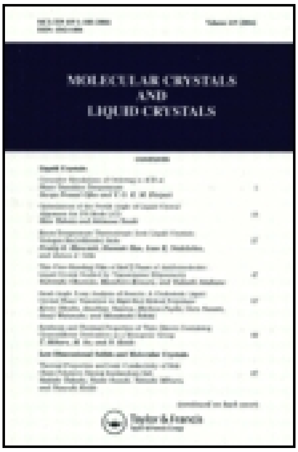

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Fabrication of CNT/Ferrocene/Glucose Oxidase/Chitosan-Layered Bioanode for Glucose/Oxygen Biofuel Cells

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Fabrication of CNT/Ferrocene/Glucose Oxidase/ Chitosan-Layered Bioanode for Glucose/Oxygen Biofuel Cells

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An enzyme-modified electrode was fabricated by entrapping glucose oxidase (GOx) and ferrocene (Fc) onto a multiwall carbon nanotube (MWCNT)-coated electrode. The MWCNT, Fc, GOx, and chitosan (CHI) were sequentially coated on a glassy carbon electrode. The MWCNT/Fc/GOx/CHI electrode was characterized by scanning electron microscopy (SEM), and cyclic voltammetry (CV). The prepared electrode exhibited good electrochemical performance for the glucose analysis with a linear range of 0–60 mM glucose. It was found that the MWCNT film on the electrode remarkably enhanced the performance of the electrode. The MWCNT/Fc/GOx/CHI electrode was integrated with a bilirubin oxidase-immobilized cathode for a biofue cell application. The maximum power density at a glucose concentration of 10 mM was 13 $\mu W/cm^2$ at a cell voltage of 0.19 V. The results of this study indicate that the MWCNT/Fc/GOx/CHI electrode could be applied in the development of biofuel cells and bisensors.

Keywords Bioanode; biofuel cells; chitosan; ferrocene; glucose oxidase; glucose sensor; mutiwalled carbon nanotubes

Introduction

Biofuel cells are devices that can convert chemical energy directly into electrical energy using biocatalysts such as enzymes and microorganisms [1–3]. Since biofuel cells are based on biological reactions, they are normally operated under mild conditions (near or at room temperature and neutral pH). These properties make biofuel cells particularly applicable as a power source for implantable and microscale electrical devices [4–6]. One of the major obstacles in enzymatic biofuel cell development

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is the lack of efficient electron transfer between redox enzymes and electrode surfaces because active sites are deeply embedded inside the enzyme. Deactivation and poor stability are also significant drawbacks in the development of practical enzymatic biofuel cells. In order to overcome these problems, electron-shuttling mediators as well as enzyme immobilization onto the appropriate matrix have been used.

Various electron mediators such as ferrocene, benzoquinone, and their derivatives have been reported to significantly enhance electron transfer between the redox enzymes and the electrodes and thus remarkably increase the electrochemical performance of the bioelectrodes [7–10]. Among the electron mediators, ferrocene and its derivatives have been reported to be the most efficient [11]. Ferrocene has good electrochemical reversibility and stability at low potential and can transfer electrons from the enzyme active site to the electrode surface through the following reactions:

$$\text{Glucose} + \text{GOx (FAD)} \rightarrow \text{Gluconolactone} + \text{GOx (FADH}_2\text{)}$$
$$\text{GOx (FADH}_2\text{)} + 2\text{Fe}^+ \text{ (ferrocinium ion)} \rightarrow \text{GOx (FAD)} + 2\text{Fe (ferrocene} + 2\text{H}^+\text{)}$$
$$2\text{Fe} \rightarrow 2\text{Fe}^+ + 2\text{e}^-$$

An electron mediator can be used in solution or entrapped into the polymer or sol–gel matrix [12–14]. Recently, chitosan has been shown to be a promising candidate for enzyme immobilization in bioelectrode applications due to its membrane forming ability, good biocompatibility, non-toxicity, and high mechanical strength. For instance, enzymatic sensors with chitin and chitosan supports were used to detect and measure of glucose concentrations [15–18]. The electron mediators are in most cases attached to the support matrix by cross-linking or covalent binding to prevent their leakage from the electrode surface into the solution. Due to their high electrical conductivity, large surface area, and chemical stability, carbon nanotubes (CNTs) have been widely used to further improve electron transfer [19–23]. CNTs can act as nanowires to create electrical bridges that facilitate electron transfer between the immobilized redox enzymes, mediator, and electrode.

