Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Journal of
Materials Chemistry B

Cite this: DOI: 10.1039/c0xx00000x

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PAPER

A polyaniline microtubes platform for direct electron transfer of glucose oxidase and biosensing applications

Lei Zhang, Cuisong Zhou*, Jiaojiao Luo, Yuyin Long, Congmin Wang, Tingting Yu and Dan Xiao*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Polyaniline (PANI) microtubes are becoming greatly significant electrochemical materials owing to their large geometric surface area, high conductivity and ideal electrocatalytic activity. In this work, glucose oxidase (GOx) used as a model redox protein, a direct electron transfer strategy based on PANI microtubes was developed for fabricating sensitive biosensors. There is a strong electrostatic interaction between the positive charged PANI microtubes and negative charged GOx, which promotes the immobilization of GOx on the PANI microtubes surface. The immobilized GOx displayed a pair of well-defined quasi-reversible redox peaks with a potential of −0.39 V (*vs*.SCE) and an ideal electron transfer rate constant (k_s) of 3.0 s^{-1} in PBS solution (0.1 M, pH = 5.5) on the PANI microtubes instead of bare glass carbon electrode (GCE). The amperometric response of GOx/PANI microtubes modified electrode was linearly proportional to the concentration of glucose in the range of 4.0 μM to 0.80 mM. The glucose detection limit was 0.8 μM at signal-to-noise ratio of 3, which was better than reported GOx/PANI film (1 mM), GOx/PANI nanowire (50 μM). The advantages might be attributed to the PANI microtubes' large geometric surface for carrying enzyme (GOx), efficient electrocatalytic activity to facilitate direct electron transfer of GOx, as well as efficient GOx biocatalyst reaction on the microtubes surface. A promising application of PANI microtubes-based biosensor was offered.

Introduction

Polyaniline (PANI) materials have been widely accepted as ideal electrochemical sensing materials because of their environmental stability, high conductivity and interesting redox properties.[1,2] There is a growing focus on PANI micro/nanotubes which play key roles as both interconnects units and functional sensing ones.[3,4] Compared to PANI micro/nanoparticles or bulk PANI materials, the micro/nanotubes have a large geometric surface area and several different areas of contact (borders, inner and outer surfaces, and structured tube walls), which entails the availability of electrical transport and a large number of active reaction sites for chemical reactions to occur.[5,6]

In-depth study of the direct electrochemistry of redox enzymes/proteins could help not only to gain further insight into enzyme-catalyzed reactions in biochemistry systems, but also to fabricate sensitive and selective biosensors without the need of expensive or harmful mediators.[7-9] Increasing efforts have been made to improve direct electron transfer (DET) efficiency of redox proteins using conducting polymers.[10] Examples are amperometric-type glucose biosensors, where the direct electrochemistry of glucose oxidase (GOx) played a leading role in the sensitive monitoring of serum and urine glucose levels. Additional advantages include high sensitivity, compatibility for miniaturization, easy operation, and low cost.[11,12] It is well known that catalytic effect of GOx relies on the presence of its cofactor flavin adenine dinucleotide (FAD). FAD exists in two different states, FAD and $FADH_2$. When a redox reaction occurs, FAD and $FADH_2$ can work as an electron acceptor and an electron donor alternatively to catalyze biochemical reactions.[3] However, the two bound redox-active FAD cofactors of GOx are deeply buried within the insulated prosthetic shells, rendering them inaccessible for direct electron transfer (DET) with bare electrodes. In addition, enzymes adsorbed on the electrode surface might be denatured resulting in the loss of their electrochemical activities.[13] Recently, considerable efforts have been made to develop alternative matrix in order to keep GOx's biological activity and promote their DET behaviors.[12-14]

PANI micro/nanotubes' electronic conductivity is several orders of magnitude higher than that of conventional forms (e.g., powders or thin films).[15] Our group have tuned PANI microtubes' diameters in a range from 0.63 to 2.93 μm and uncovered that their diameter determined the enhancement in their conductivity and electroactivity. The PANI microtubes with an optimum diameter (1.43 μm) exhibited an improved electroactive surface area and an efficient electron transfer on electrode surface, and thus an enhanced sensitivity for detecting ascorbic acid (AA).

The detection limit of AA is 0.28 μM, better than reported PANI nanotubes (1.0 μM), nanofibers (1.7 μM) and nanoparticles (8.3 μM). The advantages were attributed to the tubular structures' large geometric surface area and large electroactive surface area. Since these PANI microtubes can interconnect with each other to form a three-dimensional (3D)-structural sensing matrix, it might be expected that the 3D-structural matrix would exhibit an efficient immobilization for enzyme like GOx and promote the DET of GOx. Although considerable efforts have been made on PANI-based glucose biosensor, few papers reported that PANI microtubes would promote the DET and electrocatalytic activity of GOx until now.[16-18]

In this work, we proposed a new strategy based on a PANI microtubes platform for DET of GOx and biosensing applications. GOx can be efficiently immobilized on positively charged PANI microtubes via strong electrostatic interaction. The 3D PANI microtubes-interconnected structure with a large geometric surface area, would play important roles in having large loading of GOx, efficient charge collection and fast glucose permeation, which would be developed to a sensitive, selective and quick detection strategy. Furthermore, electron transfer rate, electroactive surface area, electrocatalytic activity of GOx/PANI microtubes were characterized in detail. Based on improved DET performance of GOx immobilized on 3D PANI microtubes, a sensitive, selective and quick sensing strategy based on the GOx/PANI microtubes would be developed. The GOx/PANI microtubes platform would be used to sensitively detect glucose in real sample. The proposed strategy could further be extended to study various DET behaviors of enzyme/protein in a range of application fields such as medical diagnosis, bioprocess monitoring and environmental monitoring.

