

Electrochemical glucose biosensor based on silver nanoparticles/multiwalled carbon nanotubes modified electrode

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Abstract A novel glucose biosensor was fabricated by immobilizing glucose oxidase (GOx) on Ag nanoparticles-decorated multiwalled carbon nanotube (AgNP-MWNT) modified glass carbon electrode (GCE). The AgNP-MWNT composite membrane showed an improving biocompatibility for GOx immobilization and an enhancing electrocatalytic activity toward reduction of oxygen due to decoration of AgNPs on MWNT surfaces. The AgNPs also accelerated the direct electron transfer between redox-active site of GOx and GCE surface because of their excellent conductivity and large capacity for protein loading, leading to direct electrochemistry of GOx. The glucose biosensor of this work showed a lower limit of detection of 0.01 mM ($S/N=3$) and a wide linear range from 0.025 to 1.0 mM, indicating an excellent analytical performance of the obtained biosensor to glucose detection. The resulting biosensor exhibits good stability and excellent reproducibility. Such bionanocomposite provides us good candidate material for fabrication of biosensors based on direct electrochemistry of immobilized enzymes.

Keywords Glucose biosensor · Ag nanoparticles · Multiwalled carbon nanotube · Electrocatalysis · Biocompatibility

Introduction

Carbon nanotubes (CNTs) as a new type of carbon material have attracted increasing interest for potential

applications in biosensors due to their special geometry and unique electronic, mechanical, chemical, and thermal properties [1]. Studies [1] also have demonstrated that the performance of CNTs as biosensors for glucose and DNA detection is much superior to those of other carbon electrodes in terms of reaction rate, reversibility, and detection limit. Because of the hydrophobicity of CNT surface, it is necessary to functionalize CNTs with conducting polymers, guest molecules, side wall substituents, or metal nanoparticles for improving the biocompatibility [2–8]. Many works have shown that the enzymatic and electrochemical activity of enzymes can be maintained when the enzymes were immobilized on rare metal nanoparticles including Pt, Au, Ag, and so on. [9–13]. Moreover, these metal nanoparticles can act as tiny conducting channels to realize the direct electron transfer between the active sites of enzyme and the electrode surface, and they will reduce the effective electron transfer distance, thereby facilitating charge transfer between electrode and enzyme [14, 15]. Furthermore, if the electrode is modified by metal nanoparticle and CNT composite, the enzyme can be immobilized through adsorption. Among these various enzyme immobilization protocols, adsorption is the simplest and involves minimal preparation. The bioactivities of the immobilized enzyme can be retained well because the adsorption needs no chemical reagents and seldom activation [16]. Zhang et al. successfully coated multiwalled carbon nanotubes with small and good dispersion Pt nanoparticles through NH_3 gas pretreatment, and the PtNP-MWNT coated electrode toward detecting H_2O_2 exhibited the best electrocatalytic property for H_2O_2 [17]. Qin et al. synthesized composites of gold nanoparticles decorated MWNTs (MWNT-GNP) by a classical chemical method, and GNP was attached on

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MWNTs through the specific interaction between –SH and Au. The electrode modified by MWNT-GNP exhibited a wide linear range, remarkable sensitivity, fast response time, good reproducibility, and long-term stability [18]. A relatively green preparative route toward Au nanoplates in aqueous solution at room temperature with the use of tannic acid as a reducing agent was presented by Zhang et al. [19]. Then a glucose biosensor was further fabricated by immobilizing glucose oxidase (GOD) into chitosan-Au nanoplate composites film on the surface of glassy carbon electrode; this sensor exhibits good response to glucose and can be used for glucose determination of real samples. Lin et al. report a one-step synthesis of silver nanoparticles/CNTs/chitosan film hybrid film as a new alternative for the immobilization of GOD and horseradish peroxidase (HRP) based on layer-by-layer technique. The proposed Ag/CNT/Ch matrix by simple one-step synthesis offered an excellent amperometric response for HRP and GOD with high sensitivity and quick response [20]. Recently, Ag nanoparticles composites decorating biosensors used for the glucose detection in human blood serum have been fabricated [21–23]. The electrodeposition method was employed by many researches to deposit Pt, Au, or Ag nanoparticles on CNT surface and further obtain a PtNP-MWNT coated electrode [13, 15, 17]. In addition, graphene, another new type of carbon materials, decorated with metal nanoparticles as immobilization matrix for glucose biosensor, exhibits best sensing performance and excellent detection limit [24–26]. However, to functionalize CNT surface through deposition of metal nanoparticles with small radius and good dispersion is still a challenge. The dispersion of metal nanoparticles on the CNT surface may affect the electrochemical behavior of the enzymes [17].

