The Fifth International Workshop on Micro and Nanotechnology for Power Generation and Energy Conversion Applications
PowerMEMS 2005
Nov. 28-30, 2005
Takeda Hall, The University of Tokyo, Tokyo, Japan

Enzyme-Based Fuel Cells for Biomedical Microdevices

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Abstract

We will briefly review the trends in enzymatic fuel cells that use enzyme as an electrocatalyst to generate electricity from biological fuel such as glucose in blood. We will then show our recent achievements mainly about the enzyme anode that is composed of a bi-layer polymer membrane, the inner layer containing diaphorase (Dp) and the outer, glucose dehydrogenase. The Dp membrane was formed from a newly synthesized Vitamin K_3-based mediator polymer. By coupling with an oxygen-selective cathode, the power generation performance was evaluated in buffer solution, serum and blood. MEMS techniques have been potentially applied to array cells and to flow the fuel continuously.

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

1 INTRODUCTION

Electric power derived from dispersed ambient energy has attracted attention as ubiquitous portable power. A potential option of portable power source is biological fuel cell that use enzyme as an electrocatalyst to generate electricity from such biological fuels as alcohols and carbohydrates [1-4]. The enzymes are the catalysts that show highly selective activity in neutral pH aqueous solution at near-room temperatures. The high reaction selectivity of enzymes would make it possible to design separator-free fuel cells that are composed of just a couple of anode and cathode electrodes exposed to solutions containing both fuel and oxidant (oxygen). Since the fuel fluid is typically aqueous neutral solution, the chemical stability of packaging does not always need to be considered. And most importantly, the high selectivity of the enzyme would allow power generation from complex natural fuel solutions without purifications, that is, direct utilization of refreshments containing sugar, plant saps, and biological fluids such as blood. Biological fuel cells have a long history in the literature and they are extensively reviewed [1-4].

The MEMS-relating techniques should greatly contribute to developing of biofuel cell. Especially, the micro-TAS has significant overlap in technical requirements, including the control of microfluids and control of biomolecules [5]. By integrating fuel cell engineering, bioengineering and microfabrication engineering, enzyme-based biofuel cells are expected to be formatted into miniature power sources for independent power-on-chip systems such as portable microdevices and implantable medical devices in future.

The enzymatic biofuel cells would mostly stand out in biomedical applications, especially for the power generation directly from biofluids, tissue fluids and blood, containing glucose (ca. 5 mM), lactate (ca. 1 mM) and oxygen (0.1 mM in arterial blood). There are many

