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Application of Hydrothermally Grown ZnO Nanorods for Electrochemical Biosensors

ZnO nanorods have been used on Au working electrodes of biosensors for enhancing biosensor characteristics. ZnO nanorods grown on working electrodes have been employed for fabricating not only glucose sensors but also electrochemical immunosensors for detecting Legionella pneumophilia. The sensitivity of these biosensors was enhanced substantially compared to typical electrochemical biosensors based on Au working electrodes.

Keywords: *ZnO, nanorod, glucose sensor, immunosensor, Legionella pneumophilia.*

Introduction

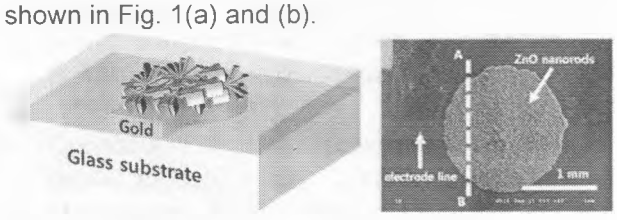
It is often important to measure quantity of an analyte precisely and electrochemical biosensors can be very useful in determining quantity of the analyte because the output of an electrochemical sensor is usually proportional to the amount of the analyte. Electrochemical sensing method has several advantages including its simplicity in detection, fast response, high selectivity, and relatively low cost [1]. Therefore, an electrochemical sensors have been under development for 50 years, ever since Clark reported upon the first enzyme electrode on which glucose ozidase (GOx) was immobilized [2].

In enzymatic electrochemical sensors, a good enzyme immobilization on the sensor electrode is of vital importance for the realization of high performance sensors. This is because this type of sensors measures the redox current of the products, such as hydrogen peroxide, which are produced by enzyme-substrate reactions. Recently, nanostructured metal oxides, such as zinc oxide (ZnO), nickel oxide (NiO), and copper oxide (CuO), have been employed as a part of the working electrode of enzyme biosensors because enzymes with a low isoelectric point (IEP, ~4.2) can be effectively immobilized on the nanostructured metal oxides with high IEPs (> 9) by electrostatic interactions [3,4].

ZnO nanorods have been considered for the biosensors due to large surface area, nontoxicity, biocompatibility, high electrochemical activity, and fast electron transfer [5,6]. The ZnO nanorods-based matrix film with large surface area has attracted considerable interest for applications of the biosensors because the sensitivity of an electrochemical biosensor is closely related to the working electrode surface area. Chen et. al. and Gupta et. al. have tried to fabricate a enzyme biosensor such as a glucose biosensor by using ZnO composite matrix film in order to improve the sensitivity [7,8]. In this paper, the effect of the matrix-based ZnO nanorods without composite material is described using both an electrochemical immunosensor for *Legionella pneumophila* detection and an electrochemical glucose sensor.

1. Glucose Biosensor Using Hydrothermally Grown ZnO Nanorods

A 500 μm thick 4″ 7740 glass wafer was used as a substrate for the fabrication of the electrochemical glucose biosensor based on the ZnO nanorod arrays and the network-shaped ZnO nanorods. The 0.02 cm^2 area Au/Ti electrodes were patterned on a glass wafer, and then a polyimide passivation layer was patterned onto the Au/Ti-patterned substrate. The photoresist was patterned on the substrate for the lift-off process and the lift-off was used for patterning the ZnO nanorods. The ZnO nanorod seed solution [9] seed solution was coated onto the substrate by a spray method. The hydrothermal growth of the ZnO nanorods was carried out by keeping the seed solution-coated substrate in a growth solution at 90°C for 4 hours [9,10]. The substrate was then rinsed with deionized water and dried with N_2 gas. The ZnO nanorods were piled on the substrate. Finally, the patterned ZnO nanorod electrode was obtained, as shown in Fig. 1(a) and (b).



a b

Fig. 1. Hydrothermally grown ZnO nanorods on Au/Ti electrode a – a schematic drawing; b – SEM picture of the fabricated electrode

In order to immobilize the GOx on the patterned ZnO nanorod electrode, a 1.5 μL GOx solution was dropped onto the patterned ZnO nanorod electrode, and then the electrode was kept at 4°C overnight. It is believed that the GOx immobilization on the patterned ZnO nanorods was enhanced by an electrostatic interaction. After the immobilization process, the electrode was washed to remove the unimmobilized GOx. The as-prepared GOx/ZnO/Au/Ti work-

ing electrode, a platinum counter electrode, and an Ag/AgCl reference electrode made up the three electrodes of the fabricated glucose biosensor. They were used for the measurement of the output redox currents by using a potentiostat.

