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The improvement of glucose bioelectrocatalytic properties of platinum electrodes modified with electrospun TiO_2 nanofibers

Hao Tang, Feng Yan*, Qidong Tai, Helen L.W. Chan

Department of Applied Physics, the Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

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ABSTRACT

Pt electrodes have been modified with electrospun titanium dioxide (TiO_2) nanofibers with controllable densities for electrochemical detection. The TiO_2 nanofiber/Pt electrodes show excellent electrocatalytic activity to electro-oxidation of hydrogen peroxide and the response current density to an addition of hydrogen peroxide is 30% higher than that for a Pt electrode. Glucose oxidase (GOx) molecules have been effectively immobilized on the surface of the TiO_2 nanofibers and the resulted chitosan/GOx/TiO_2 nanofiber/Pt electrodes with a optimum density of nanofibers exhibit excellent glucose bioelectrocatalytic performance including good amperometric current response (9.25 $\mu A\,cm^{-2}\,mM^{-1}$), short response time (10 s) and low detection limit (0.01 mM). We have found that the nanofibers modified on the Pt electrode improved the sensitivity of the enzyme electrode to glucose for 2.7 times and extended the detection limit by one order of magnitude in comparison with flat Pt electrodes. It is expected that electrodes modified with electrospun TiO_2 nanofibers will find more promising applications in high performance electrochemical biosensors.

1. Introduction

Titanium dioxide (TiO_2) has super performance and broad applications in various fields including cosmetics, additives in white paint, solar cell, photocatalysis and battery materials etc. (Fujishima and Honda, 1972; O'Regan and Gratzel, 1991; Alivisatos, 1996; Wang et al., 1997, 1998). Due to the relatively high conductivity, nontoxicity and good biocompatibility, the utilization of TiO_2 for electrocatalysis of biomolecules and electrochemical biosensing is an active research field (Yu and Ju, 2002; Nazeeruddin et al., 1993; Li et al., 2001; Milella et al., 2001). With the development of nanoscience and nanotechnology, TiO_2 nanostructural materials are attracting growing attention owing to their specific characteristics including large surface area to volume ratio, high surface adsorption and environmental safety (Tremel, 1999). TiO_2 nanomaterials including nanoparticles, nanotubes, nanofibers or their composites can be prepared by many different methods (Yao et al., 2003; Fu et al., 2005; Yuan and Su, 2004; Kasuga et al., 1998; Bavykin et al., 2004; Wang et al., 2002a,b; Armstrong et al., 2005; Varghese et al., 2003; Zhou et al., 2006), which have already shown promising applications in electrochemical sensing. For example, they illustrate good electrocatalytic activity to Meldola's blue and cytochrome *c* (Bavykin et al., 2005) and they are suitable for biomolecule immobilization and possess the ability to improve the direct electron transfer between redox proteins (particularly enzymes) and electrodes (Zhang et al., 2004a,b; Zheng et al., 2008; Zhou et al., 2005; Kang et al., 2008).

TiO_2 nanostructural materials are usually prepared by template growth (Kang et al., 2008; Sreethawong et al., 2005), sol–gel (Fu et al., 2005; Zhou et al., 2005) and alkaline hydrothermal synthesis (Tremel, 1999; Kasuga et al., 1998; Zhang et al., 2005) under carefully controlled process conditions (such as temperature, titanium concentration and pH). These methods have intrinsic advantages along with some limitations. The template method is high-cost and not suitable for mass production. Sol–gel is mainly used for the preparation of nanoparticles and the process of alkaline hydrothermal synthesis is relatively complex and difficult to control. On the other hand, the TiO_2 nanomaterial-modified electrodes reported previously are usually prepared by casting TiO_2 suspension solution on substrates, which may result in mechanically and electrically loose. Electrospinning technique (Larrondo and Manley, 1981; Sawicka and Gouma, 2006) is a simple and versatile method that offers a time and cost effective production of various nanofibers with a controllable density, which show great potential for protective clothing, catalysis, electronics, and biomedicine including tissue engineering, implants and drug delivery (Dzenis, 2004; Frenot and Chronakis, 2003; Song et al., 2006, 2008; Fan and Whittingham, 2007; Li and Xia, 2004a). Although, electrospinning obtained great progress since mid-1990s and over a hundred synthetic and natural polymers were electrospun into nanofibers, its

* Corresponding author. Tel.: +852 2766 4054; fax: +852 2333 7629.
E-mail address: apafyan@polyu.edu.hk (F. Yan).

