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Hydrogen peroxide biosensor based on microperoxidase-11 immobilized on flexible MWCNTs-BC nanocomposite film

Bingyan Zhang [a], Jianhai Zhou [b], Shaohui Li [a], Xiaofan Zhang [a], Dekang Huang [a], Yahui He [a], Mingkui Wang [a], Guang Yang [b,*], Yan Shen [a,*]

[a] Wuhan National Laboratory for Optoelectrosnics, School of Optoelectronic Science and Engineering, Huazhong University of Science and Technology, Wuhan 430074, P.R. China

[b] National Engineering Research Center for Nano-Medicine, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, P.R. China

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ABSTRACT

In the present work, we report on an experimental study of flexible nanocomposite film for electrochemical detection of hydrogen peroxide (H_2O_2) based on bacterial cellulose (BC) and multi-walled carbon nanotubes (MWCNTs) in combination with microperoxidase-11 (MP-11). MWCNTs are used to functionalize BC and provide a flexible conductive film. On the other hand, BC can improve MWCNTs' biocompatibility. The investigation shows that MP-11 immobilized on the flexible film of MWCNTs–BC can easily present a pair of well-defined and quasi-reversible redox peaks, revealing a direct electrochemistry of MP-11 on the nanocomposite film. The apparent heterogeneous electron-transfer rate constant k_s is estimated to be 11.5 s^{-1}. The resulting flexible electrode presents appreciated catalytic properties for electrochemical detection of H_2O_2, comparing to traditional electrodes (such as gold, glassy carbon electrode) modified with MP-11. The proposed biosensor exhibits a low detection limit of 0.1 μM (at a signal-to-noise ratio of 3) with a linear range of 0.1–257.6 μM, and acquires a satisfactory stability.

1. Introduction

The detection of hydrogen peroxide (H_2O_2) is of great importance in food and environmental analyses, diagnosis, industry, pharmaceutical laboratories and biological studies. H_2O_2 is harmful for biological systems and appears to be involved in the neuropathology of central nervous system diseases. It has been reported that H_2O_2 induces oxidative stress in CGNs, involving both apoptotic and necrotic death [1]. The increased production of H_2O_2 has been implicated in the pathogenesis of several neurodegenerative diseases, including Parkinson's and Alzheimer's diseases, as well as in the damage produced by ischemia and reperfusion [2]. In the past years, various methods have been developed for determination of H_2O_2, including oxidimetry [3], chemi-luminescence [4,5], surface plasmon resonance [6,7], fluorescence [8], and so on. Among these methods, the electrochemical method offers advantages including simplicity, high reliability, sensitivity, low cost and ease of use [9,10].

In recent years, great attention has been attracted to carbon nanotubes (CNTs) for biosensors application because of its unique physical, electronic and chemical properties [9–11]. Several studies have demonstrated that CNTs have excellent electro-catalytic abilities and antifouling properties and have the ability to promote electron transfer reactions when they were used as electrode material in electrochemical reactions [12–15]. CNTs have been widely used to detect several biomolecules such as dopamine, H_2O_2 and glucose [16–19]. However, most of CNTs without modification have limitation of poor dispensability and biocompatibility. In order to solve these problems, surface modification with surfactant and chitosan and other methods were introduced [20,21]. Recently, bacterial cellulose (BC) has been received much attention due to its biocompatibility, ultrafine network, and biodegradability compared with plant cellulose [22,23]. BC can be produced with different microorganisms [24]. Biosensors based on nanostructured carbon materials and BC might benefit from their complementary advantage to enhance their biocompatibility. Yoon and Zhou reported that the insulating BC could become conductive by incorporation of CNTs (1.4×10^{-1} S cm^{-1}) and graphite nanoplatelets (1.2 S cm^{-1}), respectively [25,26]. It was demonstrated that horseradish per-oxidase (HRP) immobilized on gold nanoparticles–bacteria cellulose nanofibers nanocomposite showed high performance of detecting H_2O_2 with a wide range of concentration from 0.3 μM to 1.00 mM, and a low detection limit of 0.1 μM based on $S/N=3$ [27]. Recently, flexible biosensors that can

* Corresponding authors.
E-mail addresses: yang_sunny@yahoo.com (G. Yang), ciac_sheny@mail.hust.edu.cn (Y. Shen).

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accommodate dramatic shape changes have got a fast development for the minimally invasive implantable devices and compact diagnostic platforms. Kim et al. first reported a flexible and ultrathin BC–CNTs film as a candidate to the ubiquitously employed glucose oxidase electrodes for glucose detection [28], which demonstrated a successful application for BC as a flexible matrix electrode in bioelectrochemistry.

Several proteins can be used to construct H_2O_2 biosensors, such as microperoxidase-11 (MP-11), HRP, myoglobin and hemoglobin [29,30]. MP-11 is a widely used enzyme for the construction of H_2O_2 biosensors due to its high purity, sensitivity and low cost [31,32], Herein, we communicate preliminary results on the application of MWCNTs to functionalize BC and provide a flexible conductive film. The resulting flexible MWCNTs–BC film can effectively immobilize the MP-11 and improve the stability of the biosensor compared to the bare MWCNTs, showing high performance for electrochemical detection of H_2O_2.

2. Experimental

2.1. Reagents and apparatus

MWCNTs (diameter 10–20 nm, length 2 μm, purity 97%) were purchased from Chengdu Organic Chemicals Co. Ltd. A fresh aqueous solution of H_2O_2 was prepared daily. All the reagents were of analytical grade and used without purification. The supporting electrolyte used for the electrochemical studies was 0.1 M phosphate buffered solutions (pH 7.0) which were prepared from stock solutions of 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4, 0.1 M NaCl. H_2O (18 Ω M) was obtained from a Millipore-Q water purification system.