In this study, mutiwalled-carbon nanotibubes (MWCNT), ferrocene, and glucose oxidase were coated sequentially on a glassy carbon electrode and entrapped by chitosan without using any complicated linking processes. The simply integrated MWCNT/ferrocene/glucose oxidase electrodes were characterized for the application in glucose sensors and glucose/oxygen biofuel cells.

Experimental

Materials

Glucose oxidase (GOx) from *Aspergillus niger* (Type II-S, 21000 units/g solid), bilirubin oxidase (BOD) from *Myrothecium verrucaria*, and D-glucose (99.5%) were purchased from Sigma (USA). Ferrocene (Fc) and chitosan (CHI) (high molecular weight, >75% deacetylated) were supplied from Aldrich (USA). MWCNT (CM95, >95 wt% purity, 10–15 nm in diameter) was purchased from Iljin Nanotech Co. Ltd. (Seoul, Korea) and used without any purification. Carbon black was supplied from LG Chem (Korea).

Preparation of Enzyme-Based Electrodes

The MWCNT/Fc/GOx/CHI anode was fabricated as follows: First, a polished glassy carbon electrode (disk diameter 2 mm ± 0.2 mm, Princeton Applied Research, USA) was coated with 10 μL of a MWCNT suspension (1 mg/mL in THF) and dried at room temperature. A ferrocene solution (10 μL, 15 mM in ethanol) and GOx solution (10 μL, 30 mg/mL) were sequentially dripped onto the MWCNT-covered electrode and dried at room temperature. Finally, the electrode was coated with a chitosan solution (10 μL, 5 mg/mL), which was prepared by dissolving chitosan in a sodium acetate buffer (50 mM, pH 4), and dried at room temperature. For comparison purposes, a carbon black suspension was used instead of the MWCN suspension. Similarly, a MWCNT/BOD/CHI cathode was prepared by applying a BOD solution instead of the GOx solution. This was then used as the cathode of the biofuel cell tested in this study.

Analysis

The surface morphology of the MWCNT/Fc/GOx/CHI electrode was analyzed by scanning electron microscopy (SEM) (Hitachi S-4700, Japan). The electrochemical properties were examined by cyclic voltammetry (CV) using a versatile modular potentiostat (VSP, Bio-Logic) with a conventional three-electrode system: a glassy carbon electrode as a working electrode, a platinum wire as a counter electrode, and an Ag/AgCl (saturated KCl) electrode as a reference. The glassy carbon electrode was polished using 1 μm diamond and 0.05 μm alumina slurry and then washed with distilled water prior to use. All the CVs were obtained in sodium phosphate buffer (50 mM, pH 7) at room temperature under ambient conditions. The performance of the enzymatic biofuel cell was evaluated by measuring the current-cell voltage while varying the external resistance.

Results and Discussion

In order to determine the effect of the electron mediator, ferrocene, we first tested the GOx-immobilized electrode not containing ferrocene. The CV data revealed that there were no redox peaks even in the presence of glucose, as shown in Figure 1, indicating the active site of the GOx was not electrically connected with the electrode. Usually oxidoreductases on electrode surfaces do not achieve significant electron transfer between their redox center and the conductive support, due to the electrical insulation by the surrounding protein matrixes [4]. In the case of GOx, flavine adenine dinucleotide (FAD), which is responsible for redox reactions catalyzed by GOx, is buried within the protein shell (diameter about 13 Å). Based on this preliminary experiment, the electron mediator was found to be crucial for efficient electron transfer in the enzyme based electrochemical reactions.

