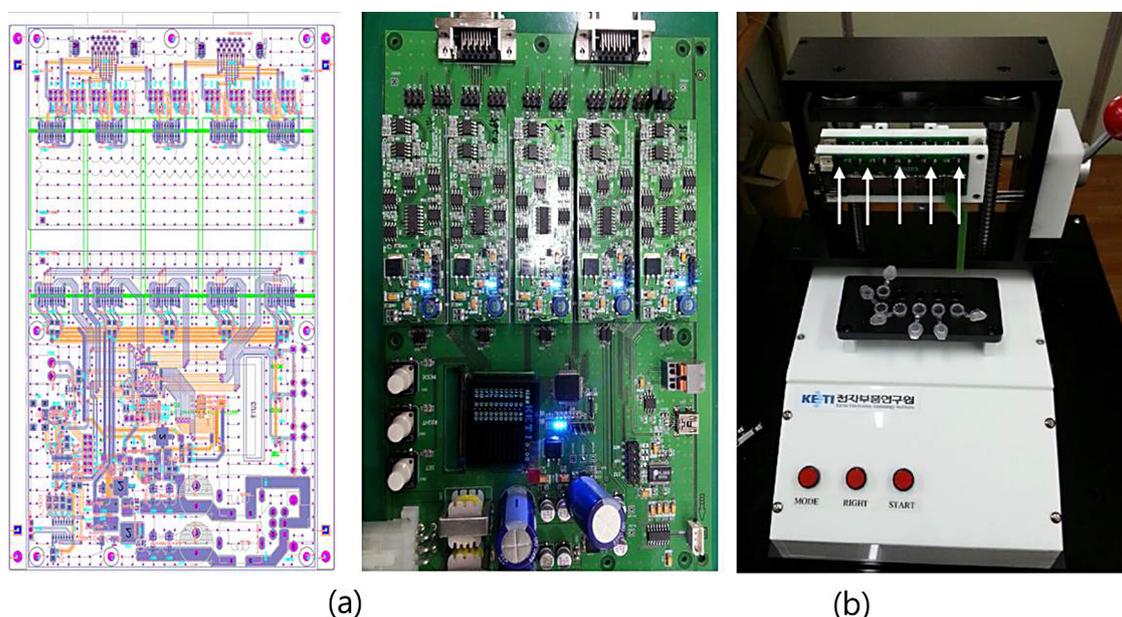


Fig. 2. (a) Layout of the read out circuit (left) and its five channel based prototype (right). (b) For this experiment, a custom-made read out platform was built. It is capable of reading five different implemented PCB chips at the same time.

passivation. As can be seen in Fig. 1(a), approximately 250 chips on a 4 in glass wafer were fabricated for each process. A central circle shaped Au electrode with an area of $\pi \text{ mm}^2$ was utilized as the working electrode (Fig 1(b)). An Au-electrode printed circuit board (PCB) chip was fabricated by an electroplating method and two types of PCB chips were made for use in other applications (Fig 1(c and d)).

Functionalization of GO nanosheets with MPHs was achieved by following a previous study Veerapandian et al. Briefly, 200 μL of MPHs and 40 μL of 3-APTES were added to a tube containing anhydrous $\text{C}_2\text{H}_5\text{OH}$ and kept for 10 h. After completion of the reaction process, FGO was drop-cast onto the oxygen plasma cleaned Au electrode PCB chip and allowed to evaporate at room temperature for 1 h. After modification of each Au electrode on the wafer, multiple layers were spin coated on the wafer. These layers were composed of a silane coupling layer on top of the FGO-Au electrode followed by GOX composites, nafion, a silane coupling layer, and a restricted permeable polymer layer to form the multilayer-FGO-Au electrode.

2.4. Instrumentation for monitoring glucose in urine

A customized reading platform was designed and built for the experiment. Fig. 2(a) shows the layout of the read out main board, the capable analog signal range of the system is between +5 and -5 V . As can be seen in Fig. 2(b), indicated with arrow, five different chips can be placed into the slots for performing simultaneous measurements of different concentrations of glucose in TES and urine and between-run tests in same concentration of glucose. All experiments were performed at room temperature in a 5 mL of collected urine samples and TES buffer for characterization. All amperometric measurements were performed at a working electrode potential of +0.6 V.

