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Short communication

A fast and sensitive potentiometric glucose microsensor based on glucose oxidase coated ZnO nanowires grown on a thin silver wire

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ABSTRACT

In this study, a potentiometric glucose biosensor was fabricated by immobilization of glucose oxidase on to zinc oxide nanowires. Zinc oxide nanowires with 250–300 nm diameters and approximately 1.2 μm lengths were grown on the surface of silver wires with a diameter of 250 μm. Glucose oxidase (GOD) was electrostatically immobilized on the surface of the well aligned zinc oxide nanowires resulting in sensitive, selective, stable and reproducible glucose biosensors. The potentiometric response *vs.* Ag/AgCl reference electrode was found to be linear over a relatively wide logarithmic concentration range (0.5–1000 μM) suitable for intracellular glucose detection. By applying a membrane on the sensor the linear range could be extended to 0.5 μM to 10 mM, which increased the response time from less than 1 to 4 s. On the other hand the membrane increased the sensor durability considerably. The sensor response was unaffected by normal concentrations of common interferents with glucose sensing such as uric acid and ascorbic acid.

1. Introduction

Glucose biosensors are by far the most widely studied type of biosensors and numerous designs have been proposed. Three generations of glucose biosensors based on glucose oxidase can be identified using (i) natural oxygen as co-substrate and generation and detection of hydrogen peroxide, (ii) synthetic electron mediator and (iii) direct electron transfer between glucose oxidase and the electrode [1]. However, converting the biological signal to an easily processed electronic signal is challenging due to the complexity of connecting an electronic device directly to a biological environment. The intrinsic advantages of electrochemical biosensors are their robustness, easy miniaturization, excellent detection limits, also with small sample volumes and ability to be used in turbid biofluids. Such biosensors including the associated microelectronic circuitry have low production cost and are easy to interface with normal electronic readout and processing.

The fast and accurate determination of glucose has widespread application since glucose concentration is a crucial indicator in many diseases, such as diabetes and other endocrine metabolic disorders. Glucose fluctuations within the normal physiological range of 110 ± 25 mg/dl (around 6 mM) are considered to be acceptable while diabetics may have values of 360 mg/dl (20 mM) or higher [2]. During several decades, researchers have been engaged in the development of glucose sensing devices including biosensors for monitoring the glucose level in biological fluids. Among these biosensors, amperometric glucose biosensors have attracted substantial interest due to good sensitivity and low detection limit. However, upon applying a high polarizing voltage (E_{app} = 0.6–0.8 V) interfering substances such as ascorbic acid and uric acid, which are commonly present in biological fluids are also oxidized, leading to nonspecific signals [3]. Several artificial redox mediators have been investigated as electron acceptors to solve these problems [4–13]. Additionally, the sensor electrodes have been modified to enhance the performance of amperometric glucose biosensors [14–18]. The interferences mentioned above are avoided in thermometric biosensors such as enzyme thermistor [19]. In addition their excellent stability makes them particularly suitable for long term monitoring. Compared to amperometric biosensors, since no extra potential is required, potentiometric biosensors have an advantage in selectivity and simplicity. However, a limitation of ion sensitive electrodes (ISEs) is that only charged molecules can be directly detected. This obstacle can be overcome by letting the analyte undergo a reaction, such as an enzyme reaction, that produces a detectable ion in an amount proportional to the concentration of the analyte in the sample. In the enzyme field effect transistor (EnFET), this is taken a step further by combining the enzyme

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reaction with an ion sensitive field effect transistor (ISFET) was first introduced by Caras and Janata [20].

In general, nanowires are attractive for their versatile roles in bioelectronics and nanoelectronics applications and they are increasingly being used as building blocks for biosensing purposes. The use of nanomaterials has allowed the introduction of many new signal transduction technologies in biosensors resulting in improved sensitivity and performance. Because of their submicron dimensions, nano-sensors, nano-probes and other nano-systems have allowed simple and rapid analyses *in vivo* [21]. Their implementation as highly sensitive electrodes is one obvious example, such as the platinum electrode network proposed by Wang et al. for glucose detection [22]. Among the nanostructures ZnO is of special interest to biological sensing due to many favorable properties. Being next to one-dimensional nanostructures ZnO nanowires have many unique advantages including high surface to volume ratio, good chemical stability, biocompatibility and easy fabrication. They are non-toxic, electrochemically active, and have a high electron communication capability [23–30]. When considering glucose detection, it is important to note that there is a large difference in the isoelectric points of zinc oxide and glucose oxidase. The isoelectric point (IEP) of zinc oxide is around 9.5, making it possible to immobilize low isoelectric point (IEP) molecules as, *e.g.* DNA or proteins by electrostatic adsorption in proper buffer solutions [31]. Glucose oxidase (GOD having IEP at pH 4.5) is widely employed in most of the glucose biosensors due to its stability and high selectivity to glucose. Various methods, such as covalent binding [32], embedding [33] and cross-linking [34–36] have been used to immobilize glucose oxidase on different supporting materials. In addition, the negative charge of glucose oxidase at neutral pH makes it feasible to immobilize it on materials with positive charge like ZnO by the physical adsorption [37,38].