In this study we successfully modified the glass carbon electrode (GCE) with Ag nanoparticle-decorated multi-walled carbon nanotube (AgNP-MWNT). The glucose oxidase (GOx) was immobilized on the AgNP-MWNT surface to detect glucose. The AgNP-MWNT composition was obtained by a green method. In a typical preparation, 0.5 g MWNTs and 3.2 g NH_4HCO_3 were put into a cylindrical ball milling container and rolled at a speed of 250 rpm for 5 h. The obtained MWNTs with functionalization surfaces were used to prepare AgNP-MWNTs composites by using silver mirror reaction. Fifty milliliters of 0.1 % sodium dodecyl sulfate (SDS) aqueous containing 200 mg functionalized MWNTs was introduced to Tollens reagent ($[\text{Ag}(\text{NH}_3)_2]^+$) solution under gentle stirring. Formaldehyde (0.5 g) as reducer was dropped to above system, and the system was constantly stirred at 60 °C for 1.5 h. The final Ag/MWNT products were collected by centrifugation and washed with water and ethanol for several times. The

AgNPs exhibit good dispersion, and the radius of the AgNPs is less than 5 nm. Its electrocatalysis and biocompatibility also showed improvement in this work.

Materials and methods

Materials and reagents

AgNP-MWNT with the AgNP content approaching 60 % was synthesized in our laboratory. It is described in details in the “Introduction”. The MWNTs with a diameter of 40–60 nm were obtained from Shenzhen Nanotech Port Ltd. Co. (China). GOx (EC 1.1.3.4, type X-S, lyophilized powder, 100–250 units mg^{-1} , from *Aspergillus niger*) and D-(+)-glucose were purchased from Sigma. All other reagents were of analytical grade and used without further purification.

Apparatus

Cyclic voltammograms (CV), differential pulse voltammograms (DPV), and electrochemical impedance spectroscopy (EIS) experiments were performed by using a CHI 660 C electrochemical workstation (Shanghai Chenhua Instruments Co., China). All experiments were carried out using a conventional three-electrode system including a 3-mm diameter GCE as the working electrode, saturated calomel electrode (SCE) as the reference electrode, and a Pt wire as the auxiliary electrode. After the GCE was modified by AgNP-MWNT film, the sample films with and without immobilized GOx were characterized using a field-emission scanning electron microscope (FE-SEM) (S-4800, Japan).

Preparation of GOx/AgNP-MWNTs modified GCE

The bare GCE was polished to a mirror-like surface with 0.3 and 0.05 μm alumina slurries and rinsed with double-distilled water for several times. Then the bare GCE was washed by using ultrasonic in ethanol and water for 5 min, respectively. After ultrasonic the electrode was rinsed with double-distilled water and allowed to dry at room temperature. Fifty milligrams of AgNP-MWNTs was added to 2-mL ethanol, and the mixture was ultrasonicated to form a homogeneous AgNP-MWNT solution. About 4- μL of AgNP-MWNT solution was dropped on the pretreated GCE surface and dried at room temperature to form AgNP-MWNTs modified GCE. To prepare GOx/AgNP-MWNTs modified GCE, the AgNP-MWNTs modified GCE was immersed in a 0.1-M phosphate buffer solution containing 10 mg mL^{-1} GOx for 20 h at 4 °C in a refrigerator. The prepared electrode was rinsed thoroughly with double-distilled water to wash away the loosely adsorbed GOx molecules. For

comparison, GOx/MWNTs (the MWNTs were functionalized by amine groups only) or GOx modified GCE was also prepared with the same procedure. All the electrodes modified by enzyme molecules were transferred to a 0.1-M phosphate buffer solution (pH 7) and stored at 4 °C in a refrigerator if not in use.

