ORDERED MOLECULAR ASSEMBLY INSIDE CARBON NANOTUBE FOREST FILMS FOR HIGH-EFFICIENCY ENZYMATIC BIOFUEL CELL

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ABSTRACT

Molecularly ordered composites of polyvinylimidazole-[Os(bipyridine) $_2$Cl] (PVI-[Os(bpy) $_2$Cl]) and glucose oxidase (GOD) are assembled inside a film of aligned carbon nanotubes. The structure of the prepared GOD/PVI-[Os(bpy) $_2$Cl]/CNT composite film is entirely uniform and stable; more than 90% bioelectrocatalytic activity could be maintained even after storage for 6 days. Owing to the ideal positional relationship achieved between enzyme, mediator and electrode, the prepared film shows a high bioelectrocatalytic activity for glucose oxidation (ca. 15 mA cm^{-2} at 25 °C) with an extremely high electron turnover rate (ca. 650 s^{-1}) comparable to the value for the GOD solutions, indicating almost every enzyme molecules entrapped within the ensemble (ca. 3 × 10^{12} enzymes in a 1× 1 mm^2 film) can work to the full. The free-standing, flexible composite film can be used by winding on a needle device, for example, a self-powered sugar monitor.

KEYWORDS

Biofuel cell, Carbon nanotube forest, Glucose oxidase, Redox polymer

INTRODUCTION

Controlling the electrical contact of redox enzymes with electrodes is a critical issue for enzymatic biodevices such as biofuel cells and biosensors. [1-5] The mutual positioning between enzyme molecules, mediator molecules (not always necessary), and electrode surface determines the efficiency, reproducibility, and stability of the bioelectrocatalysis systems.

We present herein an enzyme/mediator/electrode ordered ensemble that shows both “high turnover rate” and “large catalytic current”. In order to satisfy both of these requirements, the larger amount of enzymes than monolayer should be immobilized with keeping effective contact with electrodes. We realize such ideal condition by taking advantage of a film of well-aligned carbon nanotube forest (CNTF). [4,5] consisting of single-walled CNTs arrayed with a pitch of 16 nm. As illustrated in figure1, We have developed the stepwise process to construct molecular architecture with polyvinylimidazole-[Os(bipyridine)$_2$Cl] (PVI-[Os(bpy)$_2$Cl], MW: 15000) and GOD (EC:1.1.3.4, MW: 186 kDa).

EXPERIMENT

The CNTF film was first soaked in a stirred PVI-[Os(bpy)$_2$Cl] solution. The amount of PVI-[Os(bpy)$_2$Cl] in a CNTF film increased with the soaking time and these values are proportional to the CNTF film thickness, indicating that even the polymeric PVI-[Os(bpy)$_2$Cl] can entirely and uniformly adsorbed inside the CNTF films , as illustrated in Figure 1b. A part of the free imidazole groups of the mediator polymer would adsorb on CNT surfaces via π-π interaction.[6]

Subsequent loading of the GOD enzyme was conducted by immersing the PVI-[Os(bpy) $_2$Cl]-adsorbed CNTF Films in a stirred GOD solution. Figure 2a shows the CVs of GOD/PVI-[Os(bpy) $_2$Cl]/CNTF ensemble films. The

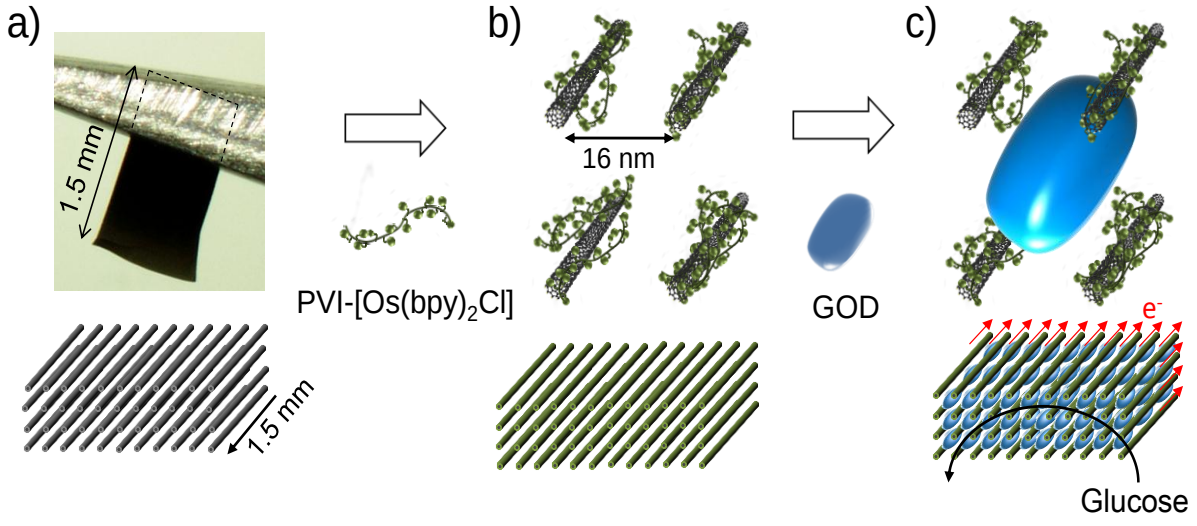

Figure 1. Photograph and schematic illustration of the CNTF-based glucose oxidation electrode prepared by stepwise modifications of PVI-[Os(bpy)$_2$Cl] and GOD inside a CNTF film.

