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One step electrochemical deposition and reduction of graphene oxide on screen printed electrodes for impedance detection of glucose

Shuang Li[a,b], Qian Zhang[a], Yanli Lu[a], Daizong Ji[a,b], Diming Zhang[a], Jiajia Wu[a], Xing Chen[a], Qingjun Liu[a,b,*]

[a] Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, 310027, PR China
[b] Collaborative Innovation Center of TCM Health Management, Fujian University of Traditional Chinese Medicine, Fuzhou, 350122, PR China

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ABSTRACT

Graphene has emerged as one of the promising tools for bio-detections as it has high surface area with strong electrochemical properties. In present work, a sensitive electrochemical sensor based on the specific boronic acid-diol binding for glucose sensing was established. The sensor was consisted of 3-aminophenylboronic acid (APBA) and reduced graphene oxide (rGO). Before the modification of electrodes, graphene oxide (GO) and APBA composites (GO/APBA) were compounded by the dehydration condensation reaction. The rGO and APBA composites (rGO/APBA) were reduced from GO/APBA and deposited onto the screen printed electrodes through cyclic voltammetry, simultaneously. With the rGO/APBA functionalized electrodes, the glucose molecule could be detected in the range from 0.1 mM to 50 mM, which could satisfy the clinical application. In order to verify the sensitivity and selectivity of the rGO/APBA functionalized electrodes, GO/APBA, and rGO alone functionalized electrodes were also explored for glucose determination. Results showed that rGO/APBA modified electrodes possessed superior electron conductivity, which promoted the detection properties in impedance spectroscopy. In our study, an easy and simple cost-effective modification method was proposed by one step electrochemical reduction and deposition technique of rGO on screen printed electrodes. With specific modification, the rGO functionalized electrodes provided a sensitive and selective detection of biological analytes.

1. Introduction

Graphene consists of a single-atom sheet of sp^2 bonded carbon atoms, which has attracted strong scientific and technological interests since it first isolated from graphite [1]. Since then, graphene has been proved to have extremely high thermal conductivity, good mechanical strength, quantum hall effect, high specific surface area, and extraordinary electrical conductivity [2,3]. Therefore, it has applied in various fields, such as electronic devices, nano-composite materials, sustainable energy storage, and biotechnologies with its unique physical and chemical properties [4,5]. At the same time, it is of great importance for scientific research and practical applications to produce graphene. Several methods have been proposed, such as: (1) micromechanical cleavage [1], (2) epitaxial graphene grown on ruthenium, Ni, and SiC [6–8], and (3) chemical or thermal reduction of graphene oxide (GO) [9,10]. However, hazardous chemicals as reductants or rapid heat-treatment at high temperature would be employed during the reduction process on the above methods. Recently, several promising research works about electrochemical reduction methods have been reported for graphene synthesis with green process [11–13]. In this way, the graphene was usually electrochemically synthesized from GO, which was more reasonable to call reduced graphene oxide (rGO). The synthesized rGO still reserved some oxygen-containing groups from GO, such as carboxyl and hydroxyl [5,14]. The carboxyl groups provide the possibility of amide bond formation with amine groups. So rGO could easily be modified by various species, such as proteins, nucleic acids, and nanoparticles [15–17]. Besides, rGO could overcome the poor electroconductivity of GO, which may have influence on the sensitivity of electrochemical detection. Therefore, rGO was widely used in electrochemical sensing due to its easy functionalization and high electronic conductivity [18,19].

* Corresponding author at: Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, 310027, PR China.
E-mail address: qjliu@zju.edu.cn (Q. Liu).

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Glucose is an elementary necessity of living creatures and plays an important role in human metabolism. Abnormality of glucose level in blood would reflect diseases like diabetes. Diabetes is a major global disease, which has been assigned next to cancer as the most leading causes of death disability [20,21]. Thus, tremendous efforts have been put into the development of efficient, reliable, and sensitive methods to determine glucose, such as: electrochemical sensing [22], aggregation induced fluorescent sensing [23], liquid crystal based glucose sensing [24], and many other enzymatic and non-enzymatic based glucose sensing [25,26]. Among them, the electrochemical based non-enzymatic biosensors have been considered as excellent analytical tools for rapid and inexpensive glucose recognition [27,28]. Besides, boronic acid derivatives were important ligands for specific recognition of *cis*-diol of glucose, which was widely used in the glucose detection [29]. Therefore, the use of boronic acid compounds, such as 3-aminophenylboronic acid (APBA) provided a novel approach in exploiting non-enzymatic electrochemical sensor designs for glucose determination [30].

Compared with traditional electrodes used in electrochemical sensing, the microfabrication of electrodes provided the possibility for the mass production of reproducible electrochemical sensing devices. In particular, screen printed electrodes were widely used in chemical and biological detections for their inexpensive, simple, rapid, and versatile characteristics [31,32]. As the functionalized materials of electrodes, rGO could be used for promoting electron transfer between the electroactive species and the electrodes, which provided a novel method for fabricating chemical sensors or biological sensors.

In this study, the rGO and APBA composites (rGO/APBA) functionalized screen printed electrodes were used as the sensitive probes for the determination of glucose. GO and APBA composites (GO/APBA) were firstly compounded by the dehydration condensation reaction. Then, a one step electrodeposition technique was proposed to prepare rGO/APBA on the electrodes, which accomplished the reduction of GO/APBA to rGO/APBA and the deposition of rGO/APBA on screen printed electrodes, simultaneously. The sensor exhibited a wide range for glucose detection from 0.1 mM to 50 mM, which covered the ranges in clinical applications. Thus, the rGO functionalized screen printed electrodes were validated as an extensive application in healthcare and bioanalytical sciences.

2. Experiments

2.1. Chemicals and reagents

In this study, GO (~99% purity) was synthesized from graphite according to the Hummers and Offerman method. 3-Aminophenylboronic acid (APBA, MW 136.96) and D-(+)-Glucose (MW 198.17) were obtained from Sigma-Aldrich. The phosphate buffer solution (PBS, 10 mM, pH 7.4) for pretreatment of screen printed electrodes was prepared by dissolving PBS tablets in deionized water. The reagents of N-hydroxysulfosuccinimide (NHS, MW 115.09) and 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC, MW 191.70) for synthesis of GO/APBA, the sodium sulfate anhydrous (Na_2SO_4, MW 142.04) for the electrochemical reduction of GO/APBA to rGO/APBA and the deposition of rGO/APBA on screen printed electrodes, and the potassium ferricyanide/ferrocyanide ($K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$) for impedance detecting were all analytical grade and purchased from Sigma-Aldrich. The human serum was purchased from InnoReagents Incorporation. NaCl (MW 58.44), KCl (MW 74.55), $CaCl_2$ (MW 110.98), L-Glutamic acid (MW 147.13), L-Cysteine (MW 121.16), bovine serum albumin (BSA, MW 66 K), human serum albumin (HSA, MW 66.47 K), Ascorbic acid (MW 176.13), Dopamine (MW 153.18), Urea (MW 60.06) and Lactic acid (MW 90.08) were used for specific detection, which were purchased from Sigma-Aldrich.