3. Results and discussion

The concentration of glucose in the TES buffer was examined by the fabricated Au-PCBs and the customized platform and the resultant images are displayed in Fig. 3. As can be seen in Fig. 3(a), the amperometric response with glucose concentration has strong

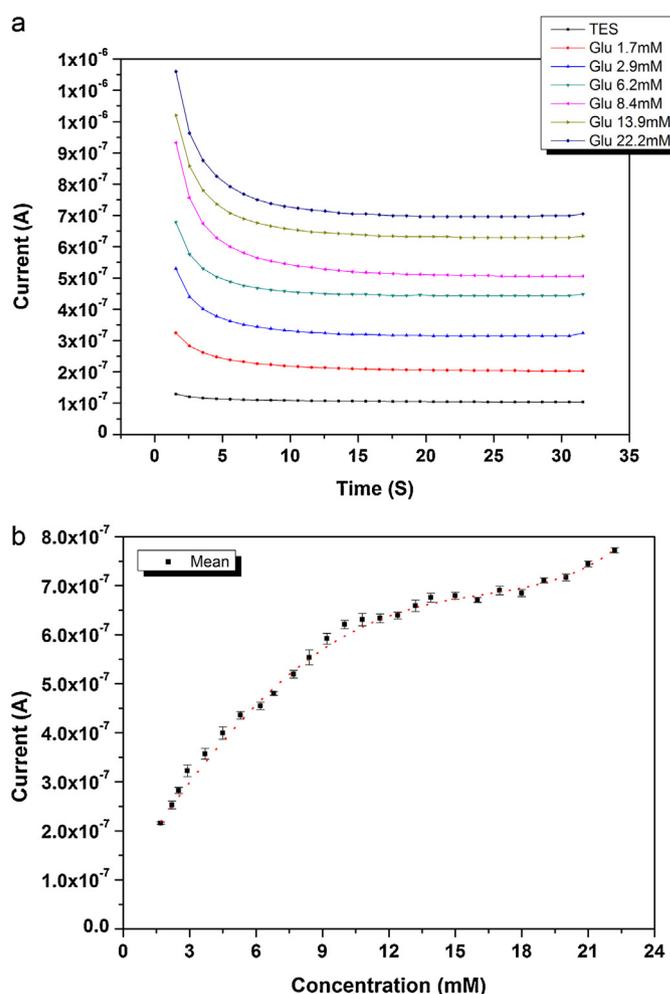


Fig. 3. (a) Amperometric response against concentration of glucose in TES buffer. (b) In between-run tests, five different PCB chips were used to measure the same concentrations in the range of 1.7–22.2 mM of glucose and their residual distribution in subfigure.

proportionality to the concentration as it increases. Fig. 3(b) shows the amperometric responses measured 7 s after the immersion of five different chips with their variations from the mean values on each concentration. The between-run results show that their variations are within 6% from their mean values.

Such materials including Ag/AgCl and Pt were not used as reference and auxiliary electrodes in this study despite these being the conventionally used materials in most electrochemical systems. Instead, we used an Au substrate as reference and auxiliary to reduce cost of chip production and increase stability during the field measurements. The results show good correlation with level of glucose in most practical ranges with minor cross reaction (0–24 mM or 0–400 mg/dL). Furthermore, this electrode has multiple layers on top permitting repeated uses after washing, these layers also provide significant durability and resistances against interferential substances in the solutions as described in previous studies [6,7].

Fig. 4(a) represents the comparisons between the amperometric responses on the first day of measurements and those after 30 days with the same chips. The chips were stored in a fridge when not being used. Compared with our previous study using FGO-Au-PCB chips without multiple layers [13], the overall level of measured current increased by 20 times as well as the long term stability was increased up to 5.6%. It was demonstrated that current generated by the multiple layer-Au-PCB drops to overall 8.7% of its initial value within 30 days. The resistant ability of the Au-PCB electrode modified with multiple layers was investigated under additions of different interferential substances, such as

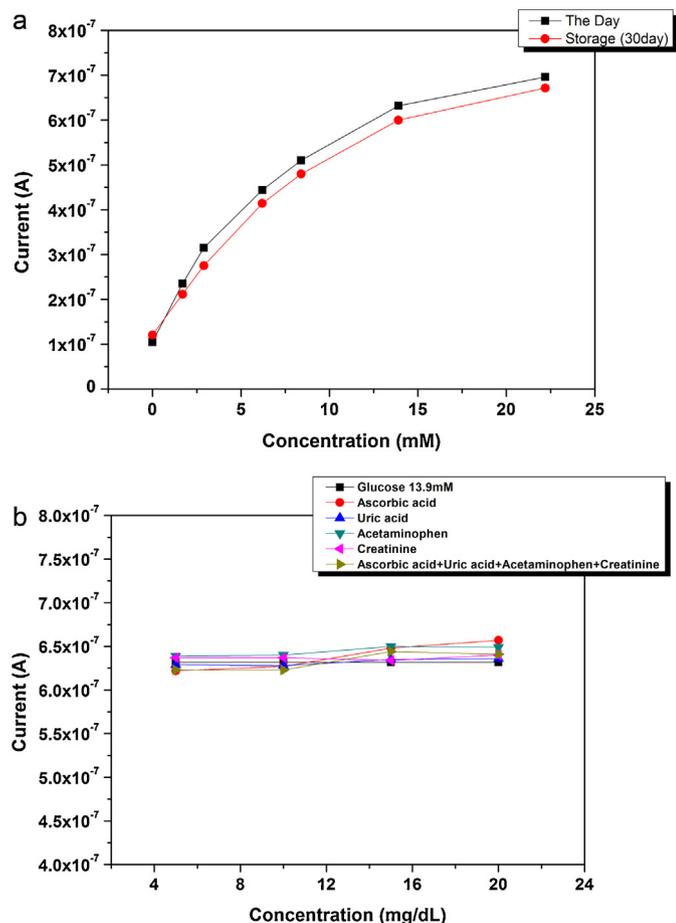


Fig. 4. (a) Amperometric responses of the PCB chip against glucose on the first day and on the same chip after 30 days. (b) The resistance of the chip against uric acid, ascorbic acid, acetaminophen, creatinine, and all these substances mixed together.