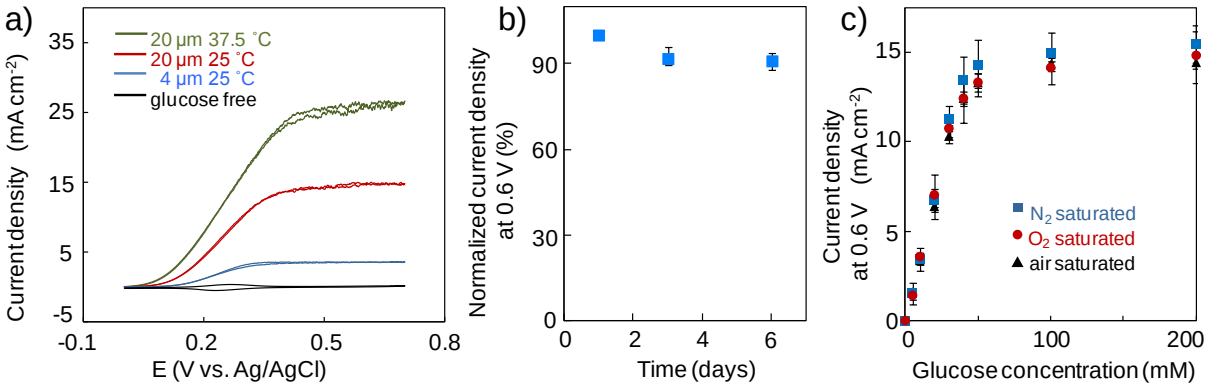

Figure 2 (a) Cyclic voltammograms of GOD/PVI-Os/CNTF ensemble films at 10 mV s^{-1} in stirred air-saturated 25˚C PBS containing 200 mM D-glucose. (b) The oxidation current densities at 0.6 V vs. Ag/AgCl for the GOD/PVI-[Os(bby)$_2$Cl]/CNTF film (20µm thickness) in a stirred 200 mM D-glucose PBS solution, periodically measured during 6 days of storage in PBS solution. (c) The oxidation current densities at 0.6 V vs. Ag/AgCl for the GOD/PVI-[Os(bby)$_2$Cl]/ CNTF film (20µm thickness) as a function of the glucose concentration.

catalytic current for glucose oxidation increased in response to the thickness of CNTF films, indicating that also GOD can entirely penetrate inside the PVI-[Os(bpy) $_2$Cl] -modified CNTF films. For example, the content of GOD incorporated in a 20µm-thick film was measured as ca. 0.86 µg by a C-6667 Protein Quantitation Kit. The current density was enhanced to as high as 26.7 mA cm^{-2} by turning up the buffer temperature to 37.5 °C. Importantly, more than 90 % of the electrode activity could be maintained even after 6 days storage in an air-saturated PBS solution (Figure 2b), proving the stability of bioelectrocatalytic architecture with the composite of PVI-[Os(bpy)$_2$Cl] polymer and GOD. The anionic GOD molecules could be stabilized by cationic Os complex of the mediator polymer anchored on the CNT surface via π-π interaction. [6]

The electron turnover rate for the 20 µm-thick film was ca. 650 s^{-1}, being comparable with that of GOD in bulk solution containing an electron acceptor of O2 (700 s-1) or ferrocene (600 s^{-1}) at 25 °C. [7] These results indicate that most of ca. 3 × 10^{12} GOD units within the film could efficiently work to the full, presumably owing to the molecularly ordered alignment of enzyme/mediator/electrode in the ensemble. Such a high efficiency of the present GOD electrode resulted in a resistance to oxygen inhibition, as shown in Figure 2c. The catalytic performance was almost identical in N_2-saturated, air-saturated and even O_2-saturated solutions. In general, glucose oxidation with GOD-modified electrodes is often disturbed by dissolved O_2, which is troublesome for glucose sensing. However, the ordered Os(bby)$_2$ groups in the present ensemble electrode could effectively accept the electron from GOD in preference to O_2, resulted in excellent O_2 resistance.

