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The application of porous ZnO 3D framework to assemble enzyme for rapid and ultrahigh sensitive biosensors

Minggang Zhao[a], Yu Zhou[a], Bin Cai[a], Ye Ma[a], Hui Cai[a], Zhizhen Ye[a,b], Jingyun Huang[a,b,*]

[a]*Department of Materials Science and Engineering, State Key Laboratory of Silicon Materials, Zhejiang University, Hangzhou 310027, China*

[b]*Cyrus Tang Center for Sensor Materials and Applications, Zhejiang University, China*

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Abstract

A porous 3 dimensional (D) ZnO framework was constructed by electrospinning. The framework was consisted of connected mesoporous nanofibers and used as excellent matrix to assemble horseradish peroxidase. A novel enzymatic biosensor based on the mesoporous ZnO nanofibers/chitosan inorganic–organic composite 3D spatial framework was prepared. The as-fabricated biosensor exhibited ultrahigh sensitivity (1910.1 μA cm^{-2} mM^{-1}), fast response (< 3 s), low detection limit (1 μ), good reproducibility and stability. The excellent performance is attributed to the 1D ZnO nanofibers with mesopores for enzyme loading, connected 3D integrated framework for excellent electrical transport properties and good biocompatibility of chitosan. The 3D porous ZnO framework provided an ideal environment for enzyme assembly.

Keywords: D. ZnO; Porous nanostrucures; Biosensor; Enzyme

1. Introduction

Electrochemical biosensors based on enzyme electrodes have potential applications in biological and chemical analysis, clinical diagnosis and environmental monitoring. The key factor for the successful operation of the biosensors is the effective immobilization of enzyme onto the electrode [1]. Nanomaterials have stimulated considerable interests due to their high specific surface for enzyme immobilization and unique optical and electrical properties. It is well known that nanomaterials with different morphologies, structures and sizes have various performances. Therefore, the design of nanomaterials with suitable morphology and structure attracts much more attention in the fabrication of enzymatic biosensors.

As a versatile semiconductor material, ZnO has a wide application such as light-emitters, chemical sensors and solar cells. ZnO nanomaterials makes many advantages of biosensing, including high-specific surface area, high Isoelectric Point (IEP~9.5), high electron communication features, nontoxicity and safe for living organisms [2–5]. Recently, various ZnO nanostructures have been synthesized to construct electrochemical biosensors and applied to detect cholesterol, glucose, protein and so on [6–9]. The determination of hydrogen peroxide (H_2O_2) is important for clinical diagnosis, food industry and environmental monitoring. However, the performance of ZnO based H_2O_2 biosensors still need to be improved [10–13]. Hence, a special ZnO nanostructure is desired to be designed for effective horseradish peroxidase (HRP) immobilization.

Electrospinning is a simple and effective technique for generating electrically active fibers with unique properties and versatile applications [14]. In this study, an integrated 3D porous framework built with 1D mesoporous ZnO nanofibers was constructed by electrospinning and subsequent annealing process. The nanostructures were used as matrixs to fabricate the biosensor. In our work, the 3D porous framework was fabricated directly on a conductive Au electrode, which ensured excellent electrical contact. For many previous works, the ZnO nanomaterials were prepared firstly and then assembled on

*Corresponding author at: Department of Materials Science and Engineering, State Key Laboratory of Silicon Materials, Zhejiang University, Hangzhou 310027, China. Tel.: +86 571 87952118; fax: +86 571 87952625.
E-mail address: huangjy@zju.edu.cn (J. Huang).

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the surface of the electrode, which resulted in a poor contact. Furthermore, the 1D nanofibers were grown connected with each other by a nature junction. As a result, an integrated electron transport network was formed by the 3D porous framework. This network ensured the fast electron transport. Moreover, the widespread mesopores in the nanofibers afford high specific surface and suitable microcell environment for effective enzyme immobilization. The porous 3D ZnO framework based H_2O_2 biosensor exhibited ultrahigh sensitivity and fast response. This nanostructure is an excellent matrix for enzyme assembly and can be extended to other biological applications.

