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ORIGINAL PAPER

Development of a biofuel cell using glucose-oxidase- and bilirubin-oxidase-based electrodes

Jinseong Kim · Jeffrey Parkey · Christopher Rhodes · Anuncia Gonzalez-Martin

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Abstract Biofuel cells have a tremendous opportunity to provide much higher energy densities and smaller footprints than batteries for powering implantable medical devices, leading to less intrusive implantable devices with longer lifetimes. This paper introduces biofuel cell anode and cathode designs based on mediated glucose oxidation by glucose oxidase and oxygen reduction by bilirubin oxidase, respectively. We report here the progress toward the development of components for biofuel cells working in physiological conditions. We have investigated enzymatic electrode formulations that have the potential to achieve higher current densities and longer stability of the electrodes: (a) high surface area by the use of multiscale carbon materials, (b) immobilization of redox mediator on the electrode surface, and (c) use of a protective biocompatible polymer coating.

Keywords Biofuel cell · Glucose oxidase · Bilirubin oxidase

Abbreviations

BOx	Bilirubin oxidase
CNTs	Carbon nanotubes
CTAB	Cetyltrimethylammonium bromide
CV	Cyclic voltammetry
CVD	Chemical vapor deposition
GC	Glassy carbon
GOx	Glucose oxidase
HQ	Hydroquinone
LP	Linear polarization
PBS	Phosphate-buffered saline
PEI	Polyethyleneimine
PEGDA	Polyethyleneglycoldiacrylate
PEGDGE	Polyethylene glycol diglycidyl ether
TBAB	Tetrabutylammoniumbromide
TP	Toray paper

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J. Kim (✉) · J. Parkey · C. Rhodes · A. Gonzalez-Martin
Lynntech, Inc.,
College Station, TX 77840, USA
e-mail: jinseong.kim@lynntech.com
URL: www.lynntech.com

Introduction

The performance of biofuel cells, in terms of power density, lifetime, and operational stability, falls far below that of chemical fuel cells [1], though exciting advances have been made since the first enzyme-based biofuel cell was reported in 1964 using glucose oxidase (GOx) as the anodic catalyst and glucose as the biofuel [2]. Most of the recent studies showing renewed interests in biofuel cells have been directed toward special applications such as implantable devices, sensors, drug delivery, micro-chips, and portable power supplies [3–5]. Implantable medical devices, including pacemakers, nerve stimulators, drug delivery pumps, glucose monitors, and biosensors, equipped with appropriate power sources offer tremendous patient benefits. However, current power sources, i.e., batteries, have a large footprint and a limited lifetime, requiring periodic surgical replacement. In addition, the development of miniature implantable devices is constrained by the size and energy density of current implantable battery technologies since the power supply is typically many times the size of the apparatus it is

powering. Therefore, new power sources with improved lifetime and reduced sizes are needed in order to allow the successful development of small-scale devices. Implantable biofuel cells have tremendous opportunity to provide higher energy densities and smaller footprints than batteries for powering implantable medical devices, leading to less intrusive implantable devices with longer lifetimes. This in turn can reduce or eliminate the need for additional surgery required for power supply replacement. Successful development of biofuel cells could provide implantable power sources that use the endogenous fuel present in the implanted host to produce power in situ. For mammalian hosts, blood is an excellent source of glucose and oxygen that can be utilized as a reactant and oxidant, respectively, in a fuel cell to produce power.

Although the idea of harvesting the readily available energy from biological sources is attractive, the development of biofuel cells as power systems faces several critical issues that need to be solved, such as lifetime, stability, electrode poisoning, design, and redox mediator potential [6]. Inorganic catalysts for glucose and oxygen are ineffective at near neutral pH's and low temperatures, and their lack of specificity and susceptibility to poisoning preclude their use in implantable biofuel cells.

Though the simplicity of direct electron transfer between an enzyme and electrode is attractive, the electron transfer rate between the active sites of enzymes and the electrode surface is low because the active site is buried deep in the protein [7]. Two possible strategies to reduce the electron tunneling distance are (a) to use carbon nanotubes to penetrate the active site of the enzymes [8–10] and (b) to incorporate immobilized redox mediators to facilitate both the anode and cathode reactions [11]. Another challenge in the development of implantable biofuel cells is the electrode stability in physiological fluids [4] as "wired" enzyme anodes and cathodes lose electrocatalytic properties rapidly in serum [12, 13]. For example, a miniature membrane-less biofuel cell operating under physiological conditions was reported to have an initial power density of 50 $\mu W/cm^2$, which decreased to 30 $\mu W/cm^2$ after 2 days of continuous operation [11]. On the other hand, the use of biocompatible polymers as protective barrier membranes has the advantages of immobilizing the enzyme, improving the stability, and avoiding adverse body reaction such as biofouling and thrombogenesis.

