Int. J. Electrochem. Sci., 3 **(2008)** 1258 - 1267

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

High Sensitivity Glucose Biosensor Based on Pt Eelectrodeposition onto Low-density Aligned Carbon Nanotubes

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Received: 29 July 2008 / *Accepted:* 21 August 2008 / *Published:* 4 October 2008

Using carbon nanotubes (CNTs) as a substrate for initial Pt coating and subsequent glucose oxidase (GOD) immobilization to detect glucose was reported. The influence of both the orientation and the density of CNTs on the electronic properties and glucose detection was also investigated by cyclic voltammogram and chronoamperometry. The results show that GC electrodes with different CNTs modification exhibit properties in the order of low-density aligned CNTs>high-density aligned CNTs>random CNTs (LD-ACNTs>HD-ACNTs>RCNTs), in terms of reversibility and electron transfer rate. The glucose biosensor with Pt nanoparticles on the LD-ACNTs can further improve the sensitivity of the glucose detection to 3.8μA/mM. The GOD/Pt/LD-ACNTs/GC biosensor has excellent stability with almost 90% of its bioactivity maintained after storage at 4 ^{o}C in phosphate buffer solution (PBS) for ten days.

Keywords: glucose biosensor; aligned carbon nanotubes; Pt nanoparticles; sensitivity; stability

1. INTRODUCTION

Carbon nanotubes (CNTs) have attracted considerable attention since their discovery [1]. With many interesting properties, such as fast electron transfer rate, high electrocatalytic activity, their small size, good electrical conductivity, strong adsorptive ability and excellent biocompatibility, carbon nanotubes are an attractive material in bioelectrochemistry [2-5], especially because they are small enough to approach the redox centers of the proteins and work as molecular wires in molecular electronics due to its good conductivity [6-10]. Carbon nanotubes modified electrodes have shown a superior performance as compared to other material modified electrodes. However, most of these studies involved the electrode modified with randomly-dispersed carbon nanotubes (RCNTs) by drop coating method. The disorder of these tangled carbon nanotubes may impair their excellent properties

used in biosensors [11]. Aligned carbon nanotubes (ACNTs), which are multi-walled and vertically aligned on a substrate, can be readily synthesized so far [12]. And these ACNTs can keep their properties as much as individual carbon nanotube does. But in order to make every nanotube work as an individual nanoelectrode, the spacing needs to be sufficiently large to prevent the diffusion layer of every nanoelectrode overlapping with the neighboring ones [13]. So the density of ACNTs may influence their inherent properties, such as electron transfer rate and electrocatalytic activity. And it may also influence the immobilization of biomolecule and the diffusion of substrate, which will finally influence the detecting performance of the biosensor modified with the ACNTs.

In this paper, low-density aligned carbon nanotubes (LD-ACNTs) and high–density aligned carbon nanotubes (HD-ACNTs) were prepared and were used to form ACNTs/GC electrode. For comparison, the randomly-dispersed CNTs (RCNTs) were also taken for CNTs/GC electrode. The advantage of ACNTs/GC was confirmed by comparing with RCNTs/GC in conductivity. The advantages of LD-ACNTs/GC were demonstrated by comparing with HD-ACNTs/GC in reversibility and effective surface area. Then platinum (Pt) nanoparticles were electrochemically deposited on LD-ACNTs/GC electrode to form Pt/LD-ACNTs/GC electrode. Finally, glucose oxidase (GOD) was immobilized to the Pt/LD-ACNTs/GC electrode and LD-ACNTs/GC electrode (for comparison) to construct GOD/Pt/LD-ACNTs/GC and GOD/LD-ACNTs glucose biosensors for glucose detection. The detecting performance of the GOD/Pt/LD-ACNTs/GC and the GOD/LD-ACNTs/GC glucose biosensors to glucose was evaluated based on its detecting sensitivity, linear range and stability. The results show that the conductivity of ACNTs is superior to that of RCNTs. The property of LD-ACNTs is better than that of HD-ACNTs in the aspect of reversibility and electron transfer rate. The deposition of Pt on LD-ACNTs can improve the detecting performance of the biosensor to glucose with high detecting sensitivity of 3.8 μA/mM. The GOD/Pt/LD-ACNTs/GC biosensor has excellent stability with almost 90% of its bioactivity maintained after storage at 4 ^{o}C in phosphate buffer solution (PBS) for ten days.