The crystallographic information and the chemical composition of the as-grown network-shaped ZnO nanorods were examined by XRD patterning and EDX spectroscopy, respectively. In Fig. 3(a), all of the diffraction peaks of the ZnO nanorod arrays and network-shaped ZnO nanorods can be indexed to the hexagonal wurtzite phase of ZnO, which matches well to the standard XRD data file. The dominant peaks for the ZnO nanorod arrays and the network-shaped ZnO nanorods are (100) and (101) peaks, in contrast to the vertically grown ZnO, where the dominant peak is (002) [11]. Figure 3(b) shows that the chemical composition of the grown ZnO nanorods is the sum of the Zn and O without any other impurities, which confirms the high purity of the grown ZnO nanorods (Zn:O=1:1.18).

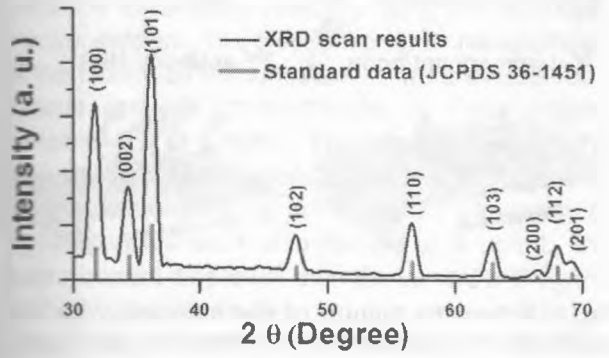


Fig. 2. The results of (a) the XRD scan and (b) the EDX spectrometry of the as-grown network-shaped ZnO nanorods

A schematic drawing of the working principle of the glucose biosensor based on the ZnO nanorod electrode is shown in Fig. 3. The electrochemical reactions for the detection of the glucose in presence of the glucose oxidase are as follows [12]:

$$\text{Glucose} + O_2 \xrightarrow{GOx} \text{Gluconolactone} + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

When a positive potential is applied to the working electrode with respect to the reference electrode, the hydrogen peroxide is oxidized on the working electrode surface, according to Eqn. (2).

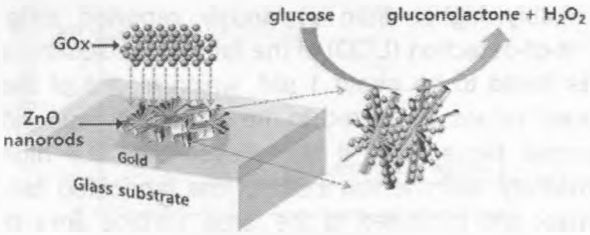


Fig. 3. A schematic view of the GOx/ZnO/Au/Ti working electrode based on the network-shaped ZnO nanorods for detecting glucose

The electrochemical experiments were carried out by using the as-prepared GOx/ZnO/Au/Ti electrode at various concentrations of glucose and GOx. At various concentrations of glucose level ranging from 0 to 50 mM, the cyclic voltammograms (CVs) of GOx/ZnO/Au/Ti electrode are shown in Fig. 4(a). It is observed from the CVs that the redox current increases with glucose concentration because the oxidation current of H_2O_2 increases with glucose concentration. Fig. 4(b) shows a calibration curve for the redox current measured at + 0.75 V versus the reference electrode at various glucose concentrations. It can be seen that the response current increases with the glucose concentration, but the response time saturates at a certain high glucose concentration due to the active sites of the enzymes saturating [13].

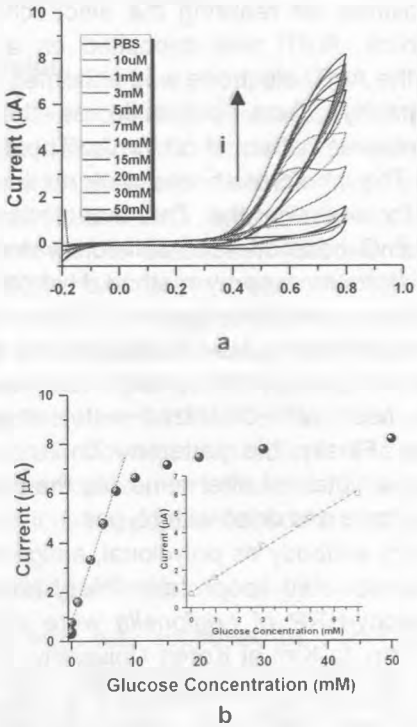


Fig. 4. a – Cyclic voltammograms and b – Calibration response current curve of the fabricated glucose biosensor at various glucose concentrations

The fabricated glucose biosensor showed a good linearity from 10 μM to 7 mM glucose concentration levels with the correlation coefficient of 0.99842 and a high sensitivity of 41.76 $\mu A \cdot cm^{-2} \cdot mM^{-1}$, which is remarkably higher than previously reported data. Limit-of-detection (LOD) of the fabricated biosensor was found to be about 1 μM, which is one of the lowest values compared to the previously reported glucose biosensors. It is believed that the high sensitivity and the low LOD of the fabricated biosensor are attributed to the large surface area of the network-shaped ZnO nanorods and to the direct electron transfer from the vertically grown ZnO nanorod arrays on the substrate.