Therefore, ferrocene was incorporated into the GOx immobilized electrode system. Since ferrocene is slightly soluble in water, ferrocene derivatives such as ferrocene carboxylic acid have been used. Ferrocene derivatives were associated with the electrodes through covalent binding to prevent leakage of the mediator from the electrode surface into the aqueous bulk solution. In this work, the ferrocene was physically embedded into the chitosan membrane without using a linking process. That is, ferrocene solubilized in ethanol and GOx were simply coated using layer-by-layer

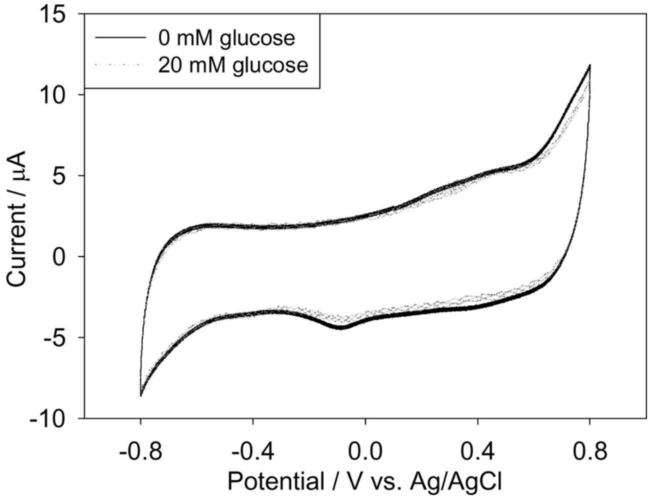

Figure 1. Cyclic voltammograms of GOx immobilized on a glassy carbon electrode in the absence (—) and presence of 20 mM glucose (...) in pH 7.0 phosphate buffer at a scan rate of 100 mV/s.

steps onto a glassy carbon electrode. The electrode was then covered with chitosan, resulting in the formation of a layered film of Fc, GOx, and CHI on the electrode. The electrochemical performance of the prepared Fc/GOx/CHI electrode was examined by CV, as shown in Figure 2. The typical oxidation and reduction peaks of glucose oxidation were observed, indicating that the embedded ferrocene mediator functioned properly relative to the electrode not containing ferrocene. The change in the anodic peak current response of the Fc/GOx/CHI electrode at different

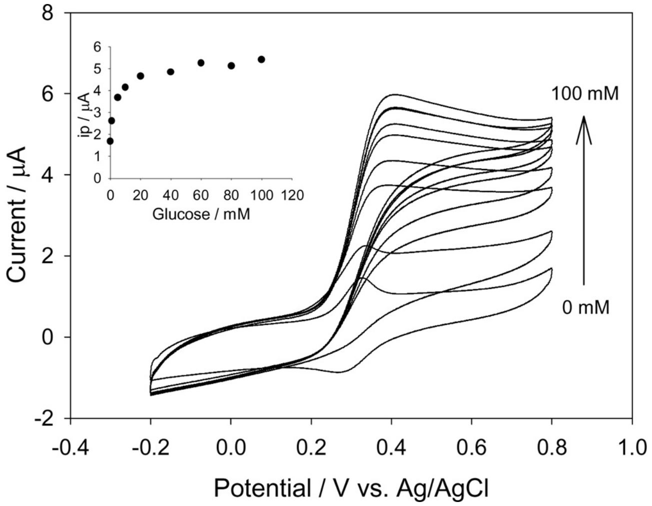

Figure 2. Cyclic voltammograms of the Fc/GOx/CHI electrode at various glucose concentrations in pH 7.0 phosphate buffer at a scan rate of 100 mV/s. Inset: plot of current vs glucose concentration.

glucose concentrations is showen in the inset of Figure 2. In the range of 0–20 mM glucose the anodic peak current increased sharply. The increasing rate of the current decreased gradually and the current remained constant when he glucose concentration was above 60 mM glucose, representing a characteristic of Michaelis–Menten kinetic mechanism [24]. This result indicates that ferrocene molecules were well embedded within the chitosan matrix on the electrode and did not leach into the aqueous buffer solution. Although further systematic leaching tests are needed, it was thought that ferrocene was stably retained within the chitosan matrix because of its insolubility in water and/or its incorporation into the chitosan membrane.

The CV analysis of the Fc/GOx/CHI electrode was carried out by varying the scan rate from 1 to 200 mV/s. In this analysis, the redox peak currents were shown to be linearly proportional to the root mean square of the scan rate in the range of 1–200 mV/s, as shown in Figure 3. According to Randles–Sevick equation [25], this is a typical characteristic of non-surface controlled electrochemical behavior, demonstrating that the electrochemical reaction of the Fc/GOx/CHI electrode was a diffusion-controlled process.

