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Enzyme-functionalized gold nanowires for the fabrication of biosensors

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Abstract

Gold nanowires were prepared by an electrodeposition strategy using nanopore polycarbonate (PC) membrane, with the average diameter of the nanowires about 250 nm and length about 10 μm. The nanowires prepared were dispersed into chitosan (CHIT) solution and stably immobilized onto glassy carbon electrode (GCE) surface. The electrochemical behavior of gold nanowire modified electrode and its application to the electrocatalytic reduction of hydrogen peroxide (H_2O_2) were investigated. The modified electrode allows low potential detection of hydrogen peroxide with high sensitivity and fast response time. Moreover, the good biocompatibility of nanometer-sized gold, the vast surface area of the nanowire-structure make it ideal for adsorption of enzymes for the fabrication of biosensors. Glucose oxidase was adsorbed onto the nanowire surface to fabricate glucose biosensor as an application example. The detection of glucose was performed in phosphate buffer (pH 6.98) at −0.2 V. The resulting glucose biosensor exhibited sensitive response, with a short response time (<8 s), a linear range of 10^{-5}–2×10^{-2} M and detection limit of 5×10^{-6} M.

Keywords: Gold nanowire; Enzyme; Functionalization; Electrodeposition; Biosensor

1. Introduction

With the development of ultrasensitive electrochemical biosensors and the need of miniaturization of sensing devices, nanomaterials have drawn considerable attentions due to their unique size and properties [1,2]. In particular, noble metal nanostructures like those of gold have gained special interest because of it conductivity, optical properties and biocompatibility [3,4]. Gold nanoparticles were one of the most widely used nanoparticles in the past years, and have been used for immobilization of enzymes for the fabrication of biosensors [5,6]. These nanoparticles can act as tiny conduction centers and can facilitate the transfer of electrons. What is more, many works have shown enzymes maintain their enzymatic and electrochemical activity when immobilized on gold nanoparticles [7].

Since the discovery of carbon nanotubes, one-dimensional nanostructures such as nanotubes, nanowires, and nanorods have been the subject of a series of investigations [8–10]. In comparison to spherical particles, nanowires have recently attracted much attention, which own a number of unique physical and electronic properties that endow them with new and important activities [11]. In the past years, many methods have been developed to prepare high-density nanowires. Among these, porous template based synthesis has been one of the most popular methods [12,13].

Enzyme immobilization was the main focus in the design and optimization of biosensors. The conventional methods for enzyme immobilization include covalent attachment with glutaraldehyde and entrapment by different substrate materials [14,15]. While covalent attachment might partially denature the activity of enzymes, the enzyme entrapped in various substrate materials made it difficult to contact its active sites with substrate [16,17]. The adsorption of enzymes on inorganic materials may improve the stability of the enzymes, which enable further practical applications [18].

In this paper, we describe a method for creating biosensing platform to achieve simple and reliable immobilization of the enzyme with enhanced detection sensitivity. First, we prepare gold nanowires by means of electrodeposition technique in polycarbonate (PC) membrane. The gold nanowires were then dispersed into chitosan (CHIT) solution and immobilized onto

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electrode surface. The modified electrode was found to have sensitive response toward hydrogen peroxide at −0.2 V with sensitivity better than that of the conventional gold electrode. The high sensitivity of the gold nanowire modified electrode toward hydrogen peroxide and the large surface area make it ideal for the absorption of enzymes for the fabrication of biosensors. Glucose oxidase was selected and absorbed onto the gold nanowire surface, and the resulting glucose biosensor enables selective determination of glucose with high sensitivity and wide linear range. The biosensor fabrication method used here have some advantages: employ mild condition allowing for large quantities of enzymes to be immobilized uniformly, the biocompatibility of metal gold provide favorable microenvironment to maintain the enzyme activity, and a large surface area for enzyme–substrate contact.

2. Experimental

2.1. Apparatus and reagents

Track-etched porous polycarbonate (PC) membrane was provided by Whatman (Anodisc 47, 0.2 μm). Glucose oxidase (GOx, from *Aspergillus niger*; EC 1.1.3.4, type VII-S; 196,000 unit g^{-1}), chitosan (CHIT, MW∼1×10^6; ∼80% deacetylation) were purchased from Sigma. A 1/15 M phosphate buffer (PB, pH 6.98) solution was prepared with Na_2HPO_4 and KH_2PO_4. All other reagents were of analytical grade, and doubly distilled water was used throughout.

