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Glucose biosensors based on a gold nanodendrite modified screen-printed electrode

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Abstract
In this study, an enzymatic glucose biosensor based on a three-dimensional gold nanodendrite (GND) modified screen-printed electrode was developed. The GNDs were electrochemically synthesized on the working electrode component of a commercially available screen-printed electrode using a solution acquired by dissolving bulk gold in aqua regia as the precursor. The 3D GND electrode greatly enhanced the effective sensing area of the biosensor, which improved the sensitivity of glucose detection. Actual glucose detections demonstrated that the fabricated devices could perform at a sensitivity of 46.76 μA mM^{-1} cm^{-2} with a linear detection range from 28 μM–8.4 mM and detection limit of 7 μM. A fast response time ($\sim$3 s) was also observed. Moreover, only a 20 μl glucose oxidase is required for detection owing to the incorporation of the commercially available screen-printed electrode.

(Some figures may appear in colour only in the online journal)

1. Introduction

Diabetes is the most prevalent cause of kidney failure, nontraumatic lower-limb amputations, and new cases of blindness among adults. It has been estimated that 25.8 million people in the United States suffer from diabetes. In 2010, the number of newly diagnosed people aged 20 years or older was approximately 1.9 million. This number is projected to increase to more than 300 million by 2030 [1]. Diabetics are required to monitor physiological blood glucose levels by taking frequent measurements to ensure effective treatment and avoid diabetic emergencies. *In vitro* diagnostic (IVD) devices for glucose detection comprise a major part of the global IVD market.

Several features are important for the development of suitable IVD devices, such as low cost, high sensitivity, specificity, ease of use, and small sample size requirement. Additionally, the units should be disposable and provide rapid and robust results. Recently, nanomaterials have been widely applied to diabetes IVD devices because of their distinguishing characteristics, such as high surface activity, high catalytic efficiency, and strong adsorbability. A nonenzymatic glucose sensor based on porous Au film electrodes, which was produced by implementing a sequential hydrogen bubble dynamic template synthesis and galvanic replacement reaction, was described by Li *et al* [2]. The device was reported to operate in a linear range from 2 to 10 mM with a sensitivity of 11.8 μA mM^{-1} cm^{-2} and detection limit of 5 μM. An enzymatic glucose biosensor adopting a gold nanoparticle (GNP)/enzyme-integrated electrode was later proposed by Yehezkeli *et al* [3]. The integrated electrode was fabricated by electropolymerizing a bis-aniline-cross-linked GNP/glucose oxidase (GOx) composite onto an Au electrode. Sensitive and selective glucose detection could be achieved by creating an effective electrical contact between the covalently

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linked GOx enzyme and the electrode. Another glucose biosensor based on a genetically modified GOx enzyme was proposed by Holland *et al* [4]. The genetic modification facilitated site-specific attachment of maleimide modified GNPs to the GOx proteins, thus enabling direct electron transfer between the enzymes and the electrode. A glucose biosensor based on a GOx–GNP/egg shell membrane (ESM) sensing electrode was developed by Zheng *et al* [5]. The sensing electrode was prepared by initial *in situ* synthesis of GNPs on the surface of an ESM followed by immobilization of GOx. A linear working range from 8.33 μM to 0.966 mM with a detection limit of 3.50 μM could be achieved. Another direct electron transfer device was subsequently developed by Lee *et al* [6]. Direct electron transfer was facilitated through GOx adsorption in an ionic liquid-derived polymer with internally organized columns of GNPs. Recently, Qiu *et al* reported a nanostructured glucose biosensor based on a Au thin film electrode that was fabricated by electrodeposition and galvanic replacement technology [7]. The nanostructured Au thin film electrode offered a favorable microenvironment so that GOx could be stably immobilized and enabled effective electron transfer between the active center of GOx and the electrodes. Two linear detection ranges at concentrations from 2.5 to 32.5 μM and from 60 to 130 μM as well as a detection limit of 0.32 μM were reported.