Results and discussion

Characterization of GCE modified by AgNP-MWNTs or GOx/AgNP-MWNTs

Figure 1 displays the scanning electron microscope (SEM) images of AgNP-MWNTs and GOx/AgNP-MWNTs coating on the GCE surface. From Fig. 1a, it can be seen that the AgNPs exhibit good dispersion on MWNT surface and the radius of AgNPs is less than 5 nm. The good dispersion of AgNPs could be attributed to the presence of $-NH_2$ providing active sites for the adsorption of AgNPs. The AgNP-MWNT composites were distributed very homogeneously on the GCE surface. The SEM image of GOx adsorbed on the AgNP-MWNT composites is shown in Fig. 1b. When the AgNP-MWNTs modified film was immersed in a GOx solution, the GOx molecules were adsorbed on the surfaces of AgNP-MWNTs and tended to aggregate into island-like structures. The GOx adsorbed on the surfaces of AgNP-MWNTs facilitated the substrate to be accessible to GOx, and good electrochemical response to glucose can be obtained.

Cyclic voltammograms of GCE modified by MWNTs and AgNP-MWNTs

Figure 2 shows the cyclic voltammograms of MWNTs and AgNP-MWNTs modified GCE in 0.1 M PBS at a scan rate of 100 mV s^{-1} . In air-saturated 0.1 M pH 6.45 PBS, there was no detectable amperometric signal for bare GCE. In nitrogen-saturated PBS, the MWNTs modified GCE showed no evident redox peak (Fig. 2a(b)). In contrast, the AgNP-MWNTs modified GCE showed one pair of redox peaks at -0.15 and 0.22 V (Fig. 2b(b)). This result suggested that the $-NH_2$ groups had been introduced on the surface of MWNTs, which was ready for the immobilization of

AgNPs. The evident redox peak could be ascribed to AgNPs decorated on MWNT surfaces. As oxygen-containing groups, the AgNPs were favorable to improving the biocompatibility for protein immobilization and enhancing the electrocatalytic activity of CNTs toward the reduction of both O_2 and the immobilized electroactive enzymes [28]. As expected, the AgNP-MWNTs exhibited better electrocatalysis toward the reduction of O_2 than MWNTs (Fig. 2(b–d)). In air-saturated PBS an obvious reduction peak of O_2 at AgNP-MWNTs modified GCE could be observed at 0.23 V , while the peak at MWNTs modified GCE occurred at -0.18 V . The 410-mV positive shift achieved at the AgNP-MWNTs modified GCE compared to MWNTs modified GCE indicated significant electrocatalytic activity of AgNP-MWNT composite toward the reduction of O_2 . The peak currents at AgNP-MWNTs modified GCE were about 15 times larger than those of MWNTs modified GCE, suggesting more AgNPs attached onto MWNTs by $-NH_2$ groups. In oxygen-saturated PBS the difference in peak current density was obvious. Similar results were reported in the literature that compared with MWNT or PtNP-MWNTs modified electrodes; PtNP-MWNT composites modified electrode showed largely increasing current signals with lower oxidation/reduction overvoltage, which means that PtNPs-MWNTs exhibited the best electrocatalytic activity [17].