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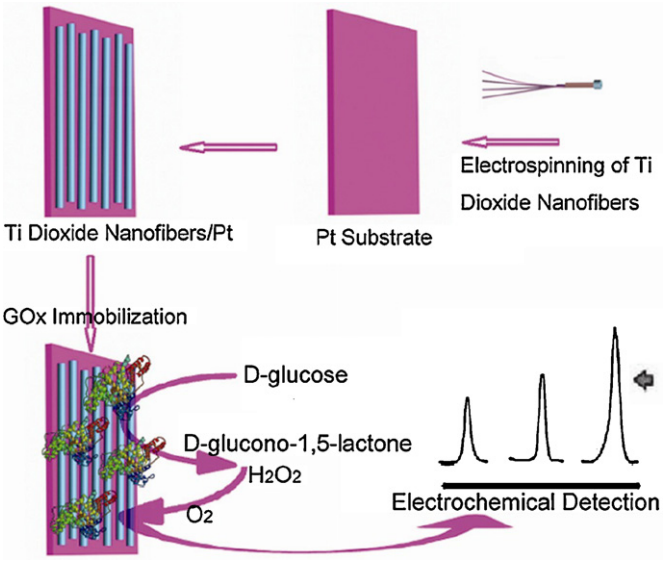

Fig. 1. Schematic diagram of the preparation of TiO_2 nanofibers by electrospinning, GOx immobilization and subsequent glucose bioelectrocatalysis.

development and application are still in an early stage. Some papers have reported the preparation of TiO_2 nanofibers or their composites by electrospinning for the application in semiconductor devices (Drew et al., 2003), dye-sensitized solar cells (Jose et al., 2008), photodecomposition of phenol and formic acid (Madhugiri et al., 2004), electrochemical capacitors (Ahn et al., 2006) and anode materials in batteries (Fan and Whittingham, 2007). However, to the best of our knowledge, the utilization of the electrospun TiO_2 nanofibers for enzyme immobilization and bioelectrocatalysis of glucose has not been investigated.

In this paper, we studied the electrospun TiO_2 nanofiber/Pt electrodes for glucose sensing. Fig. 1 shows a schematic diagram for the preparation of TiO_2 nanofibers, glucose oxidase (GOx) immobilization and subsequent characterization of glucose bioelectrocatalysis. In order to maintain a stable performance of the enzyme electrodes, a biocompatible polymer chitosan (Chit) (Wang et al., 2002a,b, 2003; Zhou et al., 2002; Schmidt et al., 2009; Zhang et al., 2004a,b) was utilized to modify the GOx/TiO_2 nanofiber/Pt electrodes. The morphologies of the TiO_2 nanofibers with and without GOx immobilization on the surface were characterized by atomic force microscopy (AFM). The electrochemical performance of the TiO_2 nanofiber/Pt electrodes and bioelectrocatalytic properties of the Chit/GOx/TiO_2 nanofiber/Pt electrodes were investigated in details by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and amperometric *i*–*t* curve.

2. Material and methods

2.1. Chemicals and apparatus

GOx (50 kU g^{-1}) was purchased from Aladdin Reagent Database Inc. and stored at −20 °C for future use. Chit was obtained from Advanced Technology & Industrial Co., Ltd, Hong Kong and used as received. Polyvinylpyrrovidone (PVP, M = 1300,000), isopropyl alcohol (IPA), acetic acid, titanium (IV) isopropoxide and titanium *n*-butoxide (>99%) were purchased from International Laboratory, USA. All other reagents were analytical grade or better. Double-distilled water and freshly prepared solutions were used throughout. Chit aqueous solution (5 mg mL^{-1}) was prepared by dissolving 0.5 g Chit in 100 mL of 50 mM acetic acid solution (pH 5–6). The solution was sonicated for an hour and then stored in a refrigerator (4 °C). GOx stocking solution (8 mg mL^{-1}) was prepared with phosphate buffered saline (PBS, pH 7.2, Invitrogen Inc.) and stored at 4 °C for future use. Glucose stocking solution was allowed to mutarotate at room temperature overnight before use.

