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Enhanced Sensitivity of a Glucose Sensor Adopting Polymer Microtubule

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Sensitivity of a glucose biosensor based on conducting polymer micro-tubule was enhanced by introducing a Pt transducer layer. Electrochemical template synthesis using polycarbonate membrane was used to fabricate the micro tubule. Glucose oxidase from Aspergillus Niger *was caged into the micro tubule structure by capping the mouth of the tubule with a nano porous polycarbonate membrane. This capped tubule structure worked as a cell-array retaining enzyme inside and as a sensing current collector for the electrochemical reaction of the species retained inside simultaneously. The nano pores of the capping membrane were filled with poly (1,3-phenylenediamine) by using electrochemical polymerization. Introducing a Pt transducer layer at the bottom side of the template enhanced the sensitivity of the sensor about sixty times compared to the one having Au layer. The larger the pore size of the template was, the faster was the response and the higher was the detection current because of the faster material transport into and out of the cells.*

Keywords: capping film; electropolymerization; glucose biosensor; microtubules; Pt transducer layer

1. INTRODUCTION

Conducting polymers have attracted great attention because of their superior properties adjustable to advanced applications including

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electrochromic displays, smart windows, sensors, capacitors, secondary batteries, etc. [1–3]. In particular, PEDOT [poly(3,4-ethylenedioxythiophene)] has been focused because it has enhanced conductivity of 300 S/cm, transparency, electrochemical and thermal stabilities, and good ion transport property compared to other materials [4–6]. Especially PEDOT/PSS [PEDOT doped with poly(4-styrenesulfonic acid)] has beneficial properties of forming easy-to-use coating dispersions and formulations for specific applications such as antistatic coatings, electrical connection, electrode materials in organic semiconductor devices [7].

Clark and Lyons evolved the modern outline of the biosensor that enzymes could be immobilized in an electrochemical detector to construct an enzyme electrode [8]. Updike and Hicks reported the fabrication of a functional enzyme electrode for glucose [9]. Martin *et al*. presented that conducting polymer micro/nano fibers and tubes were synthesized in porous membrane filter as a template [10,11]. One of their applications was demonstrated masking the opening of the structure with a non-conducting sealing agent that contained enzyme in the structure [12,13]. Recently, Son *et al*. have shown the possibility of caging reacting species and enzyme into the micro tubule array of conducting polymer without any modification of enzymes [14,15]. The advantages of enzyme caging in micro tubular cell include minimal pretreatment and need of a small sample volume. Also, the enzyme could act repeatedly as a signal transducer. But their sensitivity still needs to be improved for the micro applications: e.g., *in situ* detection of glucose level in blood vessel. In this work, we tried to insert platinum layer as a signal transducer to enhance detection current density. Because platinum electrode have been known for its catalytic activity during electrochemical oxidation of hydrogen peroxide [16]. So we attempted to utilize Pt layer as an catalytic transducer in the sensor system to enhance the detection sensitivity. A pore size dependent sensitivity was also tested to understand substrate transport within this structure.

2. EXPERIMENTAL

EDOT (3,4-ethylenedioxythiophene) was used as received from Aldrich. PVA (polyvinyl alcohol) was gratefully donated by DC Chemical (Korea). Aqueous dispersion of PEDOT/PSS (Baytron P 4083) were used as purchased from Bayer. Polycarbonate membrane filters (Isopore) having 0.05, 0.4, 1.2, and 5.0 μm pore diameter were come from Millipore and used as templates. Glucose oxidase (type II-S from *Aspergillus Niger*, 39800 units/g) and D-(+)-glucose were obtained

from Sigma-Aldrich. Phosphate buffered saline (PBS) solution consisted of 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, and 0.15 M NaCl and was adjusted to pH 7.4 with 3 M NaOH. 1,3-phenylenediamine (PDA, flakes, 99+%) was purchased from Sigma-Aldrich and used as received. The other chemicals used in this study were mostly of ACS grade. The conducting ITO glass (Samsung Corning, Korea) was utilized as a supporting electrode. Spin coating was performed with an EC101DT spin-coater (Headway Research, Inc., USA) equipped with a rotary vacuum pump. Pt layer was coated on one side of 0.4, 1.2, 5.0 μm membranes with a sputter (Crossington model 108). Gold was coated by thermal evaporation (Korea Vacuum). The electrochemical measurements were performed using a BAS 100B (BAS, USA). All electrochemical potentials in this study were referred to a Ag/AgCl (Sat'd KCl) reference electrode. SEM images and EDAX data were obtained by using JSM 6700F (JEOL, Japan) and JSM 7000F (JEOL, Japan).