In addition to GNPs, nanostructured metal oxides, such as ZnO nanorods, nanotubes, and nanofibers, have also been explored for the development of glucose biosensors. Yang *et al* synthesized highly oriented single-crystal ZnO nanotube arrays on indium-doped tin oxide (ITO) coated working electrodes on which sequential immobilization of GOx and a coating of a Nafion film was performed [8]. A low detection limit (10 μM) was achieved. The Nafion film was incorporated to eliminate possible interference by components in the blood, such as ascorbic acid and uric acid [9]. An electrospun single ZnO nanofiber was further implemented as an electrode that was subjected to GOx immobilization for glucose detection. This device demonstrated a linear detection range from 0.25 to 19 mM and a 1 μM detection limit [10]. An amperometric glucose biosensor based on an aligned ZnO nanorod film grown on ITO was developed by Liu *et al* [11]. A 3 μM detection limit was reported. A tetragonal pyramid-shaped porous ZnO (TPSP-ZnO) modified glassy carbon electrode was also used for the detection of glucose [12]. A linear detection range of 0.05–8.2 mM and detection limit of 0.01 mM was achieved under the applied potential of −0.50 V. A nonenzymatic glucose sensing device based on a nanostructured NiO modified carbon paste electrode was proposed by Mu *et al* [13]. The device was effective over a wide concentration range (1–110 μM) with a sensitivity of 43.9 nA μM^{-1} and detection limit of 0.16 μM.

Recently, carbon nanostructured electrode materials, such as carbon nanotubes (CNTs), ordered mesoporous carbons (OMCs), and graphite, have attracted substantial interest owing to their enhanced electrochemical performance compared with that of traditional materials. An enzymatic glucose biosensor using a Nafion/GOx–OMC modified glassy carbon electrode (GE) was demonstrated to operate over a large concentration range (500–15 000 μM) at high sensitivity (0.053 nA μM^{-1}) with a fast response (9 ± 1 s) and proficiency for discriminating possible interference [14]. Exfoliated graphite nanoplatelets (xGnP) were also utilized for enhancing the sensing capability of glucose biosensors [15]. A response time of less than 5 s, detection limit of 10 μM, linear detection range of up to 6 mM, and sensitivity of 14.17 μA (mM $cm^2)^{-1}$ were exemplified. In another case, a brush-like CNT nanoyarn fiber electrode was used for glucose detection [16]. The sensitivity of this device could be greatly increased by thermally annealing the CNT fiber at 250 °C prior to construction of the sensor. Furthermore, hybrid nanostructures of palladium nanoparticles and single-walled carbon nanotubes (Pd–SWNTs) were used as an electrocatalyst for the nonenzymatic oxidation of glucose [17]. In this case, a response time of 3 s, detection limit of 0.2 ± 0.05 μM, wide linear range of 0.5–17 mM, and sensitivity of ∼160 μA mM^{-1} cm^{-2} were demonstrated. The functional properties of recently developed nanostructure-based glucose biosensors are tabulated in table 1.

One of the key issues in the preparation of glucose sensing devices is maximizing the amount of GOx that can be immobilized on the sensing electrode to achieve the greatest possible sensing quality. When compared with 2D nanostructures, 3D nanodendrites with hyper-bifurcated structures can remarkably increase the surface area of the electrode for GOx absorption. Accordingly, gold nanodendrites (GNDs) that were synthesized in a solution containing a gold precursor and an inclusion complex were successfully applied to the electrochemical measurement of methanol [18]. Furthermore, GND electrodes that were synthesized by electrodeposition from a solution of $HAuCl_4$ containing iodide and ammonium chloride were reported to perform a much more sensitive detection of arsenic oxide than GNP-based electrodes [19].

In this study, an enzymatic glucose biosensor based on a GND modified screen-printed electrode is developed. The GND electrode is constructed by a simple electrochemical synthetic method using only an aqua regia solution of dissolved gold as the precursor. Since a commercially available screen-printed electrode is employed and a simple synthetic method is used for the GND electrode fabrication, it is expected that this device can closely meet current expectations for the development of efficient IVD devices.

2. Material and methods

2.1. Preparation of the solutions

GOx powder (250 U mg^{-1}; Merck, Taiwan) was dissolved in a pH 7 phosphate buffer solution (PBS) to form a 2 U μl^{-1} GOx solution. Glucose powder (100 mg; Sigma-Aldrich, USA) was dissolved in 100 ml of PBS to prepare a 100 mg dl^{-1} glucose solution. This glucose solution was then serially diluted to prepare glucose solutions of lower concentrations. A 0.5 M potassium ferricyanide ($K_3Fe(CN)_6$) solution was

Table 1. Nanostructure-based glucose biosensors and their functional properties.

