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Sensitive enzymatic glucose biosensor fabricated by electrospinning composite nanofibers and electrodepositing Prussian blue film

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ABSTRACT

In this study, a simple, novel method of preparing glucose amperometric biosensors is reported. This biosensor is based on the quantitative measurement of an intermediate product, hydrogen peroxide (H_2O_2), which in turn is oxidized by Prussian blue film in composite nanofibers. The biocomposite is composed of Prussian blue, chitosan, and polyvinyl alcohol fabricated by electrodeposition and subsequent electrospinning in enzyme-friendly conditions. The resulting biocomposite nanofibers with porous structures and good biocompatibility sustained the stability of the Prussian blue film and the efficient enzyme immobilization without additional cross-linking agents. The stability of the Prussian blue film at neutral and weak alkalescent solutions was increased after the modification. Furthermore, glucose oxidase retained the biocatalytic activities and glucose is oxidized by dissolved oxygen efficiently, which is catalyzed by glucose oxidase to produce hydrogen peroxide. The exhibited good linear behavior in glucose concentrations ranging from 3.30×10^{-6} M to 5.56×10^{-2} M with a low detection limit of 3.61×10^{-7} M. Moreover, the fabricated biosensor exhibited long-term stability, good reproducibility, and absence of interference from other co-existing electroactive species. Thus, the facile and effective methodology of sensor preparation in this study will promote further electrochemical research on proteins, biosensors, and other bioelectrochemical devices.

1. Introduction

Great efforts have been devoted to fabricate various amperometric glucose biosensors based on glucose oxidase (GOD) because of its importance in food production, fermentation, and clinical diagnostics [1–3]. Many glucose biosensors involving hydrogen peroxide (H_2O_2) quantification are based on redox mediators, which are used to perform effective electrical interaction at the redox-active sites with electrodes at low potentials. Such biosensors exhibit fast response, amplify the analytical signal, and largely diminish interference from other reduction species [4–6]. However, for most amperometric biosensors, the need for an electron transfer mediator has hindered their development and application in continuous, real-time in vivo monitoring because they are easily leached out of the electrode surface, which affects sensor performance [7].

Several artificial electron mediators have been reported. Prussian blue (PB), known as "artificial peroxidase," has been widely employed in fabricating amperometric glucose biosensors because of its good biocompatibility, well-known capabilities for enhancing electron transport, and excellent catalytic activity toward H_2O_2 reduction at a low overpotential [8–10]. However, PB is insufficiently stable in neutral and alkaline solutions [11,12], which limits PB applications in biosensor preparation. Fu et al. [13] reported the covalent immobilization of GOD by the one-pot chitosan (CS)-incorporated sol–gel process, in situ covalent cross-linking technique, and Prussian blue-deposited carbon nanotube hybrids. However, the preparation process is rather cumbersome, and the thickness of the biocomposite film is still uncontrollable. Thus, finding a simple and controllable method to construct the protective film and to improve the operational stability of PB in neutral and alkaline pH is necessary.

Electrospinning is a relatively simple and versatile method of creating polymeric fibrous membranes with a high surface area of approximately one to two orders of magnitude and a larger surface energy compared with the corresponding flat substrate [14,15]. Hence, electrospun membrane-based biosensors have been extensively used for enzyme immobilization and in the analysis of various substances such as hydrogen peroxide [16], urea [17], and glucose [18]. In the present study, we chose chitosan (CS)–polyvinyl alcohol (PVA) composite nanofibers as the protective film for improving PB stability through its abundant amino groups [19]. Moreover, CS–PVA can be used to support enzyme immobilization, not only because it is a biocompatible, biodegradable, and nontoxic natural biopolymer, but also because it can form

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long, straight, and aligned nanofibers [20]. In addition, the excellent bioaffinity and porous morphology of CS–PVA create an ideal substrate with a high surface area for the immobilization of GOD. Thus, the inherent drawbacks of enzyme sensors, such as low enzyme loading and insufficient enzyme stability due to the gradual deterioration of enzyme activity, can be overcome [21,22].

In the present study, a simple, novel glucose biosensor was fabricated via electrodeposition and subsequent electrospinning technology. PB film was electrodeposited on indium tin oxide (ITO)-coated glass plate, and CS–PVA composite nanofibers were received on the PB/ITO electrode using electrospinning in case the PB film leaches out from the surface. GOD was then immobilized on the CS–PVA/PB film by the droplet method. The pH stability of PB films and the catalysis of GOD towards glucose oxidation by dissolve oxygen were investigated further.

2. Materials and methods

2.1. Chemicals and reagents

GOD (153,100 units/G) was purchased from the Sigma Chemical Co. CS (MW = 220,000 Da, 95% deacetylated) and PVA (degree of polymerization = 1750 ± 50; 98% hydrolyzed) was purchased from the Sinopharm Chemical Reagent Co., Ltd. (China). Phosphate buffer solution (PBS) was prepared from K_2HPO_4 and KH_2PO_4. The other chemicals were of analytical grade. All solutions were prepared in deionized water.