For the formation of the capped microstructure, a piece of ITO glass was used as a substrate electrode. A home made conducting glue (PEDOT/PSS + PVA composite) was spin coated onto an ITO glass with spinning rate of 6000 rpm. Then a piece of membrane template was carefully placed on top of it before the glue was dried out completely. The composite has roles of gluing the template onto the ITO electrode and providing the electrical connection between the ITO electrode and the electrolytic solution. The electrochemical polymerization was performed by cycling the applied potential ranging from 0.3 to 1.2 V and scan rate of 50 mV/s. Glucose oxidase solution (50 mg/ml) was applied to the open mouths of the tubule array. Before drying, this microtubule array was covered with a piece of 0.05 μm porous membrane one side of which was covered with the conducting glue. Then the upper membrane was filled with the electrochemically polymerized PDA after complete drying. Polymerization was performed by cycling the potential from 0.3 to 1.3 V three times in a 0.01 M PDA/acetonitrile containing 0.1 M $LiClO_4$ as a supporting electrolyte. Most of the fabrication procedure except introducing Pt layer and pore size variation has been described elsewhere in detail [14]. The Au layer of our former sensor was replaced by Pt sputtered layer in this study [15]. The complete sensor electrode was dipped into a test solution of which glucose concentration was gradually 1 mM glucose testing solution and examined the sensitivity with an applied voltage of 0.7 V.

3. RESULTS AND DISCUSSIONS

As PEDOT was deposited as a micro tubule form, the monomer oxidation current increased during the subsequent cycles [17,18].

The micro tubules need to be wet well and not to be easily dissolved by water because the amperometric detection processes are performed in aqueous environment. Therefore, the electrochemical polymerization was conducted in 30% water containing acetonitrile electrolytic polymerization solution. Figure 1 shows the SEM images of PEDOT tubules formed in the membrane cavity. The images were taken after removing the polycarbonate membrane by immersing the electrode in methylene chloride for ten minutes. As the number of potential cycling increases, the tubule becomes stiff and stands alone firmly on the ITO surface. In this work, tubules were formed by the 15 cycles of potential scanning. The formation of conducting polymer tubule was realized by the employment of the conducting glue which offers electrical contact between base ITO electrode and monomer solution. Then, the tubule was filled with enzyme solution. The capping process was completed by putting a nano-porous membrane coated with the glue composite on one side onto the mouth opening of the micro tubule formed in the template. The 50 nm pores of the capping membrane were filled with nonconducting poly(1,3-phenylenediamine) (PPDA). PPDA was used for the purpose of quick wetting and removing interferences from ascorbic acid, acetaminophen, etc. [19]. In

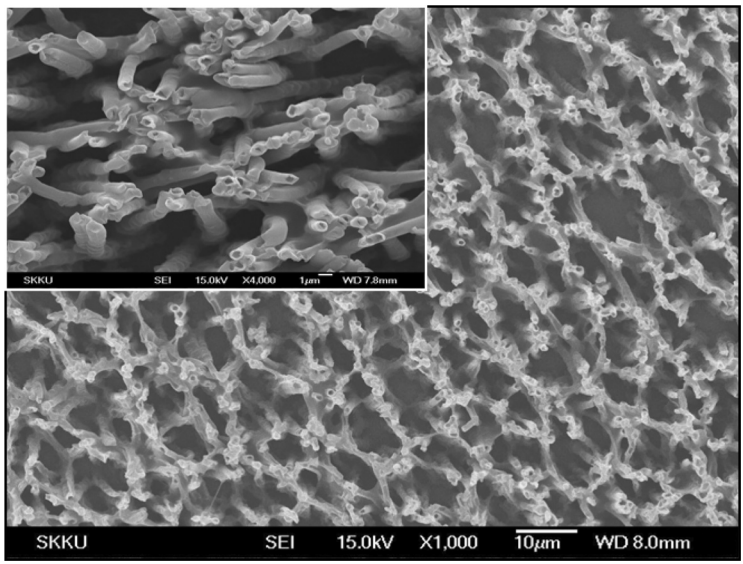

FIGURE 1 SEM images of PEDOT micro tubules after the template had been dissolved. The micro tubules were made by cycling the potential 15 times from 0.3 V to 1.2 V in 0.1 M EDOT/0.1M $LiClO_4$/acetonitrile (30% water contents).

order to confirm whether the microtubules has hollow space after capping of electropolymerization of PDA, SEM image of 45 degree slicing of the whole structure was obtained. Figure 2 shows the nano-rods of PPDA were adhered firmly on top of the main micro tubule. A thin layer of glue composite appeared between nano-rods and micro-tubule.