Experimental section

Reagents

PS watch glasses were obtained from Taizhou Kejian Medical Supplies Ltd. (Jiangsu, China). Aniline hydrochloride was purchased from Shanghai Jinshan Tingxin Chemical Reagent Factory (Shanghai, China). Ammonium persulfate ($(NH_4)_2S_2O_8$, APS) and interfaces including ascorbid acid (AA), acetaminophen (AP), salicylic acid (SA), H_2O_2, sucrose, lactose, and fructose were obtained from Chengdu Chemical Reagent Co., (Chengdu, China). N, N-dimethylformamide (DMF) was purchased from Tianjin Meilin Industry&Trade Co., Ltd. (Tianjin, China). Uric acid (UA) was purchased from Acros (New Jersey, USA). Dopamine hydrochloride (DA) was obtained from Alfa Aesar (A Johnson Matthey Company, Tianjin, China). Hydrochloric acid (HCl), concentrated sulfuric acid (98%), glucose, were purchased from Chengdu Kelong Chemical Factory (Chengdu, China). Phosphate buffer solution (PBS, 0.1 M, pH = 5.5) were prepared by mixing Na_2HPO_4 and NaH_2PO_4. GOx solution and all interference solutions were prepared using PBS immediately before each experiment. Colour system was prepared using PBS, including 0.4 mM 4-aminophenazone, 10 mM phenol, 5 mg/mL horseradish catalase, and 1 mM glucose. The glucose solution was allowed to mutarotate for at least 24 h before use. All chemicals were of analytical grade. All solutions were freshly prepared with deionized distilled water.

The preparation of PANI microtubes

PANI microtubes were prepared according to our previous work.[6] In brief, PANI microtubes (1.43 μm) were in situ polymerized using polystyrene (PS) microfibers as a template. Then the PS microfibers were removed from the PANI/PS composite microfibers using DMF solvent. After dried in a vacuum oven at 40 □ for 24 h, pure hollow PANI microtubes were obtained.

The fabrication of PANI microtubes, GOx/PANI microtubes, FAD/PANI microtubes and GOx modified electrodes.

Prior to its modification, a glass carbon (GC) electrode with a diameter of 3 mm was polished.[19] 8 mg mL^{-1} PANI microtubes solution was obtained by dispersing these PANI microtubes in the mixed solvent (DMF/PBS = 1/4) and ultrasonically treated for 10 min. Then, 3 μL of the dispersion was drop-coated onto the GC electrode. After the evaporation of solvent, the PANI microtubes modified electrode was obtained. Next, glucose oxidase solution (10 mg/mL) was mixed with 0.1% glutaraldehyde at a volume ratio of 4:1. And then, 5 μL of the mixture solution was dropped onto the PANI microtubes modified electrode. After the evaporation of solvent in air at room temperature for 5 hours, the GOx/PANI microtubes modified electrode was washed thoroughly with 0.1 M (pH = 5.5) PBS until these unimmobilized GOx was cleaned away. The electrode was stored in buffer at 4 °C when it was not in use. For control experiments, modified electrodes such as GOx/PANI microtubes and FAD/PANI microtubes were fabricated using the same dropping process (C_{FAD}: 10 mg/mL).

Apparatus

The morphology of PANI microtubes and GOx/PANI microtubes were investigated by SEM (Hitachi S-4800, Japan). To characterize the GOx/PANI microtubes, the materials can be obtained according the process of the preparation of GOx/PANI microtubes modified GCE. Then we collected the power and characterized further. The FTIR and UV–Vis spectra of the GOx/PANI microtubes were measured using the same experimental process as that of the PANI microtubes. UV–Vis spectra were measured using a UV1900 spectrophotometer (Shanghai, China) with 1 cm light path quartz cuvette. The crystal structure of PANI microtubes and GOx/PANI microtubes were analyzed by a Tongda TD-3500 XRD with Cu-Ka radiation (λ = 0.15148 nm) operating at 30.0 kV and 20.0 mA (Liaoning, China). All the electrochemical studies were performed on an Autolab PGSTAT 302 electrochemical workstation (Utrecht, the Netherlands), using a three-electrode system. The bare or modified GCE (3mm in diameter, Shanghai Chenhua, China) was employed as the working electrode, a saturated calomel as a reference electrode (SCE) and a platinum foil as a counter electrode. Electrochemical impedance spectra (EIS) were recorded in the presence of $K_3Fe(CN)_6/K_4Fe(CN)_6$ (10 mM) in the frequency range between 0.01 Hz and 100 kHz with amplitude signal of 10 mV at 0.2 V.

Electrochemical measurements

The cyclic voltammetry (CV) was recorded in 10.0 mM

$Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl supporting electrolyte by scanning in the potential range between 0.20 and 0.6 V. The EIS was scanned in the same solution at the potential of 0.2 V. In addition, the CV of investigating GOx' catalyst acitivity was also recorded in 0.1 M PBS (PH = 5.5) solution between -0.6 and -0.15 V at a scan rate of 0.1 V s^{-1}. Amperometric analysis was recorded in 0.1 M PBS (PH = 5.5) solution at a constant potential of -0.5 V.

