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Glucose Oxidase-Coated ZnO Nanowires for Glucose Sensor Applications

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Abstract Well-aligned Zinc oxide (ZnO) nanowires were synthesized on silicon substrates by a carbothermal evaporation method using a mixture of ZnO and graphite powder with Au thin film was used as a catalyst. The XRD results showed that as-prepared product is the hexagonal wurzite ZnO nanostructure and SEM images demonstrated that ZnO nanowires had been grown along the [0001] direction with hexagonal cross section. As-grown ZnO nanowires were coated with glucose oxidase (GOx) for glucose sensing. Glucose converted into gluconic acid by reaction with GOx and two electrons are generated. They transfer into ZnO nanowires due to the electric force between electrons and the positively charged ZnO nanostructures in PBS. Photoluminescence (PL) spectroscopy was employed for investigating the movements of electrons, and the peak PL intensity increased with the glucose concentration and became saturated when the glucose concentration is above 10 mM. These results demonstrate that ZnO nanostructures have potential applications in biosensors.

Keywords ZnO nanowires, glucose sensor, glucose oxidase (GOx), photoluminescense (PL)

1. Introduction

Accurate determination of glucose concentration is emerging as a critical health issue owing to the rapid increase in the number of diabetes patients and thus in recent years many researches have been focused on the development of sensitive determination of glucose concentration. Most popular glucose sensing is based on the electrochemical biosensors using enzyme electrodes[1-3] and there are only a few fluorescence-based methods for glucose sensing which are mainly using optical fibers[4,5] or fluorescent quantum dots.[6] Direct fluorimetric glucose detection using the enzyme such as glucose oxidase (GOx)[7,8] is very rare, and moreover, fluorescence-based glucose biosensors using GOx-coated nanowires have not been reported yet.

Nanocrystalline metal oxide receives great interests owing to their unique properties of high surface activity, good bio-compatibility and high stability. Nanocrystalline metal oxide, combined with various redox enzymes, have the potential for application to biosensors.[9,10] Among them, zinc oxide (ZnO) has superior biocompatible properties owing to its high isoelectric point (IEP) of about 9.5. Glucose oxidase (GOx) has the low IEP of about 4.2, which enables GOx to be combined with ZnO in phosphate buffer solution (PBS) with pH 7.4 by electrostatic interaction.[11-13] ZnO also has excellent electrical and optical properties mostly coming from high energy band gap and high exciton binding energy and the various nanostructures, namely nanorods, nanowires, nanorings, nanosprings, nanobelts, nanocombs, and nanocages, via different growth conditions.[14] These properties make ZnO nanostructure promising for its application to the biosensor.

In the presence of oxygen, glucose reacts with GOx to generate gluconic acid and hydrogen peroxidase. During this conversion, two protons and two electrons transfer from glucose substrate to flavine moiety of GOx. ZnO nanowires covered with GOx gain electrons from flavine moiety via glucose oxidation. And then extra electrons of ZnO nanowires increase with the glucose concentration.

We synthesized the ZnO nanowires coated with GOx for the glucose detection. The field emission scanning electron microscopy (FESEM, Hitachi S-4300) and X-ray diffraction (XRD, Rigaku Ultima-2000) using Cu $K\alpha$ radiation were employed to investigate their morphology and structure. Photoluminescence (PL, Dongwoo optron) was used for estimating the extra electrons of ZnO-GOx conjugates.

2. Experimental procedure

The synthesis of ZnO nanowires is schematically

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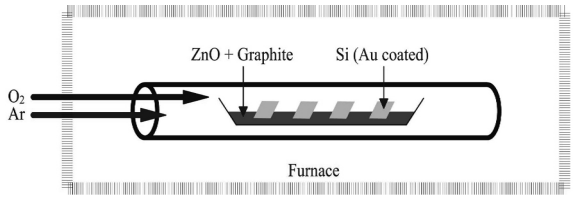

Fig. 1. Schematic illustration of ZnO nanowires synthesis.

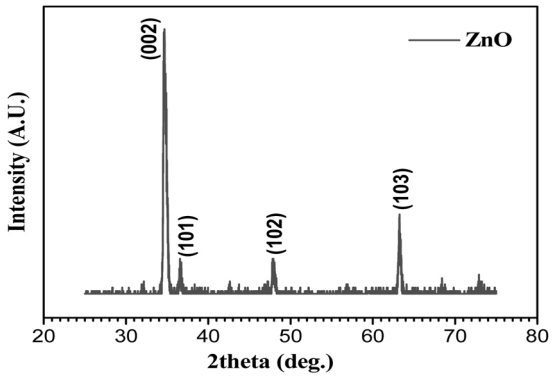

Fig. 2. XRD pattern of the ZnO nanowires.

illustrated in Fig. 1. High purity ZnO (99.99%) and graphite powder were mixed in the ratio of one to one completely and loaded into alumina boat. Au coated <111> Si substrate was laid on the alumina boat and then the boat was put into the quartz tube near the heating zone in the furnace. The experiment was carried out at 900 °C under a constant flow of not only Ar with the flow rate of 500 SCCM but also O_2 with the flow rate of 0.4 SCCM for 10 min. (SCCM denotes cubic centimeter per minute at STP) After this process, the furnace was cooled down to the room temperature.

