Surface Engineering of Graphene-Enzyme Nanocomposites for Miniaturized Biofuel Cell

Chang Liu, Zhongfang Chen, and Chen-Zhong Li

***Abstract*—A novel approach to the surface functionalization for membraneless enzymatic glucose/oxygen biofuel cell applications is described. The biofuel cell employs the gold plate electrodes modified by specific graphene-enzyme conjugations, which are immobilized by electrochemical deposition of the conducting polypyrrole polymer. The electrochemical activity of these electrodes is superior to the electrodes immobilized with sol–gel. Such enhancements can be attributed to the excellent electrical property and enzyme loading capability of the polypyrrole material. The power output and the biostability of the integrated biofuel cell are also improved.**

***Index Terms*—Biofuel cell, enzymatic fuel cell, graphene nanosheets, polypyrrole, surface engineering.**

I. Introduction

BIOFUEL cells are electrochemical power generators that are able to convert chemical energy into electrical energy through redox reactions.

In recent years, the development of enzymatic biofuel cells (EBFC) is likely to have a significant impact on homeland security, aerospace, and healthcare industries as the EBFCs can produce higher power output than other types of biofuel cells [1]–[4]. The output power of such EBFCs is well sufficient to supply some microscale electronic systems, such as cameras for remote surveillance, transmitters, actuators, or even wireless sensor networks, which paves the path for the U.S. Army's goal to eliminate all army military batteries or at least reduce the frequency of replacing batteries, thus to realize integrated soldier sensor suites as envisioned in the warfighter concept. Considerable efforts of researchers are also given to explore the potential of EBFCs for long-term space mission applications [5]. Current technologies, including solar and nuclear energies are either excessively expensive or dangerous, which motivates scientists to develop an alternative portable energy source. However, due to the poor stability caused by enzyme denaturation, the EBFCs have not been successful in practical applications. Currently, how to improve the stability and to fabricate practical EBFC devices out of theoretical concepts [6]–[10] is a key challenge. Among others, covalent binding and physical entrapment are the two main strategies for stability enhancement.

In this paper, we demonstrate a simple, practical EBFC system based on electropolymerized pyrrole immobilized enzyme/graphene sheets composite electrodes. Among different enzyme immobilization methods studied so far, encapsulating enzymes in polypyrrole film offers a facile strategy for the fabrication of EBFCs, since the process only includes the application of a fixed potential between the working electrode and the reference electrode in a solution containing the pyrrole monomer and enzymes. This technique is especially attractive for the enzymatic functionalization of EBFC electrodes, because we can estimate the amount of the immobilized enzyme and the thickness of the growing polymer film easily by measuring the charge passed through the electrode. Moreover, earlier studies on the microstructure and the conductivity of polypyrrole [11] showed that polypyrrole is a porous polymer with high conductivity and surface-to-volume ratio. These properties inspired us to examine the performance of polypyrrole as a diffusion and electron transfer medium and also as a protection of enzymes.

Besides the physical encapsulation of polypyrrole, graphene nanosheet material has also been employed to construct covalent linkage between the enzymes and the electrodes. As a novel carbon allotrope, graphene possesses a very large surface area, which is about 2630 $m^2 \cdot g^{-1}$ [12]. The electrons on the graphene surface move ballistically over the sheet without any collisions with mobilities as high as 10 000 $cm^2 \cdot V^{-1} \cdot s^{-1}$ at room temperature [13]–[16]. Recently, we demonstrated that graphene exhibits a larger I_D/I_G ratio relative to single-walled carbon nanotube, which is an indication of greater sp^2 character [17]. Note that though with high purity, the chemically coverted graphene (from graphene oxide) used in our experiments possesses a number of surface active functional moieties, such as carboxylic and ketonic groups, which are reactive and can easily bind covalently with enzymes [18]. The presence of C=C conjugation in graphene is also expected to boost the electron transfer rate, which significantly enhances the power output of the EBFCs [19]. More recently, we employed graphene and sol–gel, another common immobilization method for biofuel cell assembly. However, the power output and the stability were unsatisfied for future security and space applications [20].

