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Short communication

Use of bioelectrode containing DNA-wrapped single-walled carbon nanotubes for enzyme-based biofuel cell

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ABSTRACT

Biofuel cells that utilize enzymes are attractive alternatives to metal catalyst-based cells because they are environmentally friendly, renewable and operate well at room temperature. Glucose oxidase (GOD)/laccase based biofuel cells have been evaluated to determine if they are useful power supplies that can be implanted in vivo. However, the usefulness of GOD/laccase systems is limited because they produce low level of electrical power. The effects of DNA-wrapped single-wall carbon nanotubes (SWNTs) on the electrical properties of a fuel cell are evaluated under ambient conditions in an attempt to increase the electrical power of an enzyme-based biofuel cell (EFC). The anode (GOD) and cathode (laccase) system in the EFC is composed of gold electrodes that are modified with DNA-wrapped SWNTs. Glucose (for anode) and O_2 (for cathode) are used as the substrates. The anodic electrical properties increase significantly with a bioelectrode that contains DNA-wrapped SWNTs as an electron-transfer mediator. Furthermore, the modified bioelectrode results in increased activities and stabilities of GOD and laccase, which enhance power production (442 $\mu W\,cm^{-2}$ at 0.46 V) compared with a basic EFC.

1. Introduction

Biofuel cells are eco-friendly and attractive alternative energy sources for nano-microelectronic devices and biosensors [1–4]. Enzyme-based biofuel cells (EFCs) are capable of functioning at moderate temperatures, and biocatalysts have been found to be useful for facilitating the transfer of electrons within such cells [5,6]. The cells generate electrical energy from abundant substrates and can be constructed by means of a variety of methods [7–10]. Nevertheless, the use of oxidative biocatalysts, such as oxidases and dehydrogenases, in EFCs leads to problems in electron transfers and enzyme activity. These problems result in the production of low levels of electrical power by the fuel cell. Therefore, redox mediators must be used to enable the transfer of electrons from the substrate and an effective enzyme immobilization method must be employed to improve the enzyme activity and stability.

Poor electron transfer and enzyme activity have hindered all applications of EFC technology. To solve these limitations, some researchers have investigated biocatalysts and the environment in which they can be expected to be active. These paths overlap in that they require a significant fundamental understanding of the biocatalyst. The studies have led to the rapid development of biomimetic techniques for identifying the mechanistic aspects of biocatalysis [11–13]. The evaluation of biomimetic materials, however, has not yet yielded satisfactory biocatalysts because such synthesized materials are expensive, less active and less stable than natural biocatalysts.

Recently, many workers have investigated various types of biofuel cell for high power output. For examples, power densities of 64 $\mu W\,cm^{-2}$ at 23 °C and 137 $\mu W\,cm^{-2}$ at 37 °C have been obtained using carbon fibre (2 cm × 7 μm) electrodes that contained immobilized glucose oxidase (GOD) for the anode and laccase for the cathode [6]. Additionally, a high operating voltage (0.78 V) was obtained using laccase and modified redox polymers [14]. Furthermore, a high power output (48 $\mu W\,cm^{-2}$ at 0.60 V) was delivered under physiological conditions using the GOD/laccase system [15]. In addition, a polymer containing Vitamin-K_3 was utilized to immobilize glucose dehydrogenase (GDH) on glassy carbon [16]. The power output of an EFC containing the GDH immobilized using Vitamin-K_3 was 14.5 $\mu W\,cm^{-2}$ at 0.36 V at pH 7.0 and 37 °C, and this output remained stable for up to two weeks.

Single-wall carbon nanotubes (SWNTs) are considered to be interesting materials for use in nano-electronics. Specifically, the electrical properties of SWNTs are suitable for use in advanced biological electronics and biosensors. Unfortunately, however, SWNTs are not easily solubilized in aqueous or non-aqueous solvents. It has been reported that the sonication of DNA with SWNTs is an extremely effective method of dispersing nanotubes in aqueous

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solution [17,18]. Therefore, it is possible to modify DNA-wrapped SWNTs or other immobilization methods to enable enzyme immobilization in EFCs.

In this study, a novel bioelectrode technique that employs DNA-wrapped SWNTs is developed. The effects of these SWNTs on the electrical properties of a basic EFC based on a GOD/laccase system are investigated under ambient conditions (pH 7.0 and a reaction temperature of 25 °C).

