Electrochemistry Communications 38 (2014) 4–7

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Short communication

High performance cholesterol sensor based on ZnO nanotubes grown on Si/Ag electrodes

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ARTICLE INFO

Article history:
Received 4 October 2013
Received in revised form 25 October 2013
Accepted 28 October 2013
Available online 5 November 2013

Keywords:
ZnO nanotubes
Cholesterol biosensor
Wide linear range
High sensitivity

ABSTRACT

Zinc oxide nanotube (ZNT) arrays were grown on Si/Ag substrate by one-step chemical process in an aqueous solution and further used as a working electrode to fabricate an enzyme-based cholesterol biosensor through immobilization of cholesterol oxidase (ChOx). The fabricated biosensors exhibit high and reproducible sensitivity of 79.40 μA/mM/cm^2, wide linear range from 1.0 μM to 13.0 mM, fast response time of ~2 s and ultra-low detection limit of 0.5 nM (S/N = 3) for cholesterol sensing. The anti-interference ability and long-term stability of the biosensor were also assessed. Finally, the biosensor was applied to analyze cholesterol concentration in human serum samples.

1. Introduction

Quantification of blood cholesterol is routinely practiced for various types of medical diagnosis or screening. Thus, a simple, easy and highly sensitive cholesterol biosensor is desirable that can be useful in regular monitoring and management of cardiovascular diseases. Currently, many efforts have been focused for designing amperometric biosensor for cholesterol detection owing to its low cost, good selectivity and rapid response [1–3]. In this regard, varieties of desired nanomaterials have been exploited for constructing electrochemical biosensors, as they provide large specific surface area for higher enzyme loading and compatible microenvironment for retention of the enzyme bioactivity [4,5]. In addition, they also provide direct electron transfer between the enzyme's active site and the electrode. Nanostructured ZnO, due its unique and interesting characteristics such as high catalytic support, catalyst efficiency and strong adsorption ability has attracted enormous attention from researcher for several applications [6]. ZnO has been considered as a potential material for various enzymatic sensing applications because of its high isoelectric point (9.5), biocompatibility, and abundance in nature. The nontoxic nature, high chemical stability and electron transfer capability make ZnO even more appropriate for biomolecule immobilization without an electron mediator. Such a material can also be employed for future development of implantable biosensors [7,8]. However, most of the research groups not only used expensive materials [9–12] such as Au, GC, Ti and ITO, but also synthesized nanostructures on substrates then transferred and coated them onto electrodes, which needs additional processes such as preparation of nanostructure-containing solution, coating, network-forming for tight adhesion, and drying.

To date, ZNT have been greatly used for designing biosensors because of its porous nature, hollow interior and large specific surface area [13]. Although, various groups have reported an enhanced electrochemical response of the ZNT arrays based biosensor [14–16], but until now no ZNT based cholesterol biosensor has been fabricated. In this paper, for the first time, we report directly grown ZNT on Ag electrode via one-step low temperature solution process for highly efficient cholesterol sensor fabrication. Importantly, the cholesterol biosensor applicability was evaluated in human serum samples.

2. Experimental details

2.1. Reagents

ChOx (EC 1.1.3.6 from Streptomyces species, 20 U/mg), Nafion (5 wt.% in lower aliphatic alcohol and water mixture), cholesterol (water soluble), human blood serum (H4522), glucose (d-(+)-99.5%), ascorbic acid (AA), L-cysteine (L-Cyst), uric acid (UA), zinc nitrate hexahydrate ($ZnNO_3 \cdot 6H_2O$, 99%), hexamethylenetetramine (HMTA, 99%), and phosphate buffered saline (PBS, pH = 7.4) were purchased from Sigma–Aldrich and used without further purifications.

2.2. Fabrication of cholesterol biosensor and measurements

Firstly, Si/Ag electrode was prepared by sputtering Ag and ZnO seed layer on Si substrate. To synthesize ZNT on Si/Ag electrode, $ZnNO_3 \cdot 6H_2O$ (0.05 M) and HMTA (0.05 M) were dissolved in

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http://dx.doi.org/10.1016/j.elecom.2013.10.028

distilled water (50 mL) with pH 6.0 adjusted by 0.01 M NaOH; transferred into Pyrex glass bottle with the electrode suspended upside down; and heated at 80 °C for 3 h. After the reaction, the electrodes were rinsed with DI water and air dried. Prior to ChOx immobilization, Si/Ag/ZNT electrodes were rinsed with PBS to generate hydrophilic surfaces. A 5 μL of ChOx (1.0 mg/mL in 0.10 M PBS) was immobilized onto the Si/Ag/ZNT electrode surface by physical adsorption and air dried. Then, 1 μL Nafion solution (0.5 wt.%) was dropped onto the Si/Ag/ZNT/ChOx electrode surface, which not only provides a biocompatible environment to the enzyme without interfering on sensor response but also works as covering network (prevent possible enzyme leakage), thereby further avoids any undesirable interactions between the enzyme and interferents present in the biological medium. Also, the Nafion covered on the immobilized enzyme works as freely diffusing mediators that does not have any effect on the accessibility of ChOx to cholesterol during measurements. Finally, the electrodes were stored in dry condition at 4 °C when not in use.