2. EXPERIMENTAL PART

2.1. Chemicals and regents

Glucose oxide (GOD, 133,600 units/g, type X-S), β-D(+)-glucose (97%) were purchased from Sigma. Random arranged tangle multi-walled carbon nanotubes (MWCNTs, 10-20 nm outer diameter) were kindly provided by Chengdu Organic Chemicals Co. Ltd. All the other reagents used are analytic grade. All the reagent and material were used as received without further purification. All solutions were prepared with deionized water of a resistivity not less than 18 MΩ·cm (Milli-Q, USA).

2.2. Growth of ACNTs

The growth of ACNTs was done based on our previous work with some changes [14]. In a typical experiment for HD-ACNTs, a quartz substrate was placed in the middle of a ceramic tube (4

cm diameter) in a furnace. The furnace was first heated to 800 ^{o}C under 100 mL/min nitrogen gas and the inlet temperature of the ceramic tube was controlled at 150 ^{o}C. Then the mixture of 300 mL/min nitrogen and 50 mL/min hydrogen was flow through the reaction tube with the injection of the solution of ferrocene in xylene (0.04 mg/mL) at the injection rate of 5 mL/h. The carbon source of xylene was pyrolyzed catalytically with ferrocene as catalyst to grow ACNTs on the quartz substrate. The ACNTs prepared by this method is very compact and we denote them as HD-ACNTs.

In order to grow LD-ACNTs, the quartz substrate was firstly sputtered with Au particles that are inert to the growth of CNTs to reduce the density of active sites. The following steps are the same as the above growth of HD-ACNTs.

2.3. Preparation of CNTs/GC and ACNTs/GC electrodes

Before the modification, a glassy carbon (GC) electrode was polished by alumina powders of 0.5 μm on a carborundum paper and then was sonicated in NaOH, HCl, ethanol, and deionized water in turn for 10 minutes. For preparation of the RCNTs/GC electrode, 0.2 mg RCNTs were pressed to form a disk with diameter of 2 mm and thickness of 1mm and was attached onto the polished GC electrode with conductive adhesive. In the case of the ACNTs/GC electrode, the prepared ACNTs with diameter of 2 mm and thickness of 1 mm were attached onto the polished GC electrode with conductive adhesive. In addition, the weights of ACNTs for the HD-ACNTs/GC electrode and the LD-ACNTs/GC electrode were 0.09 mg and 0.05 mg, respectively.

2.4. Preparation of the Pt/LD-ACNTs/GC electrode

The deposition of Pt nanoparticles on the LD-ACNTs/GC electrode was carried out electrochemically. First the LD-ACNTs/GC electrode was treated by potential cycling from +1.8 to -0.4 V at scan rate of 200 mV/s in 0.5 M Na_2SO_4 solutions for 10 min to produce oxide functional groups at the defect sites located on the LD-ACNTs. Then it was treated by cyclic voltammogram under the conditions of a potential range from +0.26 to 1.4 V at a sweep rate of 5 mV/s in aqueous solutions of 2.5 mM K_2PtCl_4 and 0.1 M K_2SO_4 and then cycled from +1.6 to -0.26 V in 0.1 M H_2SO_4 solution according to the reference [15].

2.5. Construction of the GOD/Pt/LD-ACNTs/GC and the GOD/LD-ACNTs/GC biosensors

The Pt/LD-ACNTs/GC and the LD-ACNTs/GC electrodes were dipped in 1 mg/mL GOD solution overnight at 4 ^{o}C for the immobilization of GOD to construct the GOD/Pt/ACNTs/GC and the GOD/ACNTs/GC biosensors, respectively. The biosensors were stored in 0.1 M PBS (pH 7) at 4 ^{o}C in refrigerator when they were not used.

2.6. Instrumentation

The cyclic voltammogram and chronoamperometric experiments were carried out with a computer-controlled Autolab PGSTAT30 electrochemical workstation (Eco Chemie B. V., Utrecht, The Netherlands) with Gpes worksation software. A conventional three electrode electrochemical cell

was used in this work with a bare or modified glassy carbon (GC) electrode as working electrode (2 mm diameter), a platinum electrode as counter electrode and an Ag/AgCl electrode with saturated KCl as reference electrode, respectively. During the chronoamperometry experiment, a stirrer (DF-101B, Shanghai magnetic apparatus company) was used to keep the solution uniform. The morphology of ACNTs and random arranged tangle CNTs was characterized by SEM (1530VP, LEO Co.)

