

Dependence of seed layer thickness on sensitivity of nano-ZnO cholesterol biosensor

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Abstract. The anemone-like ZnO nanostructures have been synthesized by hydrothermal method and were further adsorbed immobilized cholesterol oxidase (ChOx) as a nano-biosensor. In this study, the sensitivity of biosensor were improved by varying the thickness of the ZnO seed layer. The SEM analysis showed changes in thickness of seed layer will not affect the morphologies of anemone-like ZnO nanostructures. The X-ray Diffraction patterns showed that the (002) plane of anemone-like ZnO grown on various thickness of the seed layer was more pronounced than other crystal plane. An abioelectrode (ChOx/ZnO/ITO/glass) grown on the 30nm of ZnO seed layer with high sensitivity of $57.533 \mu\text{A} \text{mM}^{-1} \text{cm}^{-2}$ ($1.488 \mu\text{A} (\text{mg/dl})^{-1} \text{cm}^{-2}$), a wide sensitive range from 25 to 500 mg/dl. It is concluded that the thinner sputtered ZnO seed layer for growing anemone-like ZnO nanostructure can effectively improve the sensitivity of the ZnO biosensor.

1. Introduction

According to the World Health Organization [1], heart and cerebrovascular diseases accounted for 20% of the world's cause of death. Many clinical diseases have a strong correlation with cholesterol concentration in the blood, such as heart disease, coronary artery disease, atherosclerosis, hypertension, thrombosis, etc. [2-3]. Excess cholesterol is a major cause of heart disease and cerebrovascular disease, and therefore it is important to monitor cholesterol levels in the blood. Development of fast and accurate cholesterol biosensor is extremely important to find those diseases. Due to their exceptional optical and electrical properties, the materials with nanostructures are receiving great attention for enzyme immobilization to improve stability and sensitivity of biosensors [4-5].

Enzymes directly immobilized on the electrode surface is easy to be deteriorated [6]. In order to improve this situation, using appropriate intermediate to modify the electrode, such as polymer, metal oxide or organic-inorganic composite film is necessary. These modifications can be used to secure the enzyme, keep the essence of the enzyme and increase the electron transfer. Therefore selection a suitable intermediate is very important for biosensor. Zinc oxide (ZnO) is an excellent intermediate in the enzyme electrode due to its unique characters [7-11]: (1) It is a semiconductor material with a wide bandgap of 3.37 eV. (2) ZnO has a high excitation binding energy of 60 mV. (3) The Zn element is easy to be obtained and cheaper. (4) Zinc oxides can be grown using various deposition methods at a low temperature. (6) It has excellent biological safety and biocompatibility. (7) Zinc oxide will be positively charged in a neutral solution due to its high isoelectric point (IEP ~ 9.5). It can let protein or enzyme adsorbed by electrostatic attraction force.



There have been a number of reports on the fabrication of cholesterol biosensor using ZnO thin films and nanostructures [12-14], but the sensitivity is too low or the detection range is too narrow. In this study, the sensitivity of ZnO biosensor was improved by varying the thickness of the ZnO seed layer. The anemone-like ZnO nanostructures had been grown on the seed layer by hydrothermal method. The cholesterol oxidase (ChOx) was immobilized on the anemone-like ZnO nanostructures as a cholesterol nano-biosensor.

2. Experimental

ZnO seeds layer was pre-sputtered on the surface of ITO ($4.4\Omega/\square$) glasses to grow a high orientation anemone-like ZnO nanostructures. Different thickness of seed layers with 30nm, 50nm and 100nm were prepared by changing sputtering time. In hydrothermal growth, all deionized water was pre-heated to a temperature of 90 °C before the hydrothermal reaction executed. The 0.05 M zinc nitrate solution made in 45 ml deionized water was mixed with 0.05M hexamethylenetetramine (HMTA) solution made in 45 ml deionized water in serum bottles. Then the electrodes were dipped in solution at 90 °C for 9 hrs. After synthesis was completed, the samples were rinsed with deionized water and dried. After drying the samples, 10 μ l of ChOx solution (1.0 mg/mL) prepared in phosphate buffer saline (PBS) (50mM, pH ~7.4, 0.9% NaCl) was spread over the surfaces of anemone-like ZnO structures and dried at room temperature for about an hour. The sample was then kept overnight for enzyme immobilization at a temperature of 4 °C by physical adsorption technique and subsequently washed with buffer solution to remove the unbound enzyme molecules. After hydrothermal growth, all the sample were stored at 4 °C. Electrochemical analysis is carried out on an Jiehan 5000 potentiostat/galvanostat using a three-electrode configuration with Ag/AgCl electrode as a reference electrode in PBS solution (50 mM, pH ~7.4, 0.9% NaCl) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The ZnO sample with nanostructures was used as the working electrode and platinum foil as a counter electrode. The cholesterol solutions have been prepared from PBS solution (50 mM, pH ~7.4, 0.9% NaCl) containing 1% of triton-X100 and stored at 4 °C.