2.2. Synthesis of GO/APBA composites

Before the synthesis, the GO (5 mg) dispersed in deionized water (5 mL) for the dispersion of GO nanosheets. The obtained solution was sonically oscillated in ice-water bath (0 °C) for 1 h and centrifuged for 15 min to obtain the dispersed GO supernatant (1 mg/mL). APBA (2 mg) was dispersed in ethanol (2 mL) and oscillated at 2800 r.p.m for 1 min with Lab Dancer (IKA, German) to obtain the APBA solution (1 mg/mL).

Combination of the GO with the APBA was achieved by linking the carboxyl groups of the GO to the amine functional groups on the APBA through dehydration condensation reaction, which was marked in red (Fig. 1A). This reaction carried out by a two-step conjugation in aqueous solvents: carboxyl firstly mixed with EDC for NHS reaction, resulting in a semi-stable NHS ester. Then amine reacted with the ester to form stable covalent bonds. In the study, EDC (2 mg) was added into the GO supernatant (5 mL) and oscillated for 1 min. Then NHS (5 mg) was added to the mixed solution and ultrasonically oscillated for 1 h to generate semi-stable NHS ester. After that, APBA (2 mL) was added into the mixture supernatant and oscillated for 1 min. The mixture was incubated at 0 °C for 4 h. Next, it was centrifuged at 12000 r.p.m for 2 h and the precipitates were the composites of GO/APBA. Finally, the GO/APBA composites were dissolved in deionized water with the concentration of 1 mg/mL. Fig. 1B showed the test principle of glucose with APBA. The APBA can bind with D-(+)-Glucose via cyclic ester bond formation.

In order to the show the nanostructures of the nano-composites, absorption spectra of the GO, APBA and GO/APBA composites were recorded on the optical device, which included a halogen cold light source (DT-MINI-2, Ocean Optics Inc., Dunodin, USA) and a spectrophotometer (USB 2000+, Ocean Optics Inc., Dunodin, USA). During the optical measurement, response events were monitored in optical absorption spectra. The range of the absorption spectra was from 200 nm to 600 nm with 0.38 nm interval.

2.3. Pretreatment of screen printed electrodes

The screen printed electrodes fabricated by continuous web-fed screen printing press technology, and represented an attractive avenue for routine electrochemical sensing. As shown in Fig. 1C, the carbon electrode was printed on 3 cm × 1 cm polyethylene glycol terephthalate substrate as working electrode and counter electrode, while the silver electrode was printed as the reference electrode. The diameter of the working electrode was 5 mm. The bottom of the screen printed electrodes were also made of silver, which were used as electrical connects. So, the electrodes could be linked to the electrochemical workstation with special socket.

Electrochemical preconditioning represented a simple and effective approach for improving the voltammetric behavior of screen printed electrodes. The electrodes were activated in 100 μL PBS solution by applying anodic potentials (ranging from +0.2 V to +2 V) for 5 min. Following activation, the electrodes were rinsed with distilled water and prepared for modification.

2.4. Functionalization of the screen printed electrodes

The electrochemical reduction of GO/APBA to rGO/APBA and the deposition of rGO/APBA on the screen printed electrodes surface were carried out by cyclic voltammetry on a CHI660E electrochemical workstation (Shanghai Chen Hua Apparatus Corporation, China). The cyclic voltammetry scanning was performed on the electrodes during −1.2 V–+1.2 V, with a scan rate of 50 mV/s. Na_2SO_4 solution of 0.1 M was employed as electrolyte to display the