We report here the progress in the development of biofuel cell components working in physiological conditions. We have investigated approaches that have the potential to achieve higher current densities and longer stability of the electrodes: (a) high surface area by the use of multiscale carbon materials, (b) immobilization of redox mediator on the electrode surface, and (c) use of a protective biocompatible polymer coating.

Experimental section

Chemicals and materials

GOx from *Aspergillus niger* and bilirubin oxidase (BOx) from *Myrothecium verrucaria* were purchased from Sigma (St. Louis, MO, USA). All chemicals, including sodium phosphate monobasic, sodium phosphate dibasic, sodium chloride, tetrabutylammonium bromide, 5% Nafion suspension, hydroquinone, ferrocene, ferrocenecarboaldehyde, chitosan, polyethleneimine, potassium ferricyanide, polyethyleneglycoldiacrylate, acetic acid, cetyltrimethylammonium bromide (CTAB), and polyethylene glycol diglycidyl ether (PEGDGE) were purchased from Aldrich (St. Louis, MO, USA) and were used without ay further purification. Oxygen, nitrogen, and purified air were purchased from BOTCO (Bryan, TX, USA).

Carbon nanotubes

Carbon nanotubes (CNTs) used in aqueous suspension were purchased from Helix Material Solutions (Richardson, TX, USA). CNTs for the multiscale support were directly grown on a Toray paper (TP) #090 purchased from E-TEK (Somerset, NJ, USA) in a high-temperature furnace via a chemical vapor deposition (CVD) technique [14]. Figure 1 shows the scanning electron microscope (SEM) images of carbon paper and CNTs grown on the carbon paper using the high-temperature CVD. Tunneling electron microscope images revealed that the CNTs prepared were multiwall carbon nanotubes.

Synthesis of redox mediators

Redox polymers poly(*N*-vinylimidazole)-[osmium(4,4′-diamino-2,2′-bipyridine)$_2$chloride]$^{+/2+}$-*N*-2-amino-ethyl (PVI-[Os(da-bpy)$_2$Cl]$^{+/2+}$-*N*-2-amino-ethyl) and polyacrylamide-poly(*N*-vinylimidazole)-[osmium(4,4′-dichloro-2,2′-bipyridine)$_2$chloride]$^{+/2+}$ (PAA-PVI-[Os(dcl-bpy)$_2$Cl]$^{+/2+}$) were synthesized according to procedures previously reported [11, 15]. Mass spectroscopy (Applied Biosystems Pulsar QSTAR), infrared (FTIR Bruker model Tensor 27), and nuclear magnetic resonance (Inova 400) were used to characterize the intermediate compounds and final products.

Electrodes

Glassy carbon electrodes were used as substrates for enzyme electrodes. This electrode was prepared as follows. Glassy carbon (GC) electrodes (4 mm diameter) were sequentially polished on an emery paper (CarbiMet® Abrasive Discs, Buehler) using 0.3 and 0.05 μm alumina suspensions. After being sonicated for 2 min in deionized

