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Miniaturized Enzyme-Based glucose / O_2 Fuel Cell Using Vitamin K3 as Electron Mediator

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Abstract

We would like to present our recent work on an enzyme-based biofuel cell, which generates electricity by coupling the anode for oxidation of glucose and the cathode for reduction of oxygen. The peculiar feature of our research is the use of diaphorase / dehydrogenase bi-enzyme system combined with the originally synthesized vitamin K3-based polymer as an electron mediator. MEMS techniques have miniaturized the cells as to be a microfluidic device.

Keywords: Biofuel Cell, Glucose Dehydrogenase, Diaphorase, Vitamin K3

1 Introduction

Fuel cells are the most promising power devices in respect to significant environmental benefits and high electrical efficiency, while most of commercial fuel cell consists of expensive materials such as noble metal catalysts and requires operating temperature over 70 ^{o}C [1]. For the power source of implanted medical devices and microrobots, the development of a cheap and safe fuel cell, which operate at physiological condition, have been expected. From this viewpoint, biofuel cells utilizing enzymes as electro-catalyst have been extensively investigated in recent years. Glucose is one of the most attractive fuel owing to its abundance and medical importance. A variety of enzyme / mediator systems have been currently studied [2-5]. For example, Heller *et al.* used glucose oxidase and Os-complex-linked polymer as mediator, and achieved excellent performance (a power density of 350 μW cm^{-2} at a 0.88 V cell potential) under physiological conditions [2, 3], while Os is a handful, most hazardous element.

We have studied the diaphorase (Dp) / dehydrogenase bi-enzyme system as the anode for glucose oxidation, as illustrated in Figure 1. Dp is a flavine-enzyme which converts NADH to NAD^{+}, coenzyme of a variety of dehydrogenase such as the glucose dehydrogenase (GDH). The electron transfer between the electrode an d Dp should be mediated by inexpensive and safe mediators for medical application, and quinine derivatives would meet these demands. Kano and Ikeda revealed that the reaction rate constant between diaphorase (Dp) and mediator is determined by the formal potential of the mediators, and that 3-methyl-1, 4-naphtoquinone (vitamin K3, VK3) is the most promising mediators to ensure the diffusion-limited maximum rate constant [6].

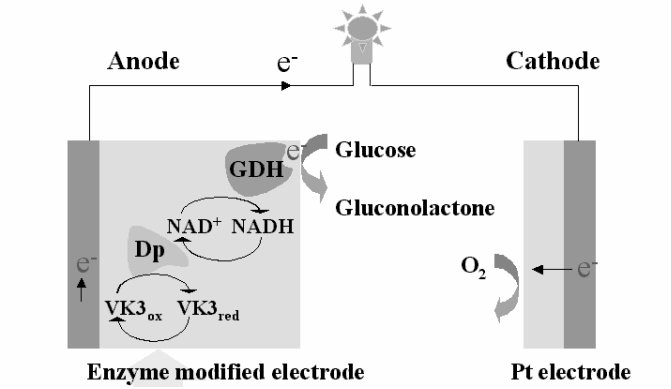

Figure 1. Schematic illustration showing the construction of Dp / GDH biofuel cell and the principle for generating electricity from biofuel (glucose in this case).

The elementary reactions are represented by eqs. (1)-(3).

$$\text{D-glucose} + NAD^{+} \xrightarrow[\text{GDH}]{} \text{D-gluconolactone} + H^{+} + \text{NADH} \quad (1)$$

$$\text{NADH} + \text{VK3(ox)} \xrightarrow[\text{Dp}]{} \text{NAD+} + \text{VK3(red)} \quad (2)$$

$$\text{VK3(red)} \rightarrow \text{VK3(ox)} + 2e^{-} \quad (3)$$

We will present herein the recent results on the anode performance of the Dp / GDH electrode combined with a VK3-pedanted polymers. The contents are as below;

1) Synthesis of a polymer of VK3 and its co-immobilization with Dp and GDH onto the anode surface

2) Characterization of an oxygen-selective cathode prepared by utilizing the gas-permeable property of PDMS thin film

3) Construction of the microfluidic biofuel cell by coupling the above functionalized electrodes

It should be noted however that the VK3 is known to be O_2-sensitive, which is a drawback for the use in fuel cell system. While the work is still preliminary, we found that the covering the anode electrode with Gox film shows the effect to eliminate dissolving oxygen from the anode. Such the layered structure containing the Gox top layer would be suit for the enzyme electrode with immobilized quinine mediators. These techniques enabled our biofuel cell to be a separator-free system.

