Silicon-Based Glucose Oxidase Working Electrode for Glucose Sensing

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ABSTRACT: We created a glucose oxidase (GOx) working electrode on a silicon-on-insulator (SOI) wafer for glucose sensing. The SOI wafer was electrically connected to a copper wire, and the GOx was immobilized onto the hydrophobilized SOI surface via silanization with aminopropyltriethoxysilane and glutaraldehyde. Electrochemical analysis (i.e., cyclic voltammetry) was employed to identify the sensing mechanism and to evaluate the performance of these SOI–GOx glucose sensors. The response of the SOI–GOx working electrode was significantly higher in the presence of oxygen than that without oxygen, indicating that a hydrogen peroxide pathway dominated in our SOI–GOx electrode. The height of cathodic peaks increased linearly with the increase of glucose concentrations up to 15 mM. The SOI–GOx working electrode displayed good stability after more than 30 cycles. On the 133rd day after the electrode was made, although the response of the SOI–GOx electrode dropped to about one-half of its original response, it was still capable of distinguishing different glucose concentrations. This work suggests that the SOI–GOx working electrode that we developed might be a promising candidate for implantable glucose sensors.

1. INTRODUCTION

The regulation and maintenance of glucose are essential for the survival of living organisms.1,2 Diabetes is a disorder that involves an individual’s inability to regulate glucose.3 Individuals affected by diabetes must tightly regulate and control their consumption of glucose. At present, diabetes patients typically monitor their glucose levels several times a day by pricking their finger,2,4 which is painful and inconvenient. Moreover, this conventional method of glucose monitoring tests only the present level of blood glucose and does not reflect an individual’s fluctuating glucose levels throughout the day. The development of a reliable and long-term method for continuous glucose monitoring has been an area of growing interest.3 Implantable glucose biosensors are continuous glucose monitoring devices that can potentially alleviate the shortcomings of conventional glucose monitoring methods.3,5,6

The development of glucose oxidase (GOx)-based enzymatic electrochemical glucose sensors that incorporate nanomaterials, polymers, and/or biocomposites has been an active research area that has attracted significant attention in recent years. Carbon-based nanomaterials, including multiwalled carbon nanotubes (MWCNTs),7–9 single-walled carbon nanotubes,10 graphene,11 electrochemically reduced graphene oxide-multiwalled carbon nanotube hybrid (ERGO-MWCNT),12 and carbon nano-onions13 have been widely used to immobilize GOx by modifying glassy carbon electrodes (GCEs), Au/Cr-coated glass substrate,10 or indium tin oxide (ITO)-coated glass plate.1 In a recent publication by Chen et al.,14 multiwalled carbon nanotube-coated carbonized silk fabric (MWCNTs/CSF) decorated with Pt microspheres was used as the working electrode for a flexible electrochemical glucose sensor. In addition to carbon-based nanomaterials, inorganic nanomaterials, such as Au nanoparticles (AuNPs),15,16 nanostructured Au thin films,17 and Au, CdS, and ZnS nanostructures,18 have also been employed to achieve GOx immobilization for glucose sensing. Moreover, glucose sensors based on polymer/GOx biocomposites, such as poly(pyrrrole-2-carboxylic acid) (PCPy),19 have also been demonstrated.

Semiconductor nanomembranes (NMs),20 owing to their high crystalline quality and compliant nature, have been demonstrated as excellent platforms for strain-engineered devices21,22 and flexible biosensors.23,24 A most convenient approach to create a flexible semiconductor NM is to release the silicon (Si) template layer of a commercial silicon-on-insulator (SOI) wafer by etching away the buried oxide sandwiched between the Si template layer and the bulk Si handling substrate. If a film with a different lattice constant, for example, a Ge film,25 is deposited on the Si template layer, a tube or “jelly roll” is formed upon releasing the Si/Ge bilayer structure from the handling substrate, due to the presence of the unbalanced strain.26 Such a tube or jelly roll has the potential to serve as the platform for a glucose sensor to be implanted into a human’s blood vessel.
In this article, we demonstrate the creation of a GOx enzyme electrochemical working electrode on an SOI wafer, an important step toward the development of semiconductor NM-based tubular-shaped implantable glucose sensors. We start from a small piece of commercial SOI wafer and create an SOI-based electrode by coating its back surface, sides, and edges of the front surface with silver epoxy and attaching a copper wire to the back surface. The hydrophilization of the SOI surface is achieved by oxygen plasma treatment, and the surface is then covered with organofunctional alkoxysilane molecules,27 a process called silanization.27 When GOx is spread on the surface, a dipeptide bond is formed between GOx and the Si template layer, enabling the immobilization of GOx on the SOI surface. This homemade SOI–GOx electrode serves as the working electrode of a three-electrode system, where commercial silver/silver chloride electrode and commercial platinum electrode are used as the reference electrode and counter electrode, respectively. We carry out cyclic voltammetry (CV) measurement using this three-electrode system in phosphate buffer solution (PBS) with glucose concentrations from 1 to 15 mM. Based on CV analysis, we identify the sensing mechanism and evaluate the performance of the SOI–GOx glucose sensor.