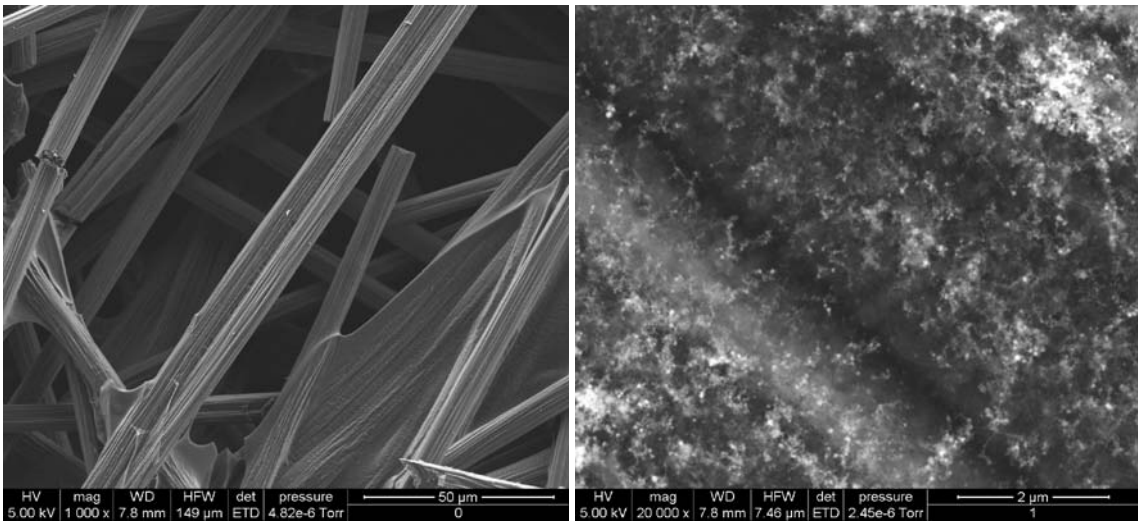

Fig. 1 SEM images of **a** carbon-based substrate (Toray paper) and **b** carbon nanotubes grown on the Toray paper

water, the glassy carbon electrode had a mirror-like finishing. On the glassy carbon electrode, a mixture of 40 mg/mL glucose oxidase (or bilirubin oxidase) in phosphate-buffered saline (PBS), 10 mg/mL CNT in water containing 0.1% CTAB, and 5% polyethyleneimine (PEI) was coated on the polished GC electrode. After the coating was dried, a casting material, one of 5% Nafion suspension, 5% tetrabutylammoniumbromide (TBAB)-modified Nafion suspension [16], chitosan in 2% acetic acid, or polyethyleneglycoldiacrylate (PEGDA) was casted and cured in ambient conditions.

Carbon cloth electrodes were prepared by attaching 3-mm-diameter Toray paper disk on the glassy carbon electrode surface using conductive carbon paint (SPI, West Chester, PA, USA). Prior to the attachment, the Toray paper was exposed to vacuum plasma for 30 s to make it hydrophilic.

For electrodes with immobilized redox mediators, multiple coatings of a mixture containing 30 μL of 30 mg/mL redox polymer, 10 μL of 40 mg/mL enzyme in a 2:1 (*v*/*v*) mixture of 0.1 M $NaHCO_3$ and 7 mg/mL $NaIO_4$ in water, and 2 μL 20 mg/mL PEGDGE were applied to the carbon cloth electrodes [11].

Instrumentation and cell

Cyclic voltammetry (CV) and linear polarization (LP) were used to evaluate the performance of different electrode components. To this end, either the anodic or cathodic enzymatic electrode was used in a water-jacketed electrochemical cell containing 80 mL of PBS (20 mM, 0.1 M NaCl, pH 6.9) at 37 °C. The measurements were performed using a potentiostat (PAR2273, Princeton Applied Research, Oak Ridge, TN, USA) interfaced with a personal computer via a software (PowerCV, Princeton Applied Research, Oak Ridge, TN, USA). The potentials were measured with respect to Ag/AgCl reference electrode (BAS, West Lafayette, IN, USA). The counter electrode was a platinum wire mesh. Nitrogen, oxygen, or purified gas was purged inside the solution for more than 30 min and then over the solution after purging to prevent ambient air diffusing inside the solution.

Results and discussion

Anode—glucose oxidase on glassy carbon

The anodic enzymatic electrode was initially prepared according to the procedure described above. CNTs were utilized within the anodic enzymatic electrode to (a) allow direct electron transfer from the carbon substrate to GOx without a mediator and (b) to provide a three-dimensional multicarbon structure for increased surface area and power density. Casting the electrode with Nafion suspension was intended to prevent (or retard at least) GOx leaching into the electrolyte. The characteristic redox peaks at around −0.4 V from the prosthetic flavin adenine dinucleotide (FAD) group of the glucose oxidase could be clearly observed (data not shown), as reported elsewhere [7, 10]. However, no current increase was observed when 25 mM glucose was added. A similar lack of glucose response, despite the observation of direct electron transfer in the absence of glucose, has been reported by other groups [7, 10, 17, 18]. The reason for the lack of glucose response is not presently clear; it is possible that the association of GOx with the CNTs results in an

enzyme orientation that blocks glucose access to the GOx active sites. The lack of a glucose response motivated the use of redox mediators to provide an electron transfer pathway from the glucose oxidation in the enzyme to the electrode surface as supported by other's work [19].