This type of glucose sensor is based on the detection of hydrogen peroxide produced during the enzymatic action of glucose oxidase with glucose. Accordingly, the responses of the Pt transducer layer of the tubule electrode to the hydrogen peroxide were examined. Figure 3 is the amperometric responses of the electrodes in 10 ml of PBS solution. After the background current was stabilized at 600 s, 1 M H_2O_2 stock solution was added in order to increase the concentration by 0.1 mM gradually. Performances of our former gold layer electrode were compared to the new Pt layer electrode. Hydrogen peroxide was added to the PBS test solution containing test electrode with the concentration increasing by 0.01 mM successively. The Pt transducer layer provided the detection current sixty times greater than that of the gold electrode. This type of enhancement was also found in recent work done by Chu *et al*. [20]. To investigate whether these electrodes

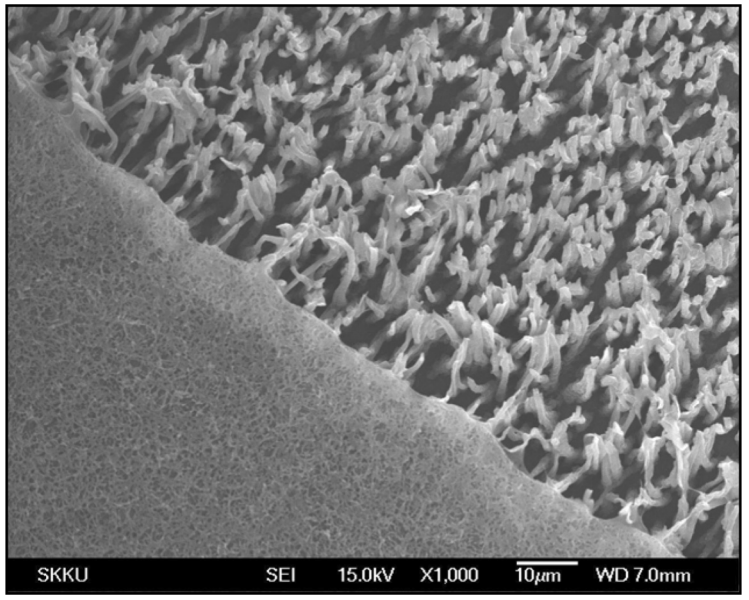

FIGURE 2 SEM image of the capped micro-tubule cut by 45 degree. Nano rods are observed at left and micro tubules are appeared at right.

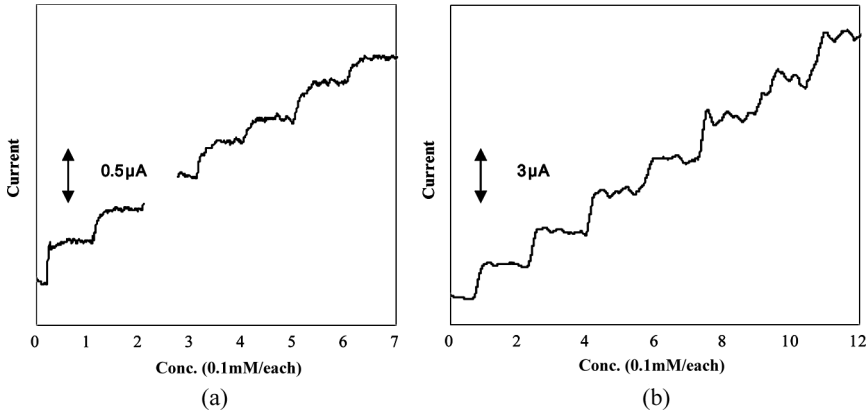

FIGURE 3 Comparison of responses to H_2O_2 between gold-treated (a) and platinum-sputtered (b) electrode. H_2O_2 was increased gradually by 0.01 mM step with the applied potential of 0.7 V and Pt plate counter electrode used.