2. Experimental section

2.1. Preparation of 3D porous ZnO framework

In a typical procedure, a polyvinyl alcohol (PVA) sol solution (8 wt%) was prepared first. Then zinc acetate (2.19 g) was added into 30 ml as-prepared PVA sol solution with stirring and heating. During the stirring time, 2 ml alcohol was added into the solution drop by drop. The viscous precursor sol solution was electrospun directly onto the Au electrode with an exposed surface of 3 mm × 3 mm. The 1D porous ZnO nanofibers were obtained after annealing at 600 °C for 2 h in air.

2.2. Fabrication of hydrogen peroxide biosensors

A quartz chip (3 mm × 1 cm) coated with an Au film was prepared and ZnO nanofibers were fabricated on one end (3 mm × 3 mm). On the other end, an exposed area (2 mm × 3 mm) was leaving. The middle area of the chip was encapsulated in wax. The HRP solution (5.0 mg ml^{-1}) was prepared by dissolving HRP in 0.067 M phosphate buffer saline (PBS PH = 7.0). The CHIT (molar weight ~1×10^6, 75–85% deacetylation) solution (0.5 wt%) was prepared by dissolving CHIT in acetic acid solution. Firstly, 10 μL of HRP solution was dropped onto the surface of ZnO nanofibers, and dried in several minutes. Then, 10 μL of CHIT solution was dropped onto the surface to prevent the leaching of the enzyme. Finally, the device was dried overnight in a refrigerator at 4 °C. The device was washed with distilled water carefully and then ready for use.

2.3. Materials characterization and electrochemical measurement

The morphology and size of as-synthesized sample were characterized with SU-70 scanning electron microscopy (SEM). All the electrochemical experiments were performed with a WPG100e electrochemical workstation (Korea). The three electrodes system was adopted for measurement, consisting of the as-prepared HRP modified electrode as working electrode, a Pt electrode as counter electrode, and an Ag/AgCl electrode as reference electrode. All the experiments were carried out in PBS (PH = 7.0) at room temperature.

3. Results and discussion

3.1. Characterization of the 3D porous ZnO framework

A high-magnification picture of a single mesoporous ZnO nanofiber is shown in Fig. 1(a). It can be observed that the surface of the nanofiber is rough and many mesopores spread all over the nanofiber. It is known that mesoporous structure has a strong physical adsorption capacity. The size of the mesopores here is larger than that of HRP [15], so the enzyme can be adsorbed inside the nanofibers. As is shown in Fig. 1(b), the pores are embedded after enzymes immobilization. Compared with other nanostructures without pores, the mesoporous structure affords large specific surface and microcell-environment for effective enzyme loading. Another peculiarity is the special junctions among the nanofiber (Fig. 1d inset), which guarantee an excellent electric contact between different nanofibers. These nanofibers are connected to each other by junctions to form an integrated 3D porous framework (Fig. 1c, d). The whole structure is like an electrified wire netting, which ensures the excellent electron transport. From many previous reports, ZnO nanomaterials with short lengths were first grown on a substrate and then taken down to stack together on the electrode for biosensors. However, the short electron transport channels and crystal grain boundaries caused a poor electrical property [16]. In our work, the nanofibers were grown directly on the Au electrode, and had long length and well contact. All these characteristic features are beneficial for the electrons transport. Furthermore, the porous 3D framework can afford enough room for enzyme molecules stretch and reactants diffusion.

A typical XRD pattern of the ZnO nanofibers is shown in Fig. 2(a). All the diffraction peaks can be indexed as the wurtzite structure ZnO (Space group: P63mc (186), $a = 0.3249$ nm, $c = 0.5206$ nm) in the standard data (JCPDS, 36-1451). The sharp peaks and strong intensity reveal a high crystallographic quality. No other diffraction peaks of impurities can be observed, indicating that a pure ZnO was obtained by this method.