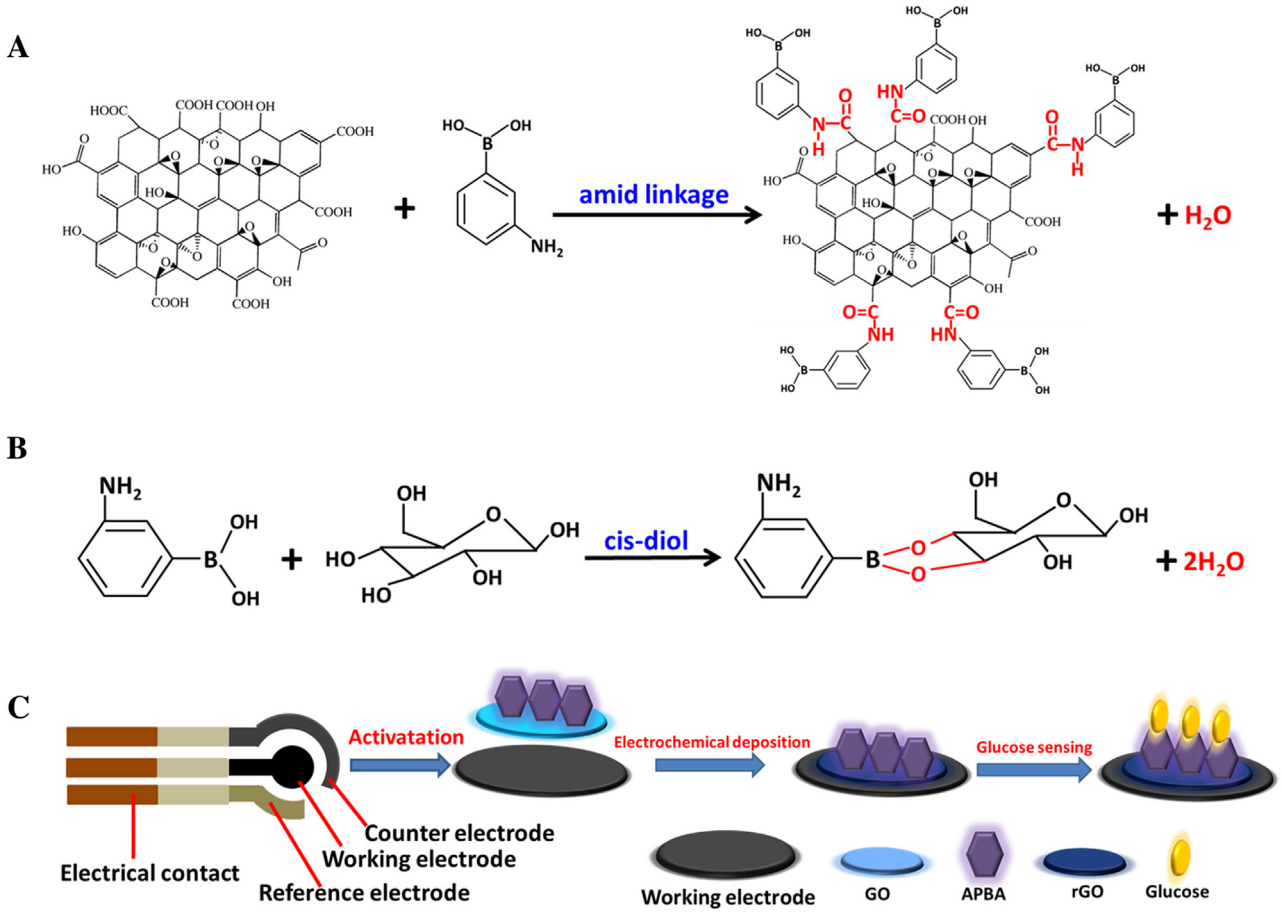

Fig. 1. Glucose detection with rGO/APBA modified screen printed electrodes. (A) Schematic illustration of the formation of GO/APBA nanosheets; (B) the mechanism of glucose linking to APBA. (C) The functionalization of the screen printed electrodes and glucose detection.

peak position of the rGO/APBA and the peak changes during the electrochemical deposition and reduction processes. As shown in Fig. 1C, 100 μL GO/APBA solutions at 1 mg/mL were spotted on the surface of working electrode. Cyclic voltammetry scanning was carried out for 40 segments to accomplish the reduction of GO/APBA to rGO/APBA and the deposition of rGO/APBA on electrodes. With the same method, the rGO modified electrodes were made for further detection.

In order to explore the stability of the electrochemical deposition, the direct physical absorptions were utilized for the modification of the electrodes with GO/APBA. 100 μL GO/APBA solutions (1 mg/mL) were spotted on the surface of working electrode. The solutions were diffused and distributed evenly on the electrodes due to the surface tension. The functionalized screen printed electrodes were dried for 12 h at room temperature (~20 °C). After the solutions dried, the GO/APBA composites were absorbed on the surface of the electrodes, which could be used for glucose detection.

Scanning electron microscopy (SEM) images of GO/APBA and rGO/APBA modified screen printed electrodes were taken on HITACHI UHR FE-SEM SU 8010, at an accelerating voltage of 3000 V. The functionalized screen printed electrodes could be stored under room temperature and kept bioactivity more than 1 week.

2.5. Glucose sensing with the impedance spectroscopy

The impedance spectra were performed by electrochemical workstation (CHI660E, ChenHua, China) to evaluate the frequency impedance properties of glucose. Before measurement, the screen printed electrodes linked to the electrochemical workstation with a special socket. Taking PBS and human serum as solvent respectively, the glucose were prepared for different concentrations, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM, and 50 mM. For the detection of glucose, the rGO/APBA modified electrodes were firstly incubated in different concentrations of glucose (100 μL) at room temperature for 30 min. After washing with PBS, the impedance spectra were recorded for glucose detection. In the impedance spectroscopy measurement, the 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1:1) mixture (100 μL) was employed as redox couple on the electrodes surface. The frequency was scanned from 1 Hz to 100 kHz with waveform magnitude of 20 mV. The PBS solution was used as blank control. After each measurement, the tested solutions were removed out of the electrodes with 500 μL PBS for three times to remove the organic residues. Parallel experiments for each concentration were tested three times. The blood glucose monitor (ACCU-CHEK Inc., China) was used as the classical approaches for sensing glucose in real biological samples. Using the needle to prick the volunteer's index finger for 200 μL blood, which were measured in the blood glucose monitor and our rGO/APBA modified electrodes.

In order to illustrate the principle of APBA in glucose detection and eliminate the influence of rGO. rGO modified electrodes were further used in glucose detection with same method as above mentioned. Besides, GO/APBA modified electrodes via physical absorption, were also explored for the glucose detection with impedance spectroscopy. We also applied the rGO/APBA modified electrodes in the detection of NaCl, KCl, $CaCl_2$, glutamic acid, cysteine, BSA, HSA, ascorbic acid, dopamine, urea and lactic acid

for specific detection of glucose. The concentrations of these substances were all 10 mM with the PBS and human serum as solvent, respectively. All of the detections were performed at room temperature (~20 °C).

In the data analysis, characteristic resistances were calculated into normalized impedance change (NIC), which was described as:

$$NIC = (Z_a - Z_b)/Z_b \quad (1)$$

In the Formula (1), Z_a and Z_b represent the impedance of working electrode after and before the glucose stimulation, respectively. The screen printed electrodes were all disposable in the measurements.

3. Results

3.1. Formation and characterization of rGO/APBA modified electrodes

Cyclic voltammetry and impedance spectroscopy were two effective techniques in probing the interface properties of surface modified electrodes. In our study, the cyclic voltammetry was utilized to accomplish the electrochemical reduction of GO/APBA to rGO/APBA and the deposition of rGO/APBA on screen printed electrodes. SEM images of GO/APBA and rGO/APBA were used to display the achievement of the reduction and deposition. Besides, impedance spectra were used to characterize the rGO/APBA modified electrodes.

Typically, the electrochemical synthesis of rGO was carried out via two steps. Firstly, GO was assembled on the electrodes by solution deposition methods, and secondly it was subjected to electrochemical reduction. Cyclic voltammetry as one of the electrochemical methods not only could achieve the deposition process, but also the reduction reaction. Therefore, in our study, the one step electrodeposition technique was proposed to prepare rGO/APBA on electrodes directly from GO/APBA dispersions, which accomplished the reduction and deposition of rGO/APBA on electrodes, simultaneously. Before the one step electrochemical deposition and reduction, absorption spectra of the GO, APBA and GO/APBA composites were measured to characterize the synthesis of the nano-composites. As shown in Fig. 2A, the absorption spectrum of APBA had a wavelength of maximum absorbance at 275.5 nm. The absorption spectrum of GO had a wavelength of maximum absorbance at 235.4 nm attributed to the π–π^* of the C—C aromatic rings [33,34]. The GO/APBA composites showed wave peaks both in 235.4 nm and 275.5 nm, which indicated the synthesis of the nano-composites. Fig. 2B showed the cyclic voltammetry recorded during the reduction process. The cathodic peak marked with red in Fig. 2B was due to the electrochemical reduction of GO [11,35]. The persistent decrease of the peak currents with successive cyclic voltammetry scanning indicated that the reduction of GO/APBA indeed was achieved. What's more, the inset (Fig. 2B) showed that after a certain number of cycles (about 20), the changes arrived at stabilization.