Anode—effect of polyethyleneimine

PEI, which is positively charged, has been reported as a binder between the negatively charged glucose oxidase and carbon nanotubes [7]. We have compared the performance of GOx electrodes with and without PEI. Figure 2 shows the decay as a function of time peak current of GOx-CNT anodes with and without PEI at around −0.4 V in deaerated PBS containing 25 mM glucose. The current was higher in the presence of PEI, indicating that the PEI layer results in higher utilization of the enzyme. In addition, the current decreased at a slightly higher rate for the GOx anode without PEI than that with PEI. These results clearly demonstrate the advantage of using PEI in the electrode casting formulation.

Anode—transition to carbon cloth

A high surface area, carbon-based electrode (Toray paper) was tested as substrate with the intention to increase the power density [19]. Prior to the electrode preparation, the carbon cloth was plasma-treated to make it hydrophilic. Since the Toray paper has much larger active surface area, the capacitive current at the potential range from −0.2 to 0.4 V was much higher at the Toray paper than at the glassy carbon electrode. Even though both electrodes have the same amount of GOx, the current associated with the redox peaks of the GOx FAD group were much higher at the Toray paper that at the glassy carbon (Fig. 3). The higher current observed for GOx on the Toray paper compared to the glassy carbon electrode may be due to a thinner layer of the enzyme, CNT, and polymer composite being distributed over the higher surface area substrate. This indicates much better utilization of the enzyme based on improved electron transfer from the FAD group to the carbon substrate in the Toray paper electrode.

The capacitive current at the Toray paper on glassy carbon electrode was as much as 50 times higher than at the glassy carbon electrode, which is in a good agreement with other reported data for oxygen reduction at laccase cathodes [19]. In addition, the glucose oxidation current at mediated GOx anode increased tenfold when using Toray on glassy carbon electrode vs. just the glassy carbon electrode. A fivefold increase was observed for the oxygen reduction current at the mediated laccase cathode [19].

Anode—optimized GOx loading

The loading of the GOx on Toray paper on a glassy carbon support was optimized. The peak current of the glucose oxidation at the potential range from 0.2 to 0.4 V in PBS containing 25 mM glucose and 1 mM hydroquinone (HQ, solution-phase mediator) was normalized to the mass of GOx. As shown in Fig. 4, the GOx loading seems to be optimal between 0.2 and 0.5 mg for a Toray paper disc of 4 mm diameter (corresponding to 1.6 to 4.0 mg of GOx per square centimeter of electrode). In addition, it was observed that the anodic peak current at the GOx-CNT anode on glassy carbon substrate was close to the current at the GOx-CNT anode on Toray paper substrate. This indicates that the oxidation peak current depends on the quantity of GOx on

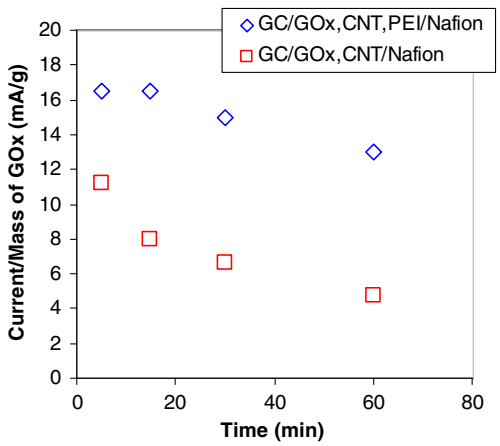

Fig. 2 Decay of cathodic peak current (normalized with respect to GOx mass) of FAD of GOx-CNT anodes with and without PEI in deaerated PBS containing 25 mM glucose

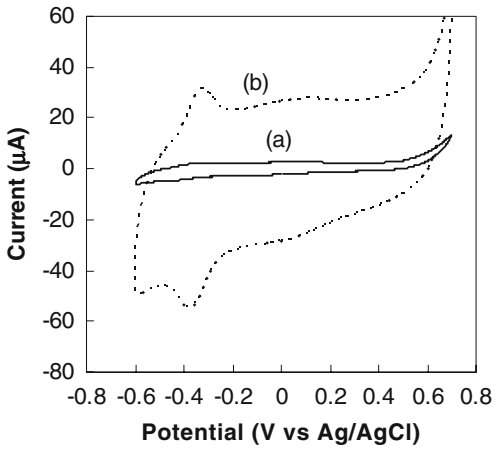

Fig. 3 Cyclic voltammetry at GOx anodes in deaerated PBS. A mixture of glucose oxidase and polyethyleneimine was coated on either (*a*) a glassy carbon electrode or (*b*) a Toray paper on a glassy carbon electrode, which was followed by casting of Nafion suspension. Scan rate=20 mV/s