Direct electrochemistry characterization of GOx-MWNTs or GOx-AgNP-MWNTs modified GCE

Figure 3 illustrates the results of direct electrochemistry of MWNTs or AgNP-MWNTs modified GCEs with immobilization of GOx in different substrate solutions. For GOx modified GCE there was no detectable amperometric signal in 0.1-M pH 6.45 PBS solution saturated with nitrogen (Fig. 3a(a)). However, a couple of redox peaks appeared for MWNTs or AgNP-MWNTs modified GCEs with absorption of GOx in nitrogen-saturated PBS solution (Fig. 3a(b), b(a)). Obviously, the presence of MWNTs or AgNP-MWNTs resulted in the direct electrochemistry of GOx. For the two types of GCEs with immobilized GOx, a pair of redox peaks also appeared in 0.1-M pH 6.45 PBS solution saturated with air or oxygen. After 1.0 mM glucose

Fig. 1 SEM images of **a** AgNPs-MWNTs and **b** GOx/AgNPs-MWNTs modified glassy carbon electrodes

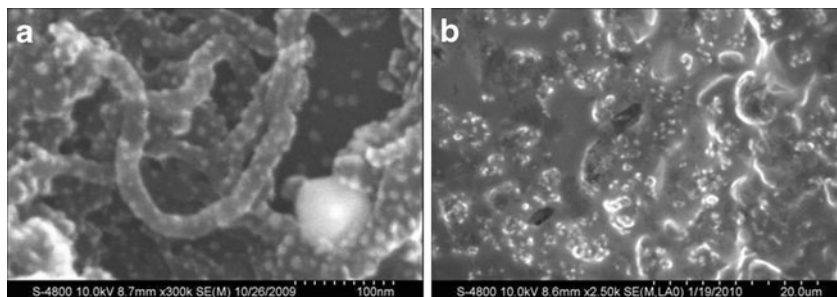
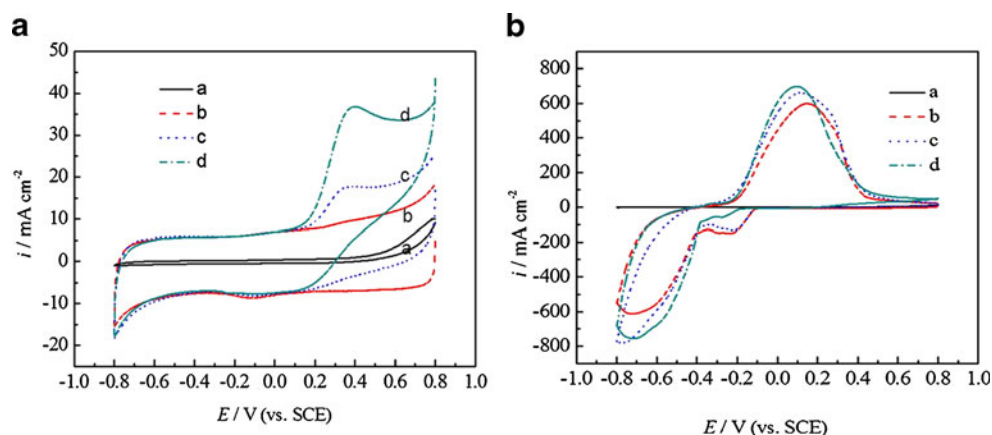


Fig. 2 CV of (a) bare GCE in air-saturated 0.1 M pH 6.45 PBS, and (b) MWNTs and (c) AgNPs-MWNTs modified GCEs in different gases-saturated 0.1 M pH 6.45 PBS. (b) Nitrogen-saturated, (c) air-saturated, (d) oxygen-saturated. Scan rate: 100 mV s^{-1}