2. EXPERIMENTAL SECTION

2.1. Preparation of SOI Electrode. SOI wafers were cut with a diamond scriber to approximately 4 mm × 4 mm in size and cleaned first using acetone and then isopropyl alcohol in an ultrasonic water bath. Once the SOI wafer was air-dried, it was coated on the back, sides, and approximately 1 mm on each edge of the front surface with silver epoxy. A copper wire was then attached to the back of the wafer with silver epoxy, as shown in Figure 1. The electrode was maintained at a temperature of 140 °C for 15 min in a muffle furnace to cure silver epoxy. It was then treated by oxygen plasma with a power of 300 W for 10 min, inside a PE25-JW plasma cleaning system, to make the surface hydrophilic.28

2.2. Immobilization of GOx on SOI Surface. We employed the following immobilization technique, modified from Subramanian and co-workers,27 to immobilize GOx on the hydrophilic SOI surface. The SOI electrode was first immersed in a 10% (v/v) solution of aminopropyltriethoxysilane (APTES) in toluene for 10 min in a water bath that is kept at 37 °C and then immersed in 1% (v/v) solution of glutaraldehyde (GA) in distilled water for 5 min. Finally, the surface of the electrode was washed with 0.1 M PBS at pH 7.4.

A solution of 1 mg/mL GOx in a 50 mM sodium acetate buffer was prepared. With a micropipette, 10 µL of GOx was spread on the exposed SOI surface and incubated at 4 °C overnight. On the following day, regular epoxy was used to cover all of the exposed silver epoxy and the portion of the copper wire that would go into the electrochemical cell, as shown in Figure 1. The regular epoxy was allowed to harden fully for 24 h before cyclic voltammetry measurement. The modification of the SOI template layer surface with oxygen plasma treatment, silanization, and GOx attachment is illustrated in Figure 2.

2.3. Electrochemical Analysis. Our homemade SOI–GOx electrode was used as the working electrode in a three-electrode system in the CHI600E electrochemical analyzer. The reference electrode used was a commercial silver/silver chloride electrode, and the counter electrode used was a commercial platinum electrode. We carried out cyclic voltammetry measurements for glucose solutions with glucose concentrations of 1–15 mM in 0.1 M PBS, with a scan rate of 0.075 V/s. All electrodes were washed with deionized water before starting a new measurement in a fresh glucose solution.

3. RESULTS AND DISCUSSION

3.1. Identification of the Sensing Mechanism. Figure 3 shows a typical CV for an SOI–GOx electrode at a glucose concentration of 3 mM, with and without the presence of oxygen. CVs at other glucose concentrations and for other SOI–GOx electrodes also exhibited similar “duck” shape,28 with two distinct redox peaks. Peaks that appeared between
−0.15 and −0.2 V were reduction peaks, and those between 0 and −0.1 V were oxidation peaks. As is evident in Figure 3, in the absence of oxygen, the response of the SOI–GOx working electrode was significantly reduced compared to the response in the presence of oxygen. This indicates that a hydrogen peroxide pathway, instead of direct electron transfer, dominates in our SOI–GOx electrode.

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glucose + GOx(FAD) \rightarrow D-glucono-\delta-lactone + GOx(FADH) + H_2O \rightarrow gluconic acid
\]

During the reaction, molecular oxygen acts as an electron acceptor, which produces hydrogen peroxide from the oxidation of the reduced flavin moiety of GOx (eq 2). The production of hydrogen peroxide, which is measured electrochemically, is proportional to the glucose concentration, allowing sensing of glucose concentration through CV measurements.