2. Experimental

2.1. Materials and reagents

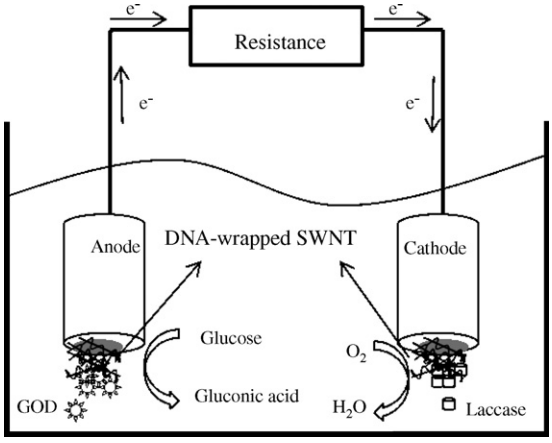
Synthetic oligonucleotides were obtained from Bionics Co., Ltd. and SWNTs were purchased from Sigma–Aldrich. GOD (113 U mg^{-1}, Bio Basic Inc.) from *Aspergillus niger* and laccase (0.72 U mg^{-1}) from *Trametes versicolor* (EC 1.1.1.27, Fluka) were used as the anode and cathode biocatalysts, respectively. N-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma–Aldrich) was used as the coupling agent, which was catalyzed for the formation of the amide bonds. Flavin adenine dinucleotide (FAD, Sigma–Aldrich) was used as the co-factor for GOD catalysis. The substrates for the anode and cathode fuels were D-glucose and O_2, respectively. Cystamine dihydrochloride was chemisorbed on the Au electrode surface via the self-assembly method (SAM). Pyrroloquinoline quinone (PQQ, Sigma–Aldrich) and 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonate)-diammonium salt (ABTs, Sigma–Aldrich) were employed as the electron-transfer mediators in the anode and cathode systems, respectively.

2.2. Preparation of DNA-wrapped SWNT

Single stranded DNA (ssDNA)-wrapped SWNTs were manufactured using a procedure similar the method described by Zheng et al. [17–19]. Briefly, modified ssDNAs with 30 base long poly-GT ($(GT)_{15}$) sequences (sequences of repeating guanine and thymine nucleotides) with amine groups at the 5′ and 3′ ends were designed. Next, 3 ml of solution that contained 0.1% SWNT, 0.1% the modified DNA, 0.2 mM ethylene diamine tetra acetic acid (EDTA), and 10 mM NaH_2PO_4 at pH 7 was sonicated for 2 h at 4 °C and 8 W to disperse the SWNTs with the modified DNA. The suspension was then centrifuged at 13 000 × *g* for 90 min, after which the pellet was discarded.

2.3. Preparation of bioelectrodes

To clean each electrode, the surface was polished to a mirror-like finish with 0.3 μm Al_2O_3 powder, followed by 0.05 μm Al_2O_3 powder. The polished electrode was immersed in Piranha solution (a solution of 30% H_2O_2 and concentrated H_2SO_4 mixed at a volume ratio of 1:3), after which it was rinsed ultrasonically with water and absolute ethanol for 3 min. The electrode was then voltammetrically cycled in 0.1 M H_2SO_4 until a stable cyclic voltammogram was obtained, at which point the potential ranged from 0.4 to 1.2 V. The electrode was then washed with sonication. Three types of bioelectrode were prepared for use in comparative experiments namely modified with DNA-wrapped SWNTs-enzyme, SWNTs-enzyme, and electron-transfer mediator-enzyme. The adsorption of DNA-wrapped SWNTs to the electrode was accomplished with a power supply at 50 V for 1 min. Immobilization of GOD (for the anode) or laccase (for the cathode) on the electrode modified with the DNA-wrapped SWNTs was conducted by sweeping the potential from −1.0 to 1.0 V at scan rates from 20 to 200 mV s^{-1}, typically for 4–5 cycles. Immobilization was conducted in a phosphate buffer solution (pH 7.0) that contained GOD (113 U mg^{-1}) [20] and was followed by incubation with 0.05 M EDC as a coupling agent for approximately 6 h.



Scheme 1. Schematic illustration of operating principle of the glucose/O_2 biofuel cell containing a bioelectrode modified by addition of DNA-wrapped SWNTs.

