



Fabrication of biofuel cell containing enzyme catalyst immobilized by layer-by-layer method



Kyu Hwan Hyun ^{a,1}, Sang Won Han ^{b,1}, Won-Gun Koh ^{b,*}, Yongchai Kwon ^{a,*}

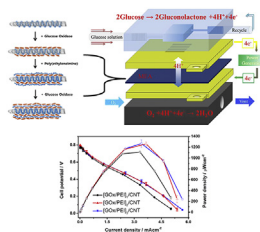
^a Graduate School of Energy and Environment, Seoul National University of Science and Technology, 232 Gongneung-ro, Nowon-gu, Seoul, 139-743, Republic of Korea

^b Department of Chemical and Biomolecular Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, Republic of Korea

HIGHLIGHTS

- We proposes a [GOx/PEI].
- Layer-by-layer method is used for formation of multiple layers of GOx/PEI on CNT.
- [GOx/PEI].
- Oxygen plays mediator role in transferring electrons and protons to FADs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 30 January 2015

Received in revised form

7 March 2015

Accepted 22 March 2015

Available online 24 March 2015

Keywords:

Enzymatic biofuel cell
Layer-by-layer structure
Poly ethylene imine
Carbon nanotube
Glucose oxidase

ABSTRACT

Enzymatic biofuel cell (EBC) employing a layer-by-layer (LbL) structure consisting of multiple layers of glucose oxidase (GOx) and poly(ethyleneimine) (PEI) at carbon nanotube (CNT) ([GOx/PEI]_n/CNT) is fabricated. The [GOx/PEI]_n/CNT serves as anode catalyst for promoting glucose reaction, while Pt is employed as cathode catalyst. To evaluate effect of [GOx/PEI]_n/CNT on EBC performance and stability, several characterizations are conducted. The optimal GOx/PEI layer is determined electrochemically, and it turns out that [GOx/PEI]₂/CNT is the best. Electron transfer rate constant of the optimal layer is 11.3 s⁻¹, its glucose sensitivity is 83 μAmM⁻¹ cm⁻², and maximum power density of EBC adopting [GOx/PEI]₂/CNT is 1.34 mWcm⁻². The values are superior to those of other reference structures, indicating that the [GOx/PEI]₂/CNT can produce excellent reactivity, followed by improved EBC performance. In terms of redox reaction mechanism of flavin adenine dinucleotide (FAD) within [GOx/PEI]₂/CNT, glucose does not affect the redox reaction of FAD, while oxygen serves as mediator in transferring electrons and protons produced by glucose oxidation into those for reduction reaction of FAD. It is also found that the [GOx/PEI]₂/CNT is confined by surface reaction and the reaction is quasi-reversible. Regarding long-term stability, [GOx/PEI]₂/CNT maintains ~83% of initial activity even after two weeks.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

At present, there are considerable interests in developing enzymatic biofuel cells (EBCs) that utilize biofuels such as glucose, ethanol and sucrose for the production of electricity [1–5]. The EBCs are a special class of fuel cells in which biocatalysts are

employed in the replacement of noble metal catalysts. Compared to conventional fuel cells, EBCs offer advantages such as biocompatibility of enzymes, mild operating parameters, superior selectivity (due to enzyme specificity), affordability of various biofuels and cost savings compared with expensive noble metals [1–3].

Due to their unique merits, EBCs have been suggested for use in

* Corresponding authors.

E-mail addresses: wongun@yonsei.ac.kr (W.-G. Koh), kwony@seoultech.ac.kr (Y. Kwon).

¹ K Hyun and S Han contributed equally to this work and should be co-first authors.

communication devices, medical implants, remote sensors and low-power electrical devices [1–3,6–8]. However, in spite of such promising availabilities, the commercialization of devices related to EBCs has been retarded to date owing to partial oxidation of the biofuel, low power density and a lack of long-term stability, which are mainly caused by low enzyme loading, low enzymatic reaction efficiency, poor enzyme stability and slow charge transfer rate [1–3].

In a bid to improve electrical performance and lifetime, establishing the enzyme system that has high electron conductivity and long-lasting enzyme structure is critical [8]. To date, significant efforts have been made to increase the electron transfer rate and maintain the rigidity of the enzyme structure as long as possible [9–12]. However, finding solutions related to how electrical performance is enhanced is still a critical subject to address.

One of the successful strategies to establish high electrical performance and long-term stability in applications including enzyme catalysts is to use a layer-by-layer (LbL) structure [13,14]. The basic concept of this LbL method is to adsorb oppositely charged species alternately from their platform. It was initially suggested by Decher in 1997, and its applications have extended and continued to make progress [15]. In bioelectrodevices using enzymes as biocatalysts, the method has been considered plausible. As a result, many groups have done research on LbL structure and its applications. For examples, Komathi and Lee et al. [16] fabricated the LbL structure consisting of Au nanoparticles (AuNP), thiol-functionalized polyaniline and glucose oxidase (GOx) and used the structure as a biosensor for glucose detection with high sensitivity. Wu et al. and Cui et al. [17,18] used CNT, AuNP and insulating polymer as the LbL layer to fabricate the glucose sensor. Hu et al. [19] evaluated the catalytic activity of hemoglobin using LbL layers of hemoglobin and didodecyldimethylammonium.