Results and discussion

Schematic illustration

In this work, GOx used as a model, a direct electron transfer strategy based on a PANI microtubes platform was developed for fabricating a sensitive biosensor. As a proof-of-concept, PANI microtubes were prepared according to our previously reported method.[6] XPS results indicated that the PANI microtubes exist in the doped emeraldine salt (ES) state and carry much positive charges, which promote their electrostatic interaction with negative charged GOx which p*I* is 4.2 in PBS buffer (pH = 5.5).[3] EIS data demonstrated that the PANI microtubes have more effective electroactive surface area and more significant electron transfer on electrode surface, compared to PANI microspheres.[6] Reasonably, the PANI microtubes are expected to have a high GOx-loading capacity and a facilitated DET of GOx on the electrode's surface. Commonly, the DET is difficult to happen on bare electrodes because redox-active FAD cofactors of GOx are deeply buried within the insulated prosthetic shells.[13] In addition, the PANI microtubes could reduce aggregation of GOx on a bare hydrophobic GCE. Importantly, the PANI microtubes have a 3D interconneted-structure with a large geometric surface area, which is essential for the GOx/PANI microtubes to get an enhanced efficient interaction with detection target glucose. Taking advantages of the PANI microtubes, a sensitive and quick biosensing strategy was developed, better than that of PANI microsphere or bulk materials.

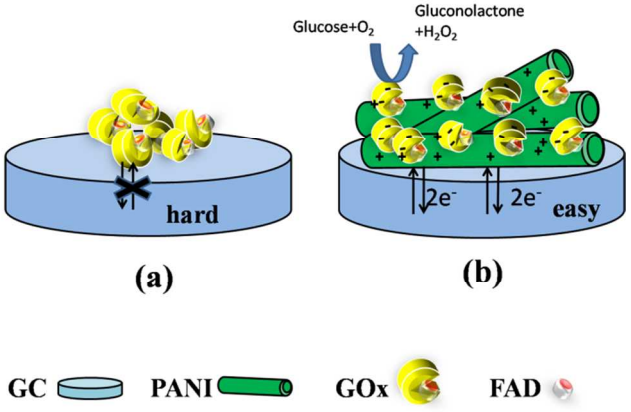


Figure 1 Schematic illustration of direct electron transfer of GOx on GCE with or without PANI microtubes.

Characterization

The PANI microtubes with outer diameter of 1.53 ± 0.16 μm were fabricated using electrospun microfibers as templates. SEM images showed that the PANI microtubes had ideal tubule structure and formed a 3D interconnected structural membrane. (Figure 2A). For the GOx/PANI microtubes, the morphology of PANI microtubes has no observable change after the immobilization of GOx (Figure 2B). The structural stability supports that the PANI microtubes can used as a promising matrix for carrying GOx and keep ideal electrochemical activity. Compared with PANI microspheres or bulk PANI materials, the PANI microtubes have large specific surface that would play important roles in efficient enzyme immobilization, high interaction efficiency, as well as favorable charge transport properties.

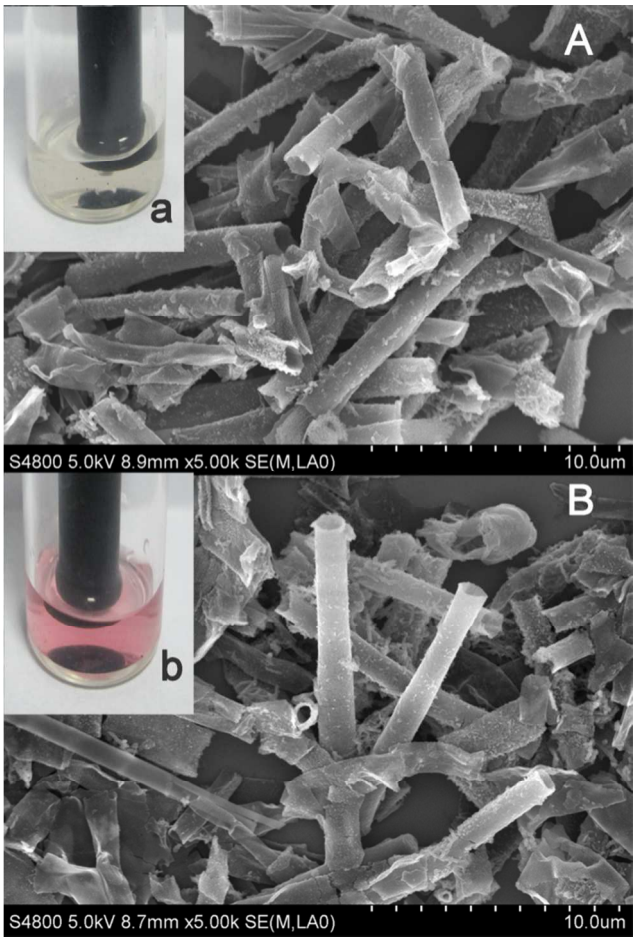


Figure 2 SEM images of PANI microtubes (A) and GOx/PANI microtubes (B). Insets show PANI microtubes modified GCE without (a) or with (b) immobilized GOx in a color system. The concentration of glucose is 5.0 mM. Color reaction time is 30 min.