Fig. 2(b) shows the UV–vis absorption spectra of the porous ZnO nanofibers at room temperature. A peak near the band edge in the exciton absorption region (370 nm) is found. Compared to the bulk excition absorption (380 nm), a blue shift is observed. The appearance of blue shift can be explained as follows: the carries are confined in a very small district, so the electron and hole moves only in a potential well. At the same time, the coupling interaction with each other is enhanced. Therefore, the blue shift is caused by the intensified excition bound and increased binding.

3.2. Assembly of enzyme

The 3D porous framework formed by mesoporous ZnO nanofibers was used successfully as matrix to fabricate the enzymatic biosensor, as is illustrated in the Fig. 3. First, the enzyme molecules were physically adsorbed inside the ZnO nanofibers, due to the strong physical adsorption capacity of mesoporous structure. Then the outer surface of the nanofiber

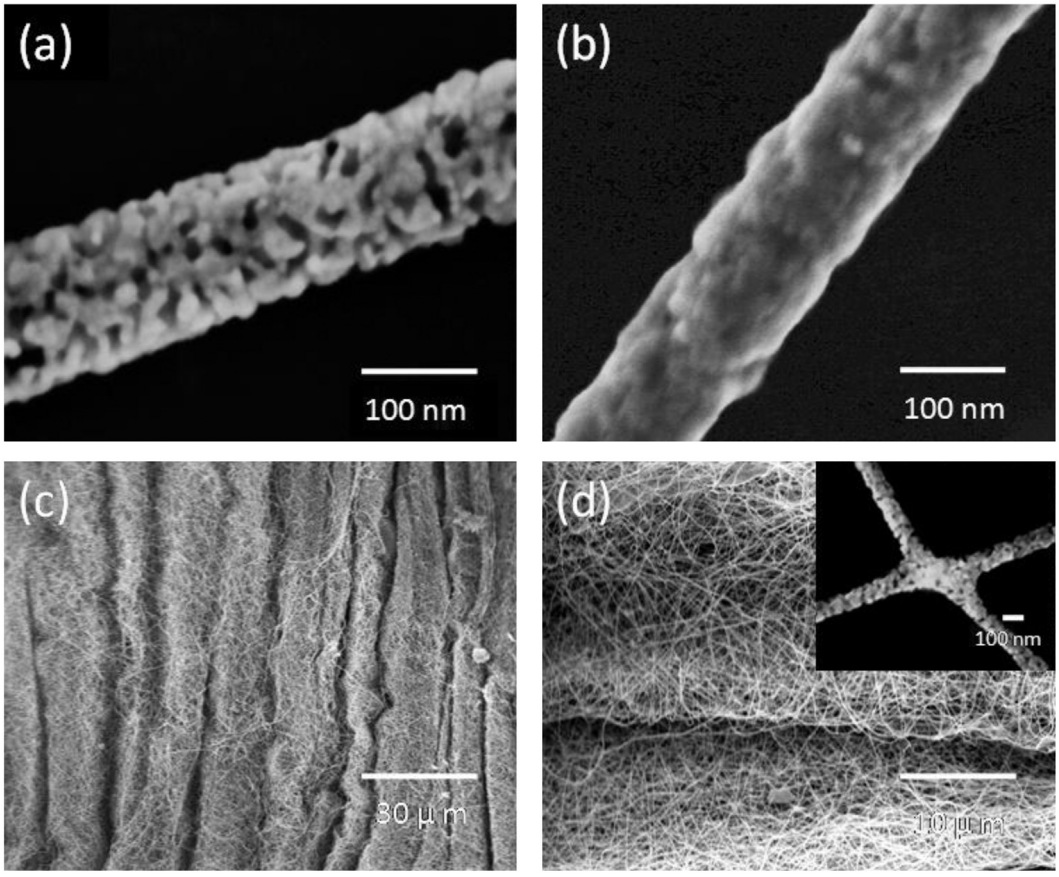

Fig. 1. SEM images of the 3D porous ZnO framework. (a), (b) Higher magnification SEM images of the ZnO nanofiber before and after immobilization of HRP. (c), (d) Lower magnification SEM images of the 3D porous ZnO framework. The inset is higher magnification SEM image of the nature junction between nanofibers.