### 3.2. Responses of the SOI–GOx Glucose Sensor at Different Glucose Concentrations.

Figure 4A shows the evolution of the cyclic voltammogram of a typical SOI–GOx working electrode as the glucose concentration was increased from 1 to 15 mM. With the increase of glucose concentration, the magnitude of the cathodic peak (i.e., reduction peak) increased monotonically. It is therefore feasible to employ the cathodic peak current as a sensing parameter of our SOI–GOx sensors. The reduction peak potential exhibited a slightly negative shift with increasing glucose concentration, which was likely due to the increased amount of reduced GOx at the electrode’s surface when the cathodic trace was scanned.

The magnitude of the anodic peak (i.e., oxidation peak) also increased with glucose concentration in general, but the change was not as significant compared to that of the cathodic peak. This could possibly be attributed to the alteration on the active site of GOx.\textsuperscript{3,31,32} The active site of GOx widened during the reaction with glucose, which made the reduced GOx more selective for hydrogen peroxide rather than oxygen.\textsuperscript{3,32} As a result, some reduced GOx shifted toward an inactive state and could not bind glucose when the anodic trace was scanned. As more GOx became inactive on the electrode surface, the sensor in PBS with glucose concentration of 7 mM, on days 7 and 133. Although the peak currents on day 133 dropped to less than 30 cycles. We also evaluated the response of an SOI–GOx electrode on the 7th and the 133rd days after it was created. Figure 5A shows the cyclic voltammograms of the sensor in PBS with a glucose concentration of 7 mM, on days 7 and 133. Although the peak currents on day 133 dropped to around one-half of the values on day 7, both anodic and cathodic peaks were still distinct on day 133, and the “duck” shape of the CV remained. The half-life (t_{1/2}) of our glucose sensor is estimated to be 4–5 months, which is considerably longer than that reported in the literature.\textsuperscript{12,14,15,17,18}

### 3.3. Stability of the SOI–GOx Glucose Sensor.

For applications in implantable sensing devices, stability and lifetime are important characteristics to be considered. To evaluate its stability, we monitored the CV of the sensor while running it continuously for multiple times, and the peak current retained nearly 97% or higher of its original value after more than 30 cycles. We also evaluated the response of an SOI–GOx electrode on the 7th and the 133rd days after it was created. Figure 5A shows the cyclic voltammograms of the sensor in PBS with a glucose concentration of 7 mM, on days 7 and 133. Although the peak currents on day 133 dropped to around one-half of the values on day 7, both anodic and cathodic peaks were still distinct on day 133, and the “duck” shape of the CV remained. The half-life (t_{1/2}) of our glucose sensor is estimated to be 4–5 months, which is considerably longer than that reported in the literature.\textsuperscript{12,14,15,17,18} Figure 5B shows cyclic voltammograms of the sensor in PBS with glucose concentrations of 3, 7, and 13 mM, on day 133. The apparent increase of peak currents with increasing glucose concentrations indicated that our glucose sensor was still capable of distinguishing glucose concentrations 133 days after the device was made.
4. CONCLUSIONS

To summarize, we have created SOI–GOx working electrodes through immobilizing GOx onto the SOI surface via silanization with APTES and GA, for glucose sensing. The significant suppression of the response of the SOI–GOx electrode in the absence of oxygen, compared to its response in the presence of oxygen, suggested that a hydrogen peroxide pathway was dominated in our SOI electrode. As revealed from the evolution of CV, the cathodic peak currents of these SOI–GOx electrodes increased monotonically and nearly linearly with the increase of glucose concentrations, in a broad range of 1–15 mM. The sensitivity of the electrodes was determined to be 0.34 μA/mM or 8.5 μA/(mM cm²). Moreover, these SOI–GOx working electrodes displayed good stability and were capable of distinguishing different glucose concentrations even 133 days after the electrode was made. These results suggest that our SOI–GOx working electrode might be a promising candidate for implantable glucose sensors.

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The authors declare no competing financial interest.

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Figure 5. (A) Cyclic voltammograms of an SOI–GOx working electrode at a glucose concentration of 7 mM, on the 7th and 133rd days after the electrode was created. (B) Cyclic voltammograms of the SOI–GOx working electrode at glucose concentrations of 3, 7, and 13 mM, on day 133.
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