3. RESULTS AND DISCUSSION

Fig.1 shows the SEM pictures of HD-ACNTs and RCNTs. It can be seen that the ACNTs is very straight and regular and the orientation of ACNTs is very good. On the contrary, the RCNTs are disordered and tangled together severely.

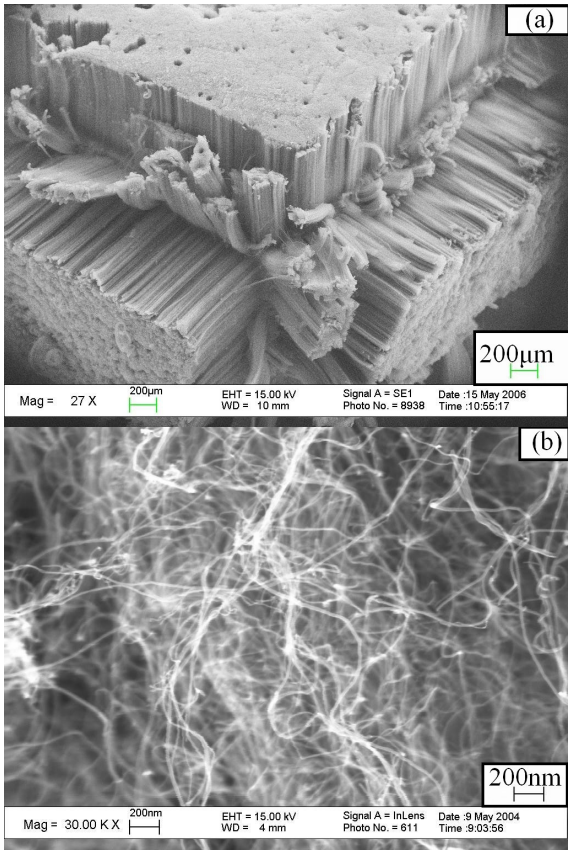

Figure 1. SEM pictures of ACNTs (a) and RCNTs (b)

In order to investigate the influence of ACNTs and random arrange tangle CNTs on conductivity, the cyclic voltammograms of electrodes modified with HD-ACNTs and random arranged tangle CNTs in PBS were shown in Fig.2. From the figure, it can be seen that the responding current of HD-ACNTs/GC is as large as that of random arranged tangle CNTs/GC, although the weight of

random arranged tangle CNTs (0.2 mg) is much larger than that of HD-ACNTs (0.09 mg). It indicates that the conductivity of ACNTs is superior to that of random arranged tangle CNTs, which is very important for CNTs to be used in amperometric biosensors. Additionally, there is a pair of redox peaks in the cyclic voltammogram of HD-ACNTs/GC. It is probably the redox signals of $Fe^{3+/2+}$ from residual ferrocene in HD-ACNTs when ferrocene is used as catalyst for the growth of HD-ACNTs.

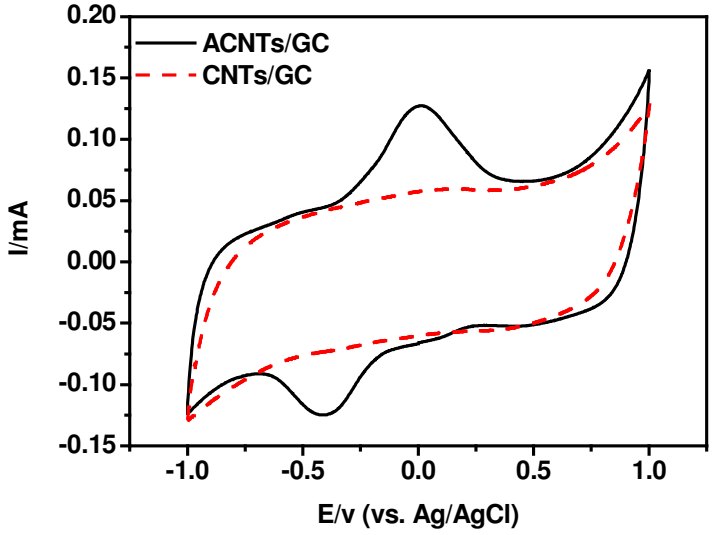

Figure 2. Cyclic voltammograms of GC electrode modified by HD-ACNTs and RCNTs in PBS