Bioelectrodes modified with a SWNT-enzyme carboxyl group or without DNA or SWNT were manufactured for comparison with the DNA-wrapped SWNTs electrode, as follows. A bioelectrode modified with a SWNT-enzyme carboxyl group was produced from SWNT using aqua regia ($HCl:HNO_3$ = 3:1). The adsorption of carboxyl-modified SWNTs to the electrode was accomplished by applying 50 V for 1 min. The modified electrode was then thoroughly rinsed with water to remove the adsorbed compounds, after which the electrode was incubated for approximately 4 h in 0.01 M N-(2-hydroxethyl) piperazine-N′-(2-ethanesulfonic acid) hemisodium salt buffer (HEPES buffer, pH 7.0) with GOD (for the anode) or laccase (for the cathode) and 20 mM EDC. During modification of the electrodes with the electron-transfer mediator-enzyme, gold electrodes were soaked in a 0.1 M aqueous cystamine solution for 4 h [21]. The disulfide group (S–S) of cystamine was sufficiently active to allow chemisorption of the cystamine on the Au electrode. The modified electrode was then thoroughly rinsed with water to remove the adsorbed compounds, after which the anode or cathode electrode was incubated for approximately 4 h in 0.003 M PQQ or ABTs solution, respectively, and 20 mM EDC in 0.01 M HEPES buffer (pH 7.0). The modified electrodes were then rinsed with water to remove the adsorbed PQQ or ABTs. For the bioelectrode without DNA or SWNT, FAD–GOD immobilization to the PQQ-modified anode electrode and Lac immobilization to the ABTs-modified cathode electrode were conducted in 0.1 M phosphate buffer using the same method that was used to immobilize PQQ or ABTs.

2.4. Preparation of a basic EFC

The electrolyte solution was composed of a 0.1 M phosphate buffer (pH 7.0) that contained D-glucose and O_2 as substrates. To analyze the power curves of a basic EFC, the modified electrodes were connected. At the GOD/DNA-wrapped SWNTs bioanode, glucose was oxidized to gluconic acid by GOD immobilized on the DNA-wrapped SWNTs. At the laccase-SWNTs biocathode, O_2 was reduced to water by the bioelectrocathalysis of laccase immobilized on the DNA-wrapped SWNT. The operating principle of a basic EFC is shown in Scheme 1. The projected surface area of the cathode and the anode was adjusted to 0.0314 cm^2 disc electrodes (gold, 1 mm radius), and the total electrolysis solution volume in the vessel was 3 ml.

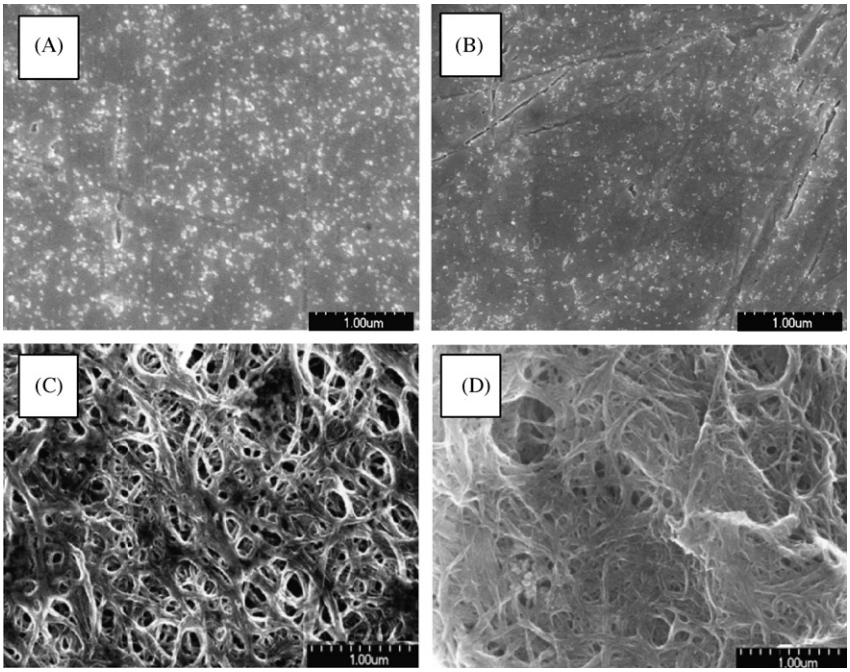


Fig. 1. FE-SEM top views of bare gold electrode (A), GOD/FAD/PQQ/gold electrode without DNA-wrapped SWNT (B), DNA-wrapped SWNT/gold electrode (C) and GOD/DNA-wrapped SWNT/gold electrode (D).

