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Bienzyme bionanomultilayer electrode for glucose biosensing based on functional carbon nanotubes and sugar–lectin biospecific interaction

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ABSTRACT

Bienzyme bionanomultilayer electrode for glucose biosensing was constructed based on functional carbon nanotubes and sugar–lectin biospecific interaction through layer-by-layer (LBL) assembly. After being functionalized by wrapping with polyelectrolyte, multiwalled carbon nanotubes (MCNTs) were water soluble and positively charged. MCNT–bienzyme bionanomultilayer electrode was then fabricated by LBL assembly of horseradish peroxidase (HRP) and glucose oxidase (GOD) on functional MCNT modified electrode. The attachment of the MCNT–bienzyme bionanomultilayer with the underlying electrode and each layer in the bionanomultilayer was based on reliably electrostatic or sugar–lectin biospecific interaction. The developed bienzyme biosensor exhibited fast amperometric response for the determination of glucose. The linear response of the developed biosensor for the determination of glucose ranged from 2.0×10^{-6} to 1.7×10^{-4} M with a detection limit of 2.5×10^{-7} M. The biosensor can be used directly to determine glucose in serum. The construction of the bienzyme biosensor showed potential for the preparation of MCNT–enzyme nanocomposite with controllability and high performance.

Owing to the clinical significance of measuring blood glucose levels, sensitive and rapid monitoring of glucose has gained significance in diagnosis [1–3]. Among developed detection techniques, amperometric enzyme biosensors have been generally considered for simple, inexpensive, and sensitive determination. A bienzyme electrochemical biosensor based on horseradish peroxidase (HRP)[1] and glucose oxidase (GOD) is inherently attractive because GOD is catalytically linked to HRP. Briefly, HRP acted as amperometric transducer for the detection of its substrate, H_2O_2, that was in situ generated by GOD-catalyzed oxidation of glucose [2–5]. To develop a reliable bienzyme biosensor, the technique used to immobilize bienzyme and efficient incorporation of functional material is of great significance for improving the performance of biosensors.

Carbon nanotubes (CNTs) represent a new class of nanomaterials. It has been proved that CNTs can act as molecular wires to accelerate electron transfer between the underlying electrode and redox centers of enzymes. Moreover, CNTs are predominant materials for biomolecule immobilization owing to the high accessible surface area [6–8]. Several studies have reported the immobilization of HRP/GOD bienzyme on CNT matrix for preparing electrochemical biosensors. For example, Sheng and Zheng [9] covalently attached HRP/GOD onto carboxyl-functionalized multiwalled carbon nanotubes (MCNTs) by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) activation for the deposition of electroactive polyaniline (PANI). Shobha Jeykumari and Sriman Narayanan [10] used neutral red (NR) to functionalize MCNTs by simple mixing to prepare reagentless bienzyme biosensors. To strengthen the attachment of CNTs to the underlying electrode, Nafion is used, and the bienzyme electrode was obtained by casting a mixture of Nafion, functionalized MCNTs, HRP, and GOD. Shobha Jeykumari and Sriman Narayanan used thionine (Th) or toluidine blue (TB) to covalently functionalize carboxylic MCNTs via EDC/NHS activation. Similar casting of mixtures containing Nafion, functional MCNTs, HRP, and GOD was then performed to make bienzyme modified electrode [11,12]. Although the use of Nafion could strengthen the attachment of CNTs and bienzymes to the underlying electrode, a time-consuming and uncontrollable process might be involved in such a manual casting or dip-coating method. Because the thickness of the resulting film was difficult to control, the biosensor fabrication was easily irreproducible. Therefore, the introduction of reliable interaction and the preparation of MCNT–bienzyme nanocomposite with controllability and high order are highly desired.

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[1] *Abbreviations used:* HRP, horseradish peroxidase; GOD, glucose oxidase; CNT, carbon nanotube; MCNT, multiwalled carbon nanotube; EDC/NHS, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide/*N*-hydroxysuccinimide; PANI, polyaniline; NR, neutral red; Th, thionine; TB, toluidine blue; LBL, layer-by-layer; ConA, concanavalin A; GCE, glassy carbon electrode; PAH–MCNT, poly(allylamine hydrochloride)-wrapped MCNT; Au, gold; PSS, poly(sodium-*p*-styrene-sulfonate); MPS, 3-mercapto-1-propanesulfonic acid, sodium salt; Si, silicon; Pt, platinum; SEM, scanning electron microscopy; PBS, phosphate-buffered saline; SCE, saturated calomel electrode; EIS, electrochemical impedance spectroscopy; CV, cyclic voltammogram; PDDA, poly(diallyldimethylammonium chloride); LOD, limit of detection; CS, chitosan; GS, graphene sheets; NP, nanoparticle; RSD, relative standard deviation.