In this work, an easily fabricated and highly sensitive potentiometric glucose biosensor based on ZnO nanowires was successfully demonstrated using a simple electrostatic process for the immobilization of GOD, in some cases combined with a cross-linking method and a Nafion membrane. The reproducibility and long term stability was evaluated by using three different sensor electrodes of both types. After more than three weeks and kept at in 4 °C temperature when not in use, the immobilized GOD/ZnO/Ag electrode lost only 10% of its initial activity, while the electrodes with a membrane preventing enzyme leakage (see Section 2.3) retained 95% of the electrocatalytic activity of GOD. The developed sensors are capable for intracellular as well as extracellular glucose measurements.

2. Experimental details

2.1. Materials

Glucose oxidase (E.C. 1.1.3.4) from *Aspergillus niger* 360 U/mg (BBI Enzymes (UK) Ltd.). Bovine serum albumin (BAS ≥ 98%), glutaraldehyde (50% solution), Nafion (5 wt.%), D-(+)-glucose (99.5%), zinc nitrate hexahydrate and hexamethylenetetramine (HMT) were purchased from Sigma–Aldrich. Phosphate buffered, 10 mM solution (PBS) was prepared from Na_2HPO_4 and KH_2PO_4 (Sigma–Aldrich) with sodium chloride in 0.135 mM, the pH was adjusted to 7.4. Glucose stock solution was kept at least 24 h after preparation for mutarotion. All chemicals used (Sigma, Aldrich) were of analytical reagent grade.

2.2. Fabrication of silver electrode with ZnO wires

A 3 cm long clean, straight piece of silver wire (250 μm in diameter) was first rinsed with acetone followed by rinsing in deionized water and then it was dried at room temperature. To grow ZnO nanowires on the silver wire a low temperature chemical approach was adopted [39]. First the wire was dipped into a seed solution for 2 min and then dried in air. This procedure was repeated twice. The seed solution contained 0.025 M zinc nitrate and 0.025 M hexamethylenetetramine (HMT) [$(C_6H_{12}N_4)$]. The solution was kept at 90 °C during the growth of ZnO nanowires. Subsequently, the wire was washed by distilled water and dried at room temperature. Typical ZnO nanowires grown on the silver wires using this procedure are shown in Fig. 1. As clearly seen from the SEM images, ZnO nanowires of 250–300 nm diameters with uniform density and spatial distribution had been grown. These nanowires were perpendicular relative to the surface of the silver wire. The morphological and structural characteristics of the grown nanowires can be controlled by adjusting the growth process parameters such as the concentration of the seed solution, the reagent stoichiometry, the temperature and the pH of the growth solution [40].

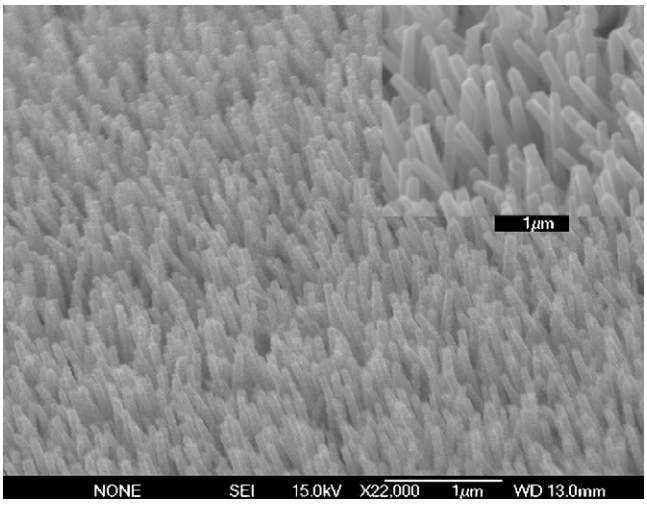

Fig. 1. A typical scanning electron microscopy SEM image of ZnO nanowires grown on 250 μm silver (Ag) wire using low temperature chemical growth. The figure shows that the diameter of the nanowires is in the range of 250–300 nm. The inset SEM image is showing the magnifying image of nanowires.

2.3. Glucose biosensor fabrication and electrochemical measurements

Two types of sensor electrodes without and with membrane were prepared for experiments. For the first type of sensor electrode, glucose oxidase (GOD) solution, 10 mg/ml, was prepared in 10 mM phosphate buffered solution (PBS) at pH 7.4, by using glucose oxidase (E.C. 1.1.3.4) from *A. niger*. Then GOD was electrostatically immobilized by dipping the ZnO nanowire-coated silver wire into the enzyme solution for 15 min at room temperature and then letting it dry in air for more than 20 min. Before the immobilization of GOD on the second type of sensor electrode, the zinc oxide electrode was rinsed with PBS to generate a hydrophilic surface. An enzyme solution was prepared by dissolving 10 mg GOD and 20 mg BSA in 200 μl PBS and the electrode was dipped into enzyme solution for 15 min and then left in air for 2 h to dry. The cross-linking procedure was carried out by adding 2 μl aqueous solution containing 2.5% glutaraldehyde and 0.5% Nafion onto the electrode surface. After drying at room temperature, 2 μl of 0.5% Nafion solution was further applied onto the electrode surface to prevent possible enzyme leakage and eliminate foreign interferences. All enzyme electrodes were stored in dry condition at 4 °C when not in use. After completing these steps, both types of sensors were initially checked potentiometrically in 100 μl of 100 μM glucose solutions with an Ag/AgCl reference electrode purchased from