The morphology of prepared materials was examined on a field emission scanning electron microscopy (FE-SEM) (Hitachi S-4800 electron microscope, Hitachi, Japan). The infrared spectra were carried out using a VERTEX 70 Fourier Transform Infrared spectrometer. Raman spectra was carried out using LabRAM HR800 Confocal Raman Spectroscopy, and UV–vis absorption spectroscopic measurements were carried out using a Hitachi U-3300 spectrophotometer.

The measurements of cyclic voltammetry (CV) and amperometric *I–t* curve (*I–t*) were carried out using a CHI 920C electrochemical analyzer (CHI). A conventional three electrode cell consisting of an Ag/AgCl (3 M KCl) reference electrode and a twisted platinum wire as an auxiliary electrode was employed. All experiments were performed at room temperature.

2.2. Preparation of MWCNTs–BC and MP-11/MWCNTs–BC film flexible electrode

Gluconacetobacter xylinum (ATCC53582) was used for the biosynthesis of BC [33]. The bacterium was cultured in a Hestrin and Schramm (HS) medium, which was composed of 2% (wt) glucose, 0.5% (wt) yeast extract, 0.5% (wt) peptone, 0.27% (wt) disodium phosphate, and 0.15% (wt) citric acid. After having been incubated statically at 26 °C for 14 days, the BC membranes were formed and then dipped in distilled water and NaOH solution, respectively. The pH was lowered to 7.0 by washing with distilled water. The purified cellulose pellicles were stored in distilled water at 4 °C to avoid drying. Then some pieces of the purified cellulose pellicles were put into the sulfuric acid solution (40%). Afterwards, the obtained BC whiskers were centrifuged and dialyzed in deionized water successively for further purified to get 0.3% BC whiskers aqueous solution. 5 mg mL^{-1} MWCNTs aqueous suspension was added into BC whiskers aqueous solution to get a uniformly mixed solution and then put it filtered. Finally, the flexible conductive MWCNTs–BC films were produced, and the mass ratio of BC and MWCNTs was 1:10. Then the film was cut into suitable shape. The obtained flexible film was immersed in the PBS solution containing 1 mg mL^{-1} MP-11 (pH 7.0) for 16 h, and then the MP-11 molecular was immobilized onto the surface of MWCNTs–BC film. The final modified flexible film was washed with deniozed water for several times.

2.3. Platelet adhesion studies

The platelet-rich plasma (PRP) was purchased from Pingrui Biotechnology Company (Beijing, China), and the experimental method has been reported by Meyerhof [23c]. Briefly, BC–MWCNTs was preincubated at 37 °C for 20 min. BC–MWCNTs film previously conditioned in phosphate-buffered saline (PBS, containing 10 mM phosphate, 138 mM sodium chloride, and 2.7 mM potassium chloride, pH 7.4) for 3 days were immersed in PRP for 2 h at 37 °C. It was then rinsed in PBS and fixed in a PBS solution containing 2.0% (w/v) glutaraldehyde for 2 h at room temperature. Samples were dehydrated by immersion in serial dilutions of ethanol (30, 50, 70, 90, and 95% v/v), two immersions in absolute ethanol.

3. Results and discussion

3.1. Characterization of the MWCNTs–BC and MP-11/MWCNTs–BC flexible film electrode

The surface morphology of MWCNTs, BC and MWCNTs–BC films were examined by field emission scanning electron microscopy (FE-SEM). The pure MWCNTs with a diameter of about 15–20 nm was evenly spread out, that result in a uniform sample as shown in Fig. 1(a). BC whisker film with ultra-fine network is shown in Fig. 1(b), which leads it to be a good candidate for flexible substrate. Fig. 1c presented the morphology of MWCNTs–BC composite film. It was observed that the diameter of MWCNTs was obviously bigger than BC, and MWCNTs were well-incorporated and distributed within the BC. The BC hydrogel itself works as a nano-sized filter. CNTs efficiently infiltrated into a network of BC when they were filtered though BC hydrogel by vacuum [28].

Fig. 1(d) shows the tensile property for the flexible film substrate tested by HY-0230 electronic universal material testing machine. The result demonstrated that the max stress was about 52.36 MPa for the flexible film with a stretch distance of 1.1 mm, although the thickness of the BC–MWCNTs film was only about 135.2 μm. It indicated that the flexible film performed a good mechanical property.

Fourier transform infrared (FTIR), Raman, and UV−visible spectrum measurements were performed to clearly characterize MWCNTs–BC film. As shown in Fig. 2a, the appearance of the peaks at 1305, 1418, 1625, 1717, 2886 and 3220 cm^{-1} were assigned to C–O, $-CH_2-$, C═C, –COOH, C–H and O–H vibrations, respectively. MP-11 molecular has functional groups, including –COOH, O–H, and $-NH_2$. It is worthy noted that the carboxyl group from MWCNTs induced by concentrated nitric acid (HNO_3, 68%) treatment is beneficial to the adsorption of MP-11onto MWCNTs surface. and the hydroxyl group from BC is beneficial to the combination to MWCNTs. Fig. 2b shows Raman spectra of MWCNTs–BC film, exhibiting two prominent peaks at 1363 and 1596 cm^{-1}, corresponding to the disorder (D band) and the crystalline (G band) graphite, respectively. This indicates the structure of MWCNTs is not affected by BC. The UV–visible spectrum revealed that MP-11/MWCNTs–BC film presents a Soret band at 408 nm. The corresponding Soret band of MP-11 in solution with 0.2 μM was found at 402 nm (Fig. 2c). The bathochromic