Fig. 2C and D showed the SEM images of GO/APBA and rGO/APBA composites, respectively. Despite GO can form well dispersed aqueous colloids with layered structure, the reduction of GO sheets could result in their agglomerate [36,37]. As can be seen, before deposition, the GO/APBA showed a smooth surface with some wrinkles (Fig. 2C). After electrochemical deposition, a rough surface could be clearly observed owing to the formation of the rGO/APBA on the electrodes surface (Fig. 2D). Therefore, it was reasonable to speculate that the rGO/APBA achieved the attachment to the electrodes surface. Considering the results showed in Fig. 2, it could come to the conclusion that the electrochemical reduction of GO/APBA to rGO/APBA and the deposition of rGO/APBA on the electrodes surface was completed.

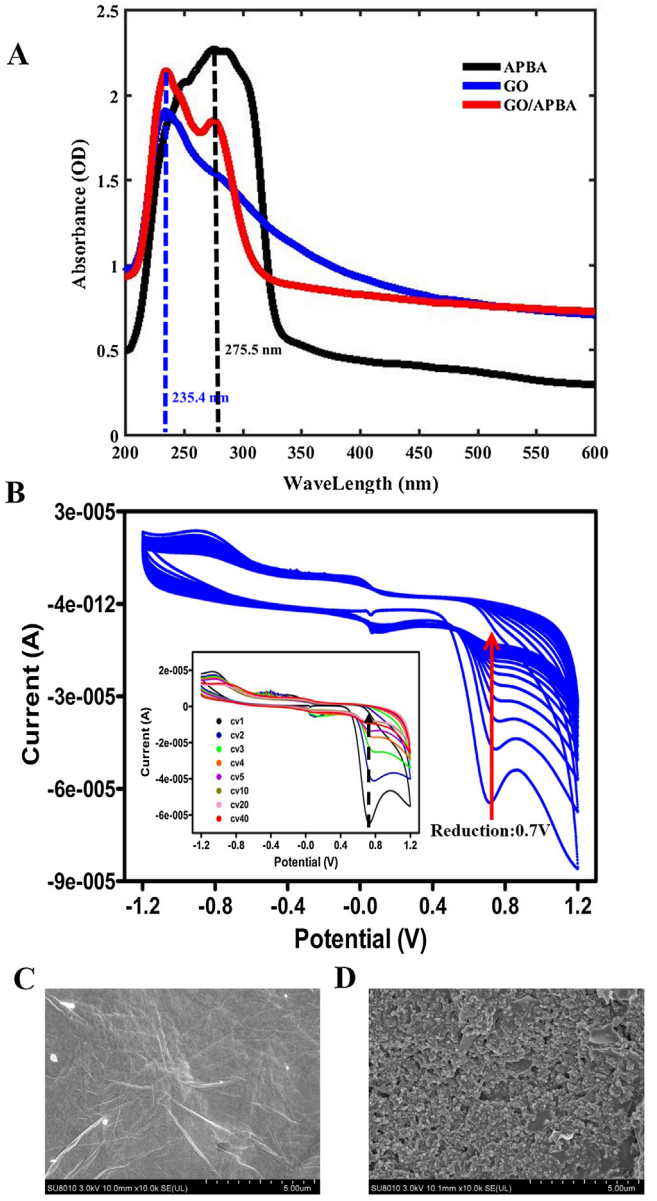

Fig. 2. The formation and characterization of rGO/APBA on electrodes. (A) The characterization of APBA, GO and GO/APBA composites in absorption spectra. (B) Cyclic voltammograms of the electrochemical reduction of GO/APBA to rGO/APBA in 0.1 M Na_2SO_4 at 50 mV/s (Segment: 40. Inset: Cyclic voltammograms at different segments). (C) SEM images of GO/APBA composites, and (D) rGO/APBA composites (magnification: 10000).

The electrochemical reduction removed partial oxygen from GO and changed electrical conductivity of the electrode. As shown in Fig. 3A, the impedance spectroscopy of the rGO/APBA modified electrodes had lower impedance than the blank electrodes. The excellent electron transfer ability of rGO, indicating that the introduction of the rGO played an important role in the increase of the electroactive surface area and provided the well conducting bridges for the electron transfer. Therefore, the rGO/APBA modified electrodes offered a high sensitivity sensing platform for further bio-detection.

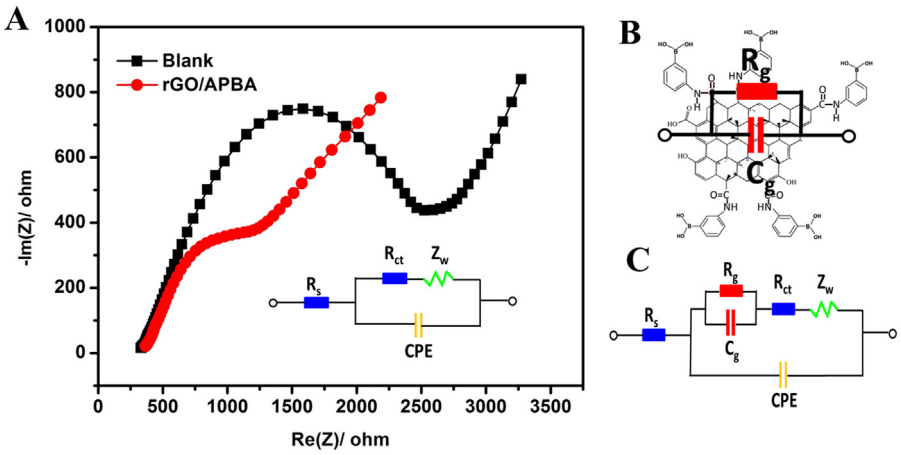

Fig. 3. (A) The electrical impedance spectra of rGO/APBA modified screen printed electrodes. (The black line with squares: bare electrodes without rGO/APBA. The red line with circle: rGO/APBA modified electrodes. Inset: Randle element for electrochemical impedance.) (B) Structural formula of the rGO/APBA with the equivalent circuit; and (C) equivalent circuit of rGO/APBA functionalized electrodes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Equivalent circuit of rGO/APBA modified electrodes