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Nowadays, layer-by-layer (LBL) assembly represents an interesting methodology due to its simplicity and versatility. Ultrathin multilayer films with high order on a molecular scale could be obtained [13–15]. Despite electrostatic interaction usually being used for LBL assembly, strongly biospecific affinity between lectin such as concanavalin A (ConA) and sugar residues of sugar proteins can be used for constructing multilayer films [16]. For example, biospecific affinity between ConA and sugar chains intrinsically located on the surface of HRP and GOD has received increasing attention during recent years. Kobayashi and Anzai directly prepared HRP or GOD multilayer films on the surface of glassy carbon electrode (GCE) through biospecific affinity between ConA and HRP or GOD. In the presence of methylene blue, the modified electrodes served as a glucose sensor [16]. Our group constructed an HRP/GOD bienzyme biosensor based on such sugar–lectin biospecific interactions for amperometric determination of phenolic compounds and aromatic amines [17]. In addition, our group used polyelectrolyte to functionalize MCNTs by a direct premixing procedure and obtained water-soluble poly(allylamine hydrochloride)-wrapped MCNTs (PAH–MCNTs). Such PAH–MCNTs were positively charged and could assemble with negatively charged HRP via LBL electrostatic assembly. The uniform PAH–MCNT/HRP bionanomultilayer showed good bioactivity for the determination of H_2O_2 and reduced thiols [18].

In this work, continuing our interest in pursuing new controllable methods for a biosensing platform, MCNT–bienzyme bionanomultilayer electrode for glucose determination was constructed based on functional CNTs and sugar–lectin biospecific interactions by the LBL technique. In the prepared bienzyme electrode, the attachment of the MCNT–bienzyme bionanomultilayer with the underlying electrode and each layer in the bionanomultilayer was based on reliably electrostatic or biospecific interaction. Efficient incorporation of functional MCNTs and bienzyme was achieved through LBL assembly via four steps. First, polyelectrolyte precursor film was prepared on negatively charged gold (Au) electrode for the attachment of water-soluble PAH–MCNTs. Second, PAH–MCNTs containing positive charge were assembled with negatively charged HRP via LBL electrostatic interaction for optimized layers. Third, ConA was deposited on the PAH–MCNT/HRP multilayers, and ConA/GOD multilayers subsequently formed based on biospecific binding between HRP–ConA and ConA–GOD. Due to the reliable interaction in the fabrication process, the as-prepared biosensor possessed good performance and could be used directly to determine glucose in serum.

Materials and methods

Chemicals and reagents

HRP (EC 1.11.1.7, 250 U mg^{-1}) was purchased from Shanghai Sanjie Biotechnology. GOD (EC 1.1.3.4, 200 U mg^{-1}) and ConA from *Canavalia ensiformis* (jack bean) were purchased from Sigma. MCNTs (95%, Shenzhen Nanotech Port) were used in this study. Poly(sodium-*p*-styrene-sulfonate) (PSS) was obtained from Acros. PAH and 3-mercapto-1-propanesulfonic acid sodium salt (MPS) were obtained from Aldrich. All other reagents were of analytical grade and used without further purification. Polyelectrolyte solutions, including polycation PAH and polyanion PSS, were 1 mg ml^{-1} in glycine–NaOH buffer (pH 12.0) containing 0.4 M NaCl.

Apparatus and electrodes

Electrochemical measurements were performed on a CHI 650 electrochemical analyzer (Shanghai CH Instrument). A conventional three-electrode system was used with bare Au electrode or modified Au electrode as the working electrode, saturated calomel electrode as the reference electrode, and platinum (Pt) disk electrode as the auxiliary electrode. Scanning electron microscopy (SEM) images were obtained at 5.0 kV on a Sirion (FEI) field emission scanning electron microscope.

Preparation of functional MCNTs and polyelectrolyte precursor film modified electrode

The treatment of MCNTs was according to the previously reported procedure [19]. Briefly, MCNTs were purified by refluxing in 3 M nitric acid for 12 h. The solution was transferred to polytetrafluoroethylene centrifuge tubes and spun at 2400*g* for 2 h. After the supernatant acid was decanted off, the resultant solid was sonicated in concentrated HNO_3 and H_2SO_4 (1:3, v/v) for 6 h, followed by extensive washing and filtrating in double deionized water until the filtrate was neutral. Then the pH was adjusted to 8.0 to achieve MCNTs containing carboxylate anions that possessed negative charge. After that, the negatively charged carboxylated MCNTs were centrifuged at 14,000*g* for 30 min to remove the supernatant and were dried in vacuum at 50 °C. The obtained MCNTs were then functionalized by wrapping with polyelectrolyte (polycation, PAH) by a direct premixing procedure. In brief, the PAH–MCNTs were prepared by adding 1 mg ml^{-1} PAH into 1 mg ml^{-1} MCNT suspension. The resulting PAH–MCNTs were water soluble and positively charged.