Electrode matrix	Sensitivity	Detection limit (μM)	Linear range	Response time (s)	Reference
Porous Au	11.8 μA cm^{-2} mM^{-1}	5	2–10 mM	—	[2]
Au nanoparticles	—	—	—	—	[3]
Au nanoparticle-coated eggshell	—	3.5	8.33 μM–0.97 mM	<30	[5]
Au-poly [$C_{10}VIm^+$][Cl^-] composite	11.8 μA mM^{-1} cm^{-2}	5	—	—	[6]
Nanostructured gold thin film	—	0.32	2.5–32.5 and 60–130 μM	—	[7]
ZnO nanotubes	30.85 μA mM^{-1} cm^{-2}	10	10 μM–4.2 mM	<6	[8]
ZnO nanofibers	70.2 μA mM^{-1} cm^{-2}	1	250 μM–19 mM	<4	[10]
ZnO nanorods	—	3	—	<5	[11]
Pyramid-shaped porous ZnO	—	10	50 μM–8.2 mM	—	[12]
Nano nickel oxide	43.9 nA μM^{-1}	0.16	1–110 μM	<5	[13]
Mesoporous carbon	0.053 nA μM^{-1}	—	500 μM–15 mM	9	[14]
Exfoliated graphite nanoplatelets	14.17 μA mM^{-1} cm^{-2}	10	10 μM–6 mM	<5	[15]
Nanoyarn CNT fiber	7.2 nA mM^{-1}	25	—	~10	[16]
Nanostructured Pd–SWNTs	160 μA mM^{-1} cm^{-2}	0.2	500 μM–17 mM	3	[17]
Au nanodendrite	46.76 μA mM^{-1} cm^{-2}	7	28 μM–8.4 mM	~3	This work

prepared by dissolving 16.462 g of potassium ferricyanide powder (Showa Chemical, Japan) in 100 ml of PBS (pH 7). A 2 wt% Nafion solution was prepared by mixing 1 g of Nafion (Sigma-Aldrich, USA) with 49 g of ethanol (1:10 ratio).

2.2. Gold-nanodendrite-based glucose biosensor fabrication

In this study, a 3D GND electrode, which was produced by electrochemical deposition of GNDs onto a commercially available screen-printed carbon electrode used as the substrate, was developed for improving glucose detection. The process for preparing the GND-based glucose biosensor includes preparation of the nanogold precursor for electrochemical deposition of 3D GNDs and fabrication of the biosensor.

(1) Nanogold precursor preparation.

Since bulk gold cannot be dissolved by any individual acid because of its low chemical activity, aqua regia was used to dissolve the bulk gold forming a solution containing $AuCl_4^-$, as shown in equation (1). The nanogold precursor is cost effective when compared with the commonly used $HAuCl_4$ solution. The prepared $AuCl_4^-$ solution was subsequently used as the nanogold precursor for electrochemical deposition of 3D GNDs.

$$Au + 3NO_3^- + 4Cl^- + 6H^+ \rightarrow AuCl_4^- + 3NO_2 + 3H_2O. \quad (1)$$

The detailed procedure includes (i) immersion of 0.5 g of bulk gold in 1 ml of hydrochloric acid for 1 min followed by its immersion in 1 ml of nitric acid for 1 min, (ii) washing the treated bulk gold with deionized water, (iii) dissolution of the isolated bulk gold in aqua regia (mixture of 1 ml of nitric acid and 3 ml of hydrochloric acid), and (iv) dilution of the resulting mixture (after the bulk gold is fully dissolved) with 50 ml of water to form the nanogold precursor solution.

(2) Glucose biosensor fabrication.

(a) GND modified electrode fabrication.

Commercially available screen-printed electrodes (Model SE 101, Zensor R & D, Taiwan) were purchased (1 US$/piece) and used as the substrates for electrochemical synthesis of the 3D GND electrode. The area of the working electrode is 0.071 cm^2.