Next, some key parameters were optimized to obtain efficient biocatalytic activity of the immobilized GOx on a PANI microtubes modified electrode. Herein, 3 μL of 8 mg mL^{-1} PANI microtubes and 5 μL of 10 mg mL^{-1} GOx solution were chosen (Figure S5). A typical colorimetric method[20] was used to confirm the immobilization of GOx on the PANI microtubes. In brief, here is a color system containing 4-aminophenazone and phenol used for determining the hydrogen peroxide produced fromglucose with GOx.[20] As shown in Figure 2B (inset b), the GOx/PANI microtubes modified electrode make the color system turn to red. In contrast, no color change is observed after the PANI microtubes modified electrode was soaked in the same color system. So, Figure 2B indicates that GOx is immobilized

well on the PANI microtubes and exhibits an efficient biocatalyst activity. In addition, UV-Vis spectrum and XRD patterns were recorded to characterize the structure of GOx/PANI microtubes. The UV-Vis spectrum of the PANI microtubes, GOx/PANI microtubes, and GOx are shown in Figure S1. PANI microtubes exhibits the absorption peaks at 340, 432, and 860 nm, which are the characteristic absorption peaks of ES state of PANI microtubes (Inset of Figure S1).[21] For the GOx/PANI microtubes, a significant UV absorption peak is observed at 277 nm, which further supported that GOx is immobilized on the PANI microtubes (Figure S1, curve b). Notably, the absorption of oxidized flavin group shifts from 452 nm to 435 nm (Figure S1, curve c). The blue-shift indicated that there is a strong interaction between the GOx and the PANI microtubes. Meanwhile, the absorption peaks around 860 nm suggest that the GOx/PANI microtubes still maintains their native tubular structure and conductive state. In addition, based on XRD data, there is no difference between the PANI microtubes and GOx/PANI microtubes (Figure S2), which also indicates that the PANI microtubes immobilized with GOx can still have high crystallinity.

The electroactivity of GOx/PANI microtubes modified GCE

In order to furthermore characterize electroactivity of the GOx/PANI microtubes, CV and EIS techniques were used. Figure 3A compared CVs of different modified electrodes measured in 10 mM $Fe(CN)_6^{3-/4-}$ with 0.1 M KCl supporting electrolyte at a scan rate of 50 mV/s. For bare GCE, one pair of well-defined redox peak is observed with a midpoint potential ($E_{1/2}$) of 0.22 V for the redox reaction of $[Fe(CN)_6]^{3-/4-}$ (Figure 3A, curve a), which is consist with previous reports.[22,23] When PANI microtubes are modified on a GCE, its peak current increases 2.7 times higher than that of bare GCE. Interesting, the GOx/PANI microtubes modified electrode got a current increase similar to that of PANI microtubes. Furthermore, their electroactive surface areas are calculated using Randles–Sevcik equation.[24]

$$I_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} \upsilon^{1/2} C$$

Where, n is the number of electrons participating in the redox reaction, A is the electroactive area (cm^2), D is the diffusion coefficient of the bulk concentration of the redox probe (cm^2/s), C is the concentration of the probe molecule in the bulk solution (mol/cm^3) and υ is the scan rate (V/s). The $[Fe(CN)_6]^{3-/4-}$ is one of the most extensively studied redox couples in electrochemistry and exhibits a heterogeneous one-electron transfer (n = 1). For this study, based on reported value for C = 10 mM, $D = 6.7\times10^{-6}$ cm^2/s and υ = 0.05 V/s, electroactive surface area of bare GCE, PANI microtubes/GCE and GOx/PANI microtubes/GCE are found to be 0.078 cm^2, 0.169 cm^2 and 0.156 cm^2, respectively. It indicates that the GOx/PANI microtubes have enlarged electroactive surface area, and would facilitate electron transfer of $[Fe(CN)_6]^{3-/4-}$ on electrode. So, the GOx immobilized on the PANI microtubes were supposed to have an efficient electrocatalytic activity, which suggest the GOx/PANI microtubes can be used as alternative electrochemical sensing surface for sensitively detection.

The enlarged electroactive surface area of the GOx/PANI microtubes was also demonstrated in EIS data (Figure 3B). The semicircle diameter equals the electron transfer resistance, R_{ct}. The resistance controls the electron transfer kinetics of redox probe ($Fe(CN)_6^{3-/4-}$) at the electrode surface.[25] As shown in Figure 3B, there is a very high R_{ct} of 137.8 Ω at a bare GCE (curve a). However, the R_{ct} of the PANI microtubes/GCE and of the GOx/PANI microtubes/GCE decreased to 18.3 Ω (curve b) and 19.2 Ω (curve c), respectively, in agreement with our previous reports that PANI microtubes have effective electroactive surface area.[6] The EIS data demonstrates that the GOx/PANI microtubes/GCE has the R_{ct} comparable to that of the PANI microtubes/GCE, and much lower than that of the bare electrode. Obviously, the GOx/PANI microtubes exhibited significant electron transfer and an effective electroactive surface area, which would improve the GOx' electrocatalyst activity. In addition, the enlarged electroactive surface area of the GOx/PANI microtubes would have a large number of active reaction sites for chemical reactions to occur.[5, 6]