The present free-standing, bioelectrocatalytic film could be used for miniature biofuel cell devices. We demonstrate here the application of the film to a self-powered sugar indicator designed for inserting into a fruit. For indicating the glucose concentration, the net performance of the biofuel cell system should be controlled by the glucose anode. Because the oxygen in fruits is limited to a lower concentration than glucose, we employ a gas-diffusion biocathode [8] for utilizing the abundant oxygen in air outside of the fruits. As shown in Figure 3, a piece of GOD/PVI-[Os(bby)$_2$Cl]/CNTF film was wound on one lead of a light-emitting-diode (LED) device, whose blinking interval is inversely proportional to the power of the biofuel cell. [8] The other lead was connected to the BOD-based gas-diffusion cathode. The blinking interval of the LED upon inserting the device to a grape was coincident with that for the extracted juice, proving that this device could serve as a sugar indicator by simply being inserted into a grape. This principle of the self-powered sensor could be applied to more important blood sugar monitoring applications. We are planning to develop a GOD/PVI-[Os(bby)$_2$Cl]/CNTF -based device structure suitable for low-invasive insertion into a blood vessel through skins.

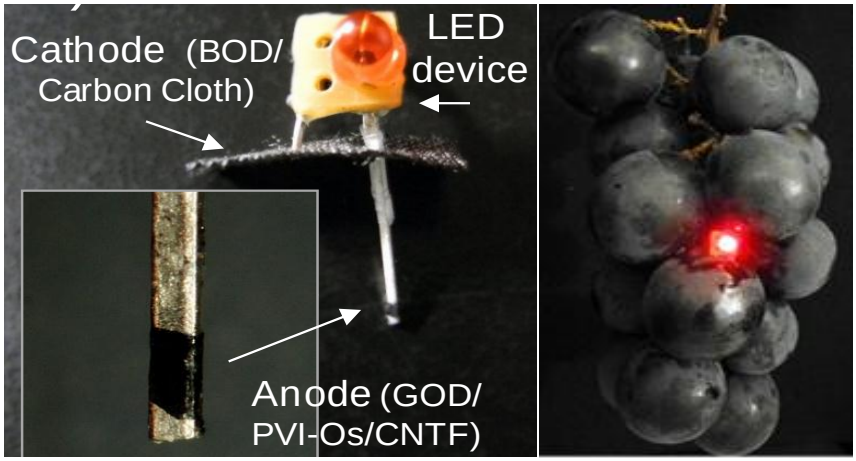

Figure 3 (Left) Photograph of the LED-based self-powered sugar indicator, at the tip of which the GOD/PVI-[Os(bby$_2$Cl]/CNTF film was wound. (Right) The device assembly was inserted in a grape to measure the LED blinking interval that correlates with the glucose concentration.

In concrusion, the larger amount of enzyme units than monolayer were successfully immobilized with keeping the effective electrical contact with the electrode (CNTs), as summarized in Figure 4. In particular, we have succeeded in forming the entirely uniform bioelectrocatalytic architecture with PVI-[Os(bby)$_2$Cl] and GOD inside a CNTF film. Owing to such ordered positional relationship between GOD, PVI-[Os(bby)$_2$Cl] and CNT, the composite film showed both "high activity" for glucose oxidation (ca. 15 mA cm^{-2}) and "high turnover rate" (ca. 650 s^{-1}), indicating almost every enzyme molecules within the film could work in the full .

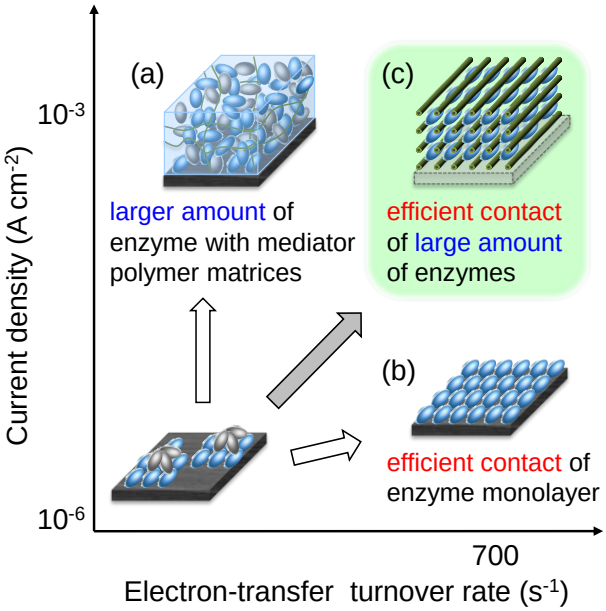

Figure 4. Scheme showing correlative characters of (a) an enzyme-film with mediator polymer matrices for larger current, (b) an enzyme-monolayer electrode for higher turnover rate, and (c) the present ensemble electrode for both large current and high turnover rate.

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