Biosensor for heavy metals using hydrothermally grown ZnO nanorods and metal-binding peptides

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ABSTRACT

We report herein a biosensor for the determination of heavy metals based on hydrothermally grown ZnO nanorods and metal-binding peptides. Metal binding peptide is immobilized on ZnO nanorods through non-specific binding. Peptide binding with heavy metal ion causes the electrical signal change, which measured and correlated to the concentration of heavy metals. The sensor performance will be optimized with respect to the operating conditions. The new biosensor format will offer great promise for real-time environmental monitoring of heavy metals.

Keywords: biosensor, zinc oxide, heavy metal binding peptide

1 INTRODUCTION

Every year, around 2.4 million tons of metal wastes from industrial sources and 2 million tons from agriculture are generated in US. Pollution caused by heavy metals poses a great danger to humans and the environment which has led to stringent regulations over the allowable limits of heavy metals in drinking water. Heavy metals such as Pb^{2+}, Hg^{2+}, and Cd^{2+} are currently ranked second, third, and seventh, respectively, on the EPA's priority list of metals that are of major environmental concern. The heavy metals mercury, lead and cadmium can be released into the environment through industrial use and leakage from dump sites, and once released can contaminate water, soil and air[1]. These heavy metals can not be broken down in the human body and their accumulation over time can result in serious health risks. Studies have indicated that long term exposure to mercury can cause permanent damage the brain, kidneys, and the developing fetus[2]. Many studies demonstrate lead's toxicity in the nervous system and its association with increased blood pressure in adults[1]. Long term exposure to cadmium can include nephropathies, emphysematous alterations in the lung, and cardiovascular diseases, whereas acute exposure is usually restricted to the lungs[3]. Because of their extreme toxicity, there are growing needs for rapid and sensitive detection of the heavy metals.

Many spectroscopic and electrochemical techniques have been developed thus far for detection of heavy metals, such as atomic absorption spectrophotometry (AAS), Auger electron spectroscopy (AES), inductively coupled plasma – mass spectrometry (ICP-MS), ICP-optical emission spectrometer (ICP-OES), ion-selective electrode (ISE), and polarography. However, their applications to the detection of environmental samples are limited due to high cost and, sometimes, to lack of real-time. Therefore, there is an urgent need to develop more sensitive and cost-effective methods to detect heavy metal concentrations in real-time format. Biosensors are good choices in this respect. A biosensor is an analytical device that combines a biological sensing element with a transducer to produce a signal proportional to the concentration of analyte. Affinity peptides, such as hexahistidines, glutathione S-transferase, maltose-binding proteins and synthetic phytochelatins $(EC)_n$ have been demonstrated to bind heavy metals even at dilute concentrations, and can be used as biological sensing elements.

Since the discovery and characterization of carbon nanostructures, extensive research has been conducted to characterize other nanostructures. ZnO is of particular interest because of its wide band gap (3.37 eV), high exciton binding energy (60 meV), high stability and high melting point. These physical properties make ZnO an attractive material for use in short-wavelength optoelectronic devices[4], such as LED, ultra-violet lasers[5], chemical and biological sensors[6,7], and solar cells[8]. Various synthesis methods are used to produce the ZnO nanostructures. Although fewer morphologies have been discovered by hydrothermal synthesis than by a solid-vapor synthesis processes, the simplicity of equipment and low temperatures required for synthesis makes the hydrothermal method a better candidate for real application. Using the hydrothermal method, techniques have been developed to produce quasi-one-dimensional ZnO structures including nanorods[9,10] and nanotubes[11]. Three-dimensional structures have also been constructed, including nanoflowers[12-14] and nanospheres[15,16]. While the morphology of ZnO nanostructures has been extensively studied, applications of ZnO in biosensors are rare[17].

We propose herein a biosensor for determination of heavy metals based on hydrothermally grown ZnO nanorods and metal-binding peptides. Heavy metal binding peptide is immobilized on ZnO nanorods. Its binding with heavy metals causes the electrical signal change which can be measured and correlated to the concentration of heavy metals. The sensor performance will be optimized with respect to the operating conditions. The new biosensor offers great promise for rapid environmental monitoring of heavy metals.

2 EXPERIMENTS

2.1 ZnO nanorods synthesis

ZnO nanorods were directly grown on a glass by hydrothermal decomposition described elsewhere[7]. Briefly, the reaction solution was prepared by mixing proper quantity of ammonia (25%) and 0.1 M zinc chloride solution in a bottle with autoclavable screw cap. The glass substrate was vertically immersed in the reaction solution. A layer of product was deposited on the substrate after the sealed bottle was heated at 95 °C for 2 hour in an oven. Then, the sample was thoroughly washed with DI water and dried in air**.** Scanning electron microscopy (SEM) was employed to examine the morphology of the hydrothermal product. Figures 1 and 2 show a typical SEM image of the as-grown product by hydrothermal decomposition. It can be seen that, the nanostructure presents a rod-like shape with a hexagonal cross-section, a typical morphology of wurtzite ZnO. It is also noted that the nanorods are primarily aligned along the perpendicular direction of the substrate. The nanorods are uniform in size with a diameter of about 300 nm and a length of 4 μm. The size uniformity is a unique feature of hydrothermally grown nanostructures compared to vapor phase transport method.