However, despite increasing demands for the LbL structure, it is still rare to use the LbL structure as a catalyst for the EBC electrode. Additionally, most LbL layers have been fabricated on the plate-type substrate, although powder type LbLs sprayed on various substrates are more flexible and applicable. To overcome such difficulties, in this study, we fabricate an enzyme catalyst comprising multiple layers of GOx/poly(ethyleneimine) (PEI)/CNT ([GOx/PEI]_n/CNT) using the powder type LbLs and employing the [GOx/PEI]_n/CNT catalyst to the anode of EBC.

To investigate the effect of [GOx/PEI]_n/CNT on EBC performance and stability, several characterizations are conducted. Cyclic voltammetry (CV) curves are measured to determine (i) the optimal number of LbL structures of [GOx/PEI]_n/CNT, (ii) electron transfer rate constant, (iii) sensitivity to glucose and (iv) pH effect of glucose, while XPS measurement is carried out to inspect whether PEI and GOx are properly incorporated onto CNT. The LbL structure is observed using TEM and enzyme activity is verified using absorbance measurement. As for EBC, its polarization curves are measured to gain maximum power density (MPD), while EIS measurements are performed to evaluate charge transfer resistance (R_{ct}) of the corresponding layers. To our knowledge, there have been few investigations of the use of powder-type LbL structures in EBC so far, and thus, our measurements are helpful to establish protocol for the quantitative characterization of the electrochemical behavior of EBCs.

2. Experimental

2.1. Chemicals

Multiwall carbon nanotubes (MWCNT), with ~95% purity, were obtained from NanoLab (Brighton, MA). Polyethyleneimine (PEI) (50wt% solution in water, MW 750,000), glucose oxidase (GOx,

from *Aspergillus niger* type X–S, 100,000 unit/g solid), β -D-glucose, peroxidase (from Horseradish type 1, 113 units/mg solid) and o-dianisidine dihydrochloride were purchased from Sigma–Aldrich (Milwaukee, WI, USA).

2.2. Fabrication of [GOx/PEI]_n/CNT layer

The (GOx/PEI)_n/CNT layer was prepared by LbL deposition of positively charged PEI and negatively charged GOx on MWCNT. Initially, PEI was adsorbed onto CNT by adding 50 mg of MWCNTs into 10 mL of PEI solution (2.5 mg mL⁻¹ in water), and the mixture underwent sonication for 10 min and was stirred for 1 h. Excess PEI was removed by deionized water washing. The PEI-coated MWCNT was then immersed in a 2.0 mg mL⁻¹ GOx solution for 20 min. Alternate deposition of PEI and GOx was repeated until the target number of polymer bilayers (n) was achieved. The formation of multilayer structure was monitored by measuring ζ -potentials using an electrophoretic light scattering spectrophotometer (ELS-8000, Otsuka Electronics Co., Japan). After building up the desired number of layers, the amount of immobilized GOx was quantified by measuring the difference between the initial and final amount of GOx within the incubation solution using a BCA standard working agent (Pierce, Rockford, IL, USA). Fig. 1 represents schematic illustrations showing the fabrication of the (GOx/PEI)_n/CNT layer.

Regarding measurements of enzyme activity and stability in the LbL structure, detailed explanations are in the SI section.

2.3. Electrochemical characterizations of glucose oxidation reaction using LbL structure

Electrochemical measurements were performed using a computer connected potentiostat (CHI 720D, CH Instrument, USA). For the three-electrode cell measurements like CV, Pt wire was used as a counter electrode, and Ag/AgCl (soaked in 3.0 M KCl) was used for a reference electrode. To make a working electrode, catalytic powder was mixed with 1 mL of H₂O. After mixing, 10 μ m of well-mingled catalytic ink was dropped on glass carbon electrodes (GCE). After the loading of the catalytic ink, the working electrode was dried for 30 m at room temperature. In turn, 5 wt% Nafion solution was again dropped on catalytic ink-loaded GCE. 1.0 M phosphate buffer solution (PBS) was used as an electrolyte to promote the redox reaction, while high purity N₂ and air gases were provided to the electrolyte to create a specific atmosphere (O₂-free state, air-saturated state). All of the tests were performed at room temperature [20,21].

As for EBC and EIS measurements using the LbL structure, a schematic of the EBC cell is shown in Fig. S1 and detailed explanations for experimental procedures of the measurements are in the SI section.

3. Results and discussion

3.1. Chemical characterization of the LbL layer

The overall process for the preparation of the [GOx/PEI]_n/CNT layer is illustrated in Fig. 1. After PEI was coated onto CNT, the presence of the PEI layer was confirmed by XPS analysis. As shown in Fig. S2, the N 1s peak was almost undetectable in the XPS spectra of bare CNT, whereas high N 1s signals were observed in the spectra of PEI-wrapped CNT due to the existence of amines in PEI.

The formation of the GOx/PEI multilayer coated onto CNT was optically inspected by TEM. According to the TEM images of Fig. 2, [GOx/PEI]_n/CNT had a larger diameter (25 nm) than bare CNT (20 nm) because of the existence of the [GOx/PEI]_n layer. These

Download English Version:

<https://daneshyari.com/en/article/7731990>

Download Persian Version:

<https://daneshyari.com/article/7731990>

[Daneshyari.com](https://daneshyari.com)