MWCNTs were used as the electrode material to further improve electron transfer. The MWCNT/Fc/GOx/CHI electrode was prepared by coating the MWCNT, Fc, GOx, and CHI sequentially on a glass carbon electrode. Figure 4 shows SEM images of the prepared electrode surfaces. Figure 4(a) depicts the MWCNT film on the electrode and shows that this electrode had a large surface area for the efficient electrical communication. Figure 4(b) shows the surface of the MWCNT/Fc/GOx/CHI electrode, indicating that the electrode was well covered by a dense membrane.

The electrochemical performance of the MWCNT/Fc/GOx/CHI electrode was evaluated by CV measurements, as shown in Figure 5. The MWCNT/Fc/GOx/CHI electrode exhibited indeed higher redox currents compared with the Fc/GOx/CHI electrode. In fact, at the glucose concentration of 20 mM, the measured current for the MWCNT/Fc/GOx/CHI electrode was about 15 times higher than that for

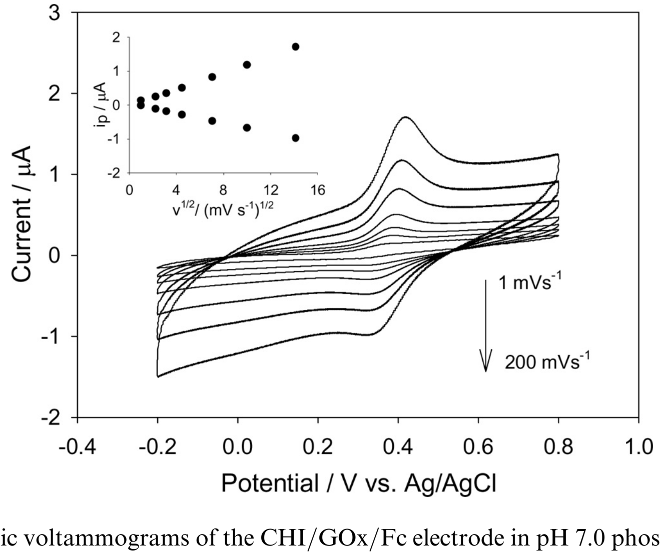

Figure 3. Cyclic voltammograms of the CHI/GOx/Fc electrode in pH 7.0 phosphate buffer at various scan rates. Inset: plot of anodic and cathodic peak currents vs $V^{1/2}$.

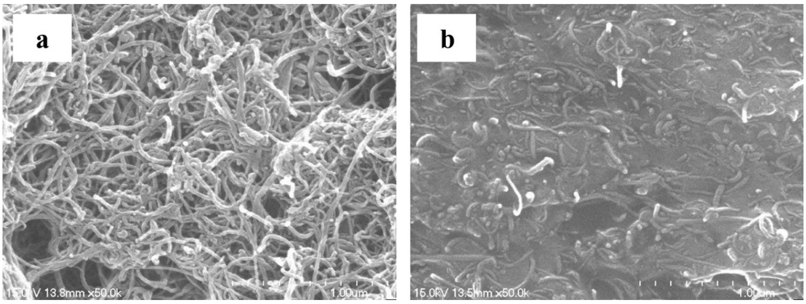

Figure 4. SEM images of (a) MWCNT-covered electrode and (b) MWCNT/Fc/GOx/CHI electrode.

the Fc/GOx/CHI electrode. In the range of 0–60 mM glucose, the anodic peak current for the MWCNT/Fc/GOx/CHI electrode increased linearly as the glucose concentration was increased. However, the current decreased when the glucose concentration was increased from 80 to 100 mM. It is not clear at this time why the current decreased at 100 mM glucose. Possible explanations could be the enzyme deactivation and/or ferrocene leakage by membrane failure under these conditions.