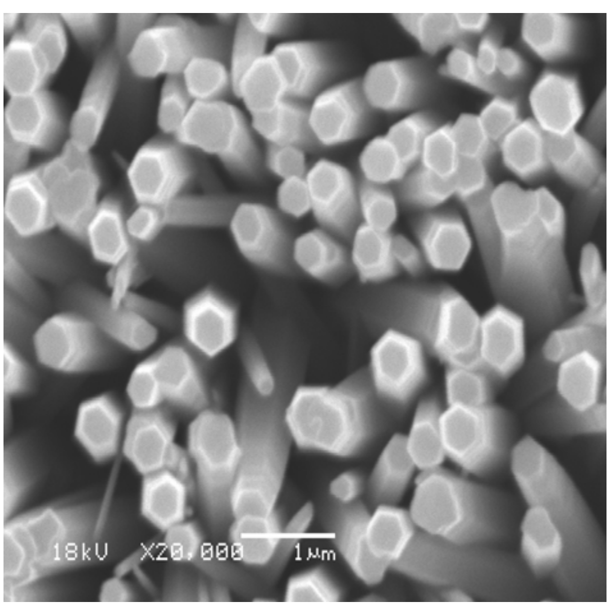

Figure 1. SEM image of the as-grown ZnO nanorods on glass substrate

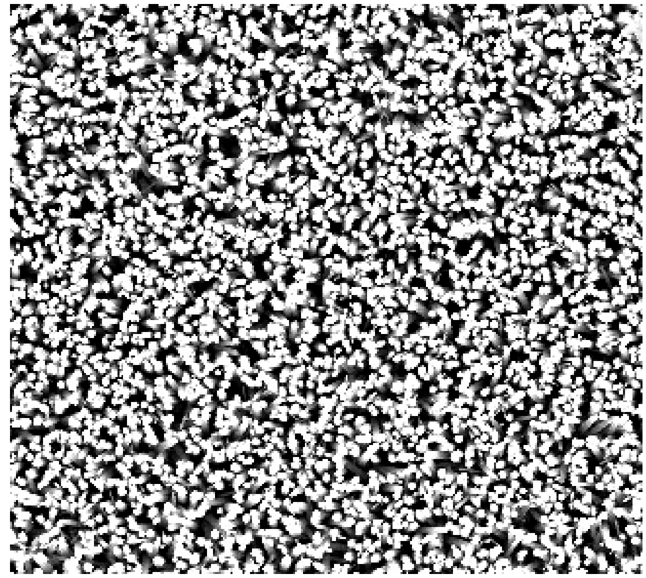

Figure 2. Large area of ZnO nanorods.

2.2 Electrode fabrication

The interdigitated electrodes (IDE) configuration (Figure 3) is chosen to enable effective electronic contact between hydrothermally grown ZnO and the electrodes over large areas. The interdigitated electrode will be fabricated using a conventional photolithographic method with a finger width of 10 μm and a gap size of 8 μm. The IDE fingers are made by thermally evaporating 20-nm Ti and 40-nm Au either on ZnO nanorods.

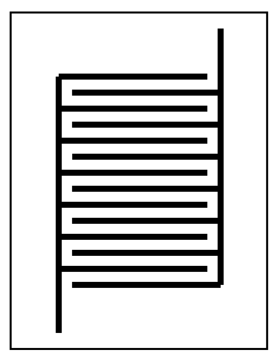

Figure 3. Schematic illustration of interdigitated electrodes on ZnO nanorods film

2.3 Metal binding peptide based biosensor

Hexahistidines, glutathione S-transferase, maltose-binding proteins and synthetic phytochelatins $(EC)_n$ can bind different heavy metal ions with different affinity. In this research, $(EC)_{10}$, which can retrieve Cd^{2+} at sub-parts per million levels, is used to develop the biosensor.

Metal binding peptide $(EC)_{10}$, purchased as a customized product, will be used to functionalize the ZnO nanorods through non-specific binding. Briefly, a small volume of metal binding peptide solution is dropped onto the ZnO for 1 hour, and then the sample is rinsed with deionized water to remove unbound peptides and dried in air. The developed biosensor is stored at 4 °C for future use. The biosensor will be challenged with simulated Cd^{2+} solution. The binding of target heavy metal with metal binding peptide will change the conductance of ZnO, which can be sensitively measured and correlated to the concentration of heavy metals.

The biosensor is still under investigation and it will be optimized and the analytical characteristics such as calibration curve, the limit of detection, selectivity, accuracy, and stability will be investigated in detail.

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