2.2. Apparatus

During electrospinning, high-voltage power (ES3OP-5w/DDP, Suzhou, China) was applied to the polymer, which was placed in a syringe using an alligator clip attached to the syringe needle. Electrochemical measurements were performed using a CHI 660C electrochemical workstation (Chenhua, Shanghai, China). A conventional three-electrode cell assembly was employed. A GOD/CS–PVA/PB/ITO electrode was employed as the working electrode. A platinum sheet was used as the auxiliary electrode, and a saturated calomel electrode (SCE) was used as the reference electrode. The electrochemical behavior of the modified electrode was studied by cyclic voltammetry (CV) in 0.1 M PBS purged with high-purity N_2 for at least 30 min prior to the experiments. A nitrogen environment was then maintained over the electrolyte. The morphology of the fibers obtained was measured using a scanning electron microscope (SEM; S-4700, Hitachi, Japan). All electrochemical experiments were carried out at 25 ± 1 °C.

2.3. Preparation of CS–PVA/PB/ITO electrode

The ITO electrode (0.5 cm × 3 cm, resistance $\leqslant 100\,\Omega$) was cleaned before sonication in acetone, ethanol, and distilled water, in series. The PB films were directly electrodeposited on the ITO electrode by potentiostatic technique in an acidic solution consisting of 2.5 mM $FeCl_3$ + 2.5 mM $K_3Fe(CN)_6$ + 0.1 M KCl + 0.1 M HCl. A constant potential of +0.4 V was applied for 120 s. In addition, the PB film was also potentiostatically deposited on the CS–PVA/ITO electrode in the same conditions.

A rigid natural polymer in CS solution (2%, w/v), and a flexible synthetic polymer in PVA solution (9%, w/v) were prepared as previously described [23]. Several key operational parameters that affect the morphology of electrospun composite nanofibers were examined and optimized, which include the volume ratio of the CS–PVA precursor (20:80, 40:60, 60:40, and 80:20) and potential (15 kV, 20 kV, and 25 kV). We operated in conditions that are optimal to the morphology of the obtained nanofibers. The CS–PVA composite solution with a 40:60 volume ratio was fabricated at a flow rate of 6 μL/min and an applied voltage of 20 kV. The collection distance between the syringe tip and the collector was set at 20 cm. The composite nanofibrous membrane was collected on the ITO electrode. Continuous and uniform CS–PVA nanofibers could be obtained after drying at 60 °C in vacuum for 12 h.

2.4. Immobilization of glucose oxidase

The GOD solution (3 mg/mL) was prepared by dissolving glucose oxidase in PBS (pH 7.0), which was stored in a refrigerator at 4 °C before use. A fraction of the solution (30 μL) was then added to the electrode by the simple droplet method, and the electrodes were then dried overnight in a 4 °C refrigerator. The positively charged CS–PVA membrane tends to bind strongly with the negatively charged GOD (pI = 4.2) [24] through electrostatic attraction. After drying, the modified electrode was used as the working electrode in all electrochemical measurements and was stored in PBS at 4 °C when not in use.

3. Results and discussion

3.1. Characterization of CS–PVA composite nanofibers

Electrodes modify materials with a large surface area per volume to increase the amount of immobilized enzyme, minimize the barriers for mass transportation between the substrate and the product, and enhance amperometric signals [25]. In optimal electrospinning conditions, the morphology of the obtained CS–PVA composite nanofibers was characterized using SEM. Nearly uniform, randomly orientated nanofibers obtained with diameters of about 500 nm (Fig. 1). The porous, three-dimensional structure of the electrospun nanofiber is propitious to immobilizing a high amount of enzyme and enhancing electron transfer.

3.2. Direct electrochemistry of CS–PVA/PB/ITO electrode and its electrocatalysis towards H_2O_2 reduction

GOD possesses a highly efficient catalytic activity toward glucose oxidation by dissolve oxygen. GOD catalyzes the oxidation of glucose into gluconic acid in the presence of oxygen and generates H_2O_2 (Eq. (1)). The H_2O_2 formed can be catalyzed (Eq. (2)) and determined by CV or chronoamperometry.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2 \quad (1)$$

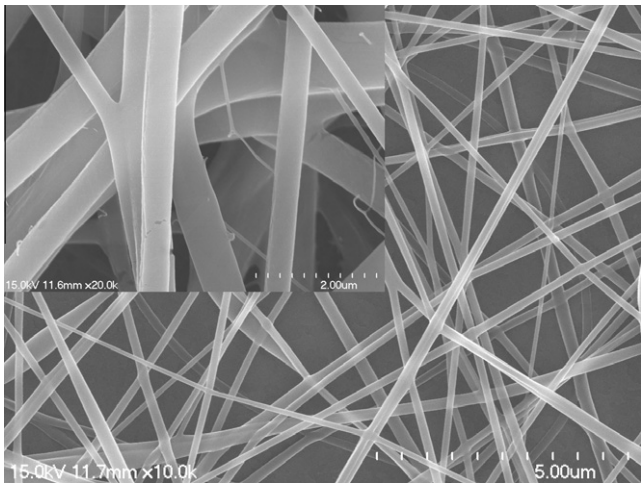

Fig. 1. SEM image of CS–PVA composite nanofibers.