ascorbic acid, uric acid, acetaminophen, creatinine and all these substances mixed together (Fig. 4(b)). The Au-PCB chip exhibited no variations with the increases of the added interferential substances, indicating that the layers on top of the electrode efficiently restrict those substances from penetrating them to reach the electrode which explains the increase of current level as well as long term stability of our fabricated chips. In addition, no changes were also observed when the interferential substances were added both in time and concentration dependent manners.

The amperometric response in urine was measured from the patients ($n=30$) with hyperglycemia and their patterns of responses were compared with the concentration of glucose in blood measured with a commercially available glucose meter. As can be seen in Fig. 5(a), the amperometric responses from a single chip, which are represented by black solid circle and left Y axis, have a similar pattern to the measured blood glucose (red solid square and right Y axis) suggesting that our system is able to measure the level of glucose in an accurate manner as well as being stable during multiple uses in real samples. Fig. 5(b) shows the high correlation between blood glucose and glucose in urine with squared R of 0.91, which means the amount of glucose in blood is likely to be linearly correlated with the concentration of glucose in urine.

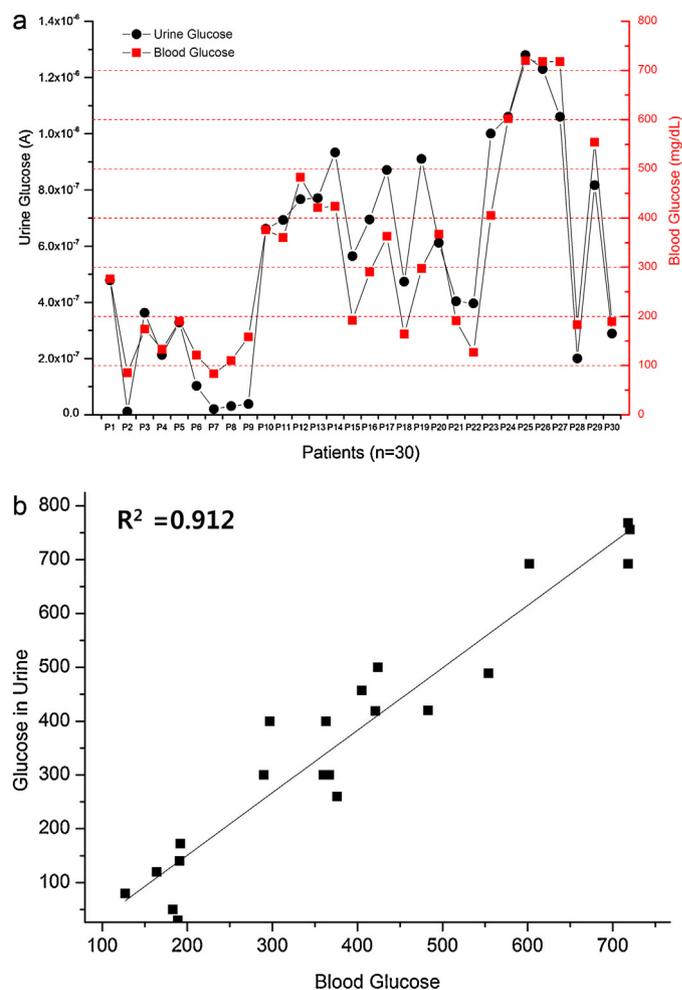


Fig. 5. (a) A plot of amperometric response to urine glucose collected from patients with hyperglycemia ($n=30$) and blood glucose of those same patients measured with the commercially available glucose meter. (b) The correlation between urine glucose and blood glucose ($R^2=0.91$).

4. Conclusions

In summary, we fabricated functionalized graphene oxide, which is an integration of metalloid polymer hybrids with oxidized graphene oxide nanosheets. Functionalized graphene oxide was then adsorbed on gold electrodes to form a FGO-Au-electrode. The FGO-Au-electrode chips with multiple layers were prepared by spin coating to form a multilayer-FGO-Au-electrode and then each of them was implemented on the PCBs. The multilayer-FGO-Au-PCB exhibited significantly high correlation with the concentration of glucose in the TES solution and stability in the presence of interferential substances such as uric acid, acetaminophen, ascorbic acid or their combination. For clinical trials, the patterns of data obtained for urine glucose from each chip was well correlated with those of glucose in blood collected from the same patients suggesting that our developed system may be able to be used in monitoring glucose levels in urine continuously while being able to give some indication of changes in the level of glucose in the blood.

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