酵素修飾ナノチューブ電極を用いた発電デバイスに関する研究

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論文内容要約

Enzymatic fuel cells (EFCs) are power devices in which enzymes are used as electrocatalysts to directly conv biochemical energy into electricity, in contrast to metallic catalysts commonly used for conventional fuel cells. The extremely hi reaction selectivity of enzymes eliminates the need for fuel purification and allows a separator-free design that consists of just a pair anode and cathode electrodes exposed to solutions containing both fuel and oxygen; thus EFCs have attracted much attention extremely safe and miniature power sources for wearable or implantable medical devices such as a health monitoring system, a dr delivery system and artificial organs. However, due to low power output (mW cm^{-2} range) and short lifetime (a few days), EFCs we thought insufficient for practical applications. In order to overcome these drawbacks, in this thesis, the author describes no techniques, e.g., nanoengineering of enzyme electrode using well-aligned carbon nanotube film. Summaries of each chapter a provided below.

Chapter 1. Introduction

In this chapter, the background and purpose of this thesis are described. The principle of an enzymatic biofuel cell, the property of components, the trends in preparation technique for enzyme electrodes are described in detail. In addition, the trends and problems of enzyme electrodes fabricated by nano-materials are reviewed.

Chapter 2. Composite of direct electron transfer-type enzyme and carbon nanotube films

A variety of nanoengineered carbon electrodes for biofuel cells have recently been developed in rapid succession. For example, the use of the carbon nanotube (CNT) or the carbon nano particles for electrode preparation resulted in a higher-power biofuel cells. However, because they are random aggregates of nano-carbons, the post-modification of enzymes to their intra-nanospace is generally hard to control. If the nanostructure of the electrode can be regulated in response to the enzyme to be immobilized, then the resultant enzymatic ensemble would avoid the difficulty in postmodification of enzymes. In this chapter, a fabrication of biofuel cell using film of carbon nanotube forest (CNTF) is described. When liquids are introduced into the as-grown CNTF (CNTs with a pitch of 16 nm) and dried, the CNTF shrinks to a close-packed structure (CNTs with a pitch of 3.7 nm) because

of the surface tension of the liquids. By using an enzyme solution as the liquid, the CNTF is expected to dynamically entrap the enzymes during the shrinkage.

Firstly, the entrapment of enzymes into CNTF through liquid-induced shrinkage is described. The in situ monitoring of electrocatalytic activity of the CNTF films on soaking in a buffer containing FDH with d-fructose showed currents corresponding to their thickness, indicating that the FDH molecule can entirely penetrate inside the CNTF films that have suitable intra-structure and size for enzyme penetration. On the other hands, the catalytic activity of the conventional random CNT electrode was a tithe of the CNTF.

Controlling the content of FDH inside a CNTF could be achieved by changing concentration of the FDH solution. Such controlled entrapment of enzymes could be also examined via the degree of CNTF shrinkage. The degree of shrinkage was found to depend on the size and content of the enzymes. These results are proof of the in situ regulation of intra-nanospace of CNTF by the amount and size of entrapped enzymes.

The electrode performance was found to depend on the content of FDH. The best performance was reproducibly obtained from the FDH-entrapped CNTF electrode prepared from 3 mg mL^{-1} FDH solution, which contains ca. 1.5 μg FDH. This value of FDH content can be modeled as a linear arrangement of FDH molecules trapped between the CNTs. The current density of FDH-entrapped CNTF for fructose oxidation reached 16 mA cm^{-2} that is one of the highest values in FDH electrodes. The preparation of a laccase (LAC)-entrapped CNTF cathode for O_2 reduction is also described. The LAC-entrapped CNTF was archived by methodology of in situ regulation of intrananospace of CNTF. The electrode film showed O_2 reduction current density of ~2 mA cm^{-2}. A further increase in current density to ~4 mA cm^{-2} was achieved by overlapping two pieces of CNTF by taking advantage of their free-standing character.

The performance of the biofuel cell constructed with the cathode of LAC-entrapped CNTF and the anode of FDH-entrapped CNTF are described. The biofuel cell performed a power density of 1.8 mW cm^{-2} (at 0.45 V) in stirred conditions, 84% of which could be maintained after continuous operation for 24 h. Additionally, The prepared enzyme-entrapped CNTF film was the first free-standing, flexible enzyme electrode which colud be patched on a flexible substrates, or could be wound on the electric leads.

The proposed "in-situ regulation" is a straightforward approach to avoid the longstanding difficulty in the post modification of enzymes into conventional nanostructured electrodes.

Chapter 3. Composite of mediated electron transfer-type enzyme and carbon nanotube films

Electrical contact between a redox enzyme and an electrode is a critical issue for designing biosensors and biofuel cells. Especially, electrodes modified with glucose oxidase (GOD) have been actively studied, since glucose is important blood sugar and is

the most abundant fuel for ubiquitous power generation. This chapter describes an enzyme/mediator/electrode nano-ensemble that shows both "high turnover rate" and "large catalytic current". In order to satisfy both of these requirements, large amounts of enzymes should be immobilized, while keeping effective contact with electrodes. This ideal condition can be realized by taking advantage of a well-ordered carbon nanotube forest (CNTF) consisting of single-walled CNTs arrayed with a pitch of 16 nm. A stepwise process for the modification of polyvinylimidazole - [Os (bipyridine)$_2$ Cl] (PVI-[Os(bpy)$_2$Cl]) and subsequently GOD have been developed.