was enwrapped by a layer of permeable CHIT film. Combined with the protection of the organic film, the enzymes were stably entrapped. Because of the existence of mesopores, the active surface area available for enzyme binding was enhanced. In addition, the porous environment was favorable for enzyme molecules stretch and retaining the essential secondary structure. On the other hand, the formed 3D porous network afforded integrated channels for excellent electrons transport and enough room for fast reactants transmission.

3.3. Electrochemical characteristics of the enzymatic biosensor

The biosensor was characterized by cyclic voltammetry between the potentials of −0.6 V and+0.6 V in an unstirred PBS (0.067 M, PH=7.0). Fig. 4(a) shows the CV curves of the CHIT/HRP/3D ZnO framework/Au electrode in the presence and absence of H_2O_2. It is observed that the electrode gives clear electrochemical behavior with a pair of typical oxidation and reduction peaks in absence of H_2O_2. The result indicated that the enzymes were effectively immobilized, and well biological activity was kept. But the electrochemical behavior was significantly changed after adding 2 mM H_2O_2. The addition of H_2O_2 caused an increase in reductive current and a decrease in the oxidative current. It indicated that the immobilized HRP enzymes exhibited fine electro-catalytic activity to H_2O_2.

Fig. 4(b) displays the typical current–time response curve for successive addition of H_2O_2 step by step under stirring. It is observed that the biosensor exhibits rapid and sensitive response to the change of H_2O_2 concentration. The current increases with the increase of H_2O_2 concentration and achieves 95% of the steady-stated current within 3 s. It indicates that the as-prepared biosensor can well catalyze the reduction of H_2O_2, which means that the HRP was effectively immobilized and retained well bioactivity. It is attributed to the fact that the porous structure of ZnO nanofibers yields a low mass transport barrier and results in a rapid diffusion from bulk solution to enzyme. The response is faster than other H_2O_2 biosensors (Table 1). The faster response is attributed to the well electrons transport channels formed by the connected 3D ZnO framework. The calibration curve of the H_2O_2 concentration versus current is shown in Fig. 4(b) inset. The linear detection range of 3D porous ZnO framework-based H_2O_2 biosensor ranges from 1×10^{-5} M to 1.56×10^{-3} M with a correction coefficient $R=0.998$. The present configuration of the biosensor shows an ultra high sensitivity of 1910.1 μA cm^{-2} mM^{-1}, which is much higher than many previous reports (Table 1). The biological activity of immobilized enzyme is generally evaluated using the apparent Michaelis–Menten constant K_M^{app}, which can be calculated according to the Lineweaver–Burk

equation:

$$1/I_{ss} = 1/I_{max}(1 + K_M^{app}(mM)/C)$$

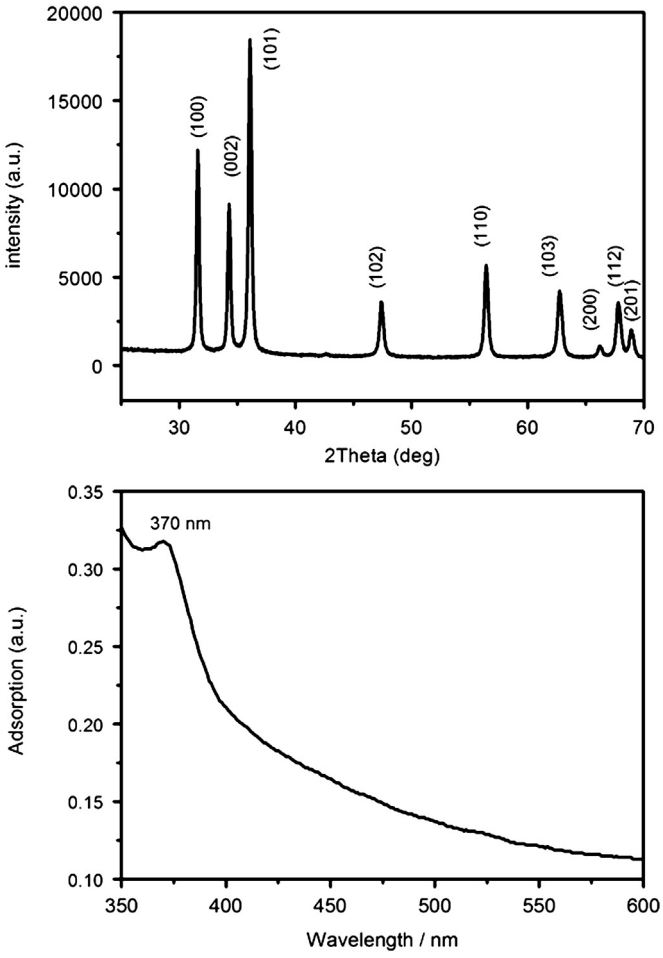

Fig. 2. (a) XRD patterns of the porous ZnO nanofibers, (b) UV–vis absorption spectra of the porous ZnO nanofibers.

where I_{ss} (μA) is the steady current after addition of H_2O_2, I_{max} (μA) is the maximum current, and C (mM) is the concentration of H_2O_2. In present study the K_M^{app} is calculated to be 1.15 mM, which is smaller than many other studies [4,11,17–19]. This indicates that the enzymes immobilized on the 3D porous ZnO framework maintain well bioactivity. The detection limit of the biosensor was estimated to be 1 μM at a signal/noise ratio of 3. The stability was also investigated. The redox peak current and peak potential of the electrode showed no obvious changes after 20 cycles. Almost 90% of its initial response current was maintained after 4 weeks.

The excellent performance of the electrode discussed above is attributed to 3 main facts: First, the mesoporous structure of the ZnO nanofiber provides a high specific surface and micro-cell environment for the enzyme loading. The combined action with permeable CHIT film ensured the stability. Second, the formed 3D porous framework constructed an integrated channel net for fast electrons transport and reactants transmission. Third, the ZnO nanofibers were grown directly on the electrode, avoiding poor electrical contact with Au electrode.

4. Conclusions

In this work, porous 3D ZnO framework were directly fabricated on Au electrode with assembling HRP to construct enzymatic H_2O_2 biosensor. The as-prepared biosensor exhibited ultrahigh sensitivity, fast response, low apparent Michaelise-Menten constant K_M^{app}, and well stability. These excellent results are attributed to the 1D mesoporous structure for effective enzyme assembly and integrated 3D porous spatial framework for well electron communication and fast reactants exchange. The porous 3D ZnO framework opens a new avenue for assembling proteins to fabricate excellent electrochemical biosensor. This fabrication strategy could be extended to other protein-based biosensors.