The influence of scan rate on the CVs of the MWCNT/Fc/GOx/CHI electrode is shown in Figure 6. In the inset of Figure 6, the anodic and cathodic peak currents were plotted as a function of the scan rate. The redox peak currents varied linearly with the scan rate in the range of 1–200 mV/s, suggesting that the electrochemical reaction of the MWCNT/Fc/GOx/CHI electrode was a surface-controlled process [26]. It should be noted that the reaction of the Fc/GOx/CHI electrode was diffusion-controlled process as discussed above. Further study should be needed to understand and verify the different reaction processes of the two electrodes; Fc/GOx/CHI electrode and MWCNT/Fc/GOx/CHI electrode.

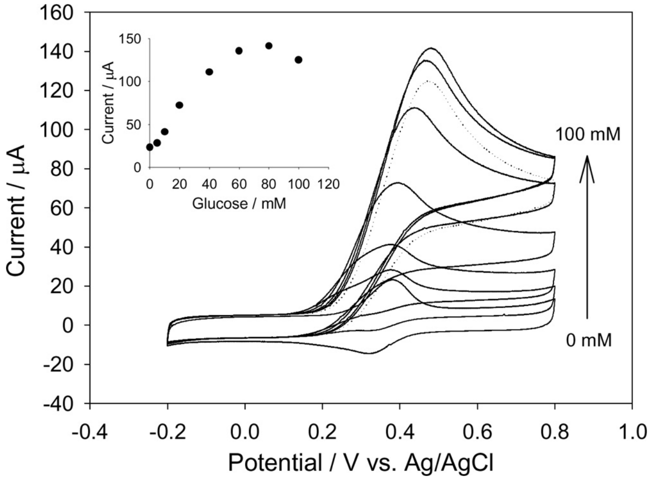

Figure 5. Cyclic voltammograms of the CHI/GOx/Fc/CNT electrode at various glucose concentrations in pH 7.0 phosphate buffer at a scan rate of 100 mV/s. Inset: plot of current vs glucose concentration.

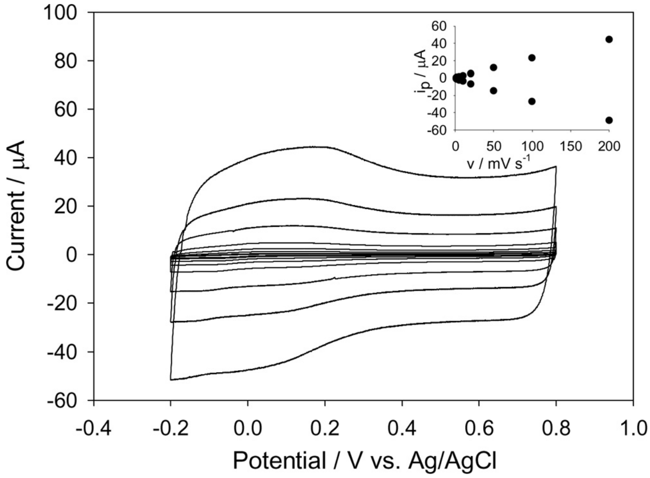

Figure 6. Cyclic voltammograms of the CHI/GOx/Fc/CNT electrode in pH 7.0 phosphate buffer at various scan rates. Inset: plot of anodic and cathodic peak currents vs V.

In order to further evaluate the performance of MWCNTs as the electrode materials, it was compared to a carbon black (CB). The CB/Fc/GOx/CHI electrode was fabricated in the same way as the MWCNT/Fc/GOx/CHI electrode. Figure 7 shows the CV of the CB/Fc/GOx/CHI electrode measured in 20 mM glucose solution under ambient conditions. The anodic peak current of the CB/Fc/GOx/CHI electrode was observed to be 27 μA. Under the same conditions, the anodic peak currents of the Fc/GOx/CHI electrode and the MWCNT/Fc/GOx/CHI electrode were 4.9 and 73 μA, respectively. The peak current for the CB electrode was about five times higher that the Fc/GOx/CHI electrode, but it was less efficient than the