In this paper, we report a novel strategy of surface functionalization and immobilization for EBFC applications using

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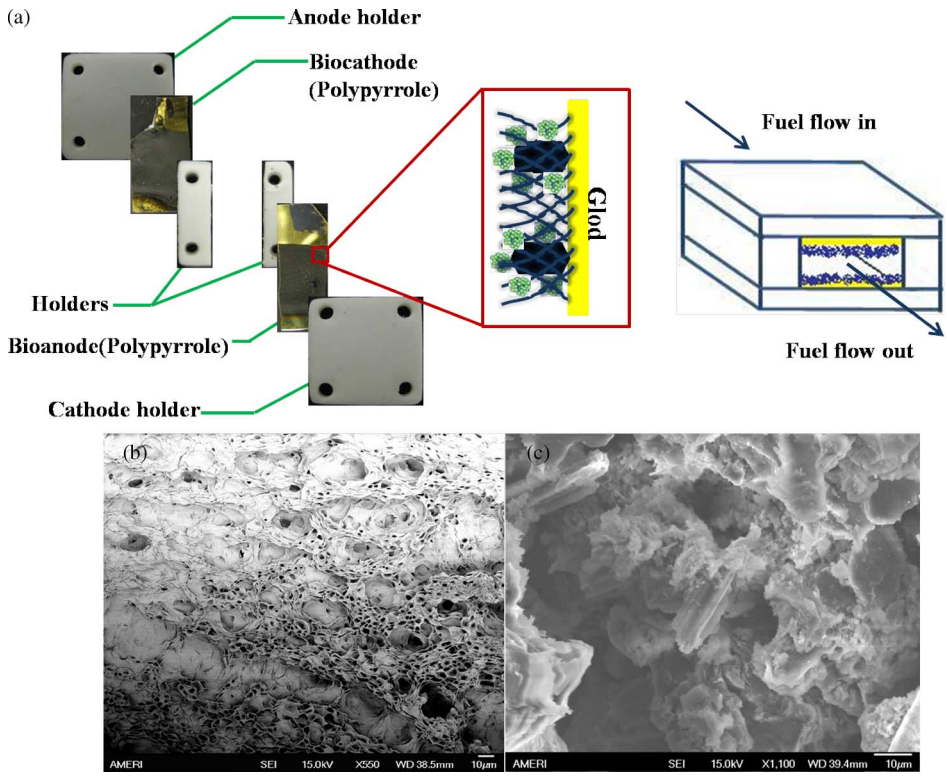

Fig. 1. (a) Polypyrrole-based electrodes microstructure and EBFC assembly diagrams. (b) SEM image of the porous structure of the polypyrrole thin film. (c) SEM image of the porous structure of silica sol–gel matrices.

electropolymerized polypyrrole thin film, and demonstrate the advantages of the electropolymerized pyrrole immobilized enzyme/graphene sheet composite electrodes for EBFC application.

II. Experimental

We first fabricated the bioelectrode by coimmobilization of enzyme and graphene using electrogenerated polypyrrole film. The bioanode was prepared as follows: 10 mg of chemically coverted graphene was mixed with 1 mL of dimethylformamide (DMF) and ultrasonicated for 10 h to yield a uniform suspension, then 50 μL of this solution was placed on a gold plate electrode surface (0.5 cm $\times$ 2 cm) and dried under IR lamp for 5 h. Afterward, 100 μL of 8 mM ferrocenemethanol (FM) phosphate buffered saline (PBS, pH 7.4) solution containing 2.5 mg glucose oxidase (E. C. 1.1.3.4, from *Aspergillus niger*; GOD) was deposited on the graphene modified electrode. After incubated for 10 h at 4 °C, the electrode was then dipped into 0.02 M pyrrole monomer and 0.1 M $NaClO_4$ PBS solution right before the electropolymerization process.

Electropolymerization and electrochemical measurement were carried out using a CHI-630 A electrochemical analyzer (CH Instruments, Inc.) with a conventional three electrode setup consisting of a modified gold electrode as the working electrode, an Ag|AgCl reference electrode (3 M KCl) and a platinum counter electrode. Polypyrrole thin layer was deposited at +0.85 V versus Ag|AgCl after 4 C electrical charge (q) passed. The film thickness (s) was estimated to be approximately 2.8 μm by Faraday's law: $s = qM/\rho AzF$, where M is the molar mass of the polymer, ρ is the density of the polymer, z is the number of electrons involved, and F is the Faraday constant. 1.5 g·cm^{-3} was used as the nominal density of the polypyrrole films (ρ) and an electron loss z of 2.25 was considered [21]. Finally, the polypyrrole immobilized graphene-enzyme composite electrode was stored in 1$\times$ PBS (pH 7.4) solution at 4 °C before test.

Similarly, the biocathode was fabricated using the same strategy except the enzyme used was 2.5 mg bilirubin oxidase (E. C. 1.3.3.5, from *Myrothecium verrucaria*; BOD) and the redox mediator was 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) [see Fig. 1(a)].