Cyclic voltammetric and amperometric measurements were carried out on CHI 760B electrochemical workstation (Shanghai, China). Scanning electron microscopy (SEM) analysis was performed using a JSM-5600LV microscope (JEOL, Ltd. Japan). A three-electrode cell (10 mL) was used with the nanowire modified glassy carbon (GC) electrode as the working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum foil electrode as counter electrode. The conventional gold electrode (4 mm in diameter) was polished with Emery paper carefully, then cleaned under bath sonication for 10 min, and finally thoroughly rinsed with distilled water. All potentials were measured and reported versus the SCE and all experiments were carried out at room temperature.

2.2. Synthesis of Au nanowires

Prior to electrodeposition, an ultrathin Au film (30 nm) was first sputtered onto one side of the polycarbonate membrane to make the template conductive. The Au-sputtered membrane, which linked with a brass wire using high purity silver paint, served as a working electrode. Meanwhile, the three electrodes were immersed into $HAuCl_4$ solution (w/w 1%) containing 0.5 M $HClO_4$, and electrodepositions were performed using chronoamperometry under a constant potential (0.18 V) at room temperature. The whole procedure accompanied continued stirring with a magnetic stirrer. After 15 min of deposition, the PC template was dissolved away by immersing in the solution of $CHCl_3$ at 4 °C for 2 h to liberate gold nanowires. Through repetitious and careful rinsing with chemical lotion and double-distilled water, the residues were removed and the desired nanowires were obtained.

2.3. Preparation of gold nanowire modified electrode

Glassy carbon electrode (4 mm in diameter) was polished with Emery paper carefully until a mirror was obtained. After 15 minute of sonication in ethanol to remove residues, the electrode was thoroughly rinsed with distilled water and dried.

A 0.5 wt.% CHIT solution was prepared by dissolving 50 mg of CHIT flake in 10 mL of 1.0% acetic acid and stirred for 3 h at room temperature until complete dissolution. The pH was adjusted to 4.0–5.0 using a concentrated NaOH solution. The nanowires were dispersed within CHIT solutions with the concentration of 2 mg/mL by using a short 15-min sonication. After that, 10 μL of the admixture was coated onto the surface of electrode to fabricate gold nanowire modified electrode. Under room temperature, the evaporation of water resulted in a uniform and thin modified film.

2.4. Preparation of enzyme-functionalized gold nanowire modified electrode

To realize functionalization of gold nanowires, the purified Au nanowires were immersed in the glucose oxidase solution (10 mg/mL) overnight at 4 °C in fridge. The functionalized gold nanowires were then dispersed in CHIT solutions under bath sonication for 15 min. The fabrication procedure for enzyme adsorbed gold nanowire modified electrode was similar to the one mentioned in previous section. In brief, the sensor was modified by casting 10 μL of the admixture onto the surface of electrode and dried overnight at 4 °C in fridge to form an infrangible film.

3. Results and discussion

3.1. The characterization of the prepared gold nanowires

The approach of the synthesis of Au nanowires using the nanopore polycarbonate template was simple and convenient. With careful choice, a deposition potential of 0.18 V and a growth time of 15 min was chosen for potentiostatic deposition. Within the process of electrodeposition, the Au nanoparticles were accumulated into the pores of the PC and grew along the pore wall to form the nanowires. After deposition of the proper time, the color of the PC template changed from its pre-electrolysis white color to yellow indicating the electrodeposition of metal within the pores of the template. The template was then dissolved in the solution of $CHCl_3$ and the desired gold nanowires were obtained. Fig. 1a is a SEM micrograph of the gold nanowires, which confirmed the formation of nanowires. As is shown in the figure, the gold nanowires grew well within the pores of PC template and display a highly regular and uniform pattern with an average diameter of about 250 nm, which corresponds to the dimension of the nanopore in the template and the length of the nanowire after 15 min of