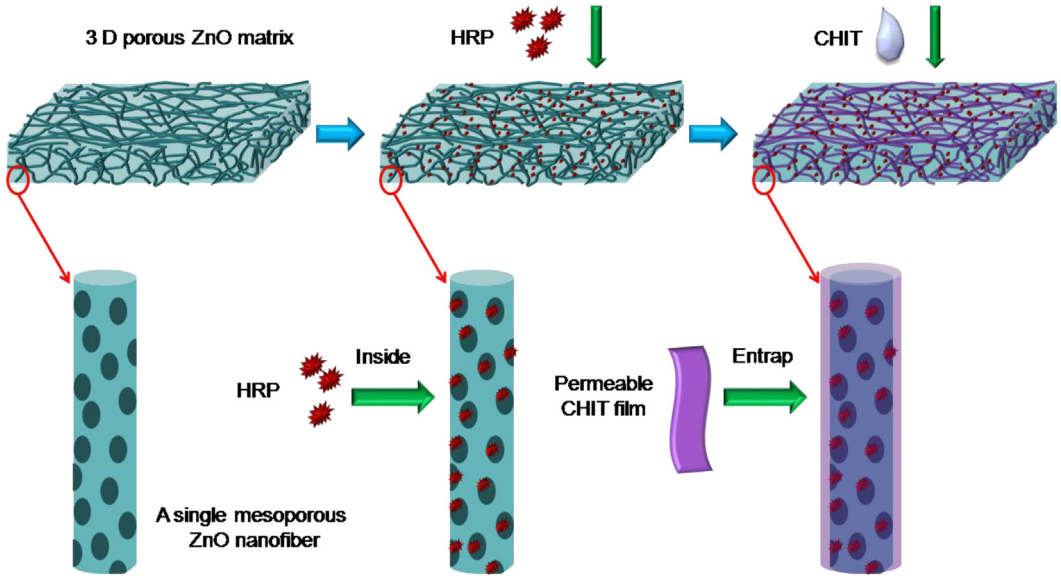

Fig. 3. Features of the 3D porous ZnO framework for assembling enzyme.

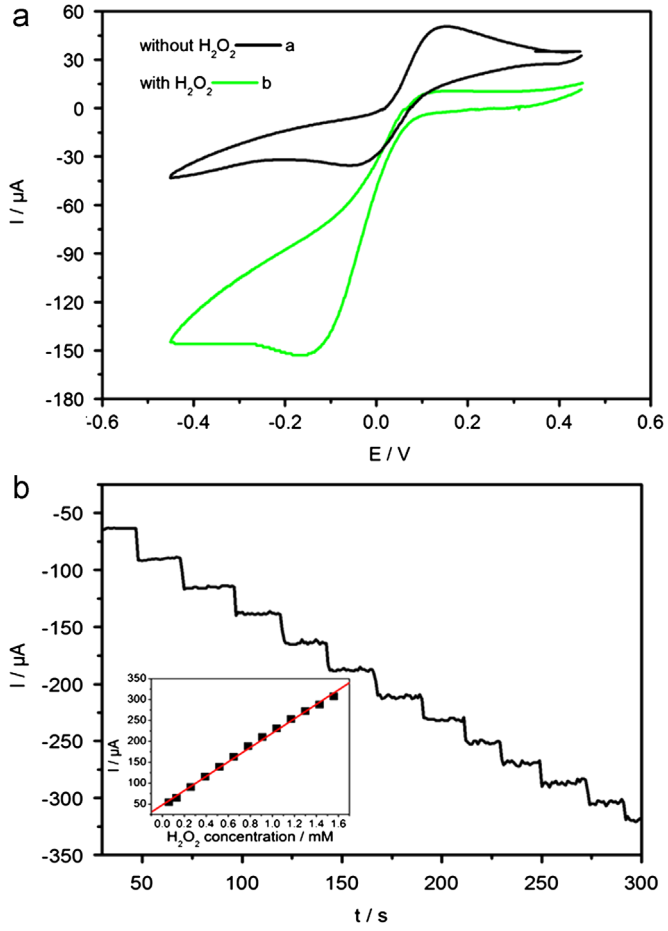

Fig. 4. Characteristics of the enzymatic biosensor. (a) Cyclic voltammograms curves of the CHIT/HRP/3D ZnO framework/Au electrode in the presence and absence of H_2O_2. (b) Typical current–time response curve for successive addition of H_2O_2 with a step of 0.13 mM in the PBS (PH=7.0) under stirring. The inset is the calibration curve of the H_2O_2 concentration versus current.

Table 1
Comparison of performances of various ZnO nanomaterials based H_2O_2 biosensors.

Nanomaterials	Sensitivities (μA/ mM cm^{-2})	Response time (s)	Linear ranges (μM)	Ref.
Flower-like ZnO	255	10	9–300	[10]
ZnO microspheres	137		10–410	[11]
ZnO nanosheets	2.0	4	1–1000	[12]
ZnO nanowire	237.8	4		[13]
ZnO nanofibers	1910.1	3	10–1560	This work

Acknowledgments

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