III. Results and Discussions

A. Surface Characterization of Polypyrrole and Sol–Gel

A field emission SEM JEOL JSM6335 (model) was used to observe the surface morphology of the graphene-enzyme composite electrode. The porous surface structure of the polypyrrole thin film deposited on the graphene modified electrode surface [22] was evidenced by the SEM image [see Fig. 1(b)]. Clearly, the polypyrrole thin film possesses a great density of pores with sizes ranging from 5 to 50 nm. In comparison, the sol–gel matrices employed for EBFC enzyme entrapment in our previous work [20] has much lower average pore density [see Fig. 1(c)]. The highly porous structure enables the polypyrrole material to retain a longer enzyme life time, while providing

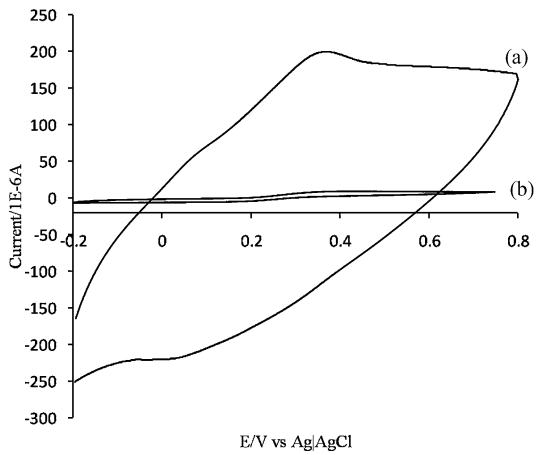

Fig. 2. Cyclic voltammogram of (a) polypyrrole-based bioanode and (b) sol–gel-based bioanode in 100 mM glucose PBS solution (scan rate: 500 mV·s^{-1}).

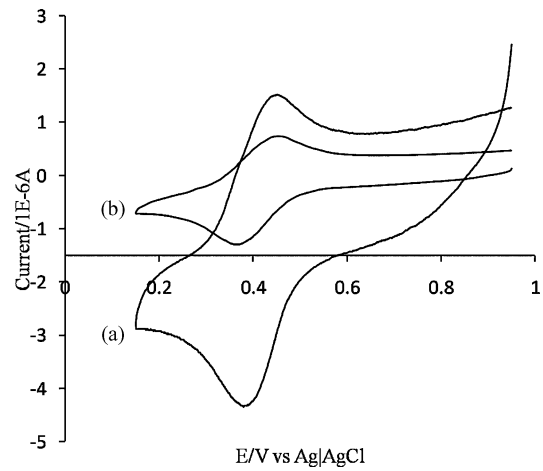

Fig. 3. Cyclic voltammogram of (a) polypyrrole-based biocathode and (b) sol–gel-based biocathode in air saturated PBS solution (scan rate: 1 mV·s^{-1}).

a more sufficient diffusion path for the fuel than its sol–gel counterpart.

B. Electrochemical Characterization of Polypyrrole and Sol–Gel-Based Enzymatic Electrodes

To evaluate the electron transfer properties of the polypyrrole immobilized graphene-enzyme composites electrodes, we initially performed the cyclic voltammetry of 100 mM glucose in 1× PBS (pH 7.4) at the graphene-GOD bioanodes immobilized by polypyrrole under room temperature. A set of control experiments was also conducted using sol–gel immobilized graphene-GOD bioanodes. Fig. 2(a) and (b) represents the cyclic voltammograms obtained at polypyrrole bioanode and sol–gel bioanode, respectively. Polypyrrole bioanodes exhibit a redox peak with a peak current density of about 15 ± 1.2 μA·cm^{-2} ($N = 4$ electrodes). However, sol–gel bioanodes show a redox peak with a peak current density of about 7.1 ± 0.5 μA·cm^{-2} ($N = 4$ electrodes). By comparing the magnitude of the polypyrrole and sol–gel electrodes current density, polypyrrole electrodes present a greater redox current. This increase of the redox current can be attributed to the massively porous surface structure and greater conductivity of the polypyrrole material [23].

To probe the performance of the polypyrrole immobilized graphene-BOD biocathodes, we then measured cyclic voltammetry of air saturated PBS solution (pH 7.4) at the polypyrrole and sol–gel biocathodes under room temperature [see Fig. 3(a) and (b)]. The polypyrrole biocathodes have a redox peak with a peak current density of about 3 ± 0.2 μA·cm^{-2} ($N = 4$ electrodes), while the sol–gel biocathodes show a redox peak with a peak current density of about 1 ± 0.1 μA·cm^{-2} ($N = 4$ electrodes). Such observation again confirmed the excellent electron transfer property and diffusion property of the polypyrrole thin film.

C. Comparison of Power Output and Stability of Polypyrrole and Sol–Gel-Based EBFCs

We also fabricated practical EBFC devices using the aforementioned bioanodes and biocathodes.