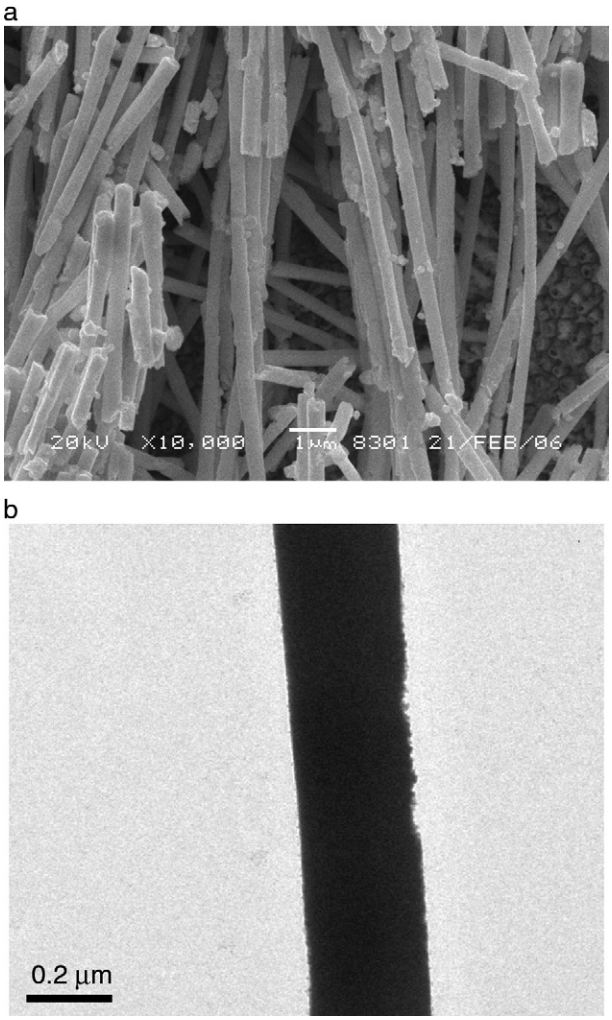

Fig. 1a. SEM micrograph of the gold nanowires. Fig. 1b TEM image of a single gold nanowire dispersed in chitosan solution.

deposition is about 10 μm. Fig. 1b shows TEM image of a single gold nanowire dispersed in chitosan solution. A straight nanowire can be seen with the diameter about 250 nm, which is in accordance with the SEM image.

3.2. Estimation of the active surface area of the gold nanowire modified electrode

The $Fe(CN)_6^{4-/3-}$ redox system is one of the most extensively studied redox couples to estimation the electroactive surface area of electrode in electrochemistry. Fig. 2 represents cyclic voltammograms (CVs) of the bare glassy carbon electrode (a) and the gold nanowire modified electrode (b) in 20 mM $K_3Fe(CN)_6$ containing 0.2 M KCl at the scan rate of 100 mV s^{-1}. The well-defined oxidation and reduction peaks due to the Fe^{3+}/ Fe^{2+} redox couple were observed at +0.145 V and 0.275 V. According to the Randles–Sevcik equation: [19]

$$I_p = 2.69 \times 10^5 \left[\mathrm{A\ s\ mol^{-1}V^{-1/2}}\right] \mathrm{Ar} D^{1/2} n^{3/2} v^{1/2} C$$

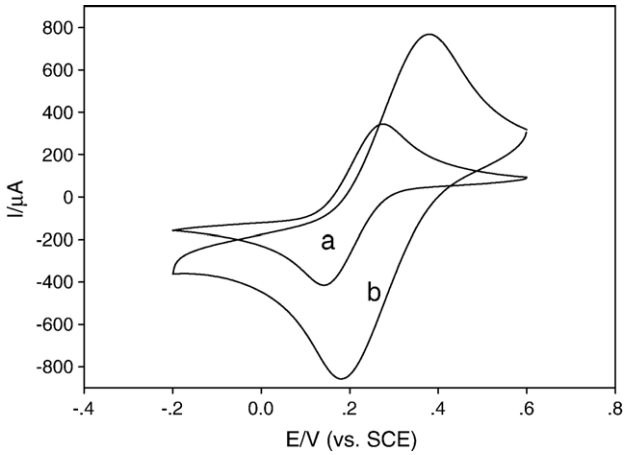

Fig. 2. Estimation of electroactive surface area by cyclic voltammograms (CVs) of the bare glassy carbon electrode (a) and the gold nanowire modified electrode (b) in 20 mM $K_3Fe(CN)_6$ containing 0.2 M KCl at the scan rate of 100 mV s^{-1}.

where n is the number of electrons participating in the redox reaction, v is the scan rate of the potential perturbation (V/s), Ar is the area of the electrode (cm^2), D is the diffusion coefficient of the molecule in solution (cm^2/s), C is the concentration of the probe molecule in the bulk solution (mol/cm^3), and the I_p is the peak current of the redox couple (A). In the equation, D, n, v and C are constant parameters values, and the electroactive surface area (Ar) is linear to the I_p value. For the Au nanowire modified electrode, the I_p value illustrated an obvious increase in the electroactive surface area. As can be seen, the electroactive surface area of the gold nanowire modified electrode is about 2.5 times larger than the bare GC electrode.