The morphology and crystallinity of as-synthesized ZNR were examined by field emission scanning electron microscopy (FESEM, Hitachi S4700), UV–visible, and X-ray diffractometer (XRD) measured with Cu-Kα radiations (λ = 1.54178Å) in the range of 30–50° with 8°/min scanning speed, respectively. Cyclic voltammetry (CV) and amperometric measurements were performed using an electrochemical analyzer connected to personal computer. All experiments were carried out using a conventional three-electrode system with the Si/Ag/ZNT/ChOx/Nafion as working electrode, platinum wire as counter electrode, and Ag/AgCl with saturated KCl solution as reference electrode. All the potentials in this work were measured with respect to Ag/AgCl reference electrode and the electrochemical measurements were carried out at room temperature in 0.10 M PBS. Each CV was performed in solution (20 mL) between −0.30 and +0.80 V. In steady state amperometric experiments, the potential was set at +0.38 V vs. Ag/AgCl electrode with magnetic stirring.

3. Results and discussion

3.1. Morphological characterization of ZNT

Fig. 1 shows the FESEM images before (a & b) and after (c & d) ChOx immobilization; (e) XRD pattern; and (f) UV–vis spectrum of directly grown ZNT on Si/Ag substrate by aqueous solution process at low

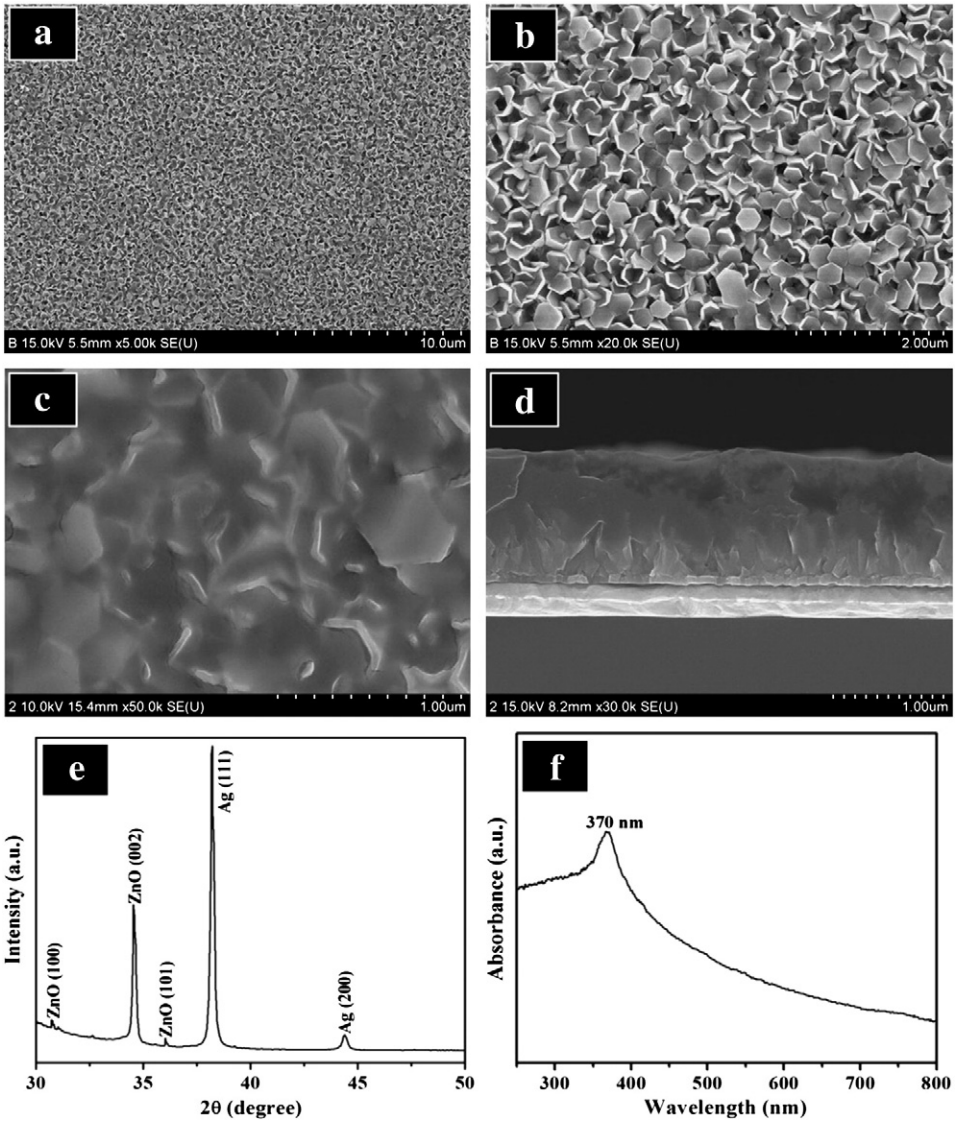

Fig. 1. Typical low (a) and high magnification (b) FESEM images of ZnO nanotubes before ChOx immobilization; top view (c) and (d) cross-section view FESEM images after ChOx immobilization; XRD (e) and UV–vis spectrum (f) of ZnO nanotubes directly grown on Si/Ag substrate.