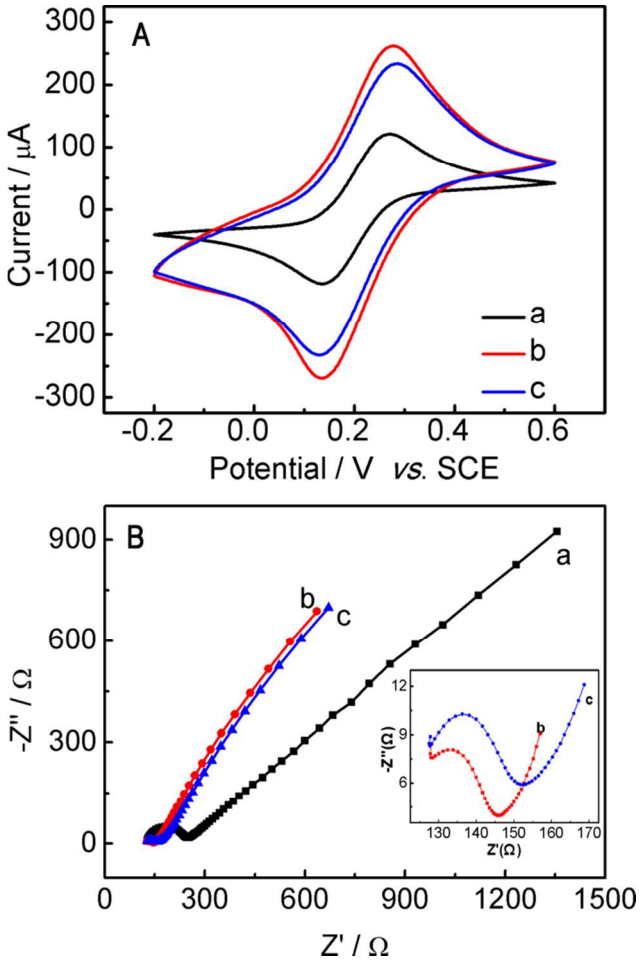


Figure 3 (A) CVs of bare GCE (a), PANI microtubes/GCE (b), and GOx/PANI microtubes/GCE (c) (B) EIS of bare GCE (a), PANI microtubes/GCE (b), and GOx/PANI microtubes/GCE (c) in 10 mM $Fe(CN)_6^{3-/4-}$ with 0.1 M KCl supporting electrolyte.

Amplified direct electron transfer of GOx and the electrocatalytic oxidation of glucose

The DET amplification of the immobilized GOx on the PANI

microtubes was confirmed using CV technique. The CV behaviors of GCE, PANI microtubes/GCE, GOx/GCE and GOx/PANI microtubes/GCE in oxygen-free PBS (0.1 M, pH = 5.5) are measured (Figure 4). No redox peak is observed at bare GCE (curve a), PANI microtubes/GCE, even and GOx/GCE (curve c). It has been reported that both the large 3D structure of enzymes and the immersed redox centers have made it generally difficult to obtain direct electron transfer between enzymes and electrode surfaces.[13] However, conducting polymers can be used to activate DET of redox enzymes.[10] As shown in Figure 4, a pair of well-defined redox peaks is observed at the GOx/PANI microtubes/GCE (curve d). The separation of peak potentials (ΔE_p) of 40 mV and the formal potential of -0.393 V are in agreement with that of FAD/$FADH_2$ (-0.4 V) in pH = 5.5 (Figure S3).[26]

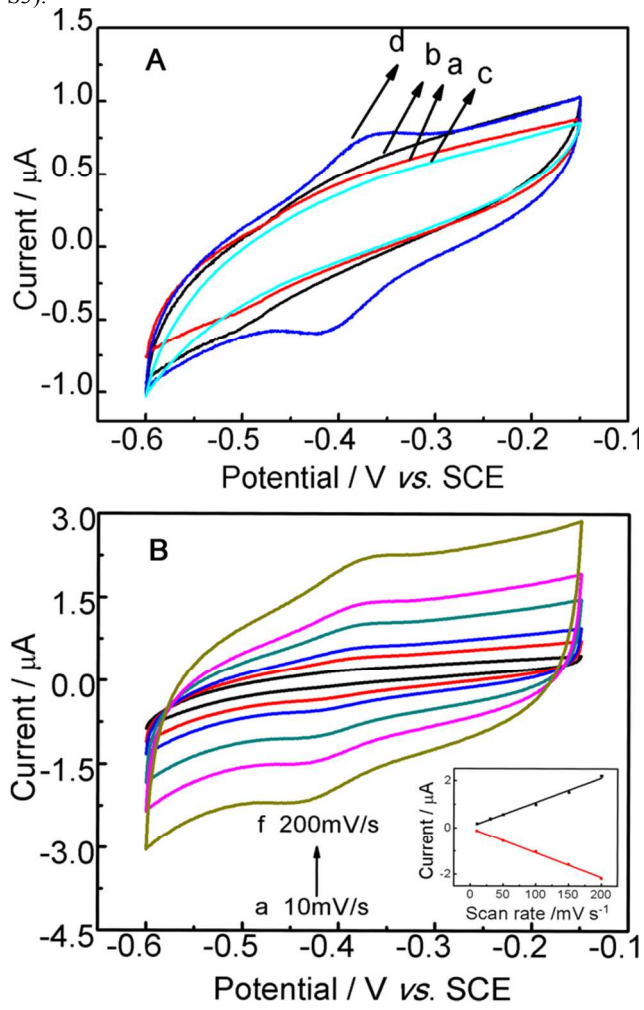
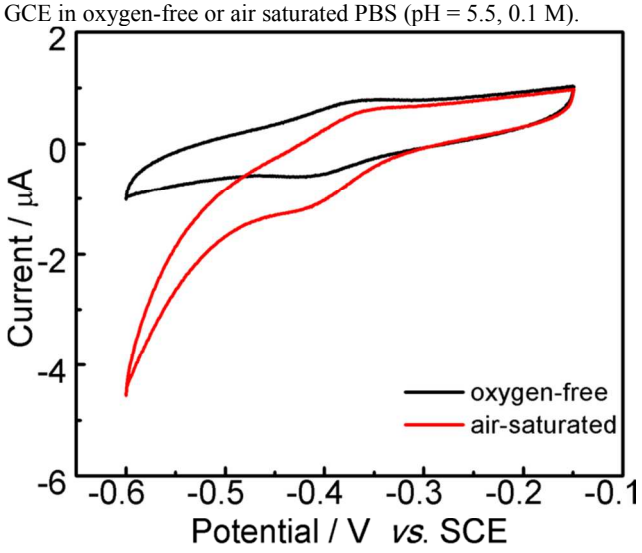