2.5. Electrochemical and FE-SEM measurements

Electrochemical measurements were conducted using a potantiostat/galvanostat (WPG100, WonATech Ltd., Korea) and a conventional three-electrode cell. The Ag/AgCl electrode and a Pt foil were used as the reference electrode and the auxiliary electrode, respectively in the cyclic voltammetry (CV cycle). The modified bioelectrode was used as the working electrode. Prior to measurement of the CV cycle of the anode, oxygen was purged from the solution by bubbling with highly purified nitrogen (99.999%) for 30 min. In addition, a nitrogen atmosphere was maintained within the cell. The cell was water-jacketted with a Teflon cover, throughout the duration of the experiment. All electrochemical measurements were conducted at an ambient temperature of $25 \pm 1\,^{\circ}C$ and all experiments were conducted in triplicate. Field-emission scanning electron microscope (FE-SEM) images of the coatings were taken (Hitachi S-4700, Hitachi Ltd., Japan). Modified electrodes with square forms ($1.0\,cm^2$) were prepared for scanning electron microscopic (SEM) analysis.

2.6. Measurement of immobilized enzymes

The amount of immobilized enzymes was measured by the Lowry method using a DC protein assay kit from Bio-Rad Ltd., USA. First, the working reagent was prepared by adding 20 μl of reagent S to each ml of reagent A (an alkaline copper tartrate solution) that was required for the run. Next, three or five dilutions of a protein standard (a bovine gamma globulin standard) containing from 0.2 to about $1.5\,mg\,ml^{-1}$ protein were prepared. Subsequently, 100 μl of standards and samples of the residues, which were not immobilized to GODs, were placed into clean and dry test tubes, after which 500 μl of reagent A were added to each test tube. The samples were then vortexed, after which 4.0 ml of reagent B (a dilute Folin reagent) were added to each test tube and the sample was immediately vortexed again. The samples were then allowed to rest for 15 min, at which time the absorbance was read at 750 nm. The amount of the immobilized GODs was calculated following Eq. (1) after the residues were measured, as follows:

$$\text{Immobilized GODs} = \text{input of GODs} - \text{residues of GODs} \quad (1)$$

2.7. Measurement of diffusion coefficient

Deionized (DI) water was used to prepare a 0.01 M solution of $K_3Fe(CN)_6$ IN 1 M KCl. The solution was deoxygenated by passing nitrogen gas through a sparging tube for 10 min. It was then deoxygenated for another 2 min. Next, the Au electrode was polished to a mirror-like finish and then thoroughly rinsed with DI water. Three electrodes (i.e.) working electrode, Ag|AgCl reference, and counter electrode, were connected to the potentiostat and 0.75 V was applied. The potential was repeatedly scanned between 0.75 and 0.15 V versus Ag|AgCl at a sweep rate of $100\,mV\,s^{-1}$. The working electrode was shacked to destroy the diffusion layer, after which the initial potential was set to 1 V versus Ag|AgCl and the potential was swept in the negative direction at $225\,mV\,s^{-1}$. The process was repeated at different scan rates (196, 169, 144, 121, . . ., $9\,mV\,s^{-1}$). The peak current (i_p) versus the square root of the scan rate ($\nu^{0.5}$) was then plotted and the diffusion coefficient (D) of the iron ion was determined from the slope of the resulting straight line.

3. Results and discussion

3.1. Surface of bioelectrode modified with DNA-wrapped SWNTs

Scanning electron microscopy was used to characterize and compare the morphologies of the different modified electrodes (Fig. 1).

On the surface of a bare electrode (Fig. 1A), a more uniform and smooth surface is observed. On the GOD/FAD/PQQ/electrode without DNA-wrapped SWNTs (Fig. 1B), the smooth surface is similar to that of a bare electrode; but there are fewer bright spots and network line structures observed due to regular distribution of the immobilized GOD molecules. Fig. 1C shows the DNA-wrapped SWNTs/electrode, which has a rough surface structure with many

Table 1
Amount of immobilized GOD and diffusion coefficients.

GOD-modified electrodes	Amount of immobilized GOD ($\mu g\,mm^{-2}$) (standard deviation)	Diffusion coefficients ($cm^2\,s^{-1}$) (standard deviation)
DNA-wrapped SWNTs	73.3 (±3.70)	5.06×10^{-5} ($\pm 6.27 \times 10^{-7}$)
Only SWNTs	19.1 (±0.61)	4.76×10^{-5} ($\pm 3.47 \times 10^{-7}$)
Without DNA-wrapped SWNTs	18.5 (±3.50)	1.05×10^{-6} ($\pm 7.48 \times 10^{-8}$)

nanosized particles or porous forms, which indicates the presence of DNA-wrapped SWNT particles. The porous forms on the surface of the electrode may be attributed to the increased immobilization of GODs. The quantity of GOD immobilized on the anode electrode modified with DNA-wrapped SWNTs appears to be greater than the amount immobilized onto the GOD/FAD/PQQ electrode (Fig. 1D). The differences between the SEM images indicate that DNA-wrapped SWNTs supply sufficient spaces on the surface of the electrode for improvement of the quantity of immobilized GODs. The quantity of immobilized GODs was measured to investigate the effect of porosity of the DNA-wrapped SWNT on the amount of immobilized GOD using a DC protein assay kit (Table 1).