Bare Au electrode was used as the supporting electrode. It was successively polished with Emery paper and 0.05 μm α-Al_2O_3 slurry. After being sonicated in deionized water for 5 min, the electrode was immersed in freshly prepared Piranha solution (30% H_2O_2 and concentrated H_2SO_4, 3:1, v/v) for 20 min. After ultrasonic rinsing, the electrode was electrochemically pretreated by cyclic potential scanning between 1.4 and −0.2 V in 0.1 M H_2SO_4 until a cyclic voltammogram of clean Au electrode was obtained. Afterward, clean Au electrode was immersed in 0.02 M MPS aqueous solution for 10 h to form a monolayer with negative charge. Then MPS modified electrode was alternately dipped into the polycation (PAH) and polyanion (PSS) solution for 20 min to form a PAH/PSS precursor film.

Construction of MCNT–bienzyme bionanomultilayer electrode

The procedure for constructing MCNT–bienzyme electrode (Au/precursor film/PAH–MCNT/HRP/ConA/GOD) is demonstrated schematically in Fig. 1. LBL assembly of $(PAH–MCNT/HRP)_n$ bionanomultilayer was formed by sequential deposition of PAH–MCNTs and HRP on precursor film modified electrode. The MCNT and HRP solutions both were 1 mg ml^{-1} in 0.04 M barbital buffer at pH 9.0 and 8.0, respectively.

Using biospecific interaction between ConA and HRP, the first layer of ConA could be assembled on $(PAH–MCNT/HRP)_n$. Typically, the electrode was dipped into a 0.5-mg ml^{-1} ConA dissolved in phosphate-buffered saline (PBS, pH 7.0) containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$ for 40 min. The obtained electrode was then immersed in GOD solution (0.5 mg ml^{-1} in PBS, pH 7.0) for 40 min to deposit GOD through biospecific ConA–sugar interaction. By alternately dipping the electrode in ConA and GOD solution, the deposition was repeated to prepare $(ConA/GOD)_n$ multilayers.

Preparation of $(PAH–MCNT/HRP)_n$ on Si wafer

The cleaned silicon (Si) wafer was first treated in 1.5 M NaOH for 20 min to create a negatively charged surface. Afterward, $(PAH–MCNT/HRP)_n$ films were assembled on the negatively charged Si wafer using a procedure identical to the one described