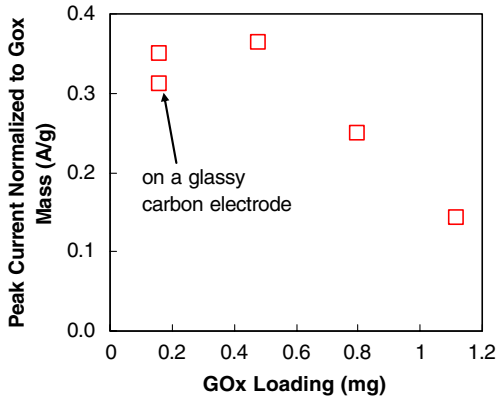

Fig. 4 Optimization of enzyme loading on the GOx-CNT anode on Toray paper

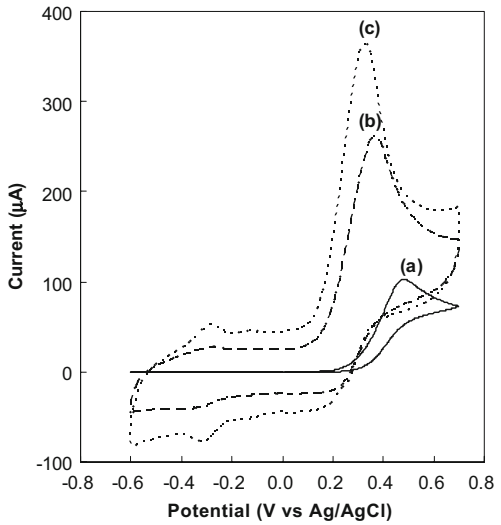

Fig. 5 Cyclic voltammetry at GOx anodes in deaerated PBS containing 25 mM glucose and 1 mM hydroquinone. A mixture of glucose oxidase and polyethyleneimine was coated on (*a*) a glassy carbon electrode, (*b*) a Toray paper on a glassy carbon electrode, or (*c*) a carbon nanotube-deposited Toray paper on a glassy carbon electrode, which was followed by duplicate casting using TBAB-modified Nafion suspension. Scan rate=20 mV/s

the electrode, not the active surface area, while the capacitive current depends on the active surface area.

As a comparison, Ref. [7] reported 1.4 mA/cm^2 at TP/CNT/GOx-PEI anode (using 0.16 mg GOx) in a solution containing 20 mM glucose at the scan rate of 50 mV/s. Similarly, we obtained 1.4 mA/cm^2 at TP/CNT/GOx-PEI anode (using 0.16 mg GOx) in 25 mM glucose at the scan rate of 20 mV/s. Both studies used HQ as mediator.

Anode—CNT grown on carbon cloth

We have investigated the effect of using CNTs on the GOx electrode performance. Two methods were used to incorporate multiscale carbon supports on the electrodes: (a) CNT in the casting formulation and (b) CNTs grown on TP. Cyclic voltammograms of the GOx anodes in deaerated PBS containing 25 mM glucose and 1 mM hydroquione are shown in Fig. 5. The positive potential for the oxidative peak is due to the hydroquinone redox potential (of 0.479 V vs. Ag/AgCl) [20]. There was a significant increase of the capacitive current (at 0 V) from 0.1 μA at the GC/GOx-PEI anode (denoted as (a) in Fig. 5) to 25 μA at the GC/TP/GOx-PEI anode (denoted as (b) in Fig. 5). The anodic peak current corresponding to glucose oxidation at the GC/TP/GOx-PEI anode was more than twice the current obtained with GC/GOx-PEI anode. At the GC/CNT-TP/GOx-PEI anode (denoted as (c) in Fig. 5), both the capacitive current and the glucose oxidation current were higher than at the GC/TP/GOx-PEI anode due to the increase of the active surface provided by the carbon nanotubes deposited on the Toray paper. Lastly, there was not much difference between the current at the anode containing CNTs grown on the Toray paper and that at the anode that has CNT in the casting formulation. Since the enzyme loadings of all anodes were the same, the improved anodic current observed with the Toray paper electrode containing carbon nanotubes, either deposited or incorporated in the casting formulation, can be attributed to the higher surface area. The higher surface area substrate may provide a thinner layer of the enzyme, CNT, and polymer composite layer being distributed over the substrate, which results in an improved exposure of the enzyme into the electrolyte. The large-scale microporosity within the multiscale carbon material allows diffusion of reactants (glucose for the anode and oxygen for the cathode) throughout the electrode architecture, and at the same time, the carbon nanotubes provide a high surface area support that allows efficient electron transfer from the enzyme and mediator to the carbon electrode.