A high amount of immobilized GODs (73.3 $\mu g\,mm^{-2}$, 8.28 U) is found to have immobilized on the electrode that contained the DNA-wrapped SWNTs, whereas electrodes modified with SWNTs alone (19.1 $\mu g\,mm^{-2}$, 2.16 U) or without DNA-wrapped SWNTs (18.5 $\mu g\,mm^{-2}$, 2.09 U), contain lower quantity of immobilized GODs. These results indicate that the adsorbed DNA-wrapped SWNTs on the electrode provide abundant spaces for immobilization of GODs while the binding of GODs to electrodes modified with SWNTs alone or FAD/PQQ is limited to covalent bonds. The effects of DNA-wrapped SWNTs on electron transfer between the electrode and an electrolyte was investigated by comparing the diffusion coefficient of iron ion at the surface of an electrode containing DNA-wrapped SWNTs, to that of electrodes modified with SWNT alone or FAD/PQQ (Table 1). To accomplish this, the redox reactions (2) and the Randles Sevcik equation (3) were used.

$$Fe(CN)_6^{3-} + e^- \Leftrightarrow Fe(CN)_6^{4-} \quad (2)$$

$$i_p = 2.69 \times 10^5 \cdot n^{1.5} \cdot S \cdot C \cdot D^{0.5} \cdot V^{0.5} \quad (3)$$

where i_p is the peak current (A), n is the number of electrons appearing in the half-reaction for the redox couple; D is the apparent diffusion coefficient; S is the surface area of the electrode (cm^2); V is the rate at which the potential is swept ($V\,s^{-1}$), C is the concentration of diffusion species ($mol\,cm^{-3}$, $K_3Fe(CN)_6$). The diffusion coefficient (D) of iron ion on the surface of the electrode was calculated from the slope of the linear regression equation of i_p versus $v^{0.5}$. For electrodes modified with DNA-wrapped SWNTs, D is $5.06 \times 10^{-5}\,cm^2\,s^{-1}$, while it is $4.76 \times 10^{-5}\,cm^2\,s^{-1}$ for those modified with SWNT alone and $1.05 \times 10^{-6}\,cm^2\,s^{-1}$ for those modified with FAD/PQQ. The diffusion coefficient is related to the electrical properties of the electrodes, such as electrical conductivity. Although the SWNTs-modified electrode does not show improved GOD immobilization, the diffusion coefficient is increased. This likely occurs because the SWNTs impart their good electrical conductivity to the anode electrode. These findings indicate that the diffusion coefficients are also impacted by the presence of SWNTs. DNA-wrapped SWNTs, which have rapid electron transfer for a $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ system, appears to function as a bridge or a mediator that supports electron transfer. It has been reported that DNA and SWNT are capable of electrical conductivity [22–24] and have an ability to transfer electrons, which is demonstrated by the good electrochemical voltammetric properties of DNA-wrapped SWNTs. In addition, DNA is rich in active functional groups, such as amino groups carbonyl groups, and carboxyl groups, that can act as media for electron transfer in redox reactions [25,26].

3.2. Effect of bioelectrodes containing DNA-wrapped SWNTs on cyclic voltammetry

An anode that contained a gold electrode, PQQ, as the electron-transfer mediator, GOD as the biocatalyst, FAD as the co-factor, and D-glucose as the substrate was constructed under ambient conditions (pH 7.0 and 25 °C) (Fig. 2).

Operation of this cell produces a wider oxidation area and an increased anodic current (about 22.0 μA at 0.90 V) in CV cycle. In addition, the use of a GOD-modified electrode that contains an amide bond results in a wider oxidation area that is superior to that of the unmodified electrode and is shifted toward the oxidation direction of the CV cycle. The inset of Fig. 2 shows a calibration curve at different concentrations of D-glucose. The location of the enzyme in a cell should be near the electrodes. This is because, even though most enzymes are used as support for the electron substrates in the electrolyte, a portion of the enzymes near the electrode can easily transfer electrons to the electrode.