3. Results and discussion

3.1. Materials analysis

Fig.1 shows the results of grown ZnO nanostructures by a modified hydrothermal method in this study. The SEM images show the morphologies of these ZnO nanostructures which look like anemones appearances. Fig.1(a) shows that each anemone-like structure has three to five elongated tentacles. There are lots of spaces among the tentacles which provide plenty of surfaces for enzyme adsorption. These anemone-like structures basically can be divided into two parts, the stubby basement structures and elongated tentacles structures, such as shown in Fig.1(b)(d)(f). These ZnO nanostructures were densely and well arranged on the surfaces of ITO substrates. The pre-sputtered ZnO seeds layer on ITO top surfaces help to overcome the activation energy for heterogeneous nucleation of ZnO nanostructures on the ITO surfaces.

Different thicknesses of ZnO seed layer were prepared by changing the sputtering time. The ZnO seed layers of 30nm, 50nm and 100nm were prepared for following hydrothermal growth. The thickness of the seed layer did not have obvious effect on the morphologies of ZnO nanostructures. The morphologies are all similar as shown in Fig.1 except with different lengths. All the appearances look like anemones. The variations of ZnO aspect ratio (length/diameter) of anemone tentacles depend on seed layer thickness. The aspect ratio decreases with decreasing the thickness of seed layer as seen in Fig.1(b),(d),(f). A lower aspect ratio leads to lower sensitivity when the ZnO nanostructures were assembled as cholesterol biosensors according to the literature [15].

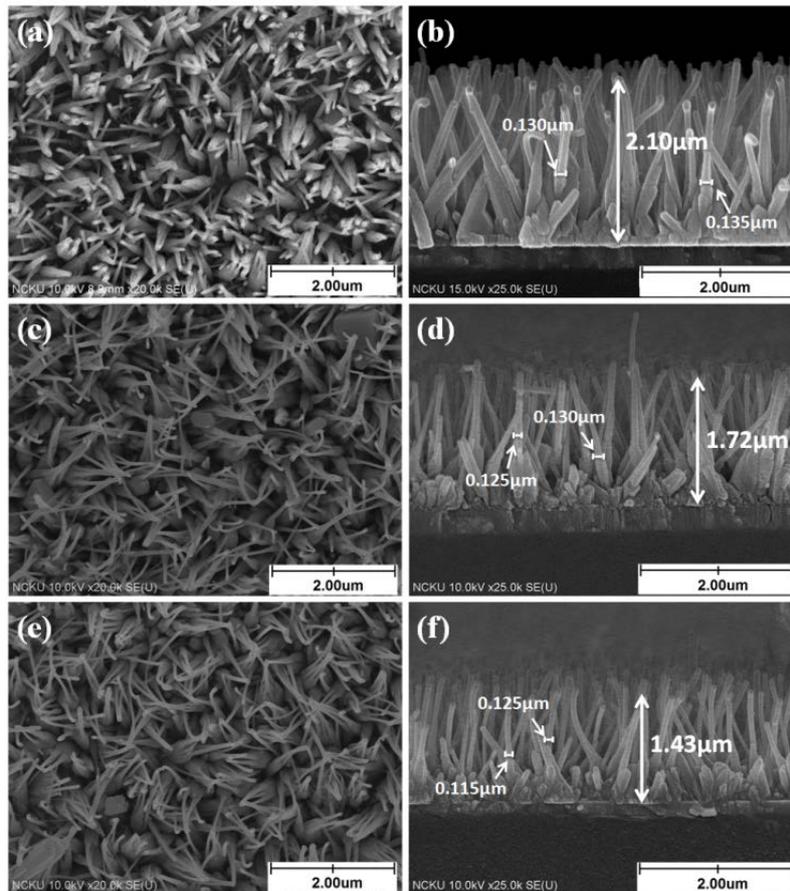


Figure 1.HR-SEM images of anemone-like ZnO nanostructures grown on 100nm ZnO seed layer, 50nm ZnO seed layer and 30nm ZnO seed layer respectively. (a),(b) with 100nm ZnO seed layer, (c),(d) with 50nm ZnO seed layer and (e),(f) with 30nm ZnO seed layer.

