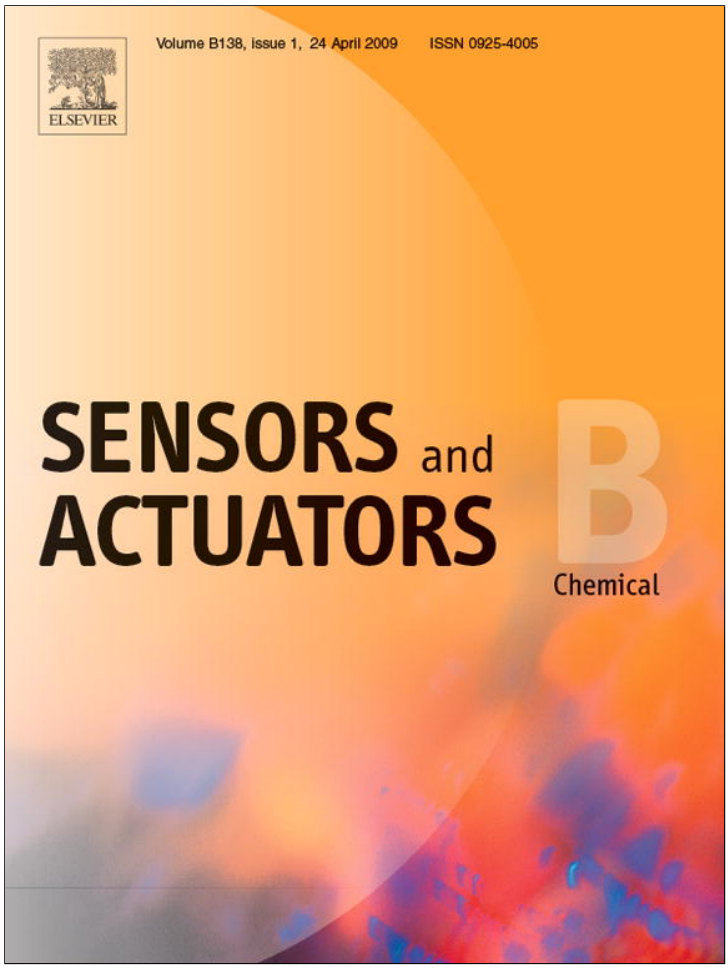

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An amperometric glucose biosensor based on the immobilization of glucose oxidase on the ZnO nanotubes

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ABSTRACT

A glucose biosensor is fabricated with immobilization of glucose oxidase onto ZnO nanotube arrays by cross-linking method. The ZnO nanotube arrays are synthesized by chemical etching of ZnO nanorods that are electrochemically deposited on the Au surface. Morphology and structure of ZnO nanotubes are characterized by FESEM, HRTEM and XRD. Fourier-transform infrared spectroscopy reveals that the glucose oxidase immobilized on the ZnO nanotubes retains its native conformation. The biosensor has a wide linear range for the detection of glucose from 50 μM to 12 mM (a correlation coefficient of 0.998) with 3 s response time. The sensitivity of the biosensor is found to be 21.7 μA/mM cm^2. Moreover, its experimental detection limit is 1 μM (S/N = 3) and the apparent Michaelis–Menten constant is calculated to be 19 mM. The anti-interference ability and long-term stability of the biosensor are also assessed. Compared with the biosensors based on the nanorod and flat structure, the proposed biosensor shows expanded linear range and sensitivity. All these results demonstrate that ZnO nanotube can provide a promising material for the biosensor designs and other biological applications.

1. Introduction

The fast and accurate determination of glucose has profound applications since glucose concentration is a crucial indicator in many diseases, such as diabetes, endocrine metabolic disorder. In recent years, many efforts have been made to develop reliable glucose biosensors using electrochemical [1], chemiluminescence [2] or other methods [3]. Among all the methods, enzyme-involved electrochemical glucose biosensor has been intensively studied because of its simplicity, high selectivity and relative low cost [4–6]. In this technique, the enzyme immobilization is considered to be one of the most important issues. Since the performance of a biosensor much relies on the supporting materials, searching of materials that provide good environment for the efficient enzyme loading and maintenance of enzyme bioactivity is highly desired.

Currently, the glucose oxidase (GOx) is widely employed in most of the glucose biosensors due to its stability and high selectivity to glucose, especially the amperometric glucose biosensors [7–9]. It contains two flavine adenine dinucleotide (FAD) cofactors and catalyzes the oxidation of glucose according to the following reaction [10]:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconolactone} + H_2O_2$$

Since the amount of glucose is proportional to that of the produced H_2O_2, the glucose concentration can be readily determined through measuring the current derived from the electrochemical reaction of H_2O_2 [11]. Many methods such as covalent binding [12], embedding method [13] and cross-linking method [14–16] have been used to immobilize the GOx onto different supporting materials. Moreover, the property of GOx with negative charge in neutral pH solutions also makes it feasible to immobilize GOx onto materials with positive charge by the physical adsorption [17,18].

Recently, zinc oxide (ZnO) nanostructures have drawn many attentions in the application of biosensors with many advantages, including nontoxicity, biological compatibility, fast electron transfer rates and easy preparation [19–26]. The isoelectric point (IEP) of ZnO is around 9.5, making it possible to immobilize low IEP DNA or proteins by electrostatic adsorption in proper buffer solutions [27]. It has been found that the microperoxidase immobilized onto the ZnO nanoparticles can enhance its catalytic ability and promote direct electron transfer [19]. In addition, a uric acid biosensor based on ZnO nanorods [20] and a cholesterol biosensor by immobilization of cholesterol oxidase onto ZnO nanoporous thin film [21] have also been reported.