Based on the higher currents obtained, Toray paper on a glassy carbon electrode was chosen as the anode substrate on which GOx-CNT-PEI mixture was coated.

Anode—immobilized mediator

Since glucose oxidation could not be achieved on the anode by direct electron transfer between the enzyme and the electrode, a redox mediator was required. In an implantable biofuel cell, it is essential to immobilize any required mediators on the electrode, so the mediators will not leach out into body fluids. Heller has reported extensive work on wired enzymatic electrodes for miniature biofuel cells using osmium-based mediators [5, 11, 21] on Toray paper (no CNT). The in-house-prepared osmium-based mediator for the anode, PVI-$[Os(da\text{-}bpy)_2Cl]^{+/2+}$-*N*-2-amino-ethyl, was immobilized on (a) a glassy carbon electrode, (b) a plasma-treated Toray paper disk glued to a glassy carbon electrode,

and (c) CNT grown on a Toray paper disk. The GOx anodes on which the Os-based mediator was immobilized were tested in deaerated PBS in the absence and in the presence of 25 mM glucose (Figs. 6 and 7). CV of the GOx anode with immobilized mediator (PVI-[Os(da-bpy)$_2$Cl]$^{+/2+}$-*N*-2-amino-ethyl) in deaerated PBS without glucose had two redox peaks: one (around −0.4 V) for FAD group of GOx and the other (around −0.2 V) for the Os-based mediator. The anodic current increased in the presence of 25 mM glucose. When the same amount of enzyme-mediator mixture was cured on the Toray papers, the glucose oxidation current increased significantly (from about 2 μA at a glassy carbon electrode to about 20 μA at a Toray paper electrode, where the glucose oxidation current was estimated by subtracting the capacitive current at 0.2 V). Using CNT grown on Toray paper was even better than the plain Toray paper in terms of redox peak currents for FAD group, redox peak currents for the immobilized mediator, and glucose oxidation current. Unlike the GOx anode with free floating mediators, GOx anode with immobilized mediator had the best performance on CNT grown Toray paper substrate (vs. CNT as part of the electrode casting formulation). This is probably because glucose oxidation at GOx anode with free floating mediator is mass-transfer-controlled, while glucose oxidation at GOx anode with immobilized mediator depends on the active surface area.

Cathode—bilirubin oxidase

In most biofuel cells, laccase enzyme has been used as the cathode. However, their performance and stability requires an acidic pH [22], and it is therefore not compatible with blood pH. On the other hand, BOx is stable and active in blood pH [23]. BOx cathode was prepared according to a protocol similar to the one used to prepare the GOx-CNT anode. As in the case of the GOx-CNT anode, it is necessary to use a mediator for the electron transfer between the electrode and

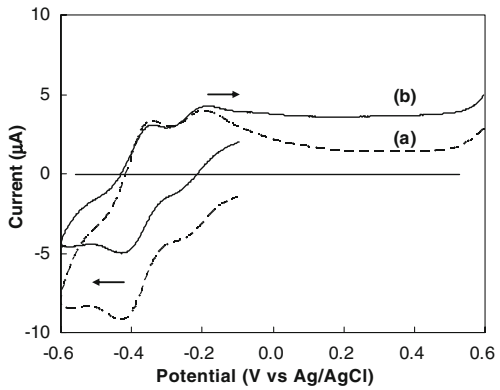

Fig. 6 Cyclic voltammetry of GOx electrode containing immobilized Os-based mediator on a glassy carbon in deaerated PBS containing (*a*) 0 mM or (*b*) 25 mM glucose. Scan rate=20 mV/s