was added into 0.1-M pH 6.45 PBS solution saturated with air, a couple of redox peaks were also found. The reduction peak current decreased (Fig. 3a(d), b(d)); thus, the glucose restrained the electrocatalytic reaction due to the enzymecatalyzed reaction between the oxidized form of GOx and glucose, which diminished the concentration of the GOx. With the increasing glucose concentration, the peak current for the electrocatalytic reduction decreased, producing a glucose biosensor [27]. The peak potential separation of 30 mV at GOx-AgNP-MWNTs modified GCE was smaller than that of 50 mV at GOx-MWNTs paste electrode, indicating the faster direct electron transfer between the redox-active site of GOx and GCE with the help of electron transfer mediator [28, 29]. It also confirmed that the AgNP-MWNTs modified electrode was advantageous for the direct electron transfer of GOx because of the excellent conductivity and large capacity for protein loading. In fact, the presence of GOx is responsible for glucose oxidation, and the Ag-Nps decorated on MWNTs are in charge of transfer of electrons resulting from glucose oxidation.

Effect of scan rates

Figure 4a shows the cyclic voltammogram curves of GOx-AgNP-MWNTs modified GCE at various scan rates. PBS

with pH 6.45 was chosen as a supporting electrolyte in order to get the maximum sensitivity and bioactivity of glucose sensor. It can be seen from Fig. 4a that the potential and peak current were dependent on the scan rates. The cathodic peak currents linearly increased with the increasing scan rate from 10 to 450 mV s^{-1} with a correlation coefficient of 0.999 (Fig. 4b), indicating that the redox process of the prepared bionanocomposites was a surface-controlled process. The MWNTs [30] especially the AgNPs decorated on the MWNT surfaces facilitate the electron transfer process between the GOx and the electrode substrate, which overcomes the difficulties of the direct electron communication with electrodes due to that the GOx is deeply embedded in a protective protein shell.

Analysis for sensitive performance of the glucose biosensor

Figure 5 displays the results of differential pulse voltammograms (DPVs) of GOx-AgNP-MWNTs modified GCE with different concentrations of glucose in air-saturated 0.1 M pH 6.45 PBS. The peak currents increased with increasing of glucose concentrations, and the increase of peak current (Δi) was linear with the concentration of glucose (inset of Fig. 5) up to 1.0 mM with a detection limit of 0.01 mM (S/N=3). Furthermore, the sensitivity of GOx-AgNP-MWNTs

Fig. 3 CV of biosensor modified by different materials in 0.1-M pH 6.45 PBS solution saturated with different gases. Scan rate: 100 mV s^{-1} . **a** (a) GOx-GCE- N_2 , (b) GOx-MWNTs-GCE- N_2 , (c) GOx-MWNTs-GCE-air, (d) GOx-MWNTs-GCE-air-glucose. **b** (a) GOx-AgNP-MWNTs-GCE- N_2 , (b) GOx-AgNP-MWNTs-GCE- O_2 , (c) GOx-AgNP-MWNTs-GCE-air, (d) GOx-AgNP-MWNTs-GCE-air-glucose