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Generally, the nanotube structure possesses lots of interesting properties. Due to its unique structure, nanotube is expected to efficiently enhance the performance of biosensors [28,29]. Recently, various nanotubes, such as titanate nanotubes [30], and platinum nanotubes [31] as well as polypyrrole nanotubes [28], have been used in the biosensor designs and fabrications. In this work, a glucose biosensor based on the ZnO nanotubes is fabricated with immobilization of glucose oxidase onto the nanotubes. Due to the good biocompatibility and intrinsic porous structure of ZnO nanotubes, the fabricated glucose biosensor shows very sensitive response and can detect as low as 1 μM glucose without any electron mediators. Compared with the biosensors based on the ZnO nanorods and flat gold film, the biosensor using ZnO nanotubes shows an efficiently expanded linear range and sensitivity. The results demonstrate that the ZnO nanotubes can offer a new and promising immobilization material for the biosensor designs.

2. Experimental

2.1. Materials

Glucose oxidase (GOx, EC 1.1.3.4 from *Aspergillus niger*, 100 U/mg), bovine serum albumin (BSA >98%) and glutaraldehyde (50% solution) were purchased from Shanghai Sangon Co., Ltd. Zinc nitrate hexahydrate ($ZnNO_3{\cdot}6H_2O$, 99%) and hexamethylenetetramine (HMT, 99%) were purchased from Sinopharm Chemical Reagent Co., Ltd. D-(+)-glucose (99.5%) and Nafion (5 wt%) were purchased from Sigma–Aldrich. 0.1 M phosphate buffer (PB) solution was prepared from K_2HPO_4 and KH_2PO_4 (Sigma–Aldrich), the pH was adjusted to 7.0 by H_3PO_4. Glucose stock solution was kept at least 24 h after preparation for mutarotation. The deionized (DI) water ($R \geq 18.2\,M\Omega\,cm$) used in all experiments was produced by the Millipore system.

2.2. Synthesis of ZnO nanotubes

In this work, ZnO nanotubes (ZnONT) were synthesized on an Au electrode which was composed of 300 nm RF sputtered Au film on a silicon wafer (2 mm × 2 mm) with 300 nm SiO_2 insulating layer. The synthesis process contained two steps [32]. Firstly, ZnO nanorods (ZnONR) were deposited with a simple electrochemical cell controlled by a CHI 660A electrochemical workstation (Shanghai Chenhua Instruments Co.). A conventional three-electrode configuration was used in this process, in which the Au electrode was working electrode while a platinum wire and saturated calomel electrode (SCE) as the counter and the reference electrode, respectively. The electrolyte was 10 ml aqueous solution containing 20 mM $Zn(NO_3)_2$ and equimolar HMT. The applied voltage was −0.8 V and the solution temperature was kept at 85 °C. After 7000 s deposition, ZnONR arrays were obtained on the Au surface. In order to attain ZnONT, the Au electrode with as-formed ZnONR was dipped into 0.125 M NaOH solution and maintained for 1.5 h at 85 °C. After the reaction, the Au electrode was rinsed by DI water for three times to remove unnecessary chemicals and then ready for the construction of glucose biosensor.

2.3. Glucose biosensor fabrication

Before the immobilization of GOx, ZnONT modified electrode was rinsed with PB solution to generate a hydrophilic surface. An enzyme solution was prepared by dissolving 10 mg GOx and 20 mg BSA in 200 μl PB solution. 1 μl of the above mixture was applied onto the surface of as-prepared electrode. The electrode was then left in air for 2 h to dry, which also allowed GOx and BSA to adsorb onto the ZnONT. The cross-linking procedure was carried out by adding 1 μl aqueous solution containing 2.5% glutaraldehyde and 0.5% Nafion onto the electrode. After dried at room temperature, 1.5 μl of 0.5% Nafion solution was further dropped onto the electrode surface to prevent possible enzyme leakage and eliminate foreign interferences. (It has been reported that Nafion, as a covering membrane, can provide biocompatible environment for enzyme and also enhance the anti-interference ability of the biosensor [33,34].) Finally, the electrode was immersed in DI water to remove unimmobilized enzymes. All prepared enzyme electrodes were stored in dry condition at 4 °C when not in use. For the comparison of the significance of the ZnONT structure, two electrodes based on the ZnONR and Au film as the supporting material were made with the identical procedure, respectively.

2.4. Biosensor characterization and electrochemical measurements

The morphology and structure of ZnONT were characterized by field emission scanning electron microscopy (FESEM, Raith e-Line, Germany), high-resolution transmission electron microscopy (HRTEM, JEOL-2010, Japan), and X-ray diffraction (XRD, D/Max-rA with Cu Kα of 1.54056 Å, Japan). Fourier-transform infrared (FT-IR) spectroscopy (Nicolet 8700, USA) was employed to evaluate the influence of ZnO on the bioactivity of glucose oxidase. In FT-IR measurement, the electrode was prepared by just adding GOx solution onto the ZnONT without BSA treatment, because BSA had the similar amide band with the GOx and complicated the result [35]. The cyclic voltammograms (CV) were acquired from −0.2 V to +0.8 V at a scan rate of 0.1 V/s in 10 ml of 0.1 M pH 7.0 PB solution. The amperometric response of biosensor to glucose was recorded in stirring (400 rpm) PB solution at +0.8 V versus SCE. All electrochemical experiments were performed at room temperature (typically, 25 °C).

3. Results and discussion

3.1. Characterization of the fabricated biosensor

Fig. 1A and B shows the SEM images of as-grown ZnONT and ZnONR arrays, respectively. It can be seen that both of the ZnONT and ZnONR are well-aligned in hexagonal structure, with an average diameter of around 250 nm. The length of ZnONT is about 1 μm and the wall thickness is estimated to be several tens of nanometers (45 ± 8 nm, 60 counts). HRTEM and corresponding selected area electron diffraction (SAED) of a single ZnONT in Fig. 1C and D reveals that the ZnONT grow uniformly along ⟨0 0 0 1⟩ direction with no obvious defects. Energy dispersive spectrometry (EDS) in Fig. 1E, only demonstrating the elements of Zn and O (Cu signal in the spectrum is derived from copper grid used in TEM experiments), further confirms the high purity of ZnONT. Moreover, the atom ratio of Zn to O is quantitatively calculated to be 1.6:1, indicating that there still exists a certain amount of oxygen vacancies in the nanotubes. With regard to the mechanism of the formation of ZnONT, it is reported that a defect-selective etching process plays an important role [32]. Briefly, upon etching by the NaOH, the etching starts preferentially from the defect-rich polar (0 0 0 1) plane of the ZnONR. Additionally, the etching rate of the metastable (0 0 0 1) plane is much faster than that of nonpolar planes. Both above factors lead to the formation of tubular ZnO nanostructure.