Immobilization of GOD on the anode electrode was conducted using DNA-wrapped SWNTs, after which the CV cycle was then evaluated for the modified bioelectrode. As shown in Fig. 3, the GOD electrode modified with DNA-wrapped SWNTs produces a wider oxidation area and an increased anodic current (about 119 μA at 1.05 V) when compared with bioelectrode that contains modified SWNTs and those that use covalent bonds to immobilize GOD with FAD/PQQ on the anode electrode (no DNA or SWNT).

The CNTs contain grapheme sheets with dense π–π stacking that are chemically stable and strongly hydrophobic. These properties make CNTs suitable redox mediators because they enable the CNTs to shuttle the electron transfer of biocatalysts [27]. In addition, CNTs produce an environment that enables the direct electron transfer of enzymes or proteins and has good conductivity, although this conductivity does vary depending on the type of CNTs used. Furthermore, CNTs can support a large contact surface

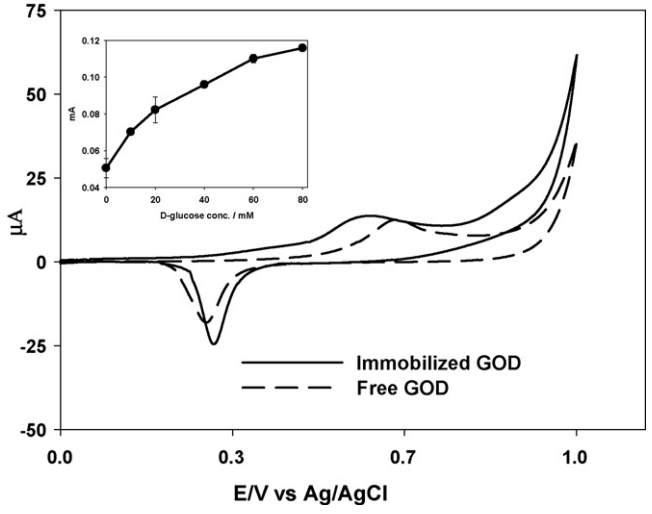


Fig. 2. Cyclic voltammetry of EFC with immobilized GOD. *Inset*: calibration curve describing electrocatalytic current at various D-glucose concentrations. All experiments are performed in a biofuel cell with a water jacket at 25 °C, phosphate buffer at a pH 7.0, and a scan rate of 50 mV s^{-1}.

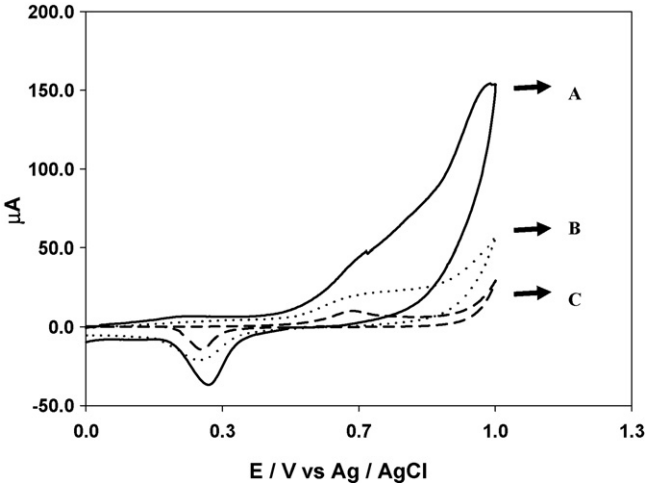


Fig. 3. Cyclic voltammetry following use of GOD bioelectrodes that contain DNA-wrapped SWNTs (A), SWNTs (B), or covalent bonds that are used for GOD immobilization with PQQ/FAD on the anode electrode (without DNA or SWNT) (C). All experiments performed in a biofuel cell with a water jacket at 25 °C, phosphate buffer (pH 7.0), and a scan rate of 50 mV s^{-1}.

for enzyme and electrode reactions. Therefore, biofuel cells that employ anode electrodes that contain SWNTs produce better CV cycles than those that do not contain SWNTs. The direct adsorption of GOD on the SWNT surface that is employed in this study appears to reduce the GOD activity. This is probably because the firm structure of the SWNTs produces a shear stress that prevents GOD from being converted into its active form. It has been reported [28] that the DNA interface between the enzyme and CNT improves the enzyme activity, but that this usually decreases when the enzymes are bound directly to the CNT. Wrapping DNA around SWNTs produces a structural balance with GOD, which leads to an increase in the activity and stability of GOD. The modified ssDNAs are composed of 30 base long poly-GT ($(GT)_{15}$) sequences (sequences of repeating guanine and thymine nucleotides). $(GT)_{30}$ is used because it retains a helical wrapping that forms a viable ssDNA structure around SWNT [29]. The modified DNAs also contain amine groups at the 5′ and 3′ ends that give rise to the production of the ionic charges that enable the SWNTs to be dispersed in polar solution.