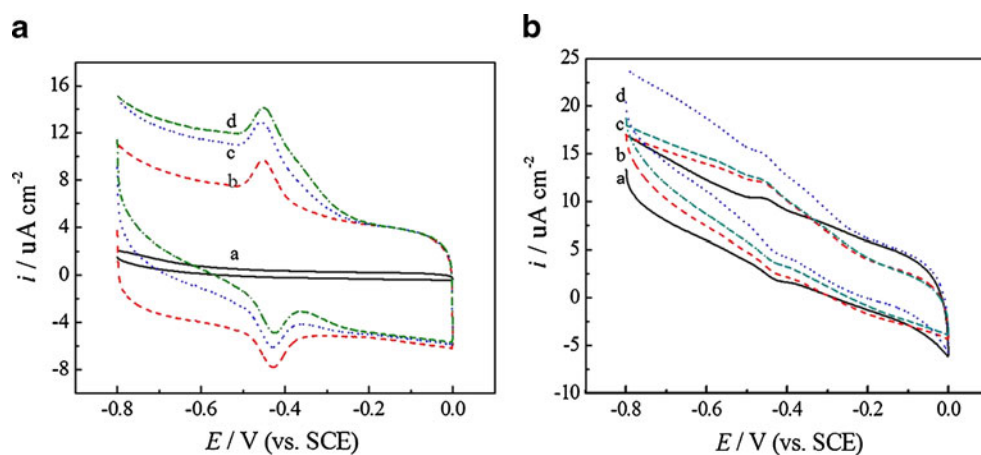
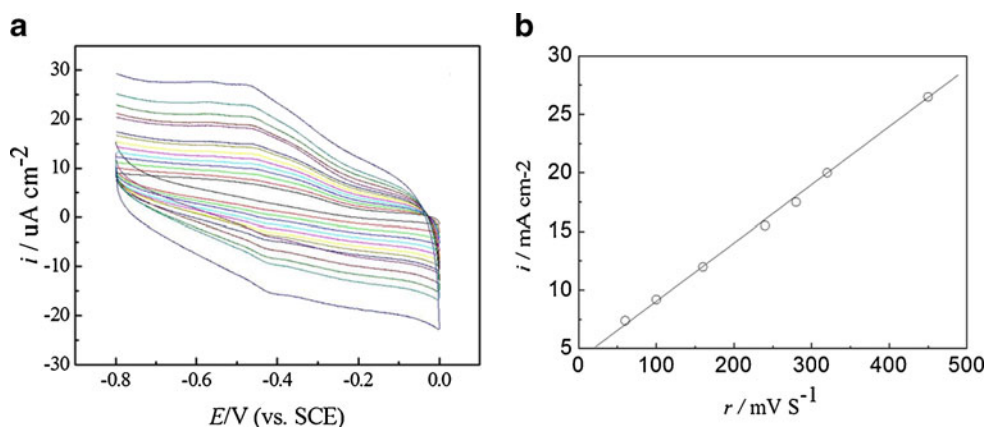


Fig. 4 **a** CV of GOx-AgNPs-MWNTs modified GCE in 0.1-M pH 6.45 PBS solution at different scan rates from 10 to 450 mV s⁻¹. **b** Plots of cathodic peak currents vs. scan rate



modified GCE was calculated to be 14.0 μA mM⁻¹ cm⁻², which was similar to 14.0 μA mM⁻¹ cm⁻² and was higher than 0.2, 1.06, and 0.053 μA mM⁻¹ cm⁻² for the glucose biosensors based on CN_x-MWNTs [28], MWCNTs [31], single-walled carbon nanohorns [32], and highly ordered mesoporous carbon foams [33]. Compared with the results reported in the literature [34–37], the glucose biosensor of this work showed a lower limit of detection and a wide linear range. In a word, the prepared glucose sensor had good analytical performances for glucose detection.

Electrochemical impedance characterization of GOx-AgNP-MWNTs modified electrode

The electrochemical impedance spectroscopy (EIS) was applied to monitor the whole procedure in the modification of the electrode, and the results are shown in Fig. 6. The redox couple K₃[Fe(CN)₆]/K₄[Fe(CN)₆] was chosen as the EIS probe. At a bare GCE the redox process of the probe showed an electron transfer resistance of about 11000 Ω (curve a), which was much larger than that of AgNP-MWNTs

modified GCE (curve b), indicating that AgNP-MWNTs could act as a good electron transfer interface between the EIS probe and the electrode. When GOx were modified on bare GCE, the resistance increased dramatically to about 26000 Ω (curve c), suggesting that the electron transfer between redox probe and electrode surface was hindered by the bulky GOx molecules. After GOx adsorbed on AgNP-MWNTs modified GCE, the semicircle diameter, being equal to the electron transfer resistance, in the EIS curve was lower than that of GOx modified GCE (curve d), indicating that the presence of AgNP-MWNTs acted as enhancing the electron transfer of redox probe. It was also shown that GOx molecules were immobilized on the surface of AgNP-MWNTs modified GCE, and the presence of AgNP-MWNTs could also accelerate the electron transfer between electroactive sites embedded in enzymes and electrode.