Both the bioanode and biocathode were glued on two self-designed Teflon holders, which were then clamped together and separated using spacers in between them [see Fig. 1(a)]. Afterward, the assembly was placed inside 100 mM air saturated glucose solution (fuel) taken in a 25 mL beaker for power output testing. Measurements were obtained by applying varying external loads (500 Ω–500 kΩ) across the anode and cathode.

As shown in Fig. 4(a), the open-circuit potential ($V_{\rm oc}$) and the maximum current density of the polypyrrole-based EBFCs were 0.79 ± 0.1 V ($N = 3$ EBFCs) and 434 ± 28 μA ($N = 3$ EBFCs), respectively. For comparison, the sol–gel-based EBFCs were tested under the same condition. The $V_{\rm oc}$ and the maximum current density were 0.58 ± 0.05 V ($N = 3$ EBFCs) and 156.6 ± 15 μA ($N = 3$ EBFCs), respectively. In addition to the aforementioned excellent conductivity of polypyrrole, the observed 0.21 V increase in the $V_{\rm oc}$ of the polypyrrole-based EBFC related to the sol–gel counterpart can also be attributed to the greater amount of enzyme immobilized on the same surface area due to the special electropolymerization process. In this process, the enzyme was immobilized during the growth of the polypyrrole film, instead of a 2-D enzyme layer on the electrode surface, a 3-D matrix, which possesses a greater amount of enzyme was formed in the porous structure of polypyrrole.

Furthermore, the power output densities were calculated by $P = U \times I$, where P is the power output, U is the potential between the anode and the cathode, and I is the current output. The polypyrrole-based EBFC exhibits a higher magnitude of the maximum power output density [78.3 ± 7 μW ($N = 3$) at 0.5 V (load 15 kΩ), Fig. 4(b)], while the power output density of the sol–gel-based EBFC is much lower [24.3 ± 4 μW ($N = 3$) at 0.38 V (load 15 kΩ)].

We then evaluated the biostability of the polypyrrole EBFC. The measurement was taken every 24 h continuously with 15 kΩ external load. The life time is shown in the inset of Fig. 4. The power output of the polypyrrole EBFC dropped to 50% of its original power output after 28 days, whereas it only took 7 days for the sol–gel EBFC.

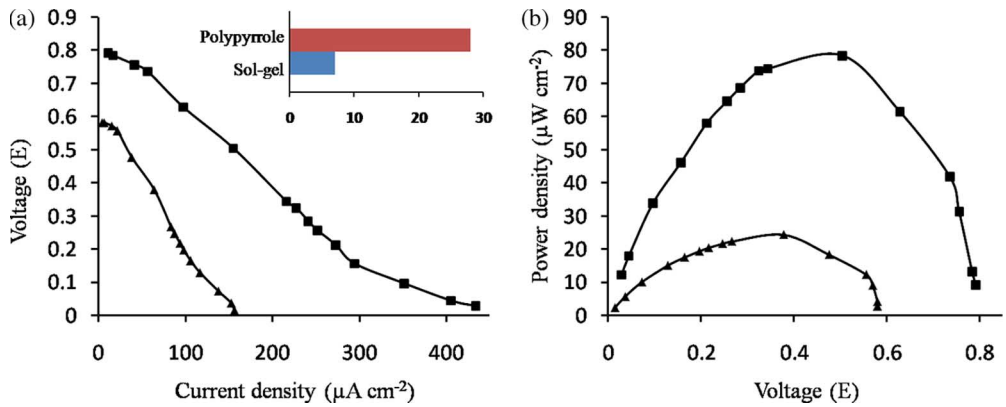

Fig. 4. (a) Current–voltage behaviors of (■) polypyrrole-based EBFC and (▲) sol–gel-based EBFC with different external loads in 100 mM glucose PBS solution. (b) Power densities at different cell voltage for (■) polypyrrole-based EBFC and (▲) sol–gel-based EBFC in 100 mM glucose PBS solution.

IV. Conclusion

In this paper, we developed a novel strategy of surface functionalization and immobilization for EBFC applications using electropolymerized polypyrrole thin film. Initially, the surface characteristics of polypyrrole were evaluated by SEM imaging, which indicated a highly porous structure of this material. Moreover, cyclic voltammetry results indicated polypyrrole-based electrodes possess better electron transfer and diffusion properties in comparison with sol–gel immobilization-based electrodes. Finally, we also measured the power output and life time of the assembled EBFCs. In all experiments, polypyrrole exhibited a better surface immobilization performance than the sol–gel. The observed marked performance of the polypyrrole electrode is mainly due to the porous surface structure and excellent conductivity of the polypyrrole material. Further work involving the EBFC packaging using low-temperature cofired ceramic (LTCC) for *in vivo* application will be conducted.

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