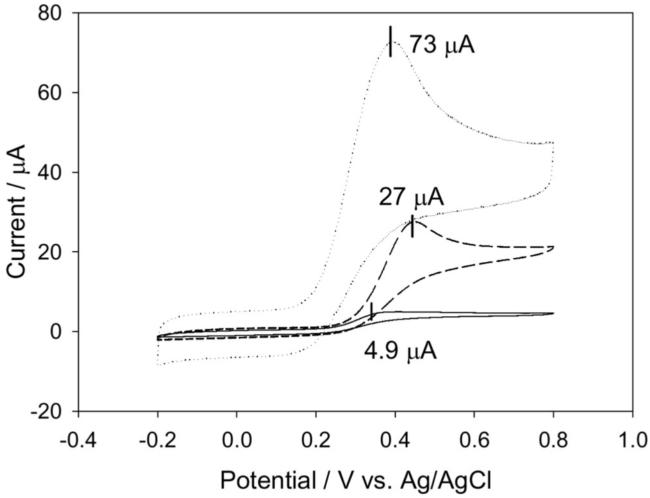

Figure 7. Cyclic voltammograms of the Fc/GOx/CHI electrode (solid line), the CB/Fc/GOx/CHI electrode (dashed line), and the MWCNT/Fc/GOx/CHI electrode (dotted line) in the presence of 20 mM glucose at a scan rate of 100 mV/s.

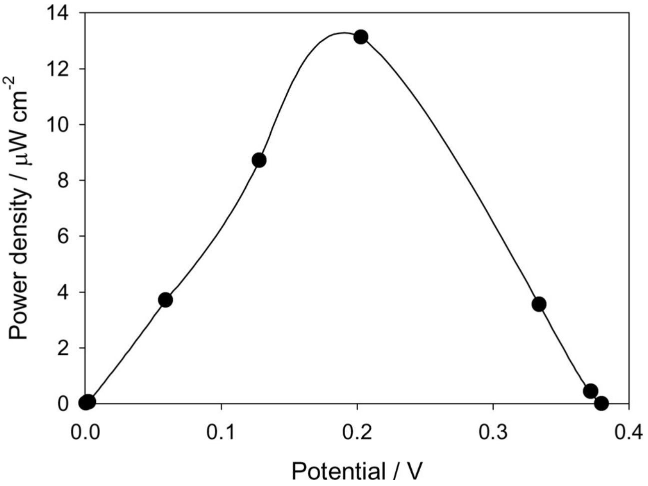

Figure 8. The power density curve of the biofuel cell with the MWCNT/Fc/GOx/CHI anode and the MWCNT/BOD/CHI cathode in a phosphate buffer (pH 7) containing 10 mM glucose at room temperature.

MWCNT electrode. Both MWCNT and CB have good electrical conductivity. Therefore, the better electrode performance of the MWCNT relative to CB was thought to have resulted from a better incorporation of ferrocene within MWCNT probably due to the π-stacking along with stronger van der Waals interactions [27].

The MWCNT/Fc/GOx/CHI electrode was tested as an anode in a glucose/oxygen biofuel cell. The enzyme based cathode was prepared by direct loading of bilirubin oxidase on the MWCNT-coated electrode and then entrapping them with chitosan membrane. The current-potential curve was measured by connecting an external variable resistance between the bioanode (MWCNT/Fc/GOx/CHI electrode) and biocathode (MWCNT/BOD/CHI electrode). The power density was calculated by multiplying the current and the cell voltage. As shown in Figure 8, at a glucose concentration of 10 mM, the maximum power density was 13 $\mu W/cm^2$ at a cell voltage of 0.19 V and the open circuit voltage (OCV) was 0.38 V. This result indicates that the MWCNT/Fc/GOx/CHI electrode could be used in a biofuel cell after optimization of the CNT, Fc, GOx, and CHI properties.

Conclusions

We have demonstrated that carbon nanotubes, ferrocene, and glucose oxidase could be effectively entrapped using a chitosan membrane for fabrication of a bioanode. The MWCNT/Fc/GOx/CHI electrode prepared by sequential deposition of each compound exhibited good electrochemical performance as an anode in a biofuel cell. The MWCNT film remarkably improved the performance of the electrode. After integrating the MWCNT/Fc/GOx/CHI electrode with the BOD-immobilized cathode, the maximum power density was obtained to be 13 $\mu W/cm^2$ at a cell voltage of 0.19 V when the glucose concentration was 10 mM. The result of this study indicates that the MWCNT/Fc/GOx/CHI electrode could be applied in the development of biofuel cells and biosensors.

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