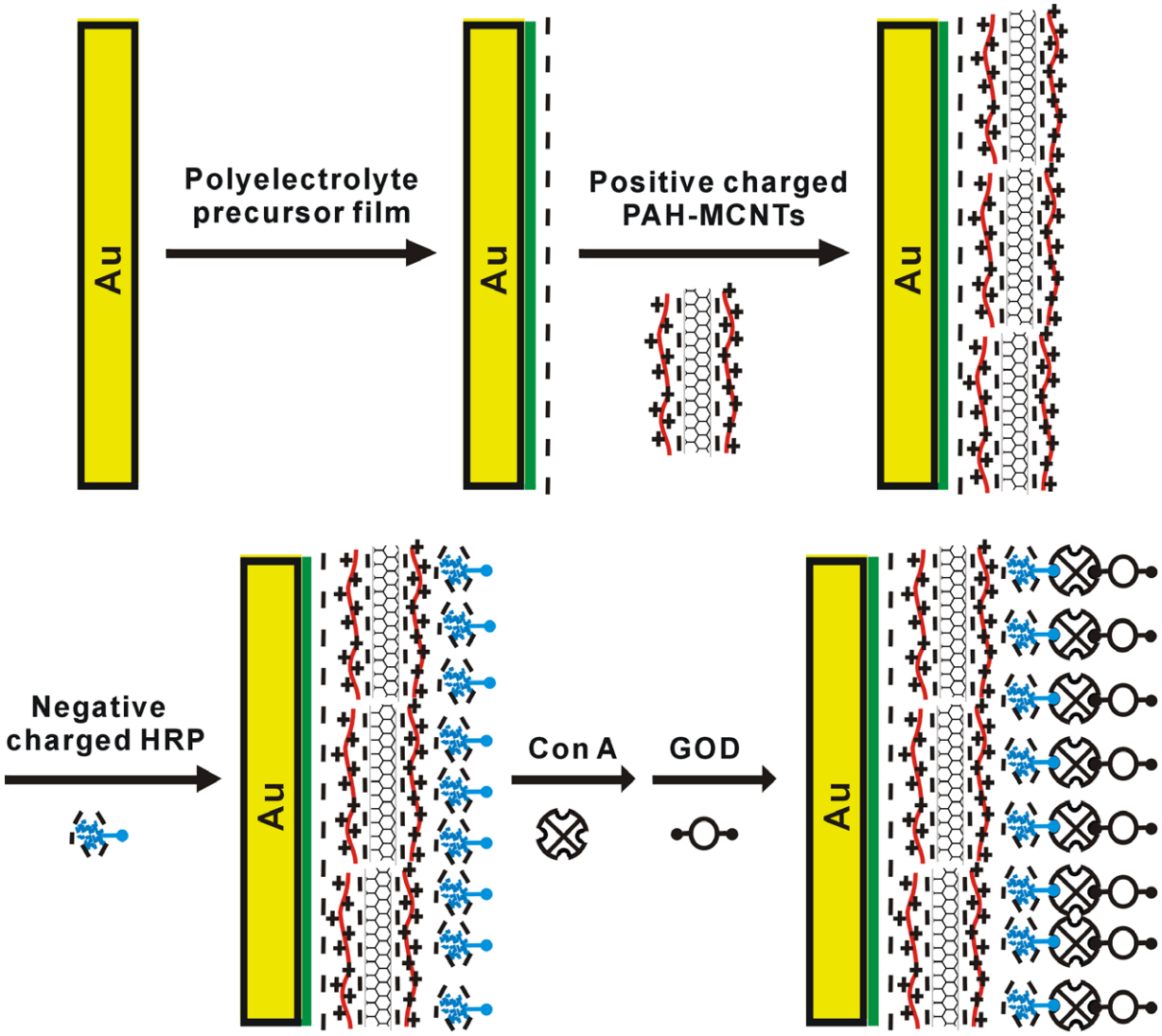

Fig. 1. Schematic illustration of the construction of bienzyme bionanomultilayer electrode based on functionalized CNTs and sugar–lectin biospecific interactions.

above. The modified Si wafer obtained after each assembly was used for SEM characterization.

Electrochemical measurements

The MCNT–bienzyme bionanomultilayer modified electrode was immersed in the supporting electrolyte (PBS, pH 6.5) containing an appropriate concentration of glucose at room temperature. Amperometric response was obtained at −0.15 V versus saturated calomel electrode (SCE), and typical steady-state response of the biosensor to successive injection of glucose was recorded with stirring. Electrochemical impedance spectroscopy (EIS) was performed in a 5.0-mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) mixture with the frequencies ranging from 10^4 to 10^{-1} Hz.

Results and discussion

Preparation of bienzyme bionanomultilayer electrode

In the current work, MCNT–bienzyme bionanomultilayer electrode for glucose determination was constructed based on functional CNTs and sugar–lectin biospecific interactions by controllable LBL technique. The attachment of the MCNT–bienzyme bionanomultilayer with the underlying electrode and each layer in the bionanomultilayer was based on reliably electrostatic or biospecific interaction.

The strategy for the preparation of bienzyme bionanomultilayer electrode involved four steps, as shown in Fig. 1. In this figure, the repeating cycle in LBL assembly is not included and the modified electrode is simply demonstrated as Au/precursor film/PAH–MCNT/HRP/ConA/GOD. First, interaction between Au electrode and mercapto compound, MPS, was used to form a uniform and tightly attached monolayer on Au electrode. Due to the presence of sulfonic groups, negative charge modified Au electrode was obtained. Second, PAH/PSS polyelectrolyte precursor film was subsequently prepared on MPS modified electrode via electrostatic assembly. Third, positively charged PAH–MCNTs assembled with negatively charged HRP for optimized multilayers. HRP contained net negative charges at assembly buffer (pH > p*I*). Fourth, ConA was deposited on PAH–MCNT/HRP multilayers, and ConA/GOD multilayers were subsequently formed based on biospecific binding.