CHI660B electrochemical workstation (CH Instruments, Inc.) and a standard three-electrode electrolytic cell were employed for the electrochemical studies. TiO_2 nanofibers were electrospun onto one side of flat Pt substrates and the TiO_2 nanofiber/Pt electrodes were used as working electrodes. Each electrode was sealed with silicon sealant with only a square effective area (0.16 cm^2) in the center of the side with TiO_2 nanofibers exposed to electrolytes. A platinum wire served as a counter electrode and an Ag/AgCl (sat. KCl) electrode was used as a reference electrode. All potential values given below are referred to the Ag/AgCl electrode. All experiments were performed at room temperature.

AFM (NanoScope® IV, Digital Instruments) was used to characterize the morphology of the electrospun TiO_2 nanofiber/Pt electrodes with and without GOx immobilization.

2.2. Electrospinning of TiO_2 nanofibers and preparation of enzyme modified electrodes

TiO_2 nanofibers were prepared by electrospinning reported by others (Kumar et al., 2007; Li and Xia, 2003, 2004b; Li et al., 2006). Briefly, a thoroughly mixed precursor solution containing PVP (0.035 g mL^{-1}), titanium (IV) *n*-butoxide (0.1 g mL^{-1}), IPA (0.16 g mL^{-1}), 2-methoxyethanol (0.66 g mL^{-1}) and acetic acid (0.01 g mL^{-1}) was electrospun onto Pt substrates at a rate of 0.1 mL h^{-1} under an electric field of 40 kV m^{-1}. To obtain uniaxially aligned nanofibers, a small rotating drum controlled by a motor was placed between the syringe and a grounded aluminum foil and the Pt substrates were fixed to the drum (Teo and Ramakrishna, 2006). Then the PVP-TiO_2 composite nanofiber/Pt substrates were sintered at 500 °C for 5 min by rapid thermal processing to obtain TiO_2 nanofiber/Pt electrodes. For comparison, a TiO_2 film/Pt electrode was also prepared by spin coating. Briefly, a titanium *n*-butoxide (0.06 g mL^{-1}) and isopropyl alcohol (0.74 g mL^{-1}) mixed solution was spin coated on a Pt substrate at 2000 rpm for four times and subsequently sintered at 750 °C by rapid thermal processing for 3 min to obtain the TiO_2 film/Pt electrode. The thickness of the TiO_2 film is about 200 nm.

The TiO_2 nanofiber/Pt electrodes were rinsed carefully with ethanol and double-distilled water. Then GOx (8 mg mL^{-1}) PBS solution with a volume of 20 μL was drop coated onto the surface of the TiO_2 nanofiber/Pt electrodes with a pipette and dried at 4 °C. In order to maintain the immobilized GOx molecules to improve the performance of the enzyme electrode, 10 μL of 0.25 mg mL^{-1} Chit acetic acid solution, which exhibits excellent film-forming ability, high water permeability, susceptibility to chemical modifications (Zhang et al., 2004a,b), was drop coated on the surface of each GOx/TiO_2 nanofiber/Pt electrode. After the Chit film was formed, the Chit/GOx/TiO_2 nanofiber/Pt electrodes were rinsed thoroughly with double-distilled water, dried and stored at 4 °C for future use. For comparison, the enzyme electrodes Chit/GOx/Pt and Chit/GOx/TiO_2 film/Pt were also fabricated by the same procedure as described above.

2.3. Electrochemical measurements

Before electrochemical measurement, all of the as-prepared electrodes were immersed in PBS (pH 7.2) for 15 min to remove residua. The TiO_2 nanofiber/Pt electrodes were investigated by CV and EIS in PBS (pH 7.2) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ redox probe, which was usually utilized to characterize the surface feature of the electrodes (Ruan et al., 2002). The impedance, including real (Z_{re}) and imaginary ($-Z_{im}$) components, was recorded at 0.2 V vs. Ag/AgCl in the frequency range of 0.1 Hz to 100 kHz with a signal