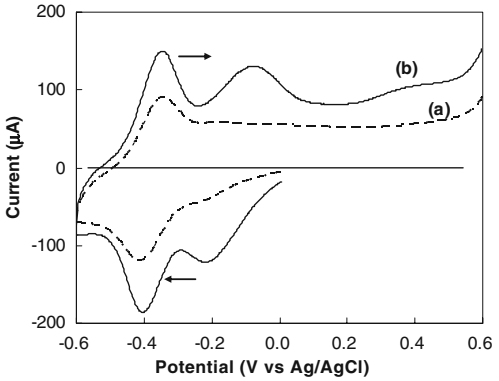

Fig. 7 Cyclic voltammetry of GOx electrode containing immobilized Os-based mediator on (*a*) a plain Toray paper or (*b*) CNT grown Toray paper in deaerated PBS containing 25 mM glucose. Scan rate=20 mV/s

the reduction reaction at the enzyme [24]. We could not get any evidence of direct electron transfer under the conditions tested here, although direct electron transfer between BOx and modified spectrographic graphite electrode has been reported [25]. Three available mediators (HQ, ferrocenecarboxylic acid, and ferricyanide) with redox potentials close to that of BOx were tested in oxygenated PBS containing one of the solution-phase mediator (1 mM in pH 6.9 buffer). Among the three solution-phase mediators, ferricyanide was the best mediator in terms of cathodic current, while the redox potential of HQ was more anodic than other two mediators. Based on the higher current, ferricyanide was used as the mediator for further BOx-CNT cathode characterization. Similar to the oxidation of glucose on the GOx-CNT anode, oxygen reduction at BOx-CNT cathode was significantly increased in the presence of a mediator.

Cathode—BOx on Toray paper

BOx electrodes prepared on a glassy carbon and Toray paper were examined using LP technique in oxygenated PBS containing 1 mM potassium ferricyanide (Fig. 8).

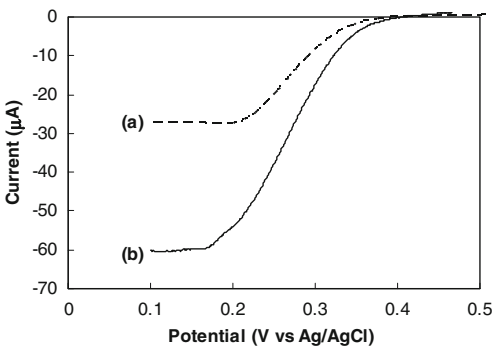

Fig. 8 Linear polarization curves of BOx-CNT cathodes on (*a*) glassy carbon or (*b*) Toray paper substrates in oxygenated PBS in the presence of 1 mM $K_3Fe(CN)_6$ at a scan rate of 1 mV/s

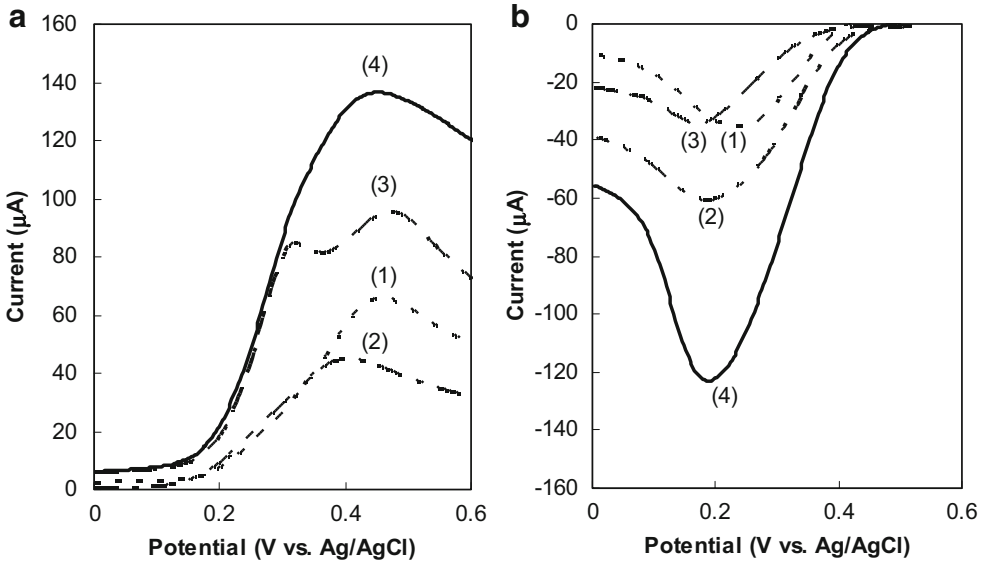

Fig. 9 Effect of biocompatible polymers as casting material: (*1*) Nafion, (*2*) chitosan, (*3*) TBAB-treated Nafion, and (*4*) PEGDA on **a** the glucose oxidation at GOx-CNT anodes in deaerated PBS containing 1 mM HQ and on **b** the oxygen reduction at BOx-CNT cathodes in oxygenated PBS containing 1 mM $K_3Fe(CN)_6$. CV at a scan rate of 20 mV/s

Enzyme loadings were 0.16 mg for the BOx-CNT on glassy carbon and 0.48 mg for the BOx-CNT on Toray paper substrate. The oxygen reduction current at the Toray paper was more than two times higher than at glassy carbon, which contained three times more BOx than the glassy carbon electrode. Thus, the oxidation currents observed in these systems seem to be proportional to the BOx loading.