2 Experimental

2.1 Chemicals and Materials

Polyallylamine-linked vitamin K3 (PAA-VK3) was newly synthesized by Daiichi Pure Chemicals Co., Ltd. Poly (ethylene-glycol) diglycidyl ether (PEGDGE; average molecular weight is 526) was purchased from Aldrich. Diaphorase (Dp) (from *Bacillus stearothermophilus*, [EC 1.6.99.-], 1090 U/mg) was from Unitika. Glucose dehydrogenase (GDH) (from *bacillus sp.*, [EC 1.1.1.47], 68 U/mg) and Glucose oxidase type-2 (GOD) (from *Aspergillus niger*, [EC 1.1.3.4], 47 U/mg) were from SIGMA. D-(+)-Glucose, potassium nitrate and sodium dihydrogen-phosphate were from Wako. $NADH{\cdot}Na_2$ was from Calzyme. These chemicals were used as received. 10 mM phosphate containing 100 mM KNO_3 (PBS) was used as a buffer solution.

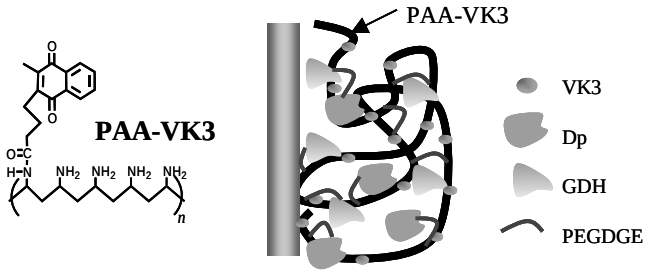

Figure 2. The molecular structure of PAA-VK3, and an illustrated structure of the composite film of Dp, GDH and PAA-VK3 cross-linked by PEGDGE.

2.2 Preparation of Electrodes and Microfluidic Cells

The anode is prepared by dropping PAA-VK3, PEGDGE, Dp and GDH mixture onto the electrode and dried in a desiccator at room temperature for typically 30 min. The epoxide groups of PEGDGE react with amino-groups of the polymer and enzymes and crosslink these elements to form hydrogel film (Figure 2). Anode evaluation was conducted using GC-disk electrode (diameter 3 mm) in a three-electrode system (Hokuto Denko HSV-100 electrochemical analyzer). We used Ag/AgCl (sat. KCl) as a reference electrode and platinum wire as a counter electrode. All measurements were carried out on room temperature (ca. 20 ^oC).

We used photolithography-based microfabrication techniques to prepare electrode substrates. The microfluidic cell consists of the channel (2 x 28 mm) made of silicon film (0.05 mm in thickness) sandwiched by Au (anode) and Pt (cathode)-sputtered glass plate having an electrode surface area of 0.56 cm^2. A fuel solutions were flowed through the microchannel, and the cell performance was analyzed by measuring cell voltage with changing an inserted variable resistance (0-3 MΩ), as shown in Figure 3.