2. Electrochemical immunosensor based on ZnO nanorods for detecting *Legionella pneumophila*

This part describes the fabrication of an electrochemical immunosensor based on ZnO nanorods matrix for *L. pneumophila* detection as a rapid and sensitive method for on-site diagnosis. In this study, a primary antibody was immobilized on the ZnO nanorods matrix and a second antibody conjugated to an enzyme label such as horse radish peroxide (HRP) was used as the detection antibody. The fabricated immunosensor was evaluated in acetate buffer containing 3,3',5,5'-tetramethylbenzidine (TMB), which is a good electron transfer mediator, with H_2O_2 by using an electrochemical measurement system.

A 800 μm thick 3" 7740 glass wafer was used as the substrate for realizing the electrochemical immunosensor. Au/Ti was deposited on a glass wafer and the Au/Ti electrode was patterned by the photolithography. Then, polyimide as the passivation layer was patterned on the Au/Ti-patterned substrate. The photoresist was patterned on the substrate for realizing the ZnO nanorods-based electrode. ZnO nanorod seed solution was coated on the substrate by a spray method. Hydrothermal ZnO nanorods growth was carried out by suspending the seed solution-coated substrate in a growth solution at 90°C for 4 h in an oven. The substrate was then rinsed with deionized water and dried with N_2 gas. Finally, the patterned ZnO nanorods electrode was obtained after removing the photoresist with acetone and dried with N_2 gas.

A primary antibody as polyclonal, antigen (peptidoglycan-associated lipoprotein, PAL), and polyclonal antibody-HRP of *Legionella* were obtained from Prof. Min Ja Kim at Korea University. For immobilizing the primary antibody on the ZnO nanorods matrix-based electrode, various primary antibody solutions (1 ~ 4 μg/mL) were prepared by dilution. A primary diluted antibody solution was dropped on the ZnO nanorods matrix-based electrode for immobilizing the antibody on the electrode, and then the electrode was kept at 37 °C for 1 hour. After washing step, the primary antibody of various concentrations was immobilized on the matrix-based ZnO. Then, a BSA/PBS solution was dropped on the electrode for blocking the blank space of the electrode, on which the primary antibody was immobilized, and then the electrode was kept at 37 °C for 1 hour for immobilizing the BSA on the ZnO nanorods. Afterward, various concentration antigen solutions as PAL proteins were prepared by the dilution. An antigen solution was dropped on the electrode in order to bind the antigen to the antibody. The electrode was kept at 37°C for 1 hour and washed in the PBS. A polyclonal antibody-HRP was dropped on the electrode in order to bind the antibody conjugated HRP to the antigen. The electrode was kept at 37 °C for 1 hour and washed in the PBS. Figure 5 shows schematic pictures of the fabricated electrochemical immunosensor.

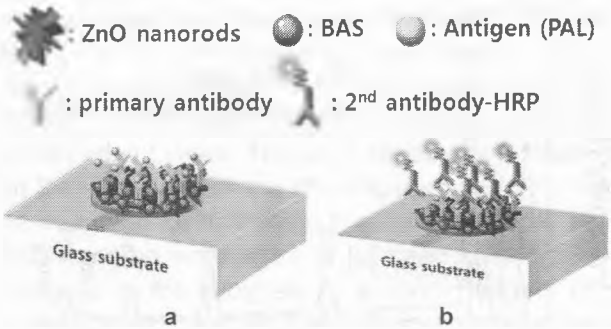


Fig. 5. Schematic picture of the fabrication for the electrochemical immunosensor based on ZnO nanorods matrix: a – Binding PAL antigen against the primary antibody; b – Binding the second antibody against the PAL antigen

The electrochemical measurements were carried out in 0.1 M acetate buffer solution using a potentiostat. According to the following Eqn. (1), electrochemical reaction for the detection of the antigen as Legionella was proposed to be following [14]:

$$TMB^+ + H^+ + e \rightleftharpoons TMB \qquad (1)$$

TMB as an electroactive molecule can be oxidized by HRP or reduced by electrochemical reaction. The oxidized TMB (TMB^+) is generated by the HRP catalytic reaction, and TMB is reduced by the electrochemical reaction. When reducing to TMB, the reduction current value would be related to the antigen concentration. A ZnO nanorod working electrode of the immonosensor, Pt wire counter electrode, and Ag/AgCl reference electrode were used in cyclic voltammetry.