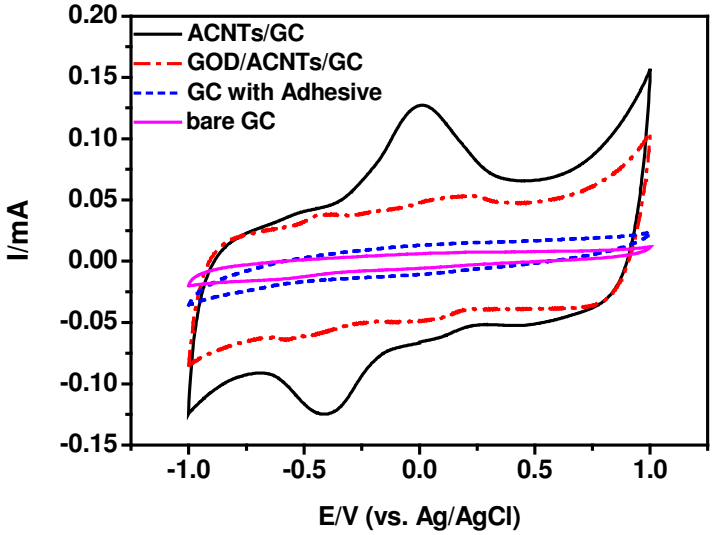

Figure 3. Cyclic voltammograms of GC electrode with different modification in PBS

In order to study the effect of the different components modified onto GC electrode on the conductivity, the cyclic voltammograms of GC electrode with adhesive, HD-ACNTs and GOD in PBS was shown in Fig. 3. From the figure, it can be seen that the responding current is in the order of HD-ACNTs/GC>GOD/HD-ACNTs/GC>GC with adhesive≈bare GC. It suggests that the adhesive has little influence on the conductivity of the GC electrode. After attaching ACNTs onto GC electrode, the conductivity is improved greatly, which indicates that ACNTs can efficiently enhance the conductivity of the electrode. After immobilized with GOD, there is a little decrease in the responding current, which is due to that GOD is nonconductive.

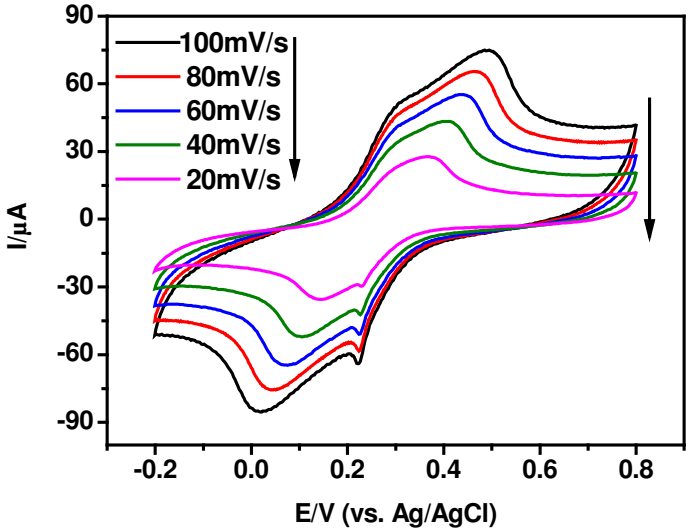

Figure 4. Cyclic voltammograms of the HD-ACNTs/GC in 5 mM $Fe(CN)_6^{4-/3-}$ with different scanning rates

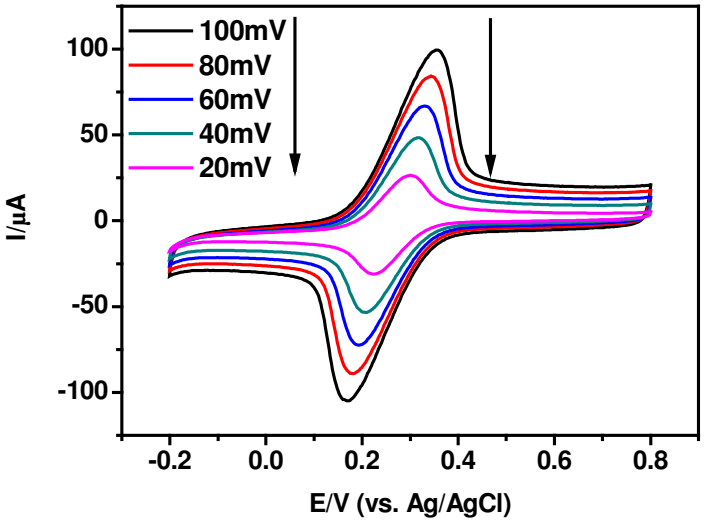

Figure 5. Cyclic voltammograms of the LD-ACNTs/GC in 5 mM $Fe(CN)_6^{4-/3-}$ with different scanning rates