Before electrochemical synthesis of the 3D GND electrode, cyclic voltammetry (CV) was performed on the substrate in a 20 ml (0.5 M) potassium ferricyanide solution at a scanning rate of 50 mV s^{-1} (approximately −1–1 V) to remove the thin film protective layer covering the electrode. The substrate was placed at the working electrode (WE). The counter-electrode and the reference electrode (RE) were a Pt film and an Ag/AgCl electrode, respectively. The screen-printed electrode was then placed in a 100 ml nanogold precursor solution (5 wt%) with its working electrode (WE) connected to a power supply unit. A Pt thin film was used as the counter-electrode. The electrochemical deposition of 3D GNDs was conducted at a specific applied voltage over 1 h under magnet agitation (120 rpm).

(b) Glucose biosensor preparation.

The procedure for glucose biosensor preparation is briefly described below.

(i) A potassium ferricyanide solution (10 μl, 0.5 M) was poured onto the GND modified electrode, and the combination was incubated at 40 °C to concentrate the potassium ferricyanide solution.

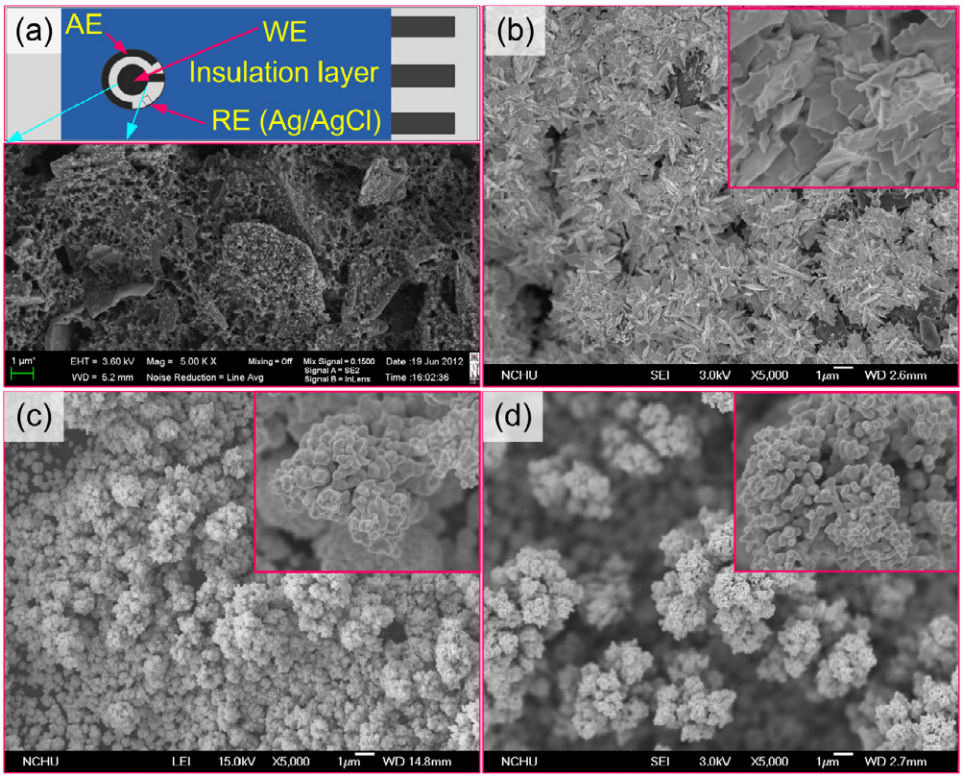

Figure 1. SEM images of (a) the screen-printed electrode and its working electrode surface and (b)–(d) the GNDs synthesized under an applied voltage of 0.7 V (b), 1 V (c), and 1.2 V (d); the insets in (b)–(d) are the ten-fold magnification of the corresponding images.

(ii) A GOx solution (20 μl, 2 U μl^{-1}) was dropped onto the biosensor, and the combination was incubated at 40 °C for 1 h.

(iii) A Nafion thin film was produced by dropping 10 μl of a Nafion solution (2 wt%) onto the GOx immobilized electrode.

An SP-150 potentiostat (Bio-Logic, USA) was implemented for determination of the effective sensing area and detection of the glucose concentration.

3. Results and discussion

3.1. Results for the synthesis of GND electrodes

Three applied voltages (0.7, 1.0, and 1.2 V) were employed for synthesis of the 3D GND electrode. Figure 1 shows SEM images of the commercially available screen-printed electrode and its WE surface and the GNDs synthesized under various applied voltages. The GNDs synthesized at the applied voltage of 0.7 V resemble nanoscale quartz, while those prepared at an applied voltage of 1.2 V look like microscale cauliflower. Furthermore, the GNDs synthesized at an applied voltage of 1.0 V are more uniform compared with those synthesized at 0.7 and 1.2 V.