XRD pattern of the as-synthesized ZnONT on Au surface is shown in Fig. 2. All diffraction peaks, except those of Au(1 1 1), Au(2 2 2) and Si(1 1 1) originated respectively from the Au film and substrate, can be well indexed as wurtzite ZnO (space group: *P*63*mc*, JCPDS Card No. 36-1451), indicating that the ZnONT are well crystallized. According to the XRD data, the ZnONT lattice constants can be calculated to be 3.247 Å and 5.204 Å, well matching those of the bulk ZnO

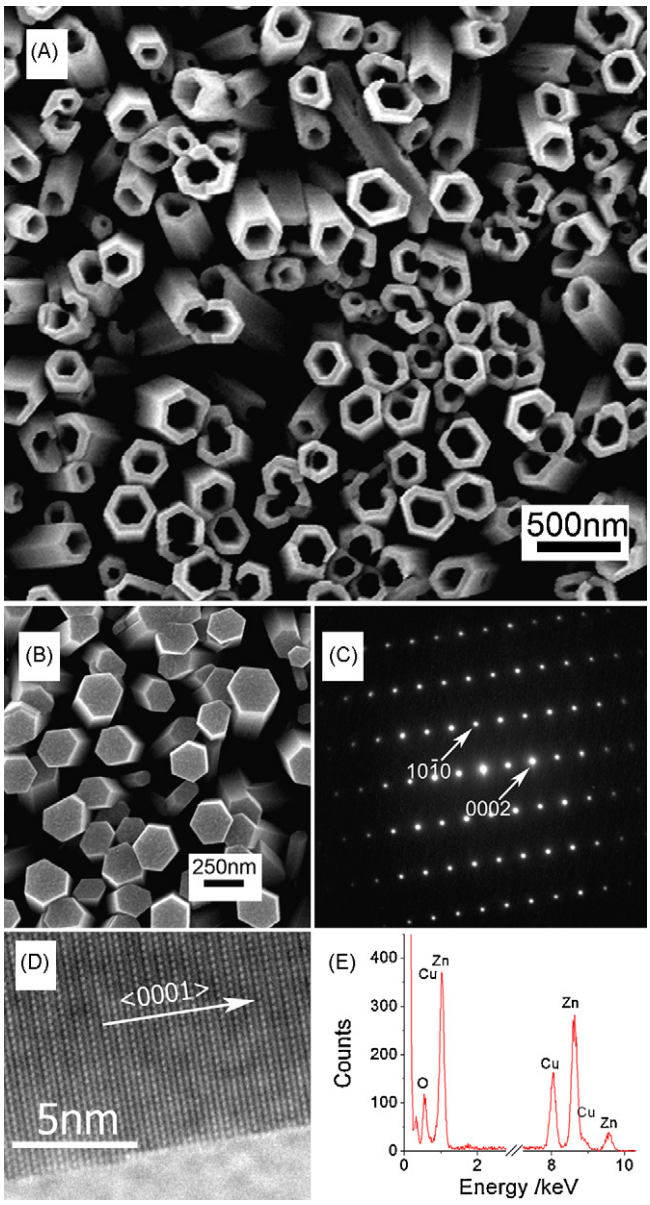

Fig. 1. FESEM images of ZnONT (A) and ZnONR (B) arrays. HRTEM (C), corresponding SAED pattern (D) and EDS spectrum (E) of a ZnO nanotube.

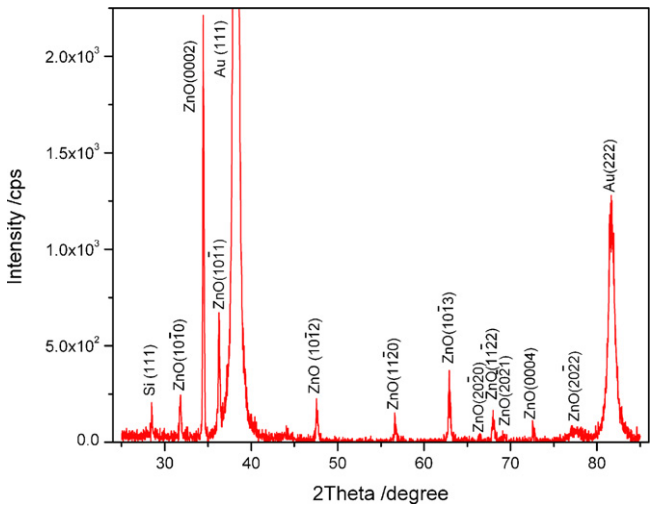

Fig. 2. XRD pattern of as-synthesized ZnO nanotubes.

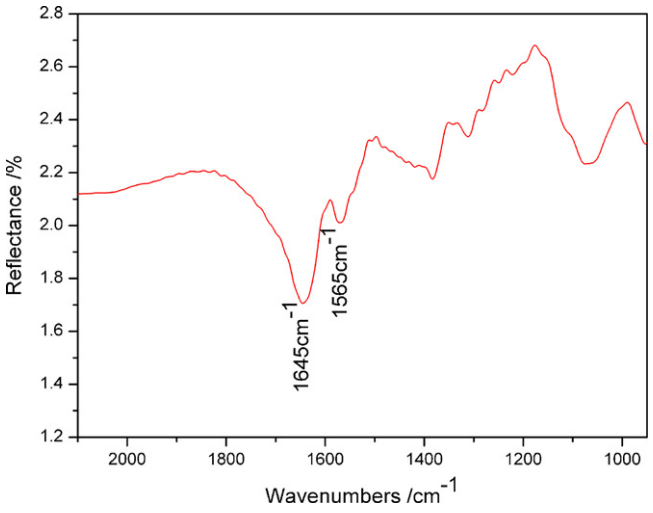

Fig. 3. FT-IR spectra of GOx immobilized on the ZnONT.

materials (a = 3.250 Å, c = 5.207 Å). Furthermore, the strongest peak ZnO (0 0 0 2) suggests that ZnONT has the preferential orientation along c-axis, which is consistent with the SEM results.