The GOx solution was prepared by dissolving 5.0 mg GOx (160 units/mg, Sigma-aldrich) in 1.0 ml, 0.01 M PBS. And this solution was droped to the ZnO nanowires and dried overnight at 4 °C in a refrigerator. After that, the sample was rinsed with de-ionized water to remove uncoated GOx.

Photoluminesccence system was employed to detect the amount of glucose in aqueous solution. Aligned ZnO on the Si substrate was attached to the cuvette wall using adhesive tape and immersed in 2.0 ml of PBS and exposed to UV light with 325 nm wavelengths at 25 °C. Glucose solution was prepared by dissolving glucose (D-(+)-Glucose, Sigma-aldrich) 900 mg in 50 ml of 0.01 M PBS. 0.05 ml glucose solution was added to PBS in the cuvette, and after 5 minutes, the emission of light was detected. The six times of successive addition and detection were carried out.

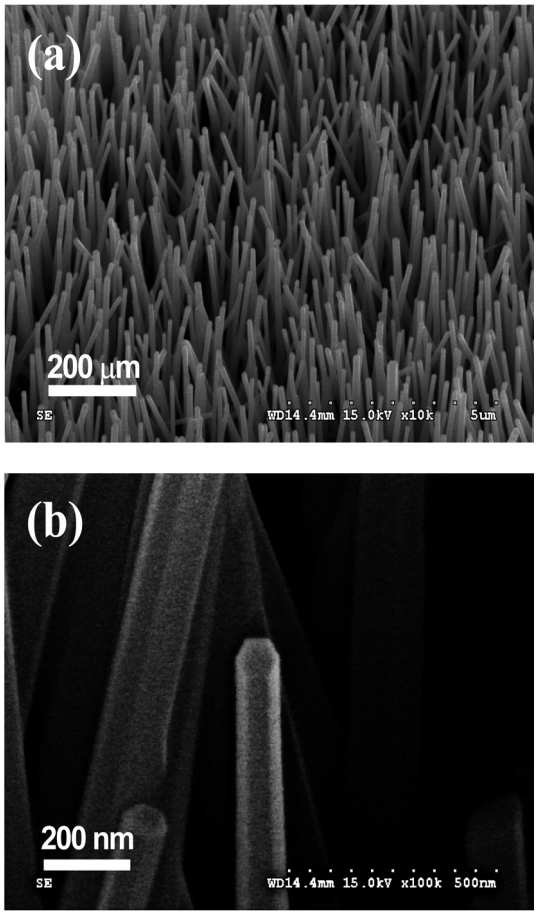

Fig. 3. (a) FESEM images shows ZnO nanowires have uniform size of 50 - 100 nm diameters and several micrometers length and (b) the faceted hexagonal shape of the ZnO nanowires.

3. Results and Discussion

XRD patterns of as-prepared ZnO nanowires are shown in Fig. 2. ZnO nanowires have hexagonal wurzite phase and other diffraction peaks of materials such as Zn and ZnO_2 were not found.

Fig. 3(a), (b) showed SEM images of ZnO nanowires in low and high magnifications, respectively. It is shown that nanowires have hexagonal cross section with the uniform size of 50 - 100 nm diameter and several micrometers length. It is also noted that the ZnO nanowires are well aligned along the perpendicular direction of the substrate.

Fig. 4(a) shows photoluminescent response of GOx attached to ZnO nanowires after the addition of successive

$$\begin{aligned} &\text{Glucose} \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2 \\ &H_2O_2 \longrightarrow 2H^+ + 2e^- + O_2 \end{aligned} \quad (1)$$

0.05 ml glucose solution by the function of the wave length of PL. In order to detect glucose accurately, oxygen and appearance time have to be considered. The glucose oxidase catalyzed reaction can be described as following.

During glucose oxidation, oxygen is generated and this can act as a quencher. Fiber optic glucose sensors are usually using this oxygen quenching effects.[15] However, without catalysts, the light quenching effect is subtle, and thus this effect is negligible. It is known that a glucose concentration is not proportional to the increase of fluorescence intensity but to the appearance time which is the gap between the moment of the glucose addition and the fluorescence change.[16] For the purpose of neglecting the appearance time, the PL values were adopted as each fluorescence intensity of samples after 5 minutes of stabilization.