3.3. Electrochemical characterization of gold nanowire modified film

To characterize the gold nanowire modified electrode, cyclic voltammograms (CVs) were performed. Fig. 3 shows CVs of the gold nanowire modified electrode in H_2SO_4 (0.5 M) at the scan rate of 50 mV s^{-1}. The GCE modified with gold nanowires

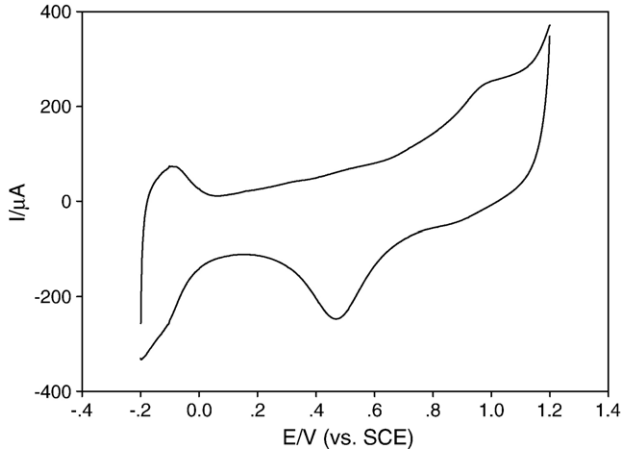

Fig. 3. Cyclic voltammograms (CVs) of the gold nanowire modified electrode in H_2SO_4 (0.5 M).

displayed hydrogen adsorption/desorption peaks around the potential range of −0.1 V together with a signature for oxide formation at 0.5 V and stripping at 1.0 V, exhibiting the characteristic features expected for metal nanostructure modified electrode [20]. The well-defined peaks also indicate that the films are highly homogeneous.

3.4. Electrochemical response of Au nanowire modified electrode toward hydrogen peroxide

As mentioned in the introduction, metal gold display excellent catalytic activity and can facilitate electron-transfer process. Here, the gold nanowire modified electrode was examined for its amperometric response toward hydrogen peroxide. Fig. 4 shows the effect of applied potential on the gold nanowire modified electrode response toward hydrogen peroxide in 1/15 M phosphate buffer. The range of potential was controlled from −0.4 to 0.4 V. As can be seen, during the potential range studied, high response current was observed and the potential of 0.3 V is a turning point. In the range of 0.3 to −0.4 V, with the negatively increasing potential, the current signal increased, and also started at 0.3 V, with the positively increasing potential, there is an increase of the current response. Compare the current response of the gold nanowire modified electrode in the negative potential range with that in the positive potential range, it can be seen that in the negative potential range, the response is much higher that in the positive range, indicating the reduction of hydrogen peroxide is much easier than it oxidation. In this study, the potential of −0.2 V was selected. At such a low applied potential, the background current decreased, the responses of common interference species can be minimized, and the oxygen reduction current can be limited.

Fig. 5 displays the typical current–time curve at the conventional gold electrode (a) and the Au nanowire modified electrode (b) to H_2O_2. The process of detection was carried out at −0.2 V under air-saturated condition and 1 mM H_2O_2 were successively added into the phosphate buffer (1/15 M, pH 6.98)

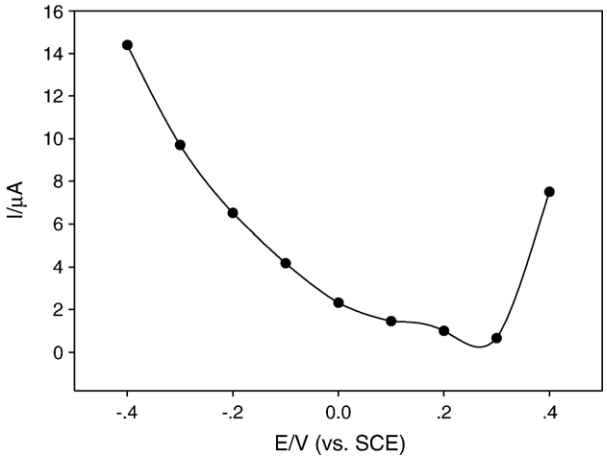

Fig. 4. Effect of potential on the response of the gold nanowire modified electrode toward 1 mM hydrogen peroxide.

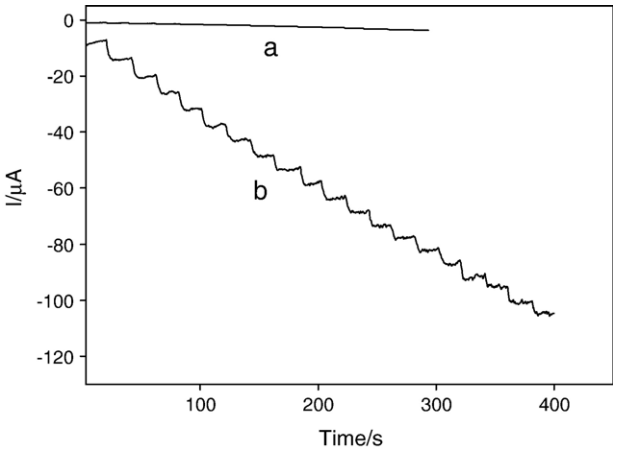

Fig. 5. Current–time recording obtained at the conventional gold electrode (a) and the gold nanowire modified electrode (b) upon increasing the concentration of hydrogen peroxide in steps of 1 mM at −0.2 V in phosphate buffer (1/15 M, pH 6.98).

[21,22]. As shown in the figure, the modified electrode responds rapidly to the changes in the level of hydrogen peroxide, producing steady signals within 5 s. The sensitivity of the gold nanowire modified electrode is about 30 times higher than conventional gold electrode.

The excellent electrochemical response observed could be ascribed to not only higher effective surface area of gold nanowire modified electrode but also the properties of Au nanowires themselves including excellent catalytic activities, unique electrical properties and high conductivity.

3.5. Electrochemical performance of the glucose biosensor fabricated by functionalized gold nanowires

The excellent electrocatalytic activity of the modified electrode toward H_2O_2 means that the electrode can be served as a new biosensing platform that provides operational access to a great deal of oxidase. In our paper, the Au nanowire modified electrode shows good electrocatalytic activity toward H_2O_2, which made it ideally to construct oxidase-based biosensors. Moreover, the good biocompatibility and large specific surface area of gold nanowires made it ideal for the adsorption of enzymes. In this paper, glucose oxidase as a model enzyme was selected. The amperometric responses of the GOx modified biosensor for successive addition of 0.5 mM glucose at −0.2 V are presented in Fig. 6, and the insert is the calibration curve. One can see that the biosensor was sensitive toward glucose and a steady-state current signal was generated within 8 s. The sensitive response means that large amount of enzymes are adsorbed onto the nanowire surface. Meanwhile, as can be seen from the calibration curve, the glucose biosensor exhibits the linear range of 0.01 mM up to 20 mM with a correlation coefficient of 0.997 and based on S/N=3, a detection limit of 5×10^{-6} M was obtained. The lower detection limit of the linear calibration range of the gold nanowire modified electrode toward glucose is 1.0×10^{-5} M. This is 200 times lower than carbon nanotube/Teflon modified electrodes (2.0×10^{-3} M) [23]. And the higher

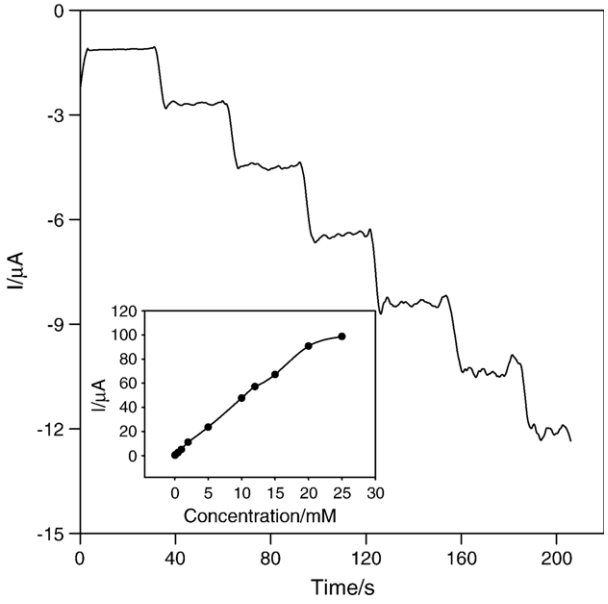

Fig. 6. Current–time recording of GOx modified biosensor for the successive addition of 0.5 mM glucose at −0.2 V. The insert is the calibration curve.

detection limit (2.0×10^{-2} M) is 8 times higher than the nano-SiO_2 film modified electrodes (2.5×10^{-3} M) [24].

The general problem in the electrochemical detection of glucose is the interference from electroactive species, such as ascorbic acid, uric acid and acetaminophen, which are existing in physiological samples. The level of endogenous ascorbic and uric acid is about 0.125 and 0.33 mM, respectively, whereas about 0.13 mM acetaminophen has been detected from blood samples [25]. At this level, the glucose biosensor exhibited negligible response to such electroactive interfering species.

The interference-free determination of glucose was demonstrated by an independent recovery test. The recovery test was carried out in 10 mL phosphate buffer containing real physiological samples (0.5 mL serum). By using standard addition method, standard glucose solutions were added to the assay solution. Five assays were performed with the recoveries in the range of 97.6–104% (Table 1), which illustrated the reliability of the results of the biosensor. The RSD were obtain thought three determination and the low values means that the GOx are stably absorbed onto the electrode surface and can be used for practical application.

Improvement of the long-term stability of biosensors has been one of the main focuses in the fabrication of biosensors.

Table 1
Recovery data for diluted serum samples added with different glucose concentrations

Added (10^{-3} mol/L)	Founded (10^{-3} mol/L)	Recovery (%)	RSD (%)
0.500	0.510	102	3.42
1.54	1.61	104	4.23
3.00	2.95	98.3	3.54
3.50	3.42	97.6	4.65
10.0	10.3	103	3.65

Values were average of three determination.

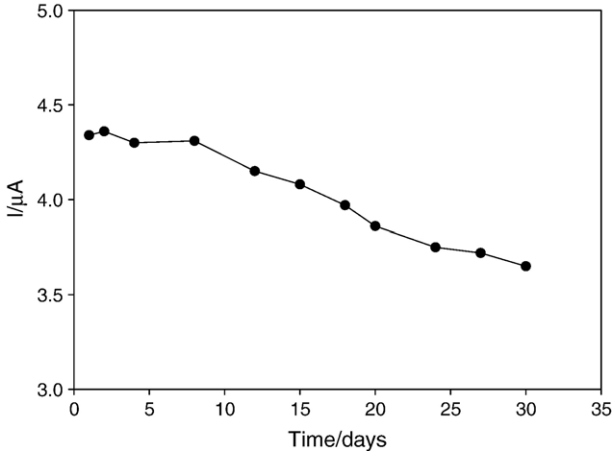

Fig. 7. Long term stability of the glucose biosensor toward 1 mM glucose.

The key factor to improve stability of biosensor is to decrease the leakage of the components and keep the bioactivity of the enzymes. In the present work, the immobilized enzyme is firmly retained on the surface of electrode after several repeated experiments and repeated use of the electrode did not affect the repeatability and long-term stability. For example, a set of 10 different amperometric measurements for 1 mM glucose with a single biosensor yield a relative standard deviation of 5.1%, indicating a good repeatability of the measurements.

The response of the biosensor to 1 mM glucose was measured intermittently and when not in used, the biosensor was stored in refrigerator under 4 °C. The results are shown in Fig. 7. In the first two weeks, little change of the response current was observed. One month later, there is a slight decrease of the response current, and still 85% of the response can be obtained. Such an excellent long-term stability can be ascribed to two aspects: the biocompatibility of the gold nanowires stabilized the enzymes that adsorbed, and, the good adhesion, excellent film-forming ability and biocompatibility of the CHIT facilitate the immobilization of the enzyme modified gold nanowires and also helped in keeping the bioactivity of the enzymes.

4. Conclusion

In this paper, we have demonstrated nanostructuring using the prepared gold nanowires improves analytical performance of the corresponding sensors compared to the conventional electrodes. The sensitivity of the nanowire modified electrode toward hydrogen peroxide is much better than that of conventional gold electrode at detection potential of −0.2 V. The biocompatibility of metal gold toward proteins and the large surface area of the nanowire made it ideal for the adsorption of proteins. In our study, glucose oxidase was adsorbed onto the nanowire surface and applied in fabrication of biosensor. The resulting biosensor exhibits good amperometric response to glucose. The biosensor fabrication method no doubt offers a promising platform for various biosensing applications.

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