As shown in Fig. 3A (the black line with squares), a typical impedance spectroscopy plot included a semicircle region and a straight line. The semicircle part observed at the high frequency, was corresponding to the electron-transfer-limited process. And the linear part at the low frequency stood for the diffusional-limited electron-transfer process. The semicircle diameter was equal to the charge transfer resistance (R_{ct}). Usually, the impedance spectroscopy could be analyzed by the Randles circuit [38,39]. It is shown in Fig. 3A (Inset), the traditional Randles circuit includes the solution resistance (R_s), charge transfer resistance (R_{ct}), warburge impedance (Z_W), and constant phase element (CPE). R_s and Z_W represented the properties of the electrolyte solution. R_{ct} and CPE were often affected by the property changes occurring at the electrode/electrolyte interface.

Graphene has drawn enormous attention in semiconductor applications [4,40]. It has been reported that graphene can be expressed using an equivalent circuit model with a combination of resistance and capacitance (RC) networks [41,42]. Therefore, in our study the rGO/APBA composites were equivalent to the parallel connection of R_g and C_g (Fig. 3B). Based on the theory and experimental results, the equivalent circuit model of the system was simulated. It is shown in Fig. 3C, the circuit consists of the traditional Randles circuit and the rGO/APBA equivalent model. During the detection, the glucose could connect to the rGO/APBA composites with cyclic ester bond, which could induce the changes of R_g. Hence, R_g might play an important role in the glucose impedance sensing. In the analysis of normalized impedance changes, R_g was extracted as the parameter with the changes of glucose concentration.

3.3. Impedance detection of glucose with rGO/APBA modified electrodes

The glucose adsorbed on the modified electrodes surface then resulted in the increase of the electrochemical impedance responses. The responses of impedance to different concentrations of glucose were examined by impedance spectroscopy.

Fig. 4A showed the impedance spectra of rGO/APBA modified electrodes in glucose detection in PBS. The impedance spectra at lower frequencies changed obviously, while that at higher frequencies almost coincided. It indicated that interactions between glucose and rGO/APBA lead the impedance of R_g to increase. Based on the equivalent circuit (Fig. 3C), the impedance of R_g was extracted. It could be found that with the concentrations of glucose increased from 0.1 mM to 50 mM, the impedance of R_g rose obviously. Fig. S1 (in the Supplementary Materials) showed the original changes of the Rg with different concentrations of glucose. Through calculating the resistance of R_g in different impedance spectra, the results of the normalized impedance changes of R_g (Fig. 4A inset) could be obtained. Moreover, the r^2 was found to be 0.9577 and the slope was 0.7448, which showed a significant linearity and sensitivity. Consecutive measurements were repeated for three times in our study. The experimental results indicated good regeneration of the biosensor. Fig. 4B showed the impedance spectra of rGO/APBA modified electrodes on glucose detection in human serum. Similar to Fig. 4A, the impedance spectra at lower frequencies changed obviously. While at higher frequencies had lower consistency compare to the detection in PBS due to the complexity of the human serum. The inset of Fig. 4B illustrated that the impedances in serum were higher than that in PBS. Fig. 4C showed the results of the normalized impedance changes of Rg on glucose detection in human serum. The r^2 was 0.9241 and the slope was 0.7242, which also showed a well linearity and sensitivity.

In order to evaluate our method, the blood glucose monitor (ACCU-CHEK Performa) was used as the control group for sensing glucose in real biological samples. The result showed in Fig. 4C (the red cross). The testing result of blood glucose showed in ACCU-CHEK Performa was 5 mM. Our impedance testing result was 4.8 mM. And the error was 4%. Therefore, our sensor can also applied in real biological samples.

3.4. Impedance detection of glucose with GO/APBA modified electrodes

In order to explore the stability and convenience of the electrochemical deposition, GO/APBA modified electrodes with the method of direct physical absorption were used in the determination of glucose as the contrast group. Comparing to the direct cyclic voltammetry deposition in about 20 segments (8 min), the GO/APBA modified electrodes needed to dry for 12 h at room temperature (~20 °C). What's more, on account of the high impedance characteristics of GO/APBA, there were not obvious impedance changes during the determination of glucose (Fig. 5A). As shown in Fig. 5A inset, the r^2 and slope of the fit curve were 0.8737 and 0.05856, respectively. They were all lower than the results showed in Fig. 4A. It could be found in Fig. 5B, the rGO/APBA modified electrodes had the lower basal impedance than the GO/APBA modified