Fig.2 was the X-ray diffraction patterns of anemone-like ZnO nanostructures grown on 100nm ZnO seed layer, 50nm ZnO seed layer and 30nm ZnO seed layer. The results show that the crystallinities of anemone-like ZnO were good because the diffraction peaks are sharp and strong. A prominent (002) peak was observed in the XRD patterns, implying the ZnO nanostructures have a strong growth direction along the c axis direction. The SEM and XRD results were consistent as presented in Fig.1 and Fig.2. In Fig.2, there are some other small peaks (denoted by triangles) observed in the figures, which are the diffraction peaks of ITO substrates.

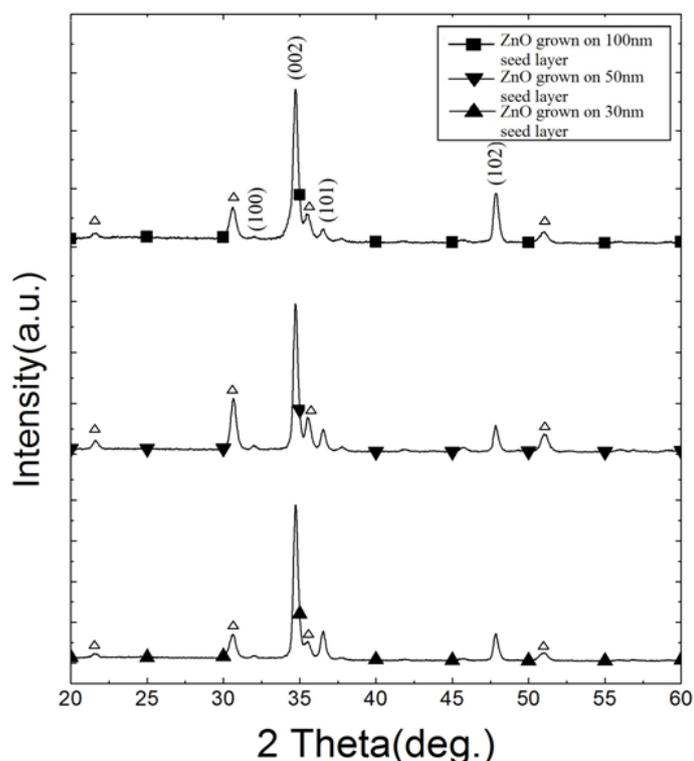


Figure 1. Shows X-ray diffraction patterns of anemone-like ZnO nanostructures grown on 100nm ZnO seed layer, 50nm ZnO seed layer and 30nm ZnO seed layer respectively

3.2. Electrochemical analysis

The anemone-like ZnO nanostructures were assembled as cholesterol biosensor. The sensitivity and linear sensing range of the biosensor were used to calculate the value of the Michaelis-Menten constant (K_m). The electrochemical measurement was employed in a PBS solution (50 mM, pH ~7.4, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$, the scan range of cyclic voltammetry from -0.2V to 0.6V and with sweep rate of 50 mV/s. Fig. 3 shows these three biosensors had good current responses. The potential difference between reduction and oxidation peaks is inversely proportional to the number of electrons transported between anode and cathode in the solution [16]. The thinner the thickness of seed layer, the less electrical resistance it is which means more electronic transportation in the solution. The oxidation current increases with decreasing the thickness of the seed layer due to less electrical resistance with thinner seed layer as seen in Fig. 3 from approximate 1×10^{-4} to around 3×10^{-4} amper. The anemone-like ZnO nanostructures grown on 30nm ZnO seed layer has the maximum amount of oxidation current will lead to a higher sensitivity as shown in Fig. 4. The sensitivity is defined as the peak oxidation current change per unit cholesterol concentration in the linear range as shown in the following equation:

$$\text{Sensitivity} = \Delta I_{pa} / (\Delta C \times A) \quad (1)$$

Where ΔI_{pa} is the variation of oxidation current peak, ΔC is the analyte concentration change, A is the working area of electrode. The sensitivity has increased significantly by reducing the thickness of the ZnO seed layer from 100nm to 30nm. The best sensitivity obtained in this study is $57.5 \mu A/mM/cm^2$ as the ZnO nanostructure prepared on the 30nm seed layer.