A design of $(PAH–MCNT/HRP)_m/(ConA/GOD)_n$ was adopted in this work [17]. The effect of the number of PAH–MCNT/HRP layers or ConA/GOD layers on the response of the biosensors was evalu-

ated using electrodes modified with $(PAH–MCNT/HRP)_m/(ConA/GOD)_n$. For $(PAH–MCNT/HRP)_m/(ConA/GOD)_5$ modified electrode, the sensitivity increased linearly when the number of HRP layers increased from one to five. Therefore, $(PAH–MCNT/HRP)_5/(ConA/GOD)_n$ was chosen. For $(PAH–MCNT/HRP)_5/(ConA/GOD)_n$ modified electrode, the same results were obtained and five GOD bilayers were applied (data not shown). As a result, the design of $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$ was used and the obtained electrode was donated as bienzyme bionanomultilayer electrode.

Characterization of LBL assembly

SEM was used to monitor the uniformly electrostatic LBL assembly of PAH–MCNTs and HRP. SEM images of PAH–MCNTs, PAH–MCNT/HRP/PAH–MCNTs, $(PAH–MCNT/HRP)_3$/PAH–MCNTs, and $(PAH–MCNT/HRP)_4$/PAH–MCNTs assembled on Si/PAH/PSS are shown in Fig. 2. When the first PAH–MCNT/HRP bilayer is assembled, MCNTs are randomly oriented and solely dispersed without wrapping with each other. After the number of bilayers increased to five, the surface displayed a dramatic increase in surface coverage. A uniform PAH–MCNT/HRP multilayer film was obtained.

Electrochemical characteristics of the developed bienzyme bionanomultilayer electrode

Cyclic voltammograms (CVs) of the $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$ in 0.1 M PBS (pH 6.5) containing 0.4 mM *o*-dihydroxybenzene at a scan rate of 100 mV s^{-1} are given in Fig. 3. Curve a was obtained without glucose, and curve b was obtained with 0.1 mM glucose. The peaks represented characteristics of redox couple of *o*-dihydroxybenzene. With the addition of glucose, the reduction peak current increased, whereas the oxidation peak current decreased. The phenomenon indicated that a catalytic reaction occurred on the biosensor. These results demonstrated that the response of the biosensor to glucose only resulted from the catalytic activity of bienzymes immobilized in the biocomposite film.

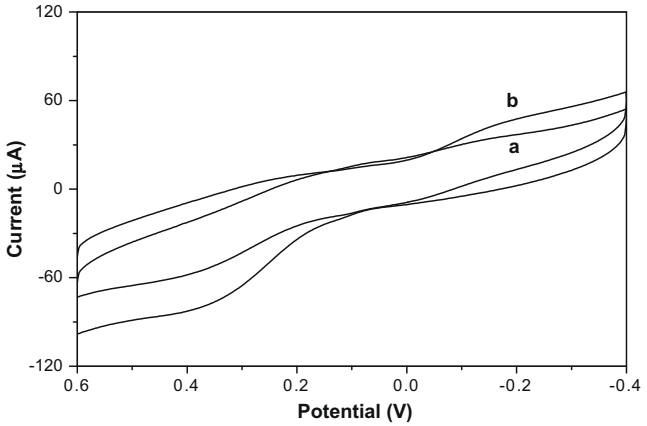

Fig. 3. CVs of the $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$ in 0.1 M PBS (pH 6.5) containing 0.4 mM *o*-dihydroxybenzene at a scan rate of 100 mV s^{-1}. Curve a was obtained without glucose, and curve b was obtained with 0.1 mM glucose.

H_2O_2 is needed for the determination of glucose. By oxidation of glucose catalyzed by GOD, the developed HRP/GOD bienzyme bionanomultilayer biosensor allows in situ generation of H_2O_2. The catalytic cycle of HRP was driven forward and gave an amperometric transducer for the detection of glucose. Briefly, H_2O_2 was formed by GOD-catalyzed oxidation of glucose. The response of H_2O_2 catalyzed by HRP and mediated by *o*-dihydroxybenzene gave the electrochemical signal.

EIS was used to characterize the interface properties of the bienzyme bionanomultilayer electrodes. Fig. 4 shows the typical results of AC impedance spectra of bare Au, $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$, and $(PAH/HRP)_5/(ConA/GOD)_5$ modified electrode. Significant differences in the impedance spectra were observed. The electron transfer resistance (R_{et}) of $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$ modified electrode was estimated to be 125 Ω. For $(PAH/HRP)_5/(ConA/GOD)_5$ modified electrode that contained no MCNTs, the value of R_{et} was remarkably high and was found to