Fig.4 and Fig.5 are the cyclic voltammagrams of the electrodes modified with high and low density ACNTs at different scan rates in 5 mM $Fe(CN)_6^{4-/3-}$. The peak potential difference of the LD-ACNTs/GC electrode is about 78 mV, which is much smaller than that of the HD-ACNTs/GC electrode (220mV). The peak potential difference between the anodic peak and cathodic peak can denote the reversibility of an electrode. The smaller the peak potential difference is, the better the reversibility of the electrode is. From the peak potential difference, it can be concluded that LD-ACNTs/GC electrode has better reversibility than the HD-ACNTs/GC electrode. So, the electron transfer rate on LD-ACNTs/GC is larger than that on HD-ACNTs/GC. According to the equation of $i_p = 269 n^{3/2} A D^{1/2} \nu^{1/2} C_0$ for reversible electrode, where i_p is the current of peak, n is the electron number involved in the electrode reaction, A is the effective surface area, D is diffusion coefficient, ν is the scan rate, Co is the substrate concentration. As to $K_3[Fe(CN)_6]$, n=1, $D=7.6\times10^{-6}$ cm^2/s [16], the effective surface area of LD-ACNTs/GC electrode is 0.11 cm^2, which is about 3.5 times compared to the geometric area of bare GC electrode (0.0314 cm^2). The larger effective surface area is favorable to the immobilization of GOD to construct glucose biosensor.

Incorporated with the above analysis, the electronic property of the electrode modified with the LD-ACNTs is superior to that of the electrode modified with HD-ACNTs and random arranged tangle CNTs. So, LD-ACNTs were used to prepare glucose biosensor in the following part of this work.

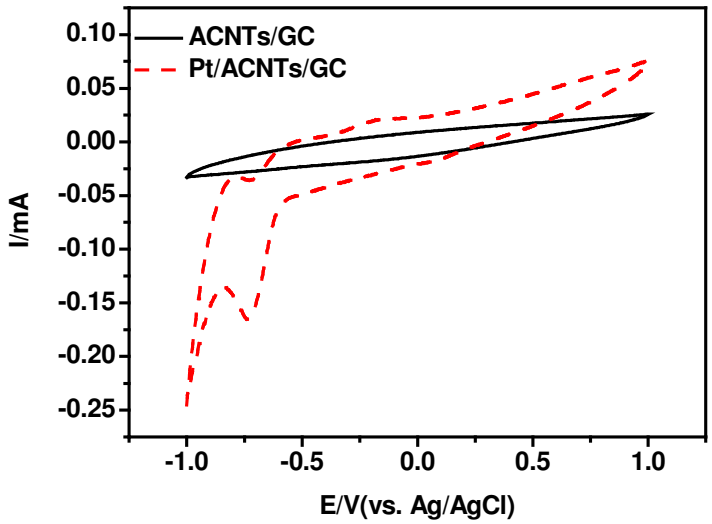

Figure 6. Cyclic voltammograms of the LD-ACNTs/GC and the Pt/LD-ACNTs/GC in PBS with the scanning rate of 0.1 V/s

Fig.6 is the cyclic voltammagrams of the electrode modified with the LD-ACNTs and LD-ACNTs with Pt deposition in PBS solution. For the electrode modified with LD-ACNTs with Pt deposition, there is a pair of redox peaks around -0.7 V. This redox peaks can be assigned to the hydrogen adsorption and desorption peaks from Pt nanoparticles, which confirms that Pt nanoparticles were successfully deposited on LD-ACNTs/GC. Therefore, we denote them as Pt/LD-ACNTs/GC.