respond directly to glucose without glucose oxidase, 1 mM glucose was added in a new buffer solution in which each electrode was placed. As shown in Figure 4, no direct responding current was observed without glucose oxidase. The amperometric responses of the biosensor of

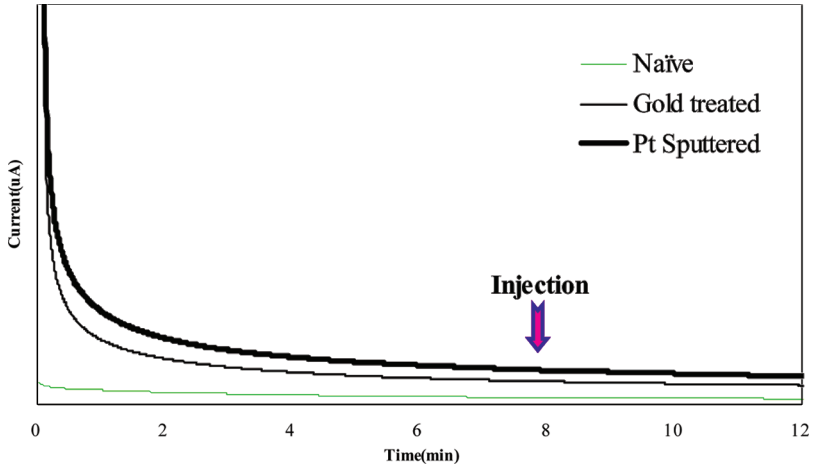

FIGURE 4 Amperometric response of microtubules to 1 mM glucose without glucose oxidase. Applied potential was 0.7 V, Pt plate counter electrode was used.

1.2 μm tubule to successive additions of 1 mM glucose appeared in Figure 5. With the increment of injection concentration, the detection current increased instantly and showed good linearity up to 18 mM. The sensitivity was calculated to be 1.173 μA/mM on average. This sensitivity is thirty times greater than that of the former Au layer one (0.04 μA/mM). Limit of detection was measured to 0.25 μM, which is much lower than that of Au layer one (0.2 mM). These are very important for the micro application perspectives of this sensor. In case fabrication method is developed, the size of the sensor can be miniaturized to less than 1 mm.

The capping of the micro-structure was performed with electropolymerization of PDA. The generation of a steady-state current during measurement took about 10 minutes, but after that the sensor responded very quickly. The capping treatment has a property of dual-aspect. Like a cage the capping can hold glucose oxidase in free-swimming state inside the cell. In the mean time it allows the substrate to transport into and out of the cell freely. The pore size is also an important factor of the sensor action. Substrate transportation is really affected by the pore size and capping thickness. One of the

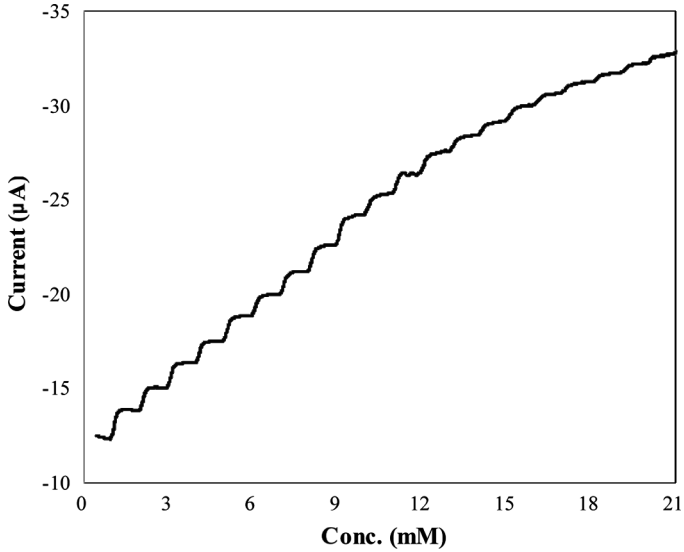

FIGURE 5 Amperometric responses, Size of 1.2 μm tubule electrode having Pt lyaer shows responding current to the successive additions of 1 mM glucose. Glucose was increased gradually from 0 to 21 mM. Applied potential was 0.7 V, Pt plate counter electrode was used.