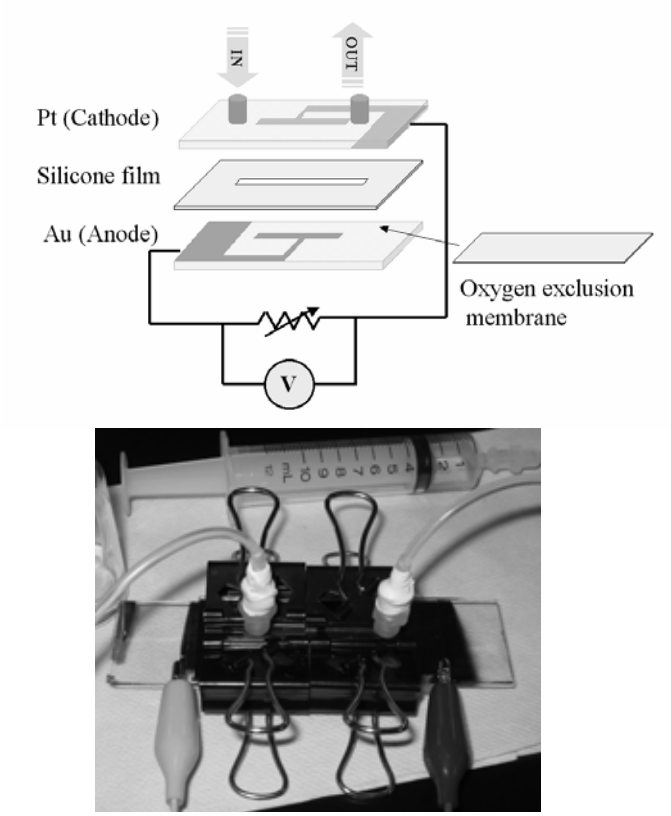

Figure 3. The illustration (upper) and photograph (lower) of the construction of the microfluidic fuel cell to evaluate the performance of enzyme electrode under the steady fuel flow.

3 Results and discussion

3.1 Redox Properties of Anode and Cathode

Figure 4 shows cyclic voltammograms of the PAA-VK3 on GC electrode under nitrogen atmosphere in PBS. Voltammograms showed reversible wave with the half wave potential of -0.25 V *vs.* Ag/AgCl (at. KCl). The inset shows that oxidation (i_{pa}) and reduction (i_{pc}) peak heights increased almost linearly with the square root of the scan rate of potential ($v^{1/2}$), indicating that the electron can diffuse through the PAA-VK3 hydrogel film by the electron-exchange between the neighboring VK3 units. These features are required for the electron–mediation for the enzymes entrapped within the hydrogel.

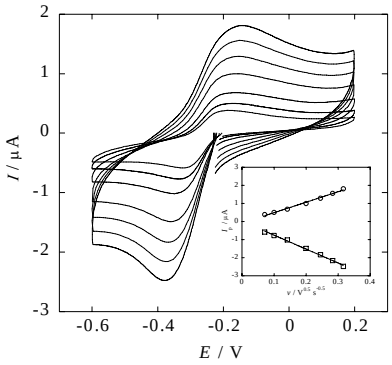

Figure 4. Cyclic voltammograms of PAA-VK3 on GC electrode under nitrogen atmosphere in PBS buffer. Scan rates were 5, 10, 20, 40, 60, 80, 100 mV/s. Inset shows dependence of oxidation and reduction peak heights on the square root of scan rates.

Cathode reaction is the reduction of dissolved oxygen on PDMS-coated Pt electrode, which would be mainly the reduction to hydrogen peroxide. The thin PDMS film on electrode serves as the O_2-selective membrane [7]. The O_2-selective property of cathode would be required upon the use of biofuel containing interfering substance such as ascorbic acid. As showed in Figure 5, peak potential of oxygen reduction was found around +0.25 V *vs.* Ag/AgCl (sat. KCl). Cell voltage comes from difference of redox potential between mediator and oxygen. Thus the expected cell voltage in roughly 0.5 V for the present system.

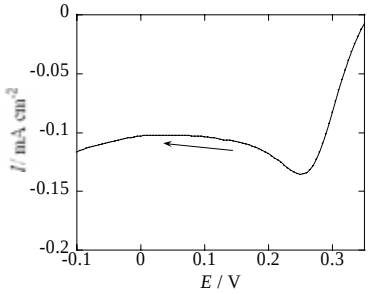

Figure 5. A linear sweep viltammogram for a PDMS-coated Pt plate in air-saturated PBS at 5 mV / s.

3.2 Catalytic Current Generation on Anode

By taking advantage of the enzymatic reactions in the anode membrane, VK3 is expected to be reduced consistently by electron transport shuttle from glucose. We prepared GDH-Dp-PAA-VK3 membrane on GC electrode and measured the cyclic voltammograms of PAA- VK3. In the presence of 0.5 mM NADH, the voltammogram of PAA-VK3 changed to a catalytic wave. Further addition of glucose (final concentration was 10 mM) increased current density of catalytic wave (Figure 6) as the result of chained enzyme reactions.