Casting with biocompatible polymers

We demonstrate here that the stability of both anodic and cathodic enzyme-based electrodes is enhanced by the use of biocompatible polymers. Four biocompatible polymers suspensions (PEGDA, chitosan-based hydrogels, 5% Nafion suspension, and modified (TBAB-treated) Nafion) were tested as casting materials in both the GOx electrode and BOx electrode. PEGDA was the best casting material for both the anode and the cathode in terms of current (Fig. 9). The study of the capability of the casting polymer to increase the lifetime of the biofuel cell electrodes, including protection from poisoning by compounds present in blood such as transition metal ions and uric acid, is currently under progress.

Initial testing of biofuel cell using separated electrode compartments

An enzymatic biofuel cell was constructed using a glucose oxidase anode with immobilized mediator and bilirubin oxidase cathode coated with PEGDA. Since the electron transfer through the cathode was mediated by a solution-phase mediator, 1 mM $K_3Fe(CN)_6$, the cathodic compartment was separated from the anodic compartment by a salt bridge. The voltage–log(current) curves for each electrode in a physiological buffer (20 mM phosphate-buffered saline, pH 6.9) containing 8 mM glucose and about 0.2 mM oxygen are shown in Fig. 10. Additional work will be aimed at significantly improving the current density and cell potential by optimizing both the anode and cathode performance, including using an immobilized redox mediator within the cathode.

Conclusion

Enzymatic electrodes were investigated that could further the goals of developing a high power density enzymatic biofuel cell that operates on glucose and dissolved oxygen in blood and provides stable long-term operation. This paper introduces biofuel cell anode and cathode designs based on mediated glucose oxidation by GOx and oxygen reduction by BOx, respectively. Direct electron transfer

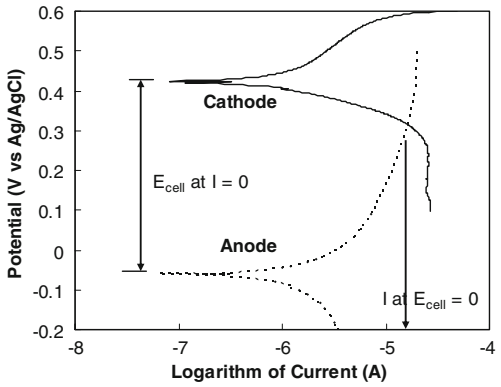

Fig. 10 Linear polarization curves of anode and cathode in aerated PBS (20 mM, pH 6.9) at a scan rate of 1 mV/s. The anode was GOx on CNT grown on a carbon cloth with an immobilized Os-based mediator, and the cathode was BOx-CNT-casted with PEGDA and with a free floating mediator, 1 mM $K_3Fe(CN)_6$

through incorporation of carbon nanotubes was negligible compared to mediated electron transfer under testing conditions. The GOx loading on the anode was optimized. An anode redox polymer, PVI-$[Os(da\text{-}bpy)_2Cl]^{+/2+}$-*N*-2-amino-ethyl, was successfully synthesized and immobilized in a GOx anode. It was demonstrated that the use of Toray paper substrate enhances the performance of both the anode and cathode electrodes. In addition, the performance of the electrodes is substantially enhanced by the use carbon nanotubes on the electrodes. When using immobilized mediator, the electrode containing the CNT grown Toray paper substrate showed better performance that the electrode containing CNT as part of the electrode casting formulation. It was also shown that the use of PEI in the electrode casting formulation resulted in higher currents and improved stability. We have also demonstrated that casting of biocompatible polymers onto the electrode further enhances the electrode performance, with PEGDA showing the best performance for both the anode and the cathode in terms of increased current. A biofuel cell consisting of GOx anode with an immobilized mediator and a separate electrode compartment containing a BOx cathode with a solution-phase mediator in a physiological buffer and containing physiological levels of glucose and oxygen was successfully fabricated and tested.

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