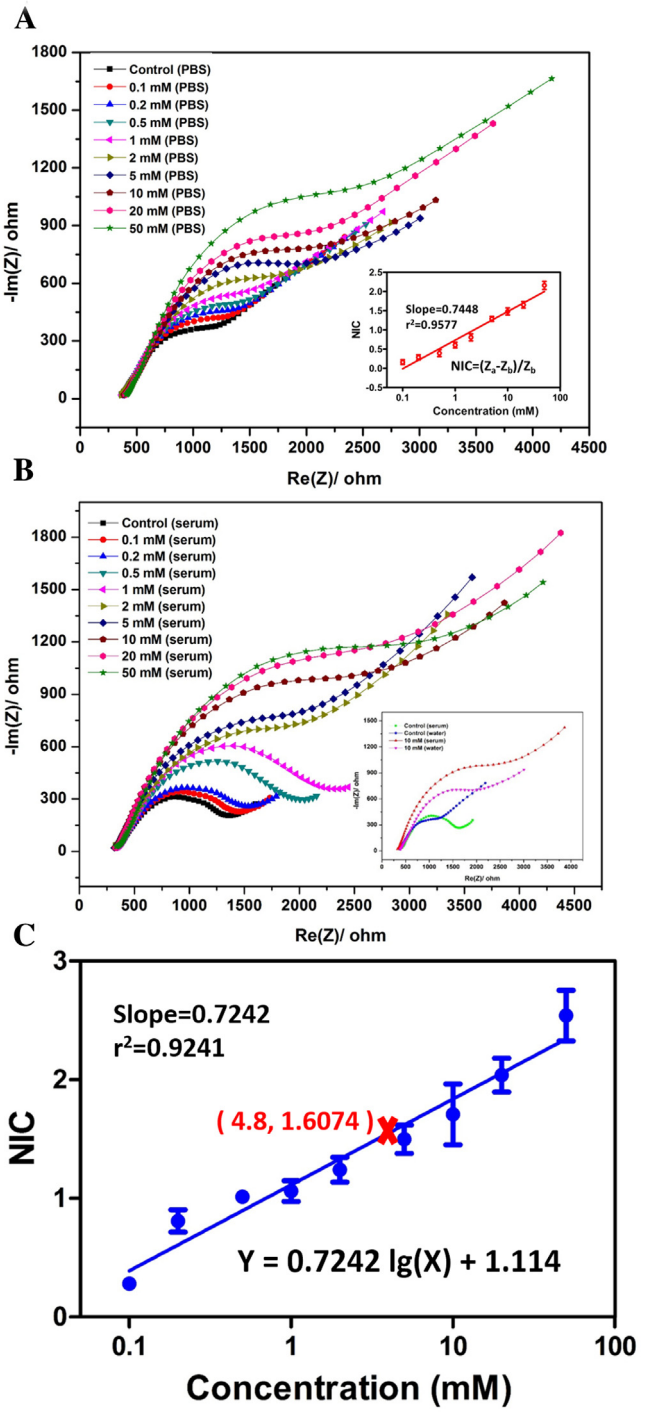

Fig. 4. Impedance spectra of rGO/APBA modified electrodes in glucose detection. (A) Glucose detection in PBS (Inset: The normalization of the impedance of Rg). (B) Glucose detection in human serum (Inset: the comparison between PBS and serum). (C) The normalization of the impedance of Rg in glucose detection (human serum) and real biological sample (the red cross). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

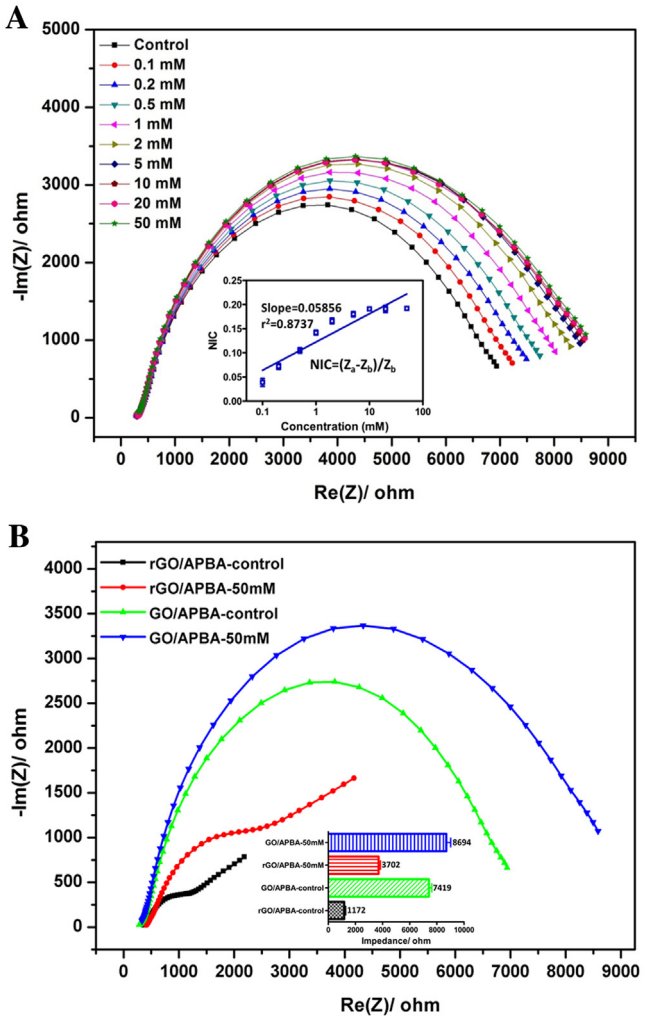

Fig. 5. (A) Impedance spectra of GO/APBA modified electrodes in glucose detection (Inset: The normalization of the impedances under different concentrations). (B) The comparison between GO/APBA and rGO/APBA modified electrodes (Inset: The equivalent impedance of the impedance spectra).

electrodes. At the same time, Fig. 5B inset showed the specific values. The control group of the rGO/APBA modified electrodes had the impedance of 1172 Ω. While the group of the GO/APBA modified electrodes had the impedance of 7419 Ω, which was six times greater than the impedance showed in rGO/APBA modified electrodes (1172 Ω). As shown in Fig. 5B inset, in the glucose detection (50 mM), the GO/APBA modified electrodes had the impedance of 8694 Ω, which increased 17.2% than the control group. While the rGO/APBA modified electrodes had the impedance of 3702 Ω in glucose detection (50 mM), which increased 215.9% than the control group. The rate of the change with the rGO/APBA modified electrodes was twelvefold greater than the GO/APBA modified electrodes.

Therefore, with the low basal impedance, rGO/APBA showed a high sensitivity in the determination of glucose. The shapes of the impedance spectra with different modified method of electrodes had significant differences. And the GO/APBA modified electrodes showed instability with repeated usage and the lack of modifier, especially in the determination of high concentration. So, it could come to the conclusion that the modification method of physical absorptions had poorer stability and sensitivity than the electrochemical modification.

3.5. Specific detection of glucose

In order to confirm the specificity of the APBA in glucose detection, rGO alone modified electrodes were used in the determination of glucose as the contrast group. As shown in Fig. 6A, the shape of the impedance spectra were similar to the impedance spectra showed in Fig. 4, while the semicircle impedance was smaller than that showed in Fig. 4. The reasons of above results were based on the same modification method and the lack of APBA, respectively.

Table 1
Comparison of our sensor with other glucose sensors (LoD: Limit of detection; SERS: Surface enhanced Raman spectroscopy; FET: Field effect transistor; LSPR: Localized surface plasmon resonances; GNPs: Gold nanoparticles; EIS: Electrochemical impedance spectroscopy; SPE: Screen printed electrodes).