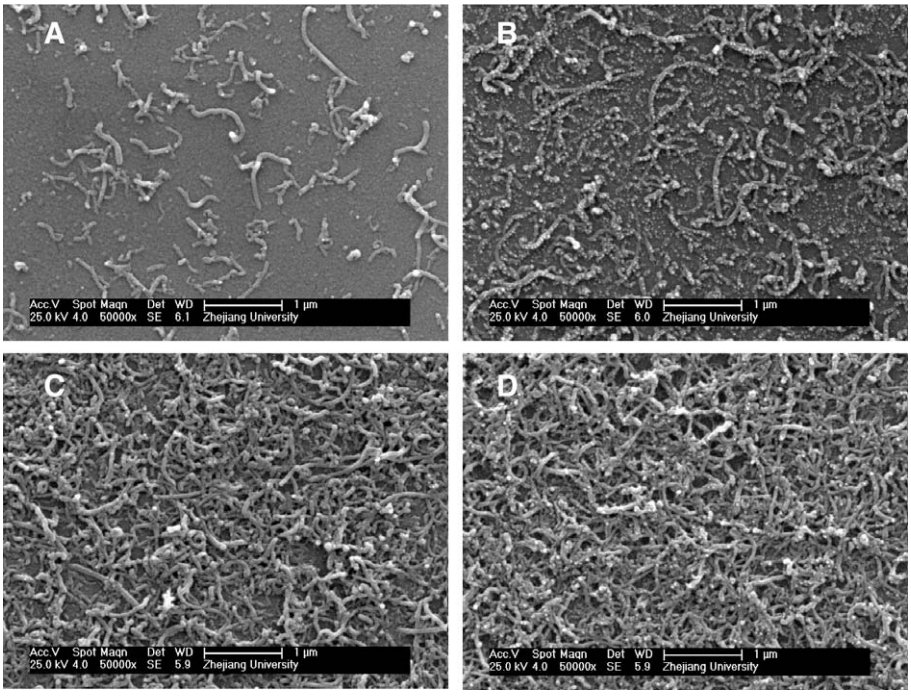

Fig. 2. SEM images of PAH–MCNTs (A), PAH–MCNT/HRP/PAH–MCNTs (B), $(PAH–MCNT/HRP)_3$/PAH–MCNTs (C), and $(PAH–MCNT/HRP)_4$/PAH–MCNTs (D) assembled on polyelectrolyte precursor film modified Si wafer (Si/PAH/PSS).

be 280 Ω. The phenomenon implied that the incorporation of MCNTs greatly facilitated the electron transfer of the electrochemical probe.

Optimization of electron mediator for determination of glucose

The electron mediator showed a significant effect on the analytical performance of the developed biosensor. The optimization of the electron mediator for the determination of glucose by the as-prepared bienzyme bionanomultilayer biosensor was investigated. Because phenolic compounds and aromatic amines could be used in the electrocatalytic cycle of HRP, five compounds were chosen: resorcinol, *o*-dihydroxybenzene, hydrochinone, *o*-phenylenediamine, and *p*-phenylenediamine. Results are given in Fig. 5. As can be seen, *o*-dihydroxybenzene gave the highest signal and was chosen for further investigation. The effect of the concentration of *o*-dihydroxybenzene on the determination of glucose by the developed bienzyme electrode was also investigated. A final concentration of 0.4 mM was chosen for further investigation.

Influence of pH and applied potential on response of bienzyme bionanomultilayer biosensor

The dependence of the biosensor response on the pH of the measurement solution was investigated. A range of pH values between 5.5 and 8.5 was studied (see supplementary material). The current response reached the maximum at pH 6.5, which is close to the optimum pH observed for free GOD [20]. Therefore, the suitable pH with the maximum performance of the biosensor was set at pH 6.5.

Studies to investigate the dependence of the biosensor response on the applied potential were also performed. The amperometric response of the bienzyme bionanomultilayer biosensor to glucose was investigated over the potential range of −0.35 to −0.05 V (see supplementary material). Determination at −0.15 V gave the highest signal-to-noise ratio. As a result, the operating potential of −0.15 V was selected to give good sensitivity for further study.