Figure 4 (A) CVs of bare GCE (a), PANI microtubes/GCE (b), GOx/GCE (c) and GOx/PANI microtubes/GCE (d) in oxygen-free PBS (0.1 M, pH = 5.5) at the scan rate of 100 mV/s. (B) CVs of GOx/PANI microtubes modified GCE in oxygen-free PBS at different scan rate. Scan rate (a-f): 10, 30, 50, 100, 150, 200 mV/s. Insert: the plot of anodic and cathodic peak current vs. scan rates.

Figure 4B shows CVs of GOx/PANI microtubes with different scan rates from 10 to 200 mV/s. Since the I_{pa}/I_{pc} ratio and the formal potential of the immobilized GOx are independent on the scan rate (Inset of Figure 4B), the DET of GOx immobilized on the PANI microtubes is a typical quasi-reversible process.[27] With the increase of the scan rate, both anodic and cathodic peak currents increase linearly (Inset of Figure 4B). These electrochemical characteristics indicate that the reaction is a surface-controlled process.[18] Using the Laviron method for a surface-controlled electrochemical system,[28] the average electron transfer rate constant (k_s) is calculated to be 3.0 s^{-1}, higher than those at a carbon nanotube materials (1.5-1.6 s^{-1}) and at a graphene electrode (2.68 s^{-1}).[29] This implies that PANI microtubes significantly facilitate the electron transfer between GOx and electrode.

Electrocatalytical behavior of GOx/PANI microtubes modified electrode

In order to study biocatalytic activity of the GOx immobilized on PANI microtubes/GCE, further electrochemical experiments about the GOx biocatalytic reaction were carried out. Figure 5 compares the CV behaviors of GOx/PANI microtubes modified GCE in oxygen-free or air saturated PBS (pH = 5.5, 0.1 M).



Scheme 1

$$GOx(FAD) + 2e^- + 2H^+ \longrightarrow GOx(FADH_2) \quad (1)$$

$$GOx(FADH_2) \longrightarrow GOx(FAD) + 2e^- + 2H^+ \quad (2)$$

$$GOx(FADH_2) + O_2 \longrightarrow GOx(FAD) + H_2O_2 \quad (3)$$

$$GOx(FAD) + Glucose \longrightarrow GOx(FADH_2) + Gluconolactone \quad (4)$$

Figure 5 CVs of GOx/PANI microtubes modified GCE in oxygen-free (black) and air-saturated (red) PBS (0.1 M, pH 5.5). Scheme 1. The mechanism for GOx electrocatalytic behavior for glucose detection.

As we expected, a pair of symmetrical redox peaks appear under an oxygen-free condition. It was indicated that the GOx undergoes a reversible direct electrochemical reaction (curve a) on the PANI microtubes modified electrode. In addition, the addition of glucose did not nearly change these redox peak value (Figure S4). When PBS is saturated with air, the oxidation peak current of the GOx decreases but the reduction peak current increases (curve b), which is consistent with previous reports.[18] However, with the addition of glucose into air-saturated PBS, the

reduction peak current of GOx (FAD) decreases (Figure 6). In addition, 1.0 mM glucose make a relative reduction peak current decrease of GOx/PANI microtubes/GCE that was 4.5 times higher that of GOx/GCE. Thus, based on the decrease of the reduction peak current, the GOx/PANI microtubes modified GCE can be used as a biosensor for detecting glucose. A mechanism for the electrocatalytic activity of the GOx/PANI microtubes toward the detection of glucose is proposed in Scheme 1: Reductive and oxidative half-reactions of GOx are described as Eqn. 1 and Eqn. 2, respectively. Due to participating of O_2, the enzymatic process (Eqn. 3) competes efficiently with the eletrochemical oxidation of $FADH_2$ and causes the decreases of the oxidation peak current of $FADH_2$, when sweeping the potential in the positive direction. Then, the FAD species that are generated by the reaction of species with dissolved oxygen can be catalytically reduced on modified GCE surface, when sweeping the potential in the negative direction. Therefore, the reduction peak current increases in air saturated PBS, compared with that in oxygen-free PBS (Figure 5). At the presence of glucose, it can be oxidized first by GOx (FAD) (Eqn. 4), which would compete with the electrochemical reduction of GOx (FAD), when sweeping the potential in the negative direction. So, reduction peak current of the GOx/PANI microtubes decreases with increasing glucose (Figure 6). At last, a new strategy based on the GOx/PANI microtubes platform can be developed for glucose biosensing applications.

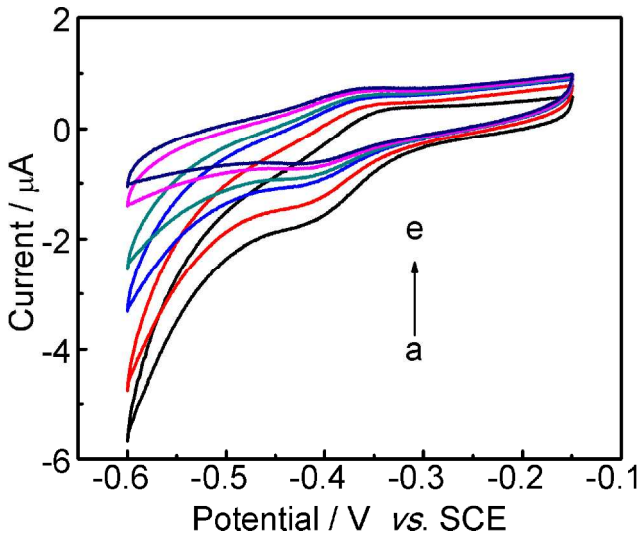


Figure 6 CVs of GOx/PANI microtubes/GCE glucose with various glucose concentration of 0 mM (a), 0.2 mM (b), 0.4 mM (c), 0.6 mM (d) and 1.0 mM (e) in air–saturated PBS (0.1 M, pH = 5.5).