A variety of electrode modification techniques have been employed to immobilize enzymes, of which covalent bonding methods are the most frequently adopted. Due to their profound bonding abilities, immobilized enzymes can be retained over a prolonged reaction period. The immobilization procedures are complicated, however, and result in a marked reduction in enzyme activity. The amino groups in the proximity of the active site of the enzyme become linked to the modified electrode during immobilization, which results in steric hindrance and thereby prevents the active sites from subsequently interacting with the substrates. This appears to occur because the electron-transferring unit of the enzyme is deeply buried within its complex structure. This conformation makes efficient electrical communication between the electrode substrate and the enzyme biocatalyst difficult [30], even with PQQ as an electron-transfer mediator.

3.3. Power curves of enzyme-based biofuel cell containing DNA-wrapped SWNTs

The power curves of a basic EFC that contained the modified electrodes using DNA-wrapped SWNTs with GOD for an anode were generated and then compared with those of an EFC modified with SWNT-GOD and without SWNTs and DNA. The production of the cathode bioelectrodes was conducted using three types of anode modifications. The cathode biocatalyst was laccase and the substrate was O_2. Power curves were generated by connecting the enzymatic anode and the cathode through an external resistance. The current–power output and cell potential relationship of a basic biofuel cell at different external resistances are given in Fig. 4A.

The maximum power output (442 μW cm^{-2} at 0.46 V) of the EFC with the DNA-wrapped SWNTs is higher than that of other EFCs, which demonstrates that the power output of the basic EFC is increased via a biofuel cell that has been modified using DNA-wrapped SWNTs. Specifically, an EFC that utilizes DNA-wrapped SWNTs produces 129 and 453% more power than an EFC that utilizes an electrode containing GOD that has been immobilized with SWNTs (193 μW cm^{-2}) and an EFC that does not contain DNA or SWNTs (80 μW cm^{-2}), respectively. The high power density of the EFC that employs DNA-wrapped SWNT appears to be affected by the high quantity (73.3 μg mm^{-2}, 8.28 U) of immobilized GODs and the higher diffusion coefficient (5.06×10^{-5} cm^2 s^{-1}) than those of other modified electrodes. The EFC using SWNTs has a low power density due to the low quantity of immobilized GODs, even though it has a high diffusion coefficient (4.76×10^{-5} cm^2 s^{-1}).

A previous study demonstrated that a D-fructose dehydrogenase (FDH)/laccase (TsLAC)-based biofuel cell had a high power density (850 μW cm^{-2}) [31]. The redox mediators in the electrolyte solution used for FDH were $(NH_4)_2OsCl_6$ and Na[Fe(EDTA)] (EDTA; ethylenediamine tetraacetic acid), where as those used for TsLAC were [Os(bpy)(5-dmbpy)$_2$] and 50 mM [Os(4-dmbpy)(5-dmbpy)$_2$]