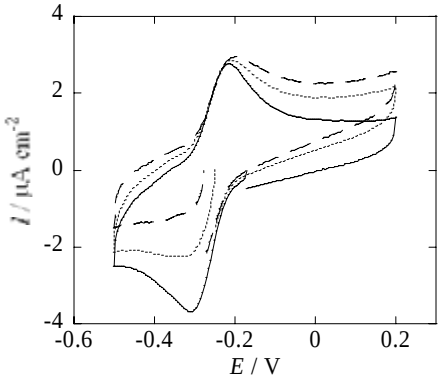

Figure 6. Cyclic voltammograms of the electrode coated with PAA-VK3 - Dp – GDH at 50 mV / s. Buffer only (solid line); 0.5mM NADH (dotted line); 0.5 mM NADH and 10 mM glucose (broken line)

3.3 Evaluation as a Fuel Cell

Using the bi-enzyme-coated anode and the PDMS-coated cathode, we constituted the microfluidic fuel cell shown in Fig. 2. Figure 5 shows dependence of the current density and the power density on the cell voltage. The cell operated at a power density of 0.13 μW cm^{-2} at a 0.15 V cell potential under ambient conditions (air saturated, [glucose] = 5 mM, [NADH] = 1 mM). The observed output potential was significantly lower than the expected value, 0.5 V, due to the O2 sensitivity of VK3. In fact, the voltammetric activity of VK3 disappears in the air-saturated condition, suggesting that oxygen interfere the electrode reaction of VK3.

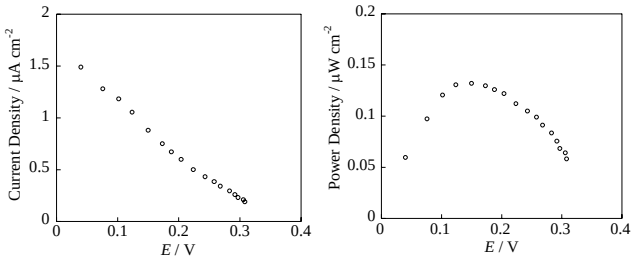

Figure 7. Dependence of the current density (left) and the power density (right) on the voltage of the biofuel cell (pH 7.0, at 25 °C). Fuel is air-saturated PBS containing 5mM glucose and 1 mM NADH.

3.4 Exclusion of Oxygen from Anode

As can be recognized from the results in Fig. 7, it is necessary to exclude oxygen from the vicinity of anode to obtain better performance. For this purpose, we are planning to coat the GDH-Dp bi-enzyme layer with a layer of an oxidase. We covered the anode with glucose oxidase (GOD; negatively charged at pH 7) -immobilized positively charged nylon membrane (Nytran® SuPerCharge), which is expected to consume oxygen with sacrifice of the corresponding amount of glucose.

In order to confirm the effect of GOD layer for eliminating oxygen, we placed it on PAA-VK3–Dp Au electrode and measured its rest potential (Figure 8). At first, we added NADH to PBS, followed by adding glucose. The lowering of the rest potential on the addition of glucose indicates the effective supply of the reduced form of VK3 to the electrode surface. The insertion of this oxidase membrane as a component of the microfluidic cell (see Fig. 1) can be expected to improve the cell performance. Experiments are under progress along this strategy.

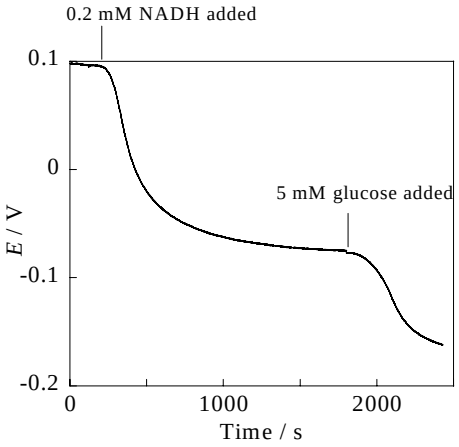

Figure 8. Time courses of the rest potential of PAA-VK3-Dp electrode, which is placed below the GOD membrane. 5 mM glucose followed by the addition of 0.2 mM NADH was added (pH 7, air saturated).

4 Acknowledgement

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