The maintenance of enzyme activity on the supporting materials is crucial in the biosensor designs, because the secondary conformational variations of the enzyme can affect its activity markedly. Herein, FT-IR is utilized to check the secondary conformation variations of the polypeptide chain of GOx on the ZnONT. As shown in Fig. 3, two infrared bands of GOx with the center positions at 1645 cm^{-1} and 1565 cm^{-1} can be observed. The former band is related to C=O stretching vibrations of the peptide linkages in the backbone (Amide I) and the latter one is ascribed to the combination of C–N stretching and N–H in-plane bending (Amide II) [36–38]. This result reveals that the secondary structure and bioactivity of GOx molecules can be reserved when immobilized on the ZnONT.

The electrochemical characterization of the biosensor is investigated by cyclic voltammetry between −0.2 V and 0.8 V versus SCE in pH 7.0 PB solution at scan rate of 0.1 V/s. Fig. 4 shows the cyclic voltammogram of the biosensor without glucose (curve A) and upon addition of 2 mM glucose (curve B). It can be found that the reduction current is suppressed while the oxidation current increases significantly, which relates to the oxidation of glucose by GOx catalysis. Moreover, a broad shoulder peak shows up appar-

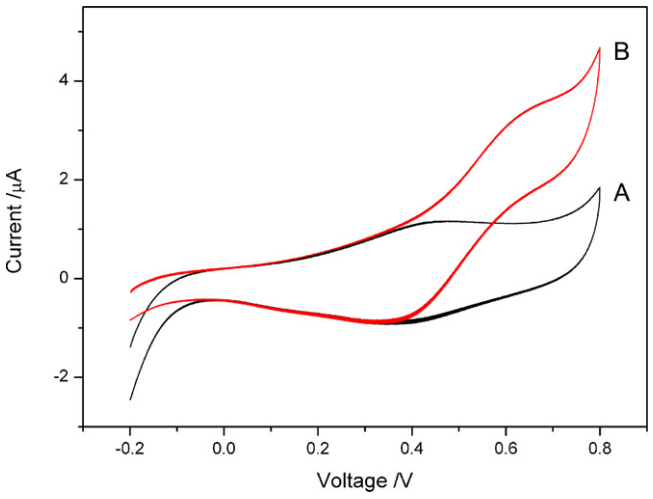

Fig. 4. Cyclic voltammograms of the biosensor without (A) and with 2 mM glucose (B) in 0.1 M pH 7.0 PB solution at scan rate of 0.1 V/s.

ently at around 0.6 V which can be ascribed to H_2O_2 generated during the oxidation of glucose [23]. These results indicate that the GOx can be well affined to ZnONT and the fabricated biosensor has good detection ability to glucose.

3.2. Response behavior of the glucose biosensor

Various experimental parameters, such as pH value, applied voltage and temperature, may affect the performance of a biosensor. In this work, these parameters were first studied to ascertain the optimal working conditions. The relationship between the response current of fabricated biosensor and the applied voltage is shown in Fig. 5A. It can be found that the current increases very slowly when the voltage is below 0.5 V. However, if the voltage excesses 0.5 V, the current first rises drastically and then shows the saturation behavior when voltage reaches at +0.8 V. Based on the above observations, the applied voltage was set to be +0.8 V in the following experiments.

The pH effect on the biosensor performance was investigated by measuring the current response to 1 mM glucose at +0.8 V. Because the ZnO is a kind of amphoteric compound and not stable in both strong acid and base solutions, the pH value ranging from 4.0 to 8.0 was adopted in this study. As seen in Fig. 5B, the biosensor shows an optimal response at pH 7.0. In this case, the buffer solution was adjusted to pH 7.0 and used in all experiments below.

The effect of varying temperature on the biosensor response was also examined between 20 °C and 65 °C. As illustrated in Fig. 5C, the current response gradually increases with the increasing of temperature and reaches to its maximum value at 50 °C. This is because the enzyme activity increases at higher temperature. After 50 °C, the response decreases which is caused by the natural thermal degradation of the enzymes. Although the biosensor shows a maximum response at 50 °C, room temperature (25 °C) is still chosen for this work in order to prevent possible solution evaporation at higher temperature and ease of operations.

Under the optimized conditions, the steady-state amperometric response of the glucose biosensor is investigated by successively adding various concentrations of glucose into PB solution. As shown in Fig. 6A and its inset, the biosensor has a fast and sensitive response to glucose, and it reaches 95% of the maximum steady-state current in 3 s. Fig. 6B illustrates the calibration plot of response current to different glucose concentrations. The linear response range is obtained from 50 μM to 12 mM and the linear regression equation is I (μA) = 0.298 + 0.866C_g (mM) (R = 0.998), where I is current and C_g is the glucose concentration. The sensitivity is calculated to be 21.7 μA/mM cm^2 as well. Additionally, the experimental limit of detection (LOD) of the biosensor is measured to be 1 μM based on a signal–noise ratio of 3 (inset of Fig. 6B). With respect to the benefit of the ZnO nanotube structure in the construction of biosensors, comparison was performed by measuring the amperometric response of biosensors using the ZnONR and Au film as immobilization materials. From Fig. 6B, the ZnONR modified biosensor shows a sensitivity of 7 μA/mM cm^2 and the linear range is up to 4.3 mM, while the Au film modified biosensor only shows a sensitivity of 5.5 μA/mM cm^2 and its linear range is up to 2 mM. Since the nanotube structure has higher surface area than nanorod and flat surface, the introduction of ZnO nanotubes in the biosensor design may increase the electrode surface area for the enhancement of GOx loading. Besides, the unique 3D structure may not only prevent the GOx from stacking together which usually happens in the flat surface case, but also allow the generated H_2O_2 diffuse to the electrode rapidly and get oxidized efficiently. Consequently, we conclude that the performance of biosensor is enhanced dramatically when ZnONT is employed.