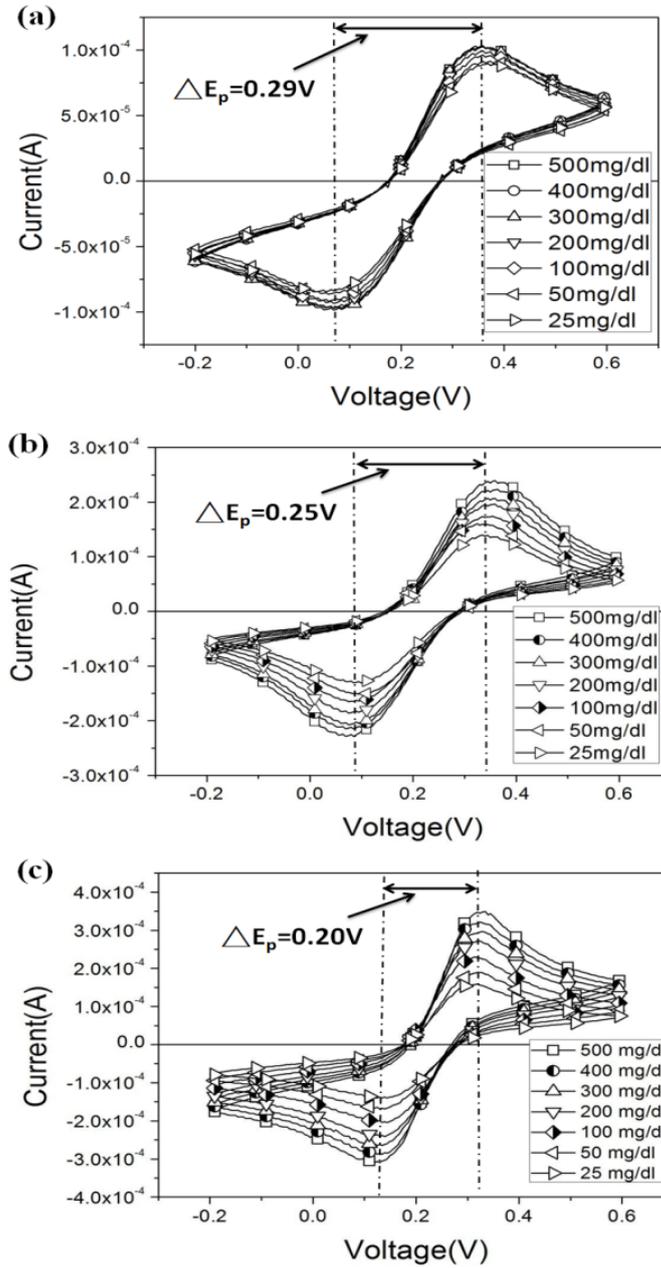


Figure 3. Cyclic voltammetry of the ChOx/ZnO/ITO/glass bioelectrode grown on 100nm ZnO seed layer, 50nm ZnO seed layer and 30nm ZnO seed layer measured at different cholesterol concentration. (a) with 100nm ZnO seeds layer, (b) with 50nm ZnO seeds layer and (c) with 30nm ZnO seeds layer

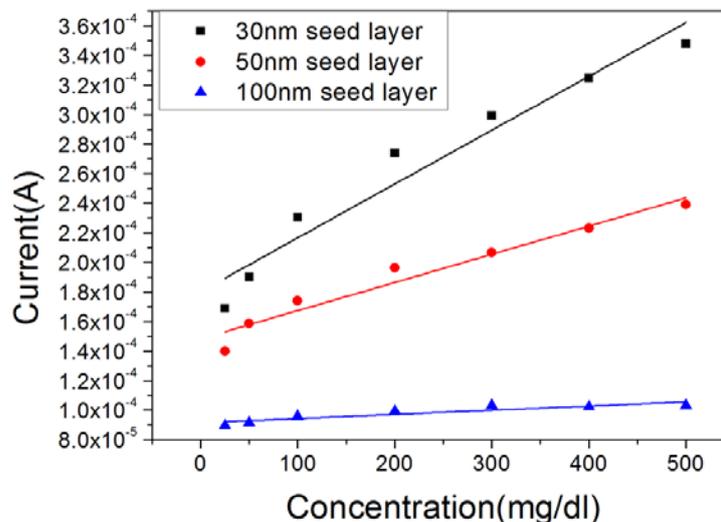


Figure 4. Variation of sensitivity of anemone-like ZnO biosensors prepared on different thickness of seed layers

4. Conclusion

In this study, the thinner sputtered ZnO seed layer for growing anemone-like ZnO nanostructure can effectively improve the sensitivity of the ZnO biosensor. The best sensitivity can be obtained in this study is 57.5 $\mu\text{A}/\text{mM}/\text{cm}^2$ as the ZnO nanostructure grown on the 30nm seed layer. The linear sensitive range is from 25 to 500 mg/dl.

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