The structure of the synthesized GNDs could be attributed to the irregularly porous structure of the screen-printed electrode, as shown in figure 1(a). During electrochemical deposition, the irregularly porous structure might induce an electric field concentration difference at different locations such that gold nanostructures were formed at the early stage followed by polycrystalline growth of the GNDs.

Figure 2 is a chart of the XRD diffractions of the synthesized GNDs. In contrast to the varying intensities, almost identical XRD patterns were observed for the GNDs synthesized at all three applied voltages. A multicrystalline structure of the synthesized GNDs was indicated by the presence of multiple peaks in the XRD diffraction chart. A possible cause for the multicrystalline structure could be attributed to the irregularly porous structure of the screen-printed electrode. In addition to the electrochemical synthetic process, growth of the reduced gold atoms along the irregularly porous structure of the screen-printed electrode also occurred, leading to 3D nanodendrites with hyper-bifurcated structures.

Figure 3 shows the current versus time charts (*i*–*t* curves) for GND synthesis at the three applied voltages used in this study. The three curves in each individual chart denote three separate experiments using different GND modified electrodes. The results indicate that the *i*–*t* curves for the GNDs synthesized at the 0.7 V applied voltage were not as consistent as those of the GNDs synthesized at 1.0 and 1.2 V. The effective sensing area of the electrode could be estimated from a current versus time chart (*i*–*t* curve) derived from the cyclic voltammogram acquired in a 1 M H_2SO_4 solution [20]. Assuming a gold atom can absorb one oxygen atom, the theoretical charge density of gold oxide reduction (Q_t) is 390 μC cm^{-2}. The measured quantity of charges (Q_m) as illustrated in figure 3(d) can be integrated from the peak

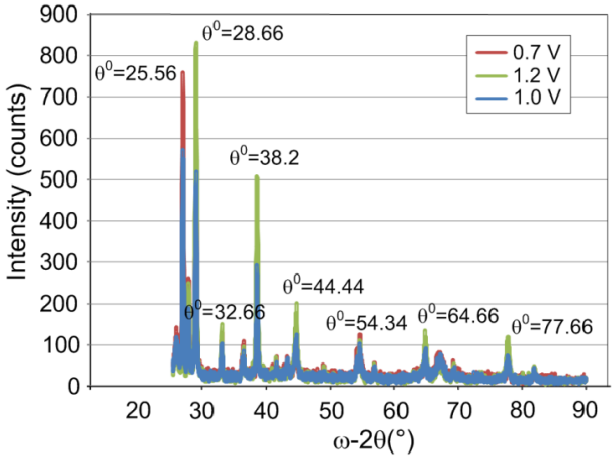

Figure 2. XRD analysis of the synthesized GNDs.

current of the current versus time chart. Hence, the effective sensing area of the electrode can be estimated by Q_m/Q_t. The effective sensing areas under the applied voltages of 0.7, 1.0, and 1.2 V are calculated to be 0.442, 0.851, and 0.989 cm^2, respectively, which are 6.23, 12.16, and 13.93 times that of a plane electrode. The results indicate that the GND modified electrodes afford a much larger effective sensing area than a plane electrode.

3.2. Glucose detection

Figure 4 illustrates several cyclic voltammograms of glucose detection for the GND modified screen-printed electrodes synthesized under various applied voltages. The cyclic voltammograms for the GNDs synthesized at 1 V under the scan rates of both 100 (figure 4(b)) and 50 mV s^{-1} (figure 4(d)) exhibit greater uniformity when compared with those of the GNDs synthesized at 0.7 and 1.2 V. Although the cyclic voltammograms for the GNDs synthesized at 0.7 V (figure 4(a)) exhibit a certain degree of uniformity; the peak cathodic currents (i_{pc}) of the 100 and 75 mg dl^{-1} analytes were very close, as were those of the 50 and 25 mg dl^{-1} samples. The results indicate that the linearity of detection for the GNDs synthesized at 0.7 V is not as good as that of the GNDs synthesized at 1 V. Figure 5 shows the *i–t* curves for the cyclic voltammogram shown in figure 4(b). It can be observed that the response time of the proposed glucose biosensor is about 3 s.