Firstly, the CNTF film was 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. A part of the free imidazole groups of the mediator polymer would adsorb on CNT surfaces via π-π interaction.

Subsequent loading of the GOD enzyme was conducted by immersing the PVI-[Os(bpy) $_2$Cl]-adsorbed CNTF films in a stirred GOD solution. The CVs of GOD/PVI-[Os(bpy)$_2$Cl] /CNTF ensemble films shows the 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. Importantly, the apparent electron turnover rate was ca. 650 s^{-1}, being comparable with that of GOD in bulk solution containing an electron acceptor of O_2 (700 s^{-1}). 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 controlled alignment of enzyme/mediator/electrode in the ensemble. Such a high efficiency of the present GOD electrode resulted in a resistance to oxygen inhibition, even under the lower glucose concentration (~ 1 mM).

Owing to the 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 electron-transfer turnover rate (ca. 650 s^{-1}), indicating almost every enzyme molecules within the film could work to the fullest extent.

Chapter 4. Fabrication of sheet-shaped biofuel cell

Enzyme-based biofuel cells have made rapid improvements in their power performance up to mW cm^{-2} levels by employing nanostructured carbon electrodes. However, the brittle carbon electrodes, which are generally aggregates of particulate or tubular nanocarbons, often limit the design and uses of such biofuel cells. In this chapter, a totally flexible, sheet-shaped biofuel cell was prepared by using a carbon fabric (CF) as the flexible, conductive base for the enzyme electrodes. The sheet-shaped biofuel cell was composed of bioanode CF strips modified with fructose dehydrogenase (FDH) for fructose oxidation, hydrogel sheets containing electrolyte and fuel (fructose), and biocathode CF strips modified with bilirubin oxidase (BOD) for O_2 reduction in the ambient air. Additionally, a CF strip was modified with Carbon nanotubes (CNT) to achieve high specific surface area that are contributed to effective enzyme immobilization.

The oxidation current density of a CF strip modified with CNT and FDH depended on the concentration of the Triton

X-100 surfactant used for the CNT dispersion. The CNT dispersion with 0.5% surfactant was capable of entirely penetrating into the CF strip. This uniform modification with CNT would be a reason for the enhanced anode performance. In addition, increasing the buffer concentration in the measurement solutions from 50 mM to 0.5 M drastically enhanced the electrode performance. This is made possible by the existence of adequate buffer capacities that prevent local pH changes caused by oxidation products. The maximum current of the optimized bioanode produced ~28 mA cm^{-2} in stirred 500 mM fructose solution.

The reduction current density of a CF strip modified with CNT and BOD reached ~4.6 mA cm^{-2}, by utilizing an oxygen supply from the ambient air through the CF, and by optimizing the buffer concentration. The FDH-modified CF strip and the BOD-modified CF strip were stacked with a double network gel film that retained an electrolyte solution and fuel (fructose) to construct a totally flexible sheet-shaped biofuel cell. This assembly allowed bending without affecting the maximum output power density, 969 μW cm^{-2} obtained at 0.36 V.

Chapter 5. Fabrication of needle-type biofuel cell

An enzyme-based biofuel cell has attracted much attention as portable and medical power supply. Among various advantages of such biofuel cell, high reaction selectivity of enzyme catalyst is quite unique because it enables to direct generation of electrical power from living organisms (e.g. animal, natural foods and raw fruits). However, natural organisms are covered by membrane. In addition, the cell operation in such organisms is subject to some limitation: e.g., low activity of the enzyme catalysts due to reaction inhibitors and limited mass flow of the biofluids.

This chapter describes the enzymatic biofuel cells designed for direct power generation from biofluids in living organisms such as animals, natural foods and raw fruits. Generally, natural organisms have a skin, and the oxygen concentration in the organisms is lower than that of biofuels like sugars. Therefore, I fabricated a new miniature assembly that consists of a needle bioanode for accessing biofuels in organisms through their skins, a gas-diffusion biocathode for utilizing the abundant oxygen in air, and an agarose hydrogel providing ion conductivity between the anode/cathode. The performance of the biocathode was fourfold improved by optimizing its hydrophobicity. The assembled device with four needle anodes for fructose oxidation was inserted into a raw grape, producing a maximum power of 26.5 μW (115 μWcm^{-2}) at 0.34 V. A light-emitting diode (LED) with the cell served as a self-powered indicator of the sugar level in the grape. Power generation from blood sugar was also investigated by inserting a needle anode for glucose oxidation into a blood vessel in a rabbit ear. Prior coating of to an anti-biofouling agent the tip of the needle anode was effective to stabilize the output power.

Chapter 6. Summary

This chapter summarized the results obtained in each chapter and mentioned the prospects of this research. The typical nano-carbon materials are generally hard to form an uniform composite with enzymes due to their own aggregation. Additionally, brittleness of carbon electrodes often limits the design and use of biofuel cells. In this thesis, a flexible and high performance biofuel

cell, which is constructed by carbon nanotube ensembles that have suitable spaces for enzyme immobilization, is developed. The sheet-type and needle-type biofuel cells, were also developed with carbon nanotubes and hydrogels, which would be suitable for generating electricity by placing the cell on surface or inside of living organisms.