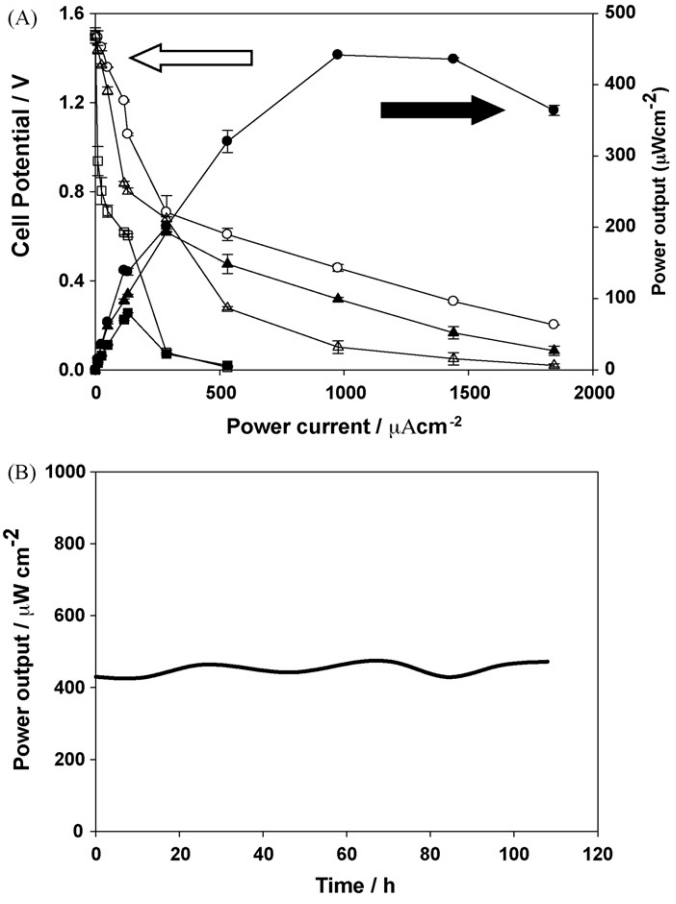


Fig. 4. Effect of use of modified bioelectrodes on power curves in a basic EFC (A) (●, power output of a bioelectrode modified with DNA-wrapped SWNTs; ▲, SWNTs; ■, without DNA or SWNT, and ○, cell potential of bioelectrode modified with DNA-wrapped SWNTs; △, SWNTs; □, without DNA or SWNT). (B) Time course of power output generated in glucose/O_2 biofuel cell at ambient conditions (pH 7.0 and 25 °C) that employ GOD and laccase electrically contacted bioelectrocatalytic electrodes.

(bpy; 2,20-bipyridine, 4-dmbpy; 4-40-dimethyl-2,20-bipyridine, 5-dmbpy;5-50-dimethyl-2,20-bipyridine), respectively [32,33]. In addition, the projected surface areas of the cathode and the anode were adjusted to 0.282 cm^2 by covering the CP electrodes with a cell vessel, and the total electrolysis solution volume in the vessel was 2 ml. Therefore, their study reported in [31] differed from that undertaken here. Specifically, in the present work, a GOD/laccase based biofuel cell is evaluated for application to implant an electrical cells in an in vivo system that contains glucose, such as a blood tube. The projected surface area of the cathode and the anode are adjusted to be 0.0314 cm^2 in disc electrodes (gold, 1 mm of radius), and the total volume of electrolyte solution in the vessel was 3 ml. Although the power output of the EFC generated here is smaller than the previous investigation [31], it is obtained in a GOD/laccase system, not in FDH/TsLAC system. In addition, the basic biofuel cell used in the present study is smaller. Furthermore, the EFC developed in this study contains no chemically synthesized redox mediators except for the DNA-wrapped SWNTs immobilized on the surface of the electrodes.

The power output of the EFC that employed the bioelectrode modified with the DNA-wrapped SWNTs is maintained at greater than 430 $\mu W\,cm^{-2}$ for approximately five days even though many factors related to electrolytes (substrates, buffers, and electron transfers) affect the electron loss and the power results (Fig. 4B). The ssDNA appear to decrease the shear stress that occurs between the immobilized GOD and SWNTs and to provide the proper structural stability, as well as to function as the primary mediator of electron transfer. When only an anode modified with the DNA-wrapped SWNTs is used in the biofuel cell, the power output is reduced significantly (data not shown). These findings indicate that the cathode electrode must be capable of accepting a sufficient amount of electrons or else it is difficult for a large volume of electrons to be transferred to the cathode from the anode substrates.

4. Conclusion

Many studies have been conducted to improve the electrical properties of EFCs using enzyme immobilization techniques to increase the stability of enzymes, which are also affected by the temperature and pH in the EFC [34]. It is expected that the bioelectrode described here, which is modified by the addition of DNA-wrapped SWNTs, will be applied to the immobilization of other unstable enzymes for use in biofuel cells. In this study, a bioelectrode for an EFC is designed using DNA-wrapped SWNTs under ambient conditions (pH 7.0 and 25 °C). When the modified electrode containing DNA-wrapped SWNTs is used in a GOD/laccase based biofuel cell, the power density is increased when compared with an EFC that employed an electrode that does not contain DNA-wrapped SWNT. Therefore, any biofuel cell system expects to obtain higher power density if employs DNA-wrapped SWNT immobilized on the surface of the electrode. These findings suggest that it is possible to apply other enzyme systems to the development of EFCs by adopting appropriate enzyme structures, buffer solutions, substrates, and co-factors. Nevertheless, further research is needed to establish the appropriate factor conditions for EFCs that contain the bioelectrode modified with DNA-wrapped SWNTs and to determine which enzymes should be employed to overcome complications such as enzyme stability, electron transfer, and power production.

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