Complete CVs for the GNDs synthesized at 1 V using glucose concentrations ranging from 0.125 mg dl^{-1} (7 μM) to 150 mg dl^{-1} (8.4 mM) were generated. Each experiment was run in quadruplicate ($n = 4$). The calibration curves are illustrated in figure 6. The R^2 value of 0.988 for the linear detection range from 28 μM–8.4 mM and the detection limit of 7 μM were obtained at a scan rate of 100 mV s^{-1}. The sensitivity of the GND-based glucose biosensor is calculated

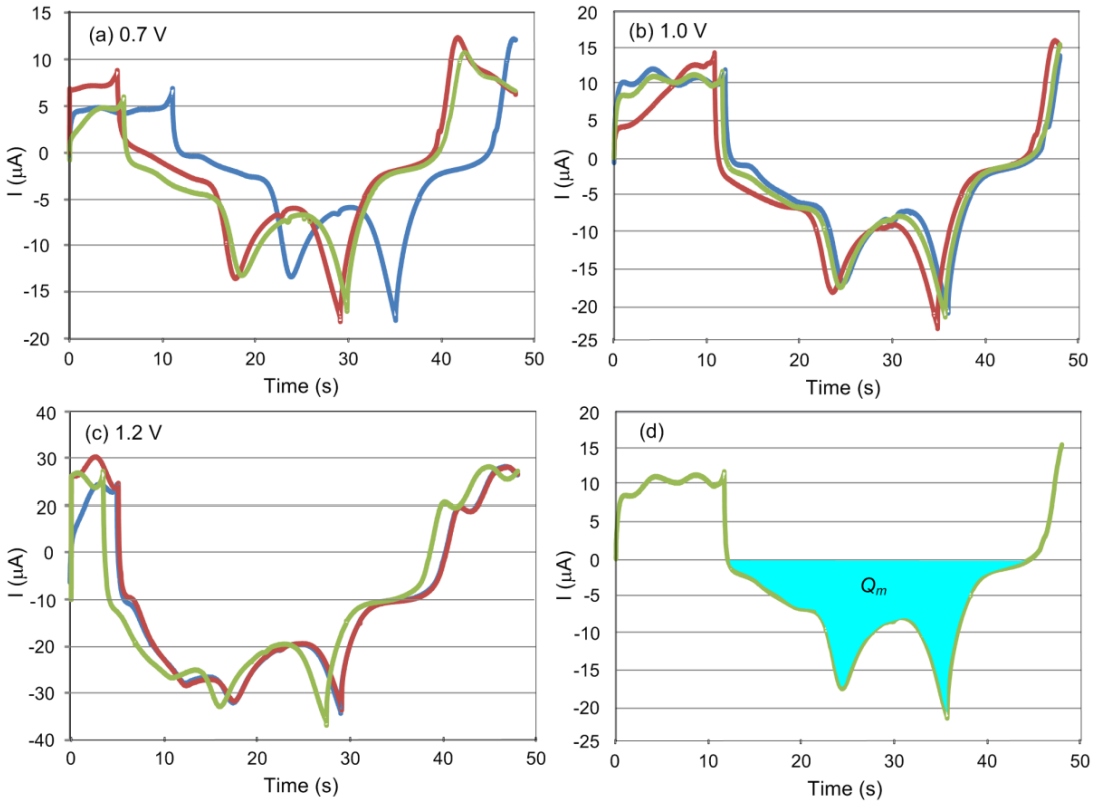

Figure 3. Current versus time charts for various applied voltages. (a) Applied voltage: 0.7 V, scan rate: 100 mV s^{-1}; (b) applied voltage: 1.0 V, scan rate: 100 mV s^{-1}; (c) applied voltage: 1.2 V, scan rate: 100 mV s^{-1}; (d) quantity of charges (Q_m) absorbed during gold oxide reduction.