Amperometric response of developed bienzyme bionanomultilayer biosensor

The amperometric response of the $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$ modified electrode to the successive addition of glucose in 0.1 M PBS (pH 6.5) containing 0.4 mM *o*-dihydroxybenzene is given in Fig. 6. As can be seen, rapid response and sharp increase were observed when glucose was added to the stirring PBS, and 95% of the steady-state current was obtained within 5 s. The time of response is less than that of those glucose biosensors that immobilize GOD on the electrode with only poly(diallyldimethylammonium chloride) (PDDA) [21–25]. The inset in Fig. 6 displays calibration curves of the two amperometric responses of the biosensor to the concentration of glucose. A well-defined current proportional to the glucose concentration was observed for $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$ modified electrode. The linear range of the biosensor for the determination of glucose was found to be 2.0×10^{-6}–1.7×10^{-4} M. A limit of detection (LOD) of 2.5×10^{-7} M was estimated using 3σ (where σ is the standard deviation of the blank solution, $n = 11$). Compared with some common amperometric glucose biosensors, it was lower than the LODs in chitosan (CS)/Au/GOD [23] and graphene sheets (GS)/GOD/Nafion systems [24] but not as good as the excellent result of the CS/CNT/Au–Pt nanoparticle (NP)/GOD system [26]. A further comparison of the performance of different electrochemical biosensors for the determination of glucose is summarized in Table 1.

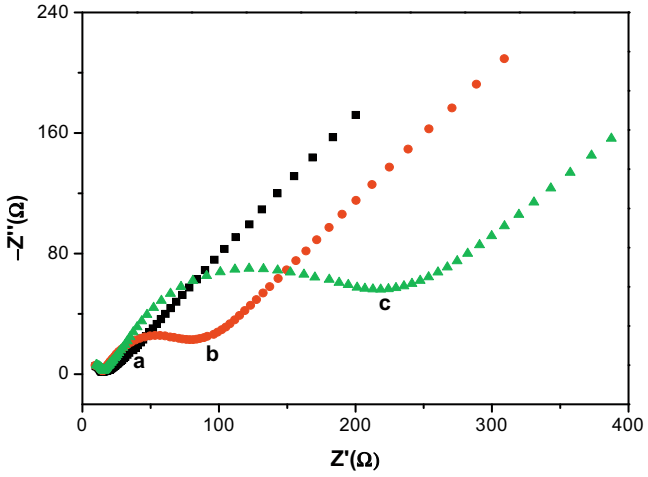

Fig. 4. EIS of bare Au (a), $(PAH–MCNT/HRP)_5/(ConA/GOD)_5$ (b), and $(PAH/HRP)_5/(ConA/GOD)_5$ (c) modified electrode.

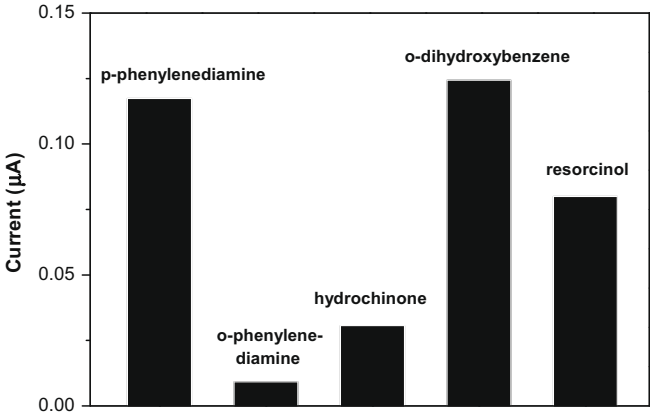

Fig. 5. Optimization of electron mediator for determination of glucose.

Effect of interferences

The possible interferences, such as ascorbic acid and urea, were studied at the developed bienzyme biosensor. As shown in Fig. 7, when 40 μM ascorbic acid and 800 μM urea were consecutively added in the supporting PBS, no significant interference was found. The developed biosensor might be used for glucose determination in serum or uric samples.

Glucose determination in serum samples

To demonstrate the practical use of the glucose biosensor, human serum samples were assayed. The recovery was investigated by standard additions of glucose to the serum sample in the concentration range from 2 to 6 mM. The samples were diluted 100 times before determination. The values of the recovery were in the range of 96.9–103.8%. These results indicate that the biosensor can be used to determine glucose in serum. The phenomenon might be ascribed to the low working potential and the high stability of the biosensor due to the introduction of interaction between the underlying electrode and each layer.

Stability of developed bienzyme bionanomultilayer biosensor

The stability of the bienzyme biosensor was evaluated by monitoring the response currents in the presence of 40 μM glucose over