The enhanced linear range and sensitivity expands the applications of the biosensor, especially in the glucose determination in serum samples. Besides, our biosensor exhibits a much wider linear range than reported multi-wall carbon nanotubes modified polymer electrode (0.02–2.2 mM) [39] and nano-SiO_2/Pt nanoclusters modified platinum electrode (0.27–4.08 mM) [40]. The LOD is significantly lower than previous reports using mesocellular silica–carbon nanocomposite foam (34 μM) [41] and Au/Pt heterostructure (39.8 μM) [42] as immobilization materials.

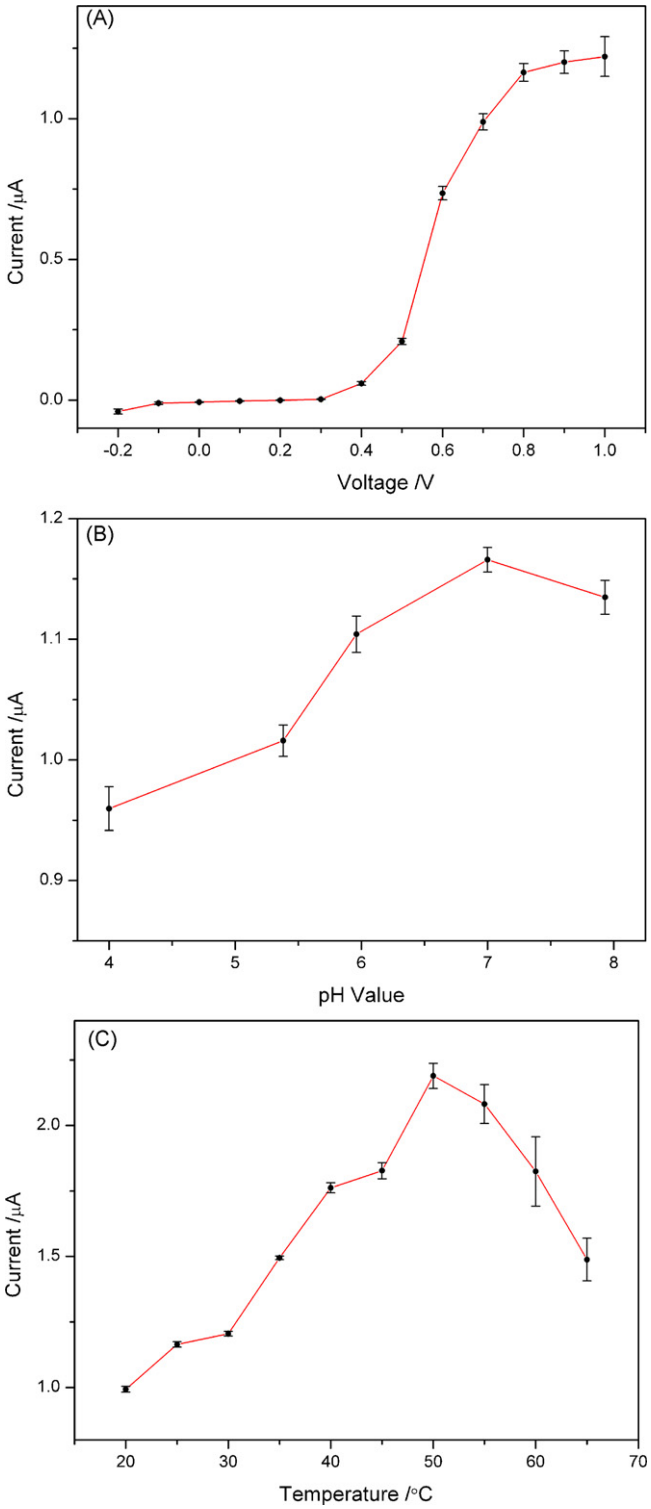

Fig. 5. The amperometric current of the biosensor varies with the applied voltage (A), pH value (B), and temperature (C).

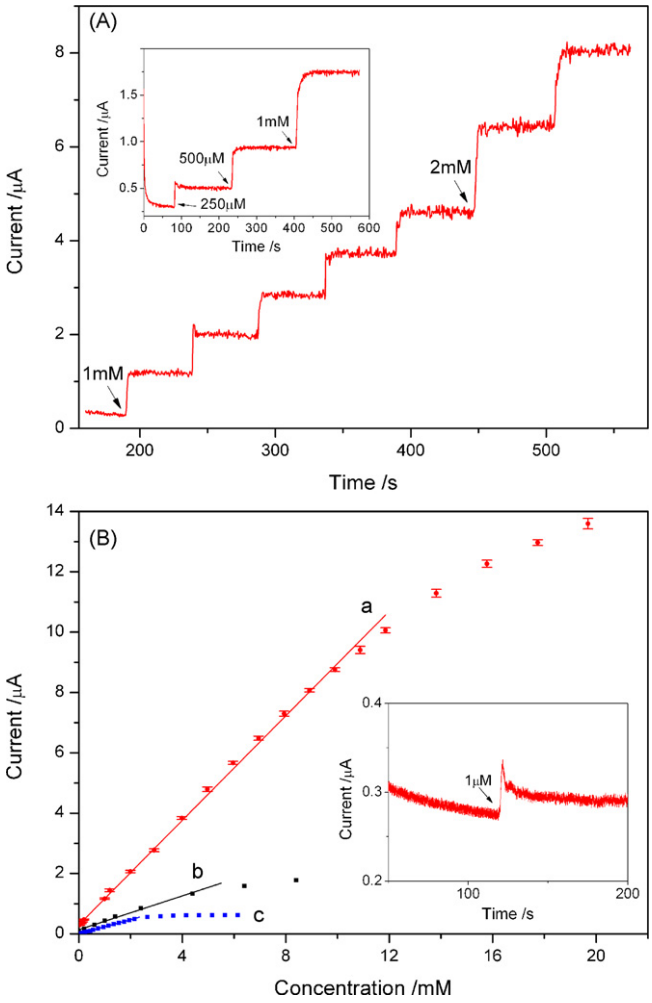

Fig. 6. Amperometric response of the biosensor based on ZnONT to different concentrations of glucose at 0.8 V in stirring pH 7.0 PB solution (A). Calibration curves for the response of the biosensors using ZnONT (a), ZnONR (b) and Au film (c) as enzyme immobilization materials (B). Inset of (A) shows the biosensor response to different concentrations of glucose; inset of (B) shows the biosensor response to the 1 μM glucose.