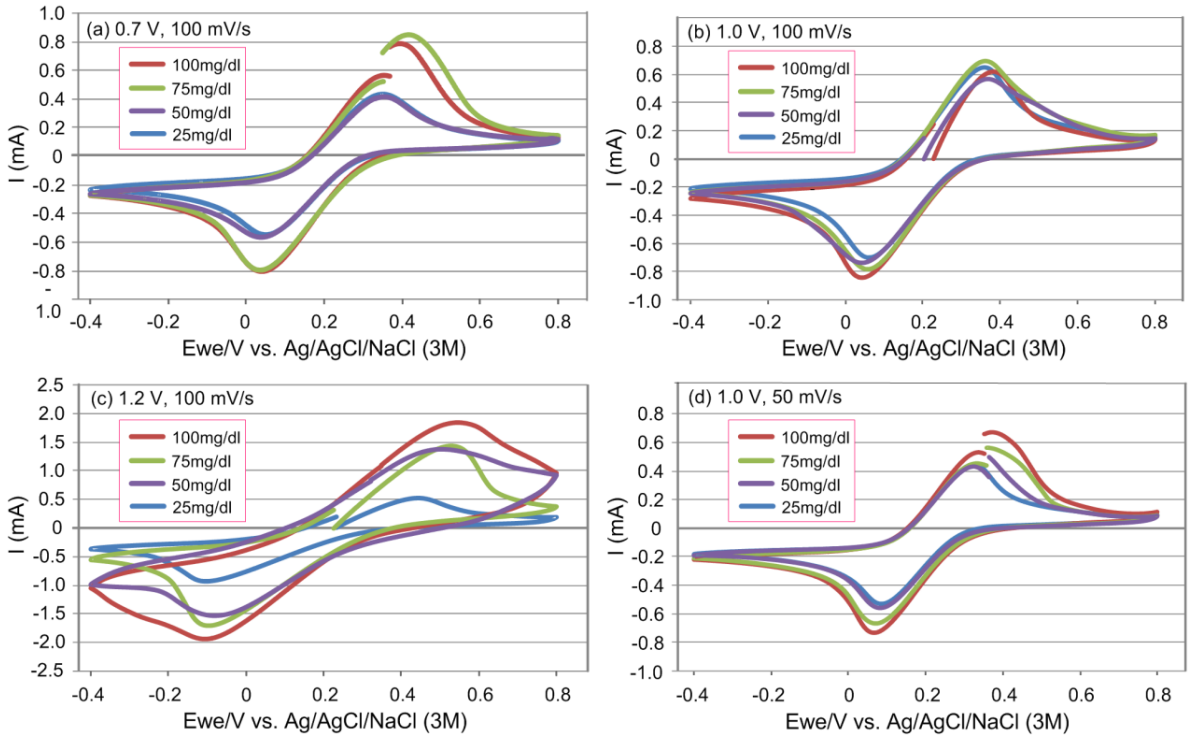

Figure 4. Glucose detection cyclic voltammograms acquired at (a) applied voltage: 0.7 V, scan rate: 100 mV s^{-1}; (b) applied voltage: 1.0 V, scan rate: 100 mV s^{-1}; (c) applied voltage: 1.2 V, scan rate: 100 mV s^{-1}; (d) applied voltage: 1.0 V, scan rate: 50 mV s^{-1}.

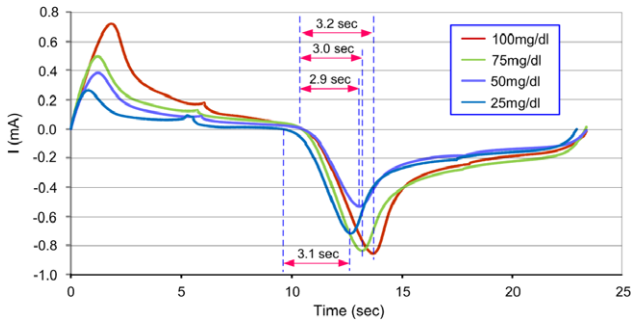

Figure 5. Current versus time charts for applied voltage of 1.0 V and scan rate of 100 mV s^{-1}.