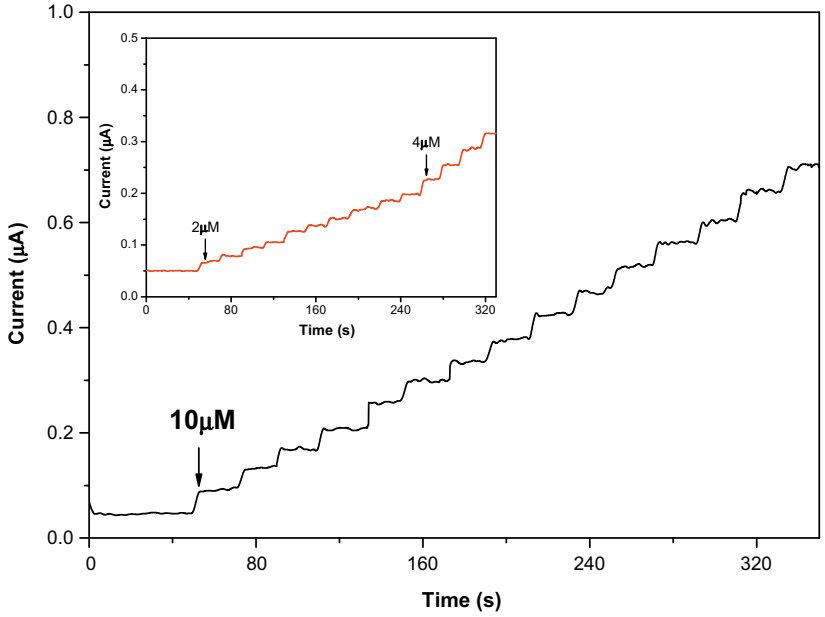

Fig. 6. Amperometric response of the bienzyme bionanomultilayer electrode to successive addition of glucose in 0.1 M PBS (pH 6.5) containing 0.4 mM *o*-dihydroxybenzene. The main panel shows the amperometric response of 10.0 μM glucose. The inset shows the amperometric response of 2.0 and 4.0 μM glucose.

Table 1
Comparison of performances of different electrochemical biosensors for determination of glucose.

Glucose biosensor	Linear range (mM)	Response time (s)	LOD (μM)	Ref.
GCE/CNT/Au/PDDA–GOD	0.5–5.0	10	–	[21]
GCE/CS/MCNT–Fc/GOD	0.012–3.8	<5	3.6	[22]
Pt/PAA/CS/Au/GOD	0.5–16.0	8	7	[23]
Au/GS/GOD/Nafion	0.015–5.8	5	5	[24]
Ti–Au/$(PDDA/MCNT)_n$/$(PDDA/GOD)_n$	0.02–2.2	20	10	[25]
GCE/CS/CNT/Au–Pt NP/GOD	0.001–7.0	<5	0.02	[26]
$(PAH–MCNT/HRP)_n$/$(ConA/GOD)_n$	0.002–0.17	<5	0.25	This work

Note. Fc, ferrocene; PAA, poly(allylamine); Ti, titanium.

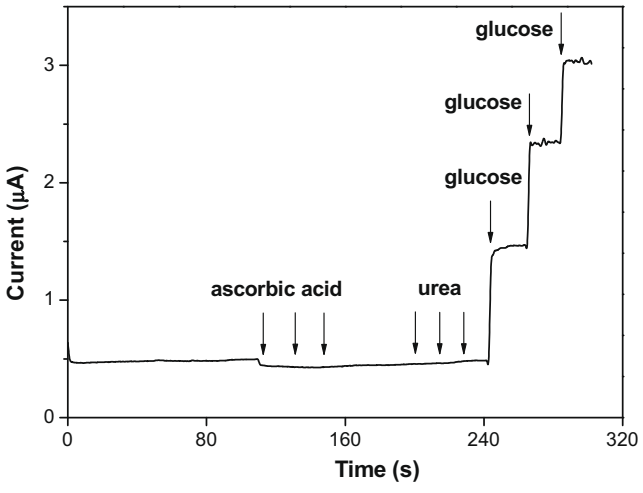

Fig. 7. Effect of 40 μM ascorbic acid and 800 μM urea on determination of glucose.

8 days. The electrode was stored at 4 °C in a refrigerator when not used. The biosensor retained approximately 93% of its original response. The reproducibility and storage stability of the proposed biosensor also were studied. The relative standard deviation (RSD) of the biosensor response to 80 μM glucose was 2.9% for seven successive measurements. The fabrication reproducibility was investigated by preparing six biosensors independently. Reproducibility with an RSD of 3.8% for the detection of 80 μM glucose was obtained.

Conclusions

An amperometric bienzyme bionanomultilayer electrode was constructed by the LBL technique for glucose biosensing based on functional CNTs and sugar–lectin biospecific interactions. The attachment of the MCNT–bienzyme bionanomultilayer with the underlying electrode and each layer in the bionanomultilayer was based on reliably electrostatic or biospecific interaction. Ascorbic acid and urea exhibited no interference in the determination, and the biosenser could be used for the determination of glucose in serum.

Acknowledgment

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