Technique	Probes	Devices	Linear range	LoD	Reference
Fluorescence	Boronic acid	Carbon Dots	9 μM – 900 μM	1.5 μM	[50]
SERS	Alkyne/Boronic acid	Planar substrate	100 μM – 100 mM	100 μM	[36]
Electricity	APBA	FET	1 μM – 100 mM	300 nM	[51]
LSPR	APBA/GNPs	Glass substrate	1 mM – 50 mM	50 μM	[52]
Fluorescence	rGO/PBA	Nanosensor	2 mg/mL – 75 mg/mL	2 mg/mL	[29]
EIS	rGO/APBA	SPE	0.1 mM – 50 mM	30 μM	Present work

It could also be found in Fig. 6A inset, there was no corresponding relevance between the concentration and the impedance. Comparing with rGO/APBA modified electrodes, the statistical results showed in Fig. 6B illustrated the rGO modified electrodes hardly had any specificity in glucose detection. Besides, the detections of NaCl, KCl, $CaCl_2$, glutamic acid, cysteine, BSA, HSA, ascorbic acid, dopamine, urea, and lactic acid were also applied on the rGO/APBA modified electrodes. Among them, NaCl, KCl and $CaCl_2$ were inorganic salt; glutamic acid and cysteine were amino acid; BSA and HSA were protein; ascorbic acid, dopamine and urea were important biological molecules; lactic acid was the product of the sugar metabolism. As shown in Fig. 6C, under the same concentration (10 mM), the glucose had the obvious response of NIC. The results showed in serum were higher than that in PBS. The ascorbic acid also had some response of NIC. While, because of the instability of ascorbic acid, the response was also instability. The NaCl, KCl, and $CaCl_2$ had the negative values of the NIC, which could attribute to the decrease of the impedance. Because the redox couple also contained the KCl, the decrease degree of impedance of KCl was the most obvious among NaCl and $CaCl_2$. The inset of Fig. 6C showed that lactic acid had little influence on the glucose detection both in PBS and human serum. Sum up, the rGO/APBA modified electrodes by electrochemical reduction technique showed well sensitivity and specificity for glucose determination.

4. Discussion

4.1. Electrochemical reduction and deposition of rGO on electrodes

GO is composed of sp^2 and sp^3 carbon atoms with some oxygen-containing groups such as carboxyl, hydroxyl, and epoxide [5,14]. The hydroxyl and epoxy functional groups are on the hexagonal network of carbon atoms and the carboxyl groups are at the edges. The carboxyl groups provide the possibility of amide bond formation with amine groups. So GO could easily be modified by various species, such as proteins, nucleic acids, and nanoparticles [15–17]. However, the electroconductivity of GO was poor, which may have influence on the sensitivity of detection.

rGO possesses a unique combination of electrical, mechanical, optical, and thermal properties, which has been widely used in biochemical sensing [22,43]. In order to obtain rGO, GO is reduced by strong reductant such as hydrazine or $NaBH_4$, thermal annealing, electrochemistry, which result in the reconstruction of the sp^3 carbon atoms to sp^2 carbon atoms on GO sheets. However, the use of toxic substances, long time, many steps, high temperatures, and harsh conditions may increase the difficulties of reduction process. In our study, the cyclic voltammetry was proposed for the reduction of GO/APBA to rGO/APBA, with Na_2SO_4 as the electrolyte solution. The results showed in Fig. 2B, indicated that the reduction time of GO/APBA to rGO/APBA was about 20 segments (8 min). Thus, the reduction method of cyclic voltammetry could be explored as the fast and green reduction method of GO.

On the other hand, it is of equal importance on the modification of the electrodes. Traditional methods were based on the physical absorptions, which were of low controllability and reusability [44,45]. The electrochemical deposition method was used to modify the electrodes, which was time saving and stable. Fig. 2C and D showed the achievement of the attachment of rGO/APBA to the electrodes surface. The GO/APBA showed a smooth surface, which was easy to exfoliate. It was not beneficial for the analytes detection. While, a rough surface showed in the rGO/APBA modified electrodes could enhance the reaction bonding during the biodetection. In glucose detection, results also showed that the rGO/APBA functionalized electrodes via electrochemical deposition had well reusability and linearity (Fig. 4). However, the GO/APBA functionalized electrodes via physical absorptions were unstable when detected high concentrations of glucose (Fig. 5). Therefore, the electrochemical reduction and deposition showed an extensive application prospect in biodetection.

4.2. rGO/APBA nano-composites for glucose detection

Numerous methods have been tried for the detection of glucose. Most of the traditional approaches employ the oxidation of glucose by the enzyme glucose oxidase or glucose dehydrogenase [46,47]. Although there was a good selectivity of high sensitivity of enzymatic electrochemical glucose sensors, significant constraints such as chemical instability of glucose oxidase, troublesome production of electrodes, and difficult storage of enzymes have led to the development of non-enzymatic glucose sensors [48,49]. In recent years, boronic acid and various molecules containing the boronic acid have drawn an immense interest in the fabrication of glucose sensors based on fluorescence, surface enhanced raman spectroscopy, electricity, and electrochemistry (Table 1). Further, nanoparticles, graphene and its derivatives have also been explored to develop non-enzymatic glucose sensors with different types of electrodes.

In our study, the rGO/APBA nano-composites were applied as sensitive probes for the determination of glucose. The rGO/APBA came from the electrochemical reduction of GO/APBA. GO possesses modifiable oxygen containing groups, which appears as an interesting route to develop nano-composites. It has been demonstrated that covalent attachment of GO to APBA has a more significant effect than physical mixtures, because the filler particles become an integral part of nano sheet. However, the reduction of GO would removes the oxygen groups, which could reduce the covalent attachment between nano-composites. In order to confirm the functionalization of the APBA on electrodes, the GO/APBA nano-composites were synthesized firstly. Then, the GO/APBA composites were reduced to rGO/APBA on electrodes by cyclic voltammetry. The results showed in Fig. 4, illustrated that rGO/APBA functionalized electrodes had well linearity response to glucose.