most important thing controlling the enzyme reaction rate is the acting volume of the sensor. The acting volume of each sensor is given by the product of pore density and pore volume. The membrane used as a template has different pore size of 0.4, 1.2, and 5.0 μm in diameter. All of membranes used have the same thickness of 10 μm. The acting volume involved in the sensor reaction was calculated by using pore density of each template. The pore densities are observed as $1.28/\mu m^2$ (0.4 μm), $0.23/\mu m^2$ (1.2 μm), and $0.004/\mu m^2$ (5.0 μm), respectively. From the pore size, the volume of each tubule can be also calculated and the pore volume ratio is 16:144:2500 for these three membranes. So the acting volume ratio is estimated to be 2:3:1. Results in Figure 6 are a little different from what one can expect from the acting volume calculations. Sensitivities obtained from the figure are 0.10 mA/mM (0.4 μm), 0.75 mA/mM (1.2 μm), 0.76 mA/mM (5.0 μm), respectively.

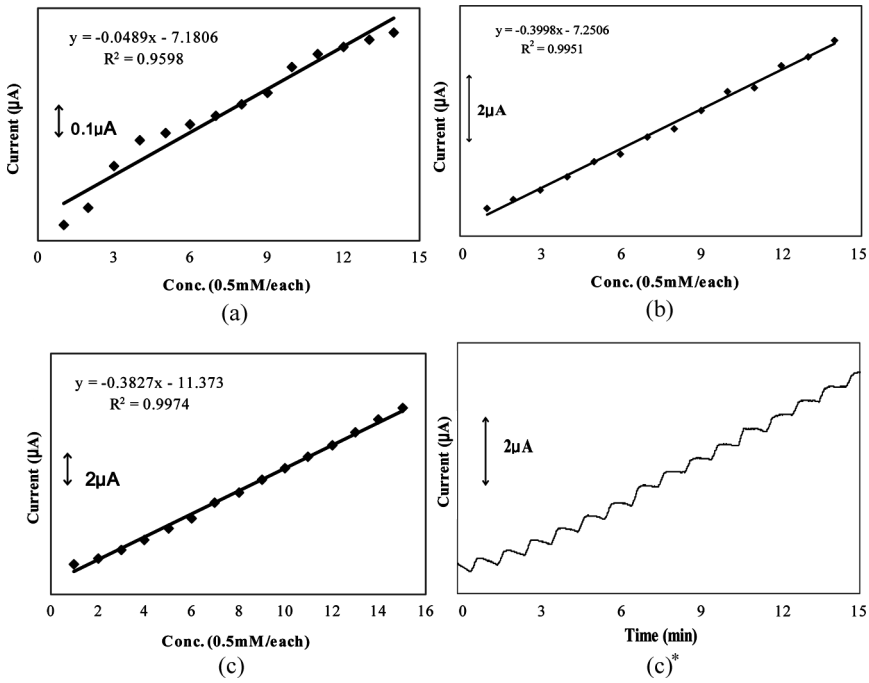

FIGURE 6 The calibration curves form sensors having (a) 0.4 μm (b) 1.2 μm (c) 5.0 μm pore sizes. Current responses to the successive additions of 0.5 mM glucose to the test solution of PBS. Supplement (c)* is the amperometric response of successive addition of 0.5 mM glucose to 5.0 μm porous membrane to evidently support for (c).

Actually the sensor holding smallest acting volumes produced the largest sensitivity (three to fifteen times greater). It is thought that the substrate transportation may explain this difference. In the capping, the only channel in which substrate can transport is the very small 50 nm rod of PPDA. The possibility of the channel to be dead end is greater in smaller pore sensor compared to that in larger pore case.

4. CONCLUSIONS

Sensitivity of a biosensor based on conducting polymer micro tubule was enhanced by using Pt transducer layer instead of Au layer. The glucose sensitivity was increased by thirty times compared to the previous one. This gives us a chance to make the sensor size reduced dramatically for mirco applications. Pore size effect to the sensitivity was examined. The sensor holding smallest acting volumes produced the largest sensitivity (3 to 15 times greater). These results were different from what one can expect from the acting volume calculations. This could be explained with substrate transportation. In the capping, 50 nm PPDA rod is the only transporting channel. And the possibility of the channel to be dead end is greater for smaller pore compared with that of larger one.

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