Amperometric detection of the GOx/PANI microtubes -based biosensor

To fabricate a highly sensitive biosensor, some key parameters were optimized including PANI microtubes amount, GOx concentration, detection potential and pH values of PBS, which were 8 mg/mL, 10 mg/mL, -0.5 V and 5.5, respectively (Figure S5). Under the optimized condition, typically steady-state amperometric response of GOx/PANI microtubes modified GCE was measured with successive addition of glucose to the stirring air-saturated PBS (pH = 5.5, 0.1 M). As shown in Figure 7, the reduction current decreases with the increase of glucose concentration. In addition, the reduction current responses quickly and reaches its steady state within 3 s after the addition of glucose. The quick response may be attributed to the PANI microtubes' effective electroactivity that facilitates direct electron transfer of GOx to electrode. The resulting calibration plot is shown in Figure 7 (insert b). The current of the biosensor is linearly related to glucose concentration between 4.0 μM and 0.8 mM with a correlation coefficient of 0.998 (n = 3). The sensitivity calculated from the linear portion of the calibration is as high as 35.6 $\mu A \cdot mM^{-1} \cdot cm^{-2}$, and the detection limit is down to 0.8 μM at signal to noise ratio of 3 that is better than that of reported GOx/PANI film (1 mM),[30] GOx/PANI nanowire (50 μM),[17] GOx/bulk PANI/PB/MWNTs (1 mM),[31] and GOx/AuNPs/GR (graphite rod) (83 μM).[32]

In order to assess the analytical performance of the proposed biosensor, the characteristics in terms of linear detection range, detection limit and sensitivity compared with earlier glucose biosensors as presented in Table 1. The results indicate that our biosensor have advantages over one-fold materials (chitosan, graphene), the different morphology of PANI (nanowire, film), and even complex composites.

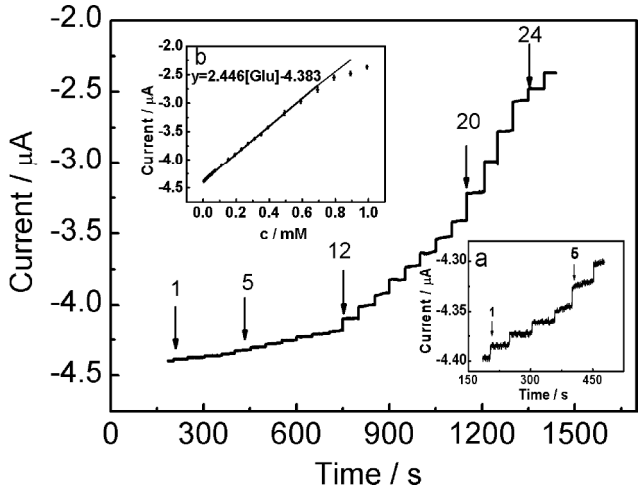


Figure 7 Amperometric response of GOx/PANI microtubes modified GCE to successive addition of glucose in air-saturated PBS (0.1 M, pH = 5.5) at applied potential of -0.5 V. The concentration of glucose: (1) 4.0 μM; (5) 24.0 μM; (12) 0.11 mM; (20) 0.49 mM; (24) 0.89 mM. Inset: (a) Magnification curve with the addition of low concentration glucose from 4.0 to 36.0 μM. (b) The calibration curve of current vs. glucose concentration.

Table 1 Comparison of amperometric glucose biosensor

Electrode	Linear range (mM)	Detection limit (μM)	Sensitivity ($\mu A \cdot mM^{-1} \cdot cm^{-2}$)	Response Time(s)	Ref
GOx/chitosan	0.6 -2.8	100	3.33	-	[32]
GOx/graphene	0.1-10	10	-	5	[29]
GOx/AuNPs/GR	0.1-10	83	101.2	-	[33]
GOx/PANI/PB[a]/MWNTs[b]	1-11	10	15.36	15	[31]
GOx/Graphene/PANI/Au	0.004-1.12	0.6	11.42[c]	4.8	[18]
GOx/PtNP/PANI/Pt	0.01-8	0.7	96.1	3	[1]
GOx/PANI (nanotubes)	0.01-5.5	0.3	97.18	3	[3]
GOx/PANI/Pec[d] NPs	0.06-4	43.5	79.49	-	[34]
GOx/PANI (microtubes)	0.004-0.8	0.8	35.42	3	this work

[a] PB: Prussion blue. [b]MWNT: multi-walled carbon nanotube. [c]11.42: calculated by this work [d] Pec :pectin

Table 2 Determination and recovery of glucose in real samples

Sample[a]		Glucose Concentration[b] (mM)	Glucose Concentration[c] (mM)	RSD[d] %	Glucose added (mM)	Glucose Found[b] (mM)	Recovery %	RSD[d] %
glucose	1	2.78	2.65	3.24	0.100	0.096	96	3.4
injection	2	0.28	0.26	3.68	0.100	0.102	102	2.9
Blood	1	-	7.92	3.72	0.100	0.095	95	2.5
serum	2	-	6.33	2.95	0.100	0.103	103	3.1

a: Two different manufacturers/people. *b*: Supplied by the instructions. *c*: Determined by this work. *d*: Three replicates were performed.