Apart from traditional glassy carbon electrodes, glucose detection was also carried out by monitoring the changes in conductivity of field effect transistor (FET) devices (Table 1). Though, they showed high sensitivity in the glucose detection, the fabrication of the devices were a little complex. What's more, the modification of the devices needed the operation of high temperature [53]. Therefore, in this study, the screen printed electrodes were utilized

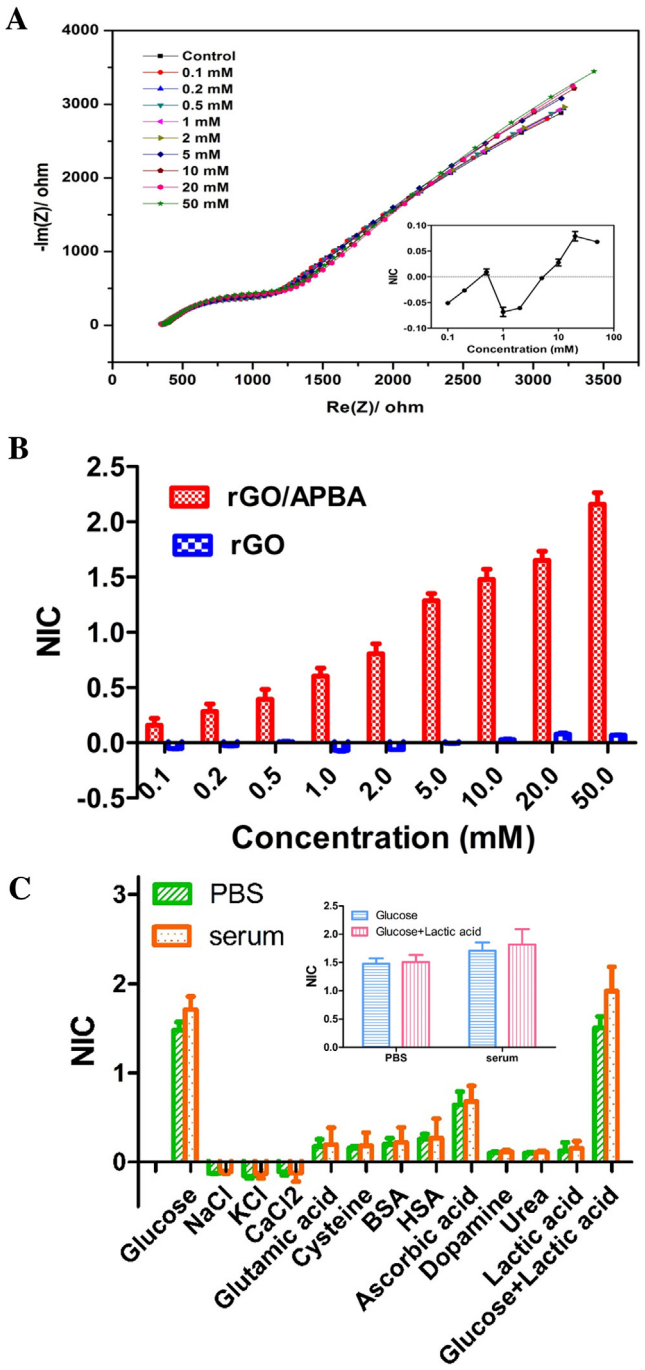

Fig. 6. (A) Impedance spectra of rGO modified electrodes in glucose detection. (Inset: The normalization of the impedances under different concentrations. The scale of the abscissa was logarithm for 10.) (B) The statistics of glucose detection with rGO/APBA and rGO modified electrodes under different concentrations. (C) The specific detection of rGO/APBA modified electrodes under different interferents (NaCl, KCl, $CaCl_2$, glutamic acid, cysteine, BSA, HSA, ascorbic acid, dopamine, urea and lactic acid) with the same concentration of 10 mM in PBS (green slash) and serum (orange dot). Inset: the comparison between "glucose" and "glucose+ lactic acid". (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with the characteristics of simplicity, portability, manufacturability, disposability, and low cost as our sensitive electrodes. Results showed the long-time stability of the electrodes with a one week period, which promoted the practicability in biodetection.

Therefore, a green and convenient method for the modification of rGO on screen printed electrodes has been proposed in our study. The method implemented the electrochemical reduction and deposition by one step. For the inexpensive and disposable characteristics of screen printed electrodes and the sensitivity of the functionalized materials, our rGO modified electrodes could be widely used in rapid and versatile chemical and biological detections

5. Conclusion

A strategy for developing non-enzyme glucose sensors was proposed based on rGO/APBA functionalized screen printed electrodes. Through cyclic voltammetry, rGO/APBA nano-composites were directly deposited onto the electrodes with the reduction of the GO/APBA colloidal solution, which realized the reduction and deposition in one step. This easily and efficient modification method made the detection process for glucose more stable, which was attractive in the electrochemical detection field. At the same time, compared with GO/APBA and rGO, the rGO/APBA functionalized electrodes showed higher linearity and sensitivity for the detection of glucose. The reasons were the high conductivity of rGO and the sensitivity of APBA in glucose determination. The proposed biosensor showed good reproducibility, selectivity, and acceptable stability. The low cost of the material and the good conductivity of the electrodes made it a promising sensor in biological and chemical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.12.142.

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Biographies

Shuang Li received her bachelor degree in Hunan normal University in 2014. Now she is a Ph.D. student of biomedical engineering of Zhejiang University. Her work includes biosensors and electronic measurement.

Qian Zhang received her bachelor degree in Zhejiang University in 2012. Now she is a Ph.D. student of biomedical engineering of Zhejiang University. Her work includes biosensors and electronic measurement.

Yanli Lu received her bachelor degree in Xi'an Jiaotong University in 2012. Now she is a Ph.D. student of biomedical engineering of Zhejiang University. Her work includes biosensors and electronic measurement.

Daizong Ji received his bachelor degree in University of Shanghai for Science and Technology in 2011. Now he is a Ph.D. student of biomedical engineering of Zhejiang University. His work includes biosensors and electronic measurement.

Diming Zhang received his Ph.D. degree in biomedical engineering from Zhejiang University in 2016. Now he is a Post doctorate in Duke University. His work includes biosensors and electronic measurement.

Jiajia Wu received her bachelor degree in Zhejiang University in 2016. Now she is a Ph.D. student of biomedical engineering of Zhejiang University. Her work includes biosensors and electronic measurement.

Xing Chen received his BS degree and Ph.D. degree in biomedical engineering from Zhejiang University, PR China in 2003 and 2008, respectively. Then, he finished his postdoctoral research in the UT M. D. Anderson Cancer Center, USA, in 2010. He is currently a professor of Biosensors National Special Lab, Department of Biomedical Engineering of Zhejiang University. His research interests include biomedical sensors and epidemiology.

Qingjun Liu received his Ph.D. degree in biomedical engineering from Zhejiang University, PR China in 2006. He is currently a professor in Biosensor National Special Laboratory, Zhejiang University. He is also a visiting scholar in the Micro and Nanotechnology Laboratory (MNTL) at the University of Illinois at Urbana-Champaign (UIUC). He published the book of Cell-Based Biosensors: Principles and Applications, by Artech House Publishers USA in October 2009. His research interests concentrate on the biosensors (e.g. living cell sensor, DNA sensor and protein sensor) and BioMEMS system.