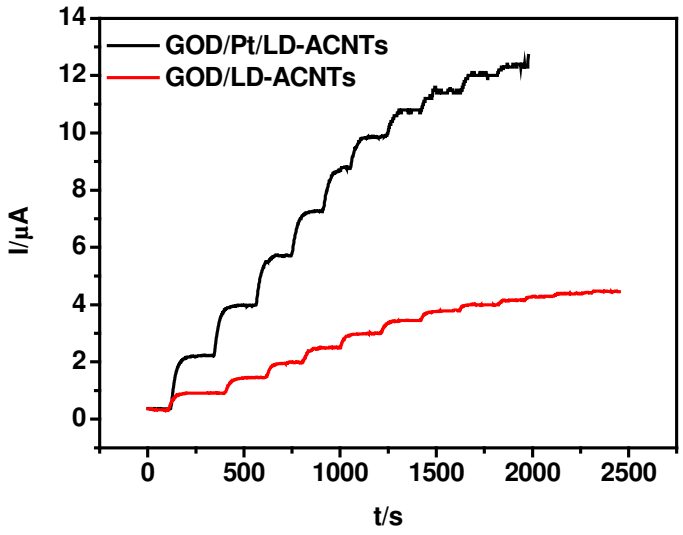

Figure 7. The chronoamperometric curves of the GOD/Pt/LD-ACNTs/GC and the GOD/LD-ACNTs/GC biosensors to glucose at working potential of 0.5 V for the former and 0.7 V for the latter

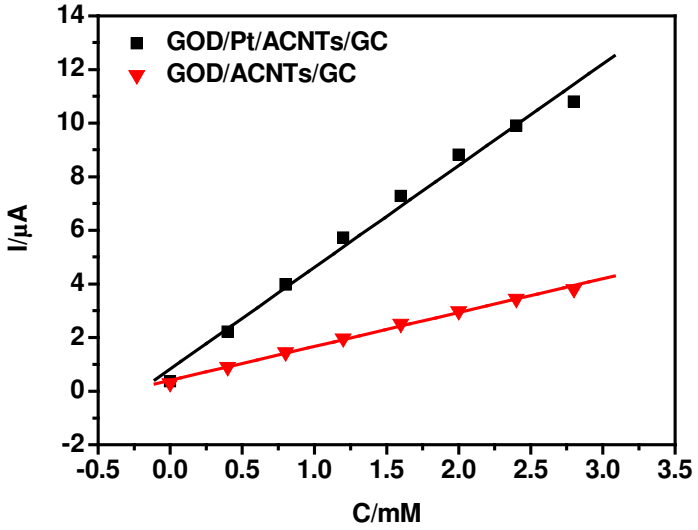

Figure 8. Calibration curves of the GOD/Pt/LD-ACNTs/GC and the GOD/LD-ACNTs/GC biosensors to glucose from figure 7

The detecting performance of the glucose biosensors modified with LD-ACNTs and Pt/LD-ACNTs were shown in Fig.7 and the calibration curves were shown in Fig.8. The linear ranges of the both biosensors modified with LD-ACNTs and Pt/LD-ACNTs are almost the same and up to almost 3 mM for glucose. But the sensitivity of GOD/Pt/LD-ACNT/GC to glucose is 3.8 μA/mM (Fig.8), which is much higher than that of the GOD/LD-ACNT/GC without Pt nanoparticles (1.3 μA/mM). It indicates that the deposition of Pt nanoparticles on LD-ACNTs can improve the detecting sensitivity

greatly, which is in agreement with other researchers' reports [17-20]. The stability test results show that the biosensor of GOD/Pt/LD-ACNTs/GC biosensor can keep almost 90% of its initial bioactivity after storage at 4 ^{o}C in PBS for ten days. The high sensitivity and excellent stability of the GOD/Pt/LD-ACNTs biosensor to glucose can be attributed to the excellent electrocatalytic activity of Pt nanoparticles and the biocompatible microenvironment around the GOD enzyme. On the other hand, the large spacing in the LD-ACNTs can make every nanotube work as an individual nanoelectrode [13], which is also contributive to the high sensitivity of the GOD/Pt/LD-ACNTs/GC biosensor to glucose. So, the detection performance of the Pt/LD-ACNT/GC glucose biosensor can be greatly improved by modifying with Pt deposited onto LD-ACNTs.

4. CONCLUSIONS

The conductivity of ACNTs is superior to that of the random arranged tangle CNTs. The property of LD-ACNTs is better than that of HD-ACNTs in the aspect of reversibility and electron transfer rate. The deposition of Pt on LD-ACNTs can improve the detecting performance of the biosensor to glucose with high detecting sensitivity of 3.8 μA/mM. The GOD/Pt/LD-ACNTs/GC biosensor has excellent stability with almost 90% of its bioactivity maintained after storage at 4 ^{o}C in PBS for ten days. This study has developed a promising and practical pathway for the construction of glucose biosensor with excellent glucose detecting performance.

ACKNOWLEDGEMENTS
Financial support from the Guangdong Provincial Science and Technology Project (No.2007A020100001-10) and the Guangzhou City Science and Technology Project (No. 2007Z3 - D2101) are greatly acknowledged.

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