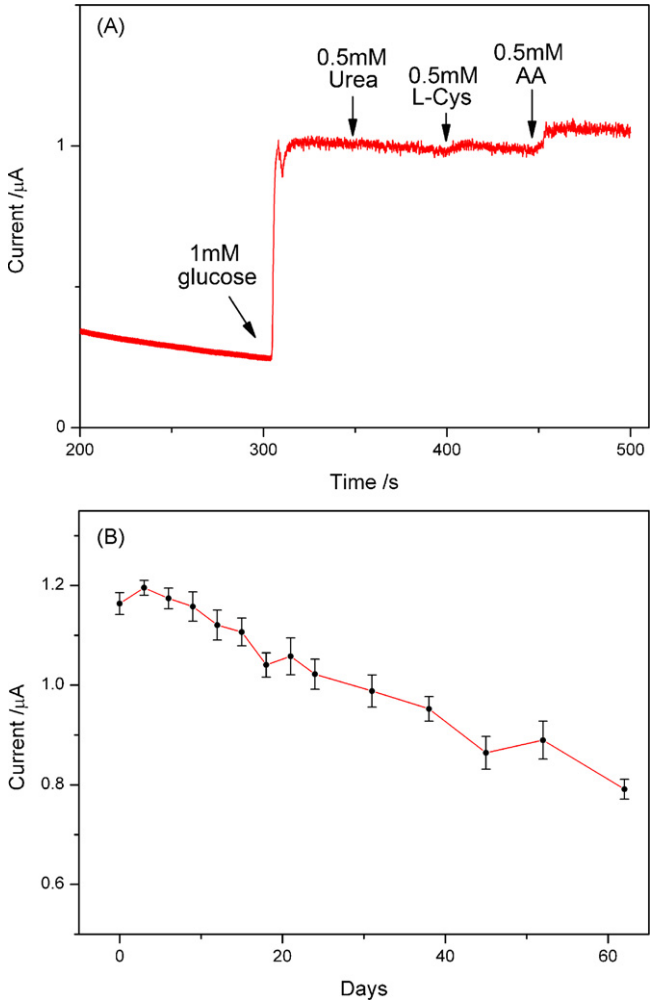

Fig. 7. (A) Effect of interfering species to the response of the biosensor. (B) Long-term stability of the fabricated biosensor.

From Fig. 6B, one can further observe that the biosensor response gradually deviates from the linear feature as the glucose concentration up to 12 mM. This is the characteristic of a typical Michaelis–Menten kinetics. The apparent Michaelis–Menten constant K_m^{app}, which depicts the enzyme–substrate kinetics of biosensor, can be calculated from the Lineweaver–Burk equation: $1/I_{ss} = (K_m^{app}/I_{max})(1/C) + 1/I_{max}$, where C is the concentration of substrate, I_{ss} is the steady-state current and I_{max} is the maximum current measured under substrate saturation [43]. Therefore the values of the K_m^{app} and I_{max} in this work can be calculated to be 19 mM and 24 μA, respectively. The obtained K_m^{app} is lower than the recent reported glucose biosensors based on ZnO:Co nanoclusters (21 mM) [22], polypyrrole films (37.6 mM) [44], and nano-$CaCO_3$ (21.4 mM) [45]. The lower K_m^{app} means the higher enzymatic activity of immobilized GOx, thus the above result further indicates that the ZnONT modified biosensor possesses a high affinity to glucose.

3.3. Anti-interference and stability of biosensor

It is well known that some electroactive species in serum, such as ascorbic acid (AA), L-cysteine (L-Cys) may influence the performance of glucose biosensor. In this work, 0.5 mM urea, L-Cys and AA were consecutively added into PB solution containing 1 mM glucose to study the anti-interference ability of the fabricated glucose biosensor. As can be seen from Fig. 7A, the urea has not any obvious effect on the biosensor, while L-Cys can cause negligible 2.1% current increment compared with that of 1 mM glucose. However, 9.0% current increment can be observed when AA is added. Considering that the concentration of AA in physiological condition is below 0.5 mM (around 0.1 mM) [46] and much smaller than that of glucose (normal range: 4–7 mM), it also brings negligible effect for the glucose detection in serum samples. All the above results imply that the constructed biosensor has a good anti-interference ability.

The reproducibility of biosensor was also examined by measuring the response towards 1 mM glucose in 10 ml pH 7.0 PB solution. Four biosensors constructed independently give a relative standard deviation (R.S.D.) of 8.2%. As for one biosensor, the R.S.D. is 2.2% for 13 continuous assays. The long-term stability of biosensor was evaluated by measuring its performance every few days. It can be seen from Fig. 7B that the biosensor retains around 90% of initial response after three weeks. After 60 days, the biosensor is still active and retains around 70% of the initial response, indicating that the immobilized GOx can efficiently retain their bioactivity for a quite long time due to the good microenvironment provided by ZnONT.

4. Conclusions

A biosensor towards the glucose detection is fabricated by immobilization of glucose oxidase onto the ZnO nanotubes via

cross-linking method. The biosensor shows a fast response to glucose and has a quite wide linear range from 50 μM to 12 mM as well as its experimental limit of detection can be achieved as low as 1 μM. It also possesses good anti-interference ability and stability. All these advantageous features can make the designed biosensor applicable in medical, food or other areas. Moreover, the investigation also exhibits that the ZnO nanotubes may be applied as a potential novel immobilization material for a variety of biosensor designs.