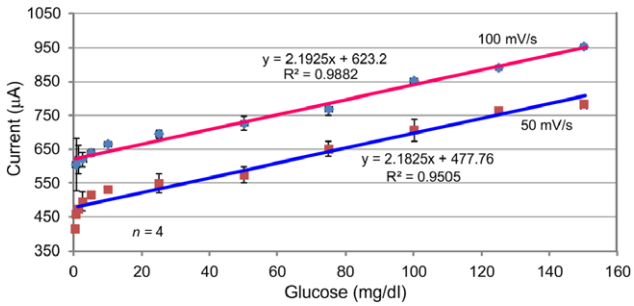

Figure 6. Calibration curves for the scan rates of 100 and 50 mV s^{-1} under an applied voltage of 1 V.

to be 46.76 μA mM^{-1} cm^{-2}. A fast response (~3 s) was also obtained. For the CV acquired at the 50 mV s^{-1} scan rate, the R^2 value for the same linear detection range was reduced to 0.95. The average overpotential (ΔE_p), which is the difference between the reduction peak (E_{pc}) and the oxidation peak (E_{pa}), for nine different glucose concentrations (1.25–150 mg dl^{-1}) were calculated to be 0.29 and 0.23 V for the scan rates of 100 and 50 mV s^{-1}, respectively. For a completely reversible electrode reaction, the overpotential is independent of the scan rate. However, quasi-reversible electrode reaction is the real situation. Redox potential slightly offsets along with the increase of the scan rate. The increase of the scan rate increases the charging potential of the double layer and reduces the ohmic potential within the solution. Hence the overpotential is increased [21].

The illustrated high sensitivity could be attributed to the large effective sensing area of the GNDs, which accommodated a considerable amount of GOx protein, hence facilitating more extensive glucose oxidation.

In addition to the characteristics such as sensitivity, detection limit, linear range, and response time, stability is another crucial factor in a glucose biosensor. Hence the stability of the proposed GND-based glucose biosensors was examined over a period of ten days. The device of GNDs synthesized at 1 V was stored dry at 4 °C and measured every day. The stability of the proposed GND-based glucose biosensors is illustrated as the long-term investigation results shown in figure 7. The measured current dropped about 16%. The possible cause of this deterioration is the decrease of the GOx activity. Actual commercially available detection chips are sterilized and vacuum packaged to prevent the decrease of the GOx activity.

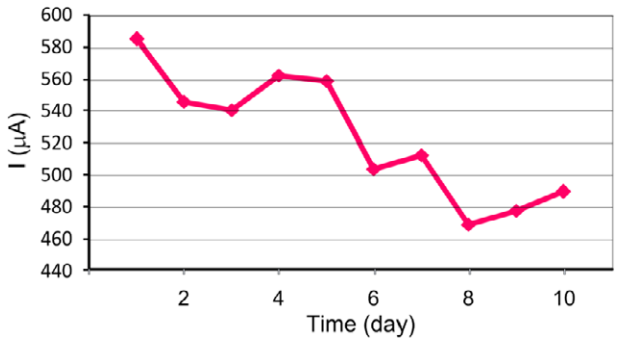

Figure 7. Long-term stability of the proposed GND-based glucose biosensor; measurement at 100 mV s^{-1} with glucose of 25 mg dl^{-1}.

Nanostructure-based glucose sensors are sensitive and inexpensive and have the advantage of a short response time. Recently, enormous efforts have been devoted to the development of nanostructure-based glucose biosensors. In table 1, we compare the functional properties of our work in this study with recently developed nanostructure-based glucose biosensors in terms of sensitivity, detection limit, linear range, and response time. Overall, the 3D GND-based glucose biosensor used in the current study exhibited good performance. Moreover, the fabrication scheme is easy, and the sensor can be readily fitted to commercially available detection devices.

4. Conclusions

A highly sensitive, fast response glucose biosensor based on a three-dimensional gold nanodendrite (GND) modified screen-printed electrode was successfully fabricated. The GNDs were formed on the screen-printed electrode by simple electrochemical deposition using a solution acquired by dissolving bulk gold in aqua regia as the precursor. Potassium ferricyanide and glucose oxidase (GOx) were subsequently coated in turn onto the GND modified screen-printed electrode for glucose measurement. Actual glucose detections demonstrated that the fabricated devices could operate in a linear range of 28 μM–8.4 mM with good sensitivity (46.76 μA mM^{-1} cm^{-2}) and a fast response time ($\sim$3 s). Moreover, only a 20 μl glucose oxidize was required for detection owing to the incorporation of the commercially available screen-printed electrode. This study suggests an effective means of producing modern IVD devices that are low cost, highly sensitive, easy to use, and disposable and afford rapid and robust results with a small sample size requirement, thereby fulfilling current expectations for these devices.

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