The hydrothermally grown ZnO nanorods were successfully patterned on the Au electrode. The diameter of ZnO nanorods was about 700 ~ 900 nm, and the length of ZnO nanorods is about 5 ~ 7

μm. The crystal structures of ZnO nanorods were analyzed by X-ray diffraction technique (XRD). All the diffraction peaks of the matrix-based ZnO nanorods can be indexed to the wurtzite ZnO with high crytallinity. The main peaks for the matrix-based ZnO nanorods are (100) and (101) peaks, in contrast to vertically grown ZnO, where the main peak is (002) [15].

The electrochemical experiments were carried out by using the fabricated immunosensors, on which the primary and second antibodies of 2 μg/mL and various antigen concentrations. When various concentrations of antigen (PAL protein) were applied to the working electrode, in case of the bare electrode, no electrochemical reaction was observed in absence of the oxidized TMB. It can be seen that the reduction current increases as the antigen concentration increased, and the reduction peak currents of the oxidized TMB were observed at + 0.25 V. The reduction currents of the oxidized TMB increased with antigen concentration.

It is important to immobilize the antibody on the electrode for antigen detection using the fabricated immunosensors. The electrochemical characteristics of the fabricated immunosensors were evaluated at various antibody concentrations by fixing antigen concentration at 5 ng/mL. The output currents of the fabricated immunosensors increased with the antibody concentration up to 2 μg/mL. However, at higher concentration of antibody, at 4 μg/mL, the output current was lower than the current at 2 μg/mL antibody as shown in Fig. 6(a). It is believed that this result was attributed to the increase of the resistance and double layer capacitance of the ZnO nanorods-based electrode because of the decrease of ratio of sensing sites in the ZnO nanorods-based electrode [16,17].

The fabricated immunosensors with the various concentrations of the antibody and the antigen showed the response currents in Fig. 6(b). The fabricated immunosensors based at 1 μg/mL and 2 μg/mL antibodies showed the response currents increasing with the antigen concentration. The response currents of the fabricated immunosensors based at 2 μg/mL antibody was higher than those based at 1 μg/mL antibody with increasing the antigen concentration. A limit of detection of the fabricated immunosensor can be detected under 1 pg/mL antigen. In case of the fabricated immunosensors based at a 4 μg/mL antibody, the response current increased with the antibody concentration up to 100 pg/mL, and it gradually decreased at higher antigen concentrations because of increasing the resistance and double layer capacitance of the ZnO nanorods-based electrode [16,17].

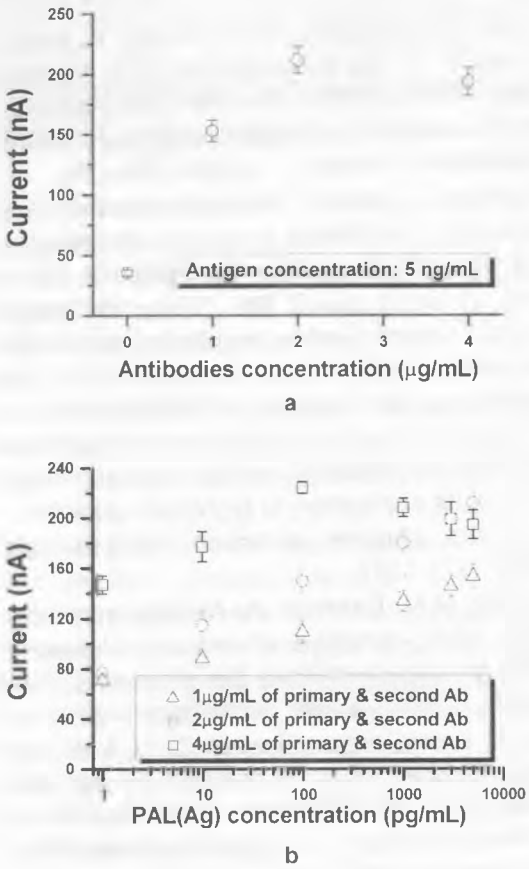


Fig. 6. Output currents of the fabricated immunosensors at various antigen and antibofy concentrations: a – 5 ng/mL antigen concentration and at 0 ~ 4 μg/mL primary/second antibody concentrations; b – 1 pg/mL ~ 5 ng/mL antigen and at 0 ~ 4 μg/mL primary/second antibody

Conclusion

An electrochemical immunosensor fabricated on matrix-based ZnO nanorods for detecting glucose or a *Legionella pneumophila* was introduced. The matrix-based ZnO nanorods were successfully grown on the Au electrode hydrothermally at low temperature and were patterned by lift-off as shown in FESEM images. XRD scan result exhibited that the ZnO nanorods were grown to the Zn and O without impurity. The antibody and GOx can be effectively immobilized on the ZnO nanorods with by electrostatic interaction. The fabricated electrochemical sensors showed higher sensitivity than previously reported electrochemical sensors.

Acknowledgements

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