Fig. 4(b) shows the peak PL intensity of the samples by the function of the glucose concentration. The fluorescence intensity increases with the glucose concentration and becomes saturated when the glucose concentration is above 10 mM. The fluorescence intensity saturation is probably caused by the inability of the GOx to react with glucose. And this result is similar to that of electrochemical ZnO biosensors.[12]

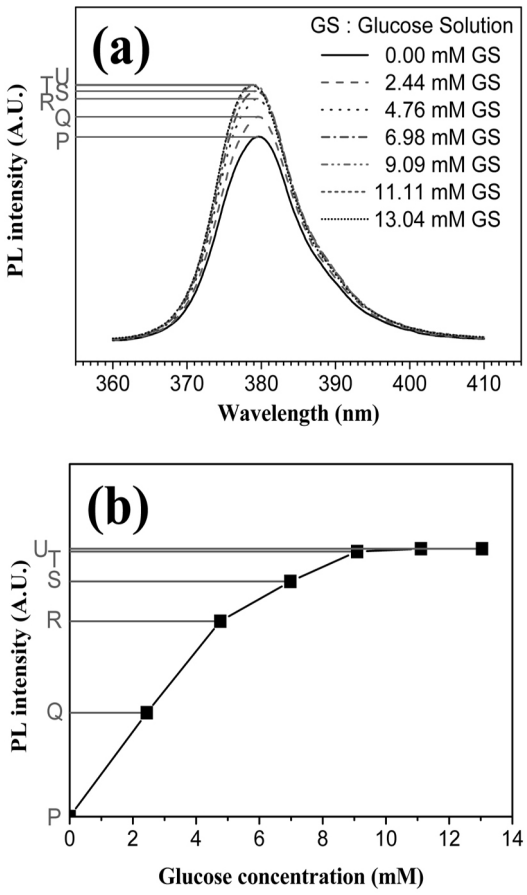

Fig. 4. (a) The photoluminescent response of GOx-attached ZnO nanowires after addition of successive 0.05 ml glucose solution by the function of the wave length of PL and (b) the peak PL intensity by the function of the glucose concentration.

GOx is homodimetric enzyme. Each enzyme has one coenzyme molecule of flavine adenine dinucleotide (FAD) and each GOx monomer have two distinct domains. The one binds to the FAD moiety and the other binds to the glucose substrate. Glucose converted into gluconic acid by catalytic reaction with GOx and the hydrogen peroxide generated. During this reaction, two protons and two electrons are transferred from the glucose substrate to the flavine moiety of GOx.[17]

IEP is the pH at which a particular molecule or surface carries a neutral charge. GOx contain both acidic and basic functional groups. When GOx are surrounded in aqueous solution with pH values below or above IEP, this can be positively or negatively charged owing to the gain or loss of protons (H^+). On the other hand, the surface of ZnO nanowires are generally covered with hydroxyl species, Zn-OH. When ZnO are immersed in aqueous solution with pH values below IEP, M-OH^+ species are dominant on the surface, while in aqueous solution with pH values above IEP, M-O^- species are dominant on the surface. In PBS with pH 7.4, GOx having the low IEP of about 4.2 are negatively charged and ZnO having the high IEP of about 9.5 are positively charged. And then they can combine with each other in PBS with pH 7.4 by the electrostatic interaction.

The electrons generated from the flavine moiety of GOx move into ZnO nanowires owing to the electric force between the electrons and the positively charged ZnO in PBS. This means extra electrons are generated in the valence band of the ZnO nanowires. When the light excited at 325 nm wavelengths exposed to the GOx-ZnO conjugates, the electrons in the valence band can be excited to the conduction band of the ZnO nanowire and the extra electrons also can be excited to the conduction band. As a result, the PL intensity increases. The schematic illustration of the movement of electrons showed in Fig. 5.

4. Conclusions

In summary, we reported glucose biosensors using ZnO nanowires coated with GOx. The PL intensity increases

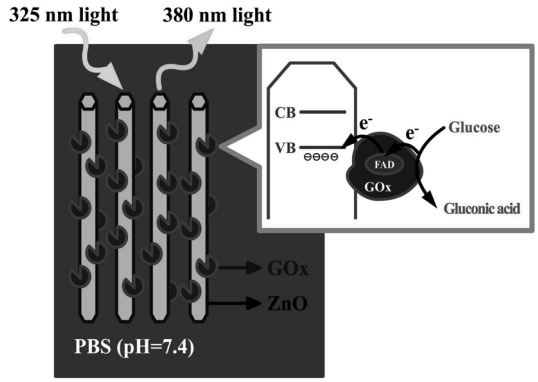

Fig. 5. The electrons generated by oxidization of glucose are transferred from glucose substrate to ZnO nanowires.

with the glucose addition due to the effective transfer of electrons coming from the enzymatic oxidation of glucose by GOx. The direct bonding between the GOx and ZnO by the electrostatic attraction plays a key role in the electron transfer. The GOx-ZnO nanowire bioconjugates reveal the strong potential to be used as a sensitive glucose biosensor.

Acknowledgements

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