The selectivity, reusability, reversibility and the long term stability of the GOx/PANI microtubes modified electrode

In biosensor applications, a key concern is interference from some coexisting electroactive species in real samples. Here, some potential interferences including DA, AA, AP, UA, SA, H_2O_2, sucrose, lactose and fructose, were tested one by one to evaluate the selectivity of the GOx/PANI microtubes-based biosensor. All these concentrations were 0.3 mM. As shown in Figure 8, none of the above interferences causes any notable response from the biosensor. However, when 0.10 mM glucose is added, a significant enhancement (0.21 μA) of the current is observed. So, the GOx/PANI microtubes exhibited a good selectivity of glucose over these molecules with similar structures or electroactivity. The reusability of the GOx/PANI microtubes was demonstrated by separately measuring its current values when it was exposed to ten cycles of 0.30 mM glucose and buffer, respectively. When the GOx/PANI microtubes is exposed to 0.30 mM glucose solution, its response time is less than 3 s and the relative standard deviation (RSD) of the measured currents for ten successive measurements was less than 2.50 %. Whenever the GOx/PANI microtubes were moved to the buffer, its current value went back to the origin. The signal changes were fully reversible. To test the long-term stability, the biosensor was stored at 4 °C in the refrigerator and measured at intervals. No obvious decrease of amperometric response during the first. After storage of 15 days and a month, the biosensor retained about 96 % and 85 %, respectively, of its original current response (Figure S6). The bioactivity of the immobilized GOx is well maintained, which indicates that the PANI microtubes provided a biocompatible microenvironment for enzyme molecules.

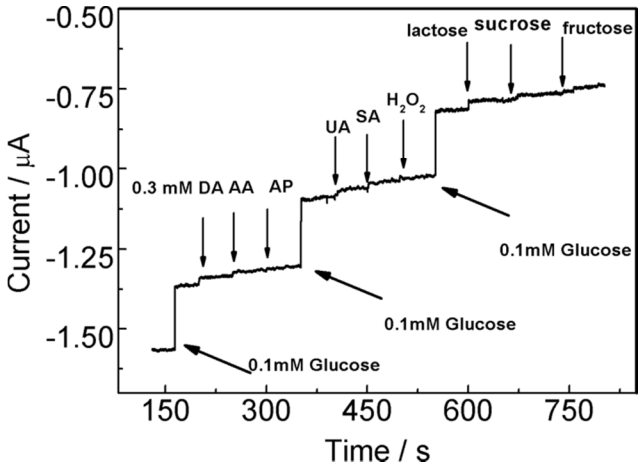


Figure 8 Amperometric response of the GOx/PANI microtubes/GCE to the addition of 0.1 mM glucose or 0.3 mM other interferences in 0.1 M air-saturated PBS at the potential of -0.5 V.

Glucose determination in real samples

To illustrate the feasibility of the GOx/PANI microtubes biosensor in biologically relevant matrix, it was employed to detect glucose in real samples. In our work, the real samples were simply diluted with an appropriate dilution ratio using the supporting electrolyte (PBS, pH 5.5) to yield testing sample solutions. In addition, the recovery test was conducted. All the data are summarized in Table 2. The results obtained by the proposed glucose biosensor are satisfactory. These results demonstrate that the GOx/PANI microtubes offer an excellent, accurate, and precise method for determination of glucose in a biologically relevant matrix.

Conclusions

A sensitive glucose biosensor based on a 3D-GOx/PANI microtubes platform has been constructed. The biosensor exhibites high sensitivity, selectivity and rapid response, and it can be used for detection of glucose in real examples that are glucose injection and blood serum. The advantages of the platform might be attributed to PANI microtubes' electrocatalytic activity for GOx' DET, large geometric surface area for carrying GOx and efficient GOx biocatalytic capability. A potential application based on the 3D- GOx/PANI microtubes platform is offered for medical diagnosis, diabetes management and environmental monitoring. In addition, the strategy based on the 3D-GOx/PANI microtubes platform could further be extended to DET of other redox enzyme/proteins for specific recognition and detection.

Acknowledgement

This work is financially supported by the National Natural Science Foundation of China (No. 21105070, No. 21475091).

Notes and references

College of Chemistry, Key Laboratory of Green Chemistry and Technology, Ministry of Education, Sichuan University, 29 Wangjiang Road, Chengdu, 610064, China.
E-mail: zcs@scu.edu.cn;
Fax: +86 28-85416029; Tel: +86-28-85415029
† Electronic Supplementary Information (ESI) available: See DOI: 10.1039/b000000x/

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A polyaniline microtubes platform for direct electron transfer of glucose oxidase and biosensing applications

Lei Zhang, Cuisong Zhou,* Jiaojiao Luo, Yuyin Long, Congmin Wang,Tingting Yu and Dan Xiao*

College of Chemistry, Key Laboratory of Green Chemistry and Technology, Ministry of Education, Sichuan University, 29 Wangjiang Road, Chengdu, 610064, China.

* Corresponding author. Email: zcs@scu.edu.cn (C.S. Zhou), Tel.: +86-28-85415029; fax: +86-28-85416029

A polyaniline microtubes platform was fabricated for direct electron transfer of glucose oxidase and biosensing applications