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References

[1] J.S. Ye, Y. Wen, W.D. Zhang, L.M. Gan, G.Q. Xu, F.S. Sheu, Nonenzymatic glucose detection using multi-walled carbon nanotube electrodes, Electrochem. Commun. 6 (2004) 66–70.
[2] L. Zhu, Y. Li, F. Tian, B. Xu, G. Zhu, Electrochemiluminescent determination of glucose with a sol–gel derived ceramic–carbon composite electrode as a renewable optical fiber biosensor, Sens. Actuat. B: Chem. 84 (2002) 265–270.
[3] K.E. Shafer-Peltier, C.L. Haynes, M.R. Glucksberg, R.P. Van Duyne, Toward a glucose biosensor based on surface-enhanced Raman scattering, J. Am. Chem. Soc. 125 (2003) 588–593.
[4] D. Lee, J. Lee, J. Kim, J. Kim, H.B. Na, B. Kim, C.H. Shin, J.H. Kwak, A. Dohnalkova, J.W. Grate, T. Hyeon, H.S. Kim, Simple fabrication of a highly sensitive and fast glucose biosensor using enzymes immobilized in mesocellular carbon foam, Adv. Mater. 17 (2005) 2828–2833.
[5] X.L. Luo, J.J. Xu, Y. Du, H.Y. Chen, A glucose biosensor based on chitosan–glucose oxidase–gold nanoparticles biocomposite formed by one-step electrodeposition, Anal. Biochem. 334 (2004) 284–289.
[6] W. Zhao, J.J. Xu, C.G. Shi, H.Y. Chen, Multilayer membranes via layer-by-layer deposition of organic polymer protected Prussian blue nanoparticles and glucose oxidase for glucose biosensing, Langmuir 21 (2005) 9630–9634.
[7] S. Liu, H. Ju, Reagentless glucose biosensor based on direct electron transfer of glucose oxidase immobilized on colloidal gold modified carbon paste electrode, Biosens. Bioelectron. 19 (2003) 177–183.
[8] S.G. Wang, Q. Zhang, R. Wang, S.F. Yoon, A novel multi-walled carbon nanotube-based biosensor for glucose detection, Biochem. Biophys. Res. Commun. 311 (2003) 572–576.
[9] X. Chen, S.J. Dong, Sol–gel-derived titanium oxide/copolymer composite based glucose biosensor, Biosens. Bioelectron. 18 (2003) 999–1004.
[10] Z. Lin, J. Chen, G. Chen, An ECL biosensor for glucose based on carbon-nanotube/Nafion film modified glass carbon electrode, Electrochim. Acta 53 (2008) 2396–2401.
[11] S.H. Lim, J. Wei, J. Lin, Q. Li, J.K. You, A glucose biosensor based on electrodeposition of palladium nanoparticles and glucose oxidase onto Nafion-solubilized carbon nanotube electrode, Biosens. Bioelectron. 20 (2005) 2341–2346.
[12] B. Piro, V.A. Do, L.A. Le, M. Hedayatullah, M.C. Pham, Electrosynthesis of a new enzyme-modified electrode for the amperometric detection of glucose, J. Electroanal. Chem. 486 (2000) 133–140.
[13] S. Cosnier, A. Senillou, M. Grätzel, P. Comte, N. Vlachopoulos, N.J. Renault, C. Martelet, A glucose biosensor based on enzyme entrapment within polypyrrole films electrodeposited on mesoporous titanium dioxide, J. Electroanal. Chem. 469 (1999) 176–181.
[14] H. Muguruma, A. Hiratsuka, I. Karube, Thin-film glucose biosensor based on plasma-polymerized film: simple design for mass production, Anal. Chem. 72 (2000) 2671–2675.
[15] Q. Yang, P. Atanasov, E. Wilkins, Development of needle-type glucose sensor with high selectivity, Sens. Actuat. B: Chem. 46 (1998) 249–256.
[16] B. Wu, G. Zhang, S. Shuang, M.M.F. Choi, Biosensors for determination of glucose with glucose oxidase immobilized on an eggshell membrane, Talanta 64 (2004) 546–553.
[17] M.K. Ram, M. Adami, S. Paddeu, C. Nicolini, Nano-assembly of glucose oxidase on the in situ self-assembled films of polypyrrole and its optical, surface and electrochemical characterizations, Nanotechnology 11 (2000) 112–119.
[18] L. Xu, Y. Zhu, L. Tang, X. Yang, C. Li, Biosensor based on self-assembling glucose oxidase and dendrimer-encapsulated Pt nanoparticles on carbon nanotubes for glucose detection, Electroanalysis 19 (2007) 717–722.
[19] X.L. Zhu, I. Yuri, X. Gan, I. Suzuki, G.X. Li, Electrochemical study of the effect of nano-zinc oxide on microperoxidase and its application to more sensitive hydrogen peroxide biosensor preparation, Biosens. Bioelectron. 22 (2007) 1600–1604.
[20] F.F. Zhang, X.L. Wang, S.Y. Ai, Z.D. Sun, Q. Wan, Z.Q. Zhu, Y.Z. Xian, L.T. Jin, K. Yamamoto, Immobilization of uricase on ZnO nanorods for a reagentless uric acid biosensor, Anal. Chim. Acta 519 (2004) 155–160.
[21] S.P. Singh, S.K. Arya, P. Pandey, B.D. Malhotra, S. Saha, K. Sreenivas, V. Gupta, Cholesterol biosensor based on rf sputtered zinc oxide nanoporous thin film, Appl. Phys. Lett. 91 (2007) 63901–63903.
[22] Z.W. Zhao, X.J. Chen, B.K. Tay, J.S. Chen, Z.J. Han, K.A. Khor, A novel amperometric biosensor based on ZnO:Co nanoclusters for biosensing glucose, Biosens. Bioelectron. 23 (2007) 135–139.
[23] J.X. Wang, X.W. Sun, A. Wei, Y. Lei, X.P. Cai, C.M. Li, Z.L. Dong, Zinc oxide nanocomb biosensor for glucose detection, Appl. Phys. Lett. 88 (2006) 233106–233108.
[24] J.F. Zang, C.M. Li, X.Q. Cui, J.X. Wang, X.W. Sun, H. Dong, C.Q. Sun, Tailoring zinc oxide nanowires for high performance amperometric glucose sensor, Electroanalysis 19 (2007) 1008–1014.
[25] A. Wei, X.W. Sun, J.X. Wang, Y. Lei, X.P. Cai, C.M. Li, Z.L. Dong, W. Huang, Enzymatic glucose biosensor based on ZnO nanorod array grown by hydrothermal decomposition, Appl. Phys. Lett. 89 (2006) 123902–123904.
[26] X.W. Sun, J.X. Wang, A. Wei, Zinc oxide nanostructured biosensor for glucose detection, J. Mater. Sci. Technol. 24 (2008) 649–656.
[27] E. Topoglidis, A.E.G. Cass, B. O'Regan, J.R. Durrant, Immobilisation and bioelectrochemistry of proteins on nanoporous TiO_2 and ZnO films, J. Electroanal. Chem. 517 (2001) 20–27.
[28] E.M.I. Mala Ekanayake, D.M.G. Preethichandra, K. Kaneto, Polypyrrole nanotube array sensor for enhanced adsorption of glucose oxidase in glucose biosensors, Biosens. Bioelectron. 23 (2007) 107–113.
[29] M. Delvaux, S. Demoustier-Champagne, Immobilisation of glucose oxidase within metallic nanotubes arrays for application to enzyme biosensors, Biosens. Bioelectron. 18 (2003) 943–951.
[30] M. Yang, J. Wang, H. Li, J.G. Zheng, N.N. Wu, A lactate electrochemical biosensor with a titanate nanotube as direct electron transfer promoter, Nanotechnology 19 (2008) 75502–75507.
[31] J. Yuan, K. Wang, X. Xia, Highly ordered platinum-nanotubule arrays for amperometric glucose sensing, Adv. Funct. Mater. 15 (2005) 803–809.
[32] G.W. She, X.H. Zhang, W.S. Shi, X. Fan, J.C. Chang, C.S. Lee, S.T. Lee, C.H. Liu, Controlled synthesis of oriented single-crystal ZnO nanotube arrays on transparent conductive substrates, Appl. Phys. Lett. 92 (2008) 53111–53113.
[33] Y. Yang, H. Yang, M. Yang, Y. Liu, G. Shen, R. Yu, Amperometric glucose biosensor based on a surface treated nanoporous ZrO_2/chitosan composite film as immobilization matrix, Anal. Chim. Acta 525 (2004) 213–220.
[34] J.J. Xu, Z.H. Yu, H.Y. Chen, Glucose biosensors prepared by electropolymerization of p-chlorophenylamine with and without Nafion, Anal. Chim. Acta 463 (2002) 239–247.
[35] T. Maruyama, S. Katoh, M. Nakajima, H. Nabetani, T.P. Abbott, A. Shono, K. Satoh, FT-IR analysis of BSA fouled on ultrafiltration and microfiltration membranes, J. Membr. Sci. 192 (2001) 201–207.
[36] S. Zuo, Y. Teng, H. Yuan, M. Lan, Direct electrochemistry of glucose oxidase on screen printed electrode through one-step enzyme immobilization process with silica sol–gel/polyvinyl alcohol hybrid film, Sens. Actuat. B: Chem. 133 (2008) 555–560.
[37] J. Li, Y.B. Wang, J.D. Qiu, D.C. Sun, X.H. Xia, Biocomposites of covalently linked glucose oxidase on carbon nanotubes for glucose biosensor, Anal. Bioanal. Chem. 383 (2005) 918–922.
[38] L. Wang, Z. Yuan, Direct electrochemistry of glucose oxidase at a gold electrode modified with single-wall carbon nanotubes, Sensors 3 (2003) 544–554.
[39] X.B. Yan, X.J. Chen, B.K. Tay, K.A. Khor, Transparent and flexible glucose biosensor via layer-by-layer assembly of multi-wall carbon nanotubes and glucose oxidase, Electrochem. Commun. 9 (2007) 1269–1275.
[40] H. Yang, Y. Zhu, Glucose biosensor based on nano-SiO_2 and "unprotected" Pt nanoclusters, Biosens. Bioelectron. 22 (2007) 2989–2993.
[41] S. Wu, H. Ju, Y. Liu, Conductive mesocellular silica–carbon nanocomposite foams for immobilization, direct electrochemistry, and biosensing of proteins, Adv. Funct. Mater. 17 (2007) 585–592.
[42] D.C. Deshpande, H. Yoon, A.M. Khaing, V.K. Varadan, Development of a nanoscale heterostructured glucose sensor using modified microfabrication processes, J. Micro/Nanolith. MEMS MOEMS 7 (2007) 23005–23010.
[43] R.A. Kamin, G.S. Wilson, Rotating ring-disk enzyme electrode for biocatalysis kinetic studies and characterization of the immobilized enzyme layer, Anal. Chem. 52 (1980) 1198–1205.
[44] Y.M. Uang, T.C. Chou, Fabrication of glucose oxidase/polypyrrole biosensor by galvanostatic method in various pH aqueous solutions, Biosens. Bioelectron. 19 (2003) 141–147.
[45] D. Shan, M. Zhu, H. Xue, S. Cosnier, Development of amperometric biosensor for glucose based on a novel attractive enzyme immobilization matrix: calcium carbonate nanoparticles, Biosens. Bioelectron. 22 (2007) 1612–1617.
[46] Z.X. Zhao, M.Q. Qiao, F. Yin, B. Shao, B.Y. Wu, Y.Y. Wang, X.S. Wang, X. Qin, S. Li, L. Yu, Q. Chen, Amperometric glucose biosensor based on self-assembly hydrophobin with high efficiency of enzyme utilization, Biosens. Bioelectron. 22 (2007) 3021–3027.

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