Stability and reproducibility of glucose biosensor

The storage stability of the glucose biosensor was studied in this work. The sensor was stored in 0.1 M pH

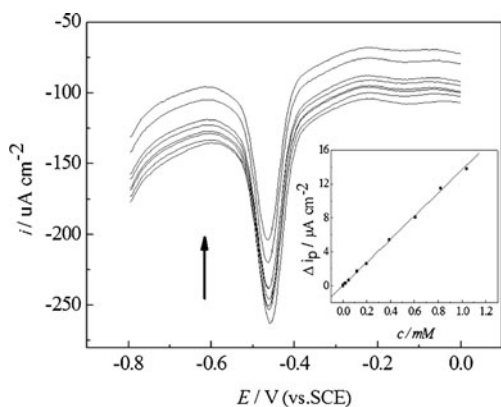


Fig. 5 Differential pulse voltammograms of GOx-AgNPs-MWNTs modified GCE in air-saturated 0.1 M pH 6.45 PBS containing 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mM glucose (from bottom to top). Inset: calibration curve at a pulse amplitude of 50 mV and a pulse width of 50 ms

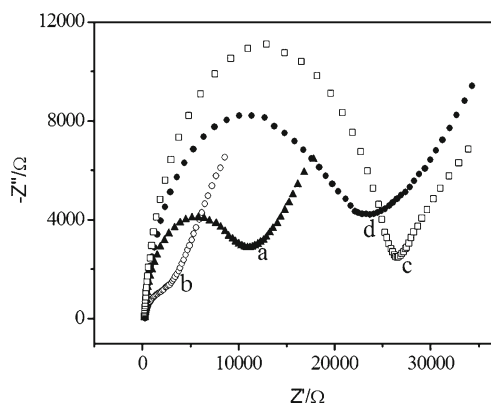


Fig. 6 Electrochemical impedance spectroscopy of (a) bare, (b) AgNPs-MWNTs, (c) GOx, and (d) GOx-AgNPs-MWNTs modified GCEs. The supporting electrolyte was 0.1-M KCl solution containing 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆]

6.45 PBS at 4 °C after the electrochemical measurement and being rinsed with doubly distilled water. The stability was examined by periodical measurements. No obvious decrease in the response to glucose was observed after 1 week of storage. It was found that the current response decreased only 7 % compared with the initial response value after being stored for 30 days. This indicated that the GOx-AgNP-MWNTs modified GCE could retain the bioactivity of the immobilized enzyme and the obtained biosensor had good storage stability.

The reproducibility of the glucose biosensor was also investigated. Five glucose sensors were fabricated independently under the same conditions and used to detect 0.1 mM glucose. The relative standard deviation (RSD) of reduction peak current from the measurement of 0.1 mM glucose at five independently prepared biosensors was only 2.7 %. In addition, the RSD for measurement of one glucose biosensor by ten times in 0.1 mM glucose was 4.3 %. All the results demonstrated good reproducibility of the biosensor preparation.

Conclusions

In summary, we have successfully fabricated a novel glucose biosensor by immobilizing GOx on AgNP-MWNTs modified GCE. The AgNPs decorating on the MWNT surfaces significantly improved biocompatibility for GOx immobilization, enhanced electrocatalytic activity toward the reduction of oxygen, and increased the effective area for protein loading. The direct electron transfer between the redox-active site of GOx and the GCE surface was also accelerated by the AgNPs with excellent conductivity and large capacity for protein loading, leading to the direct electrochemistry of GOx. The obtained glucose biosensor exhibited excellent analytical performance for glucose detection with a lower limit of detection of 0.01 mM ($S/N=3$) and a wide linear range from 0.025 to 1.0 mM. The resulting biosensor showed good storage stability and excellent reproducibility and has been used for detection of glucose in real sample with good accuracy. Such bionanocomposite as potential material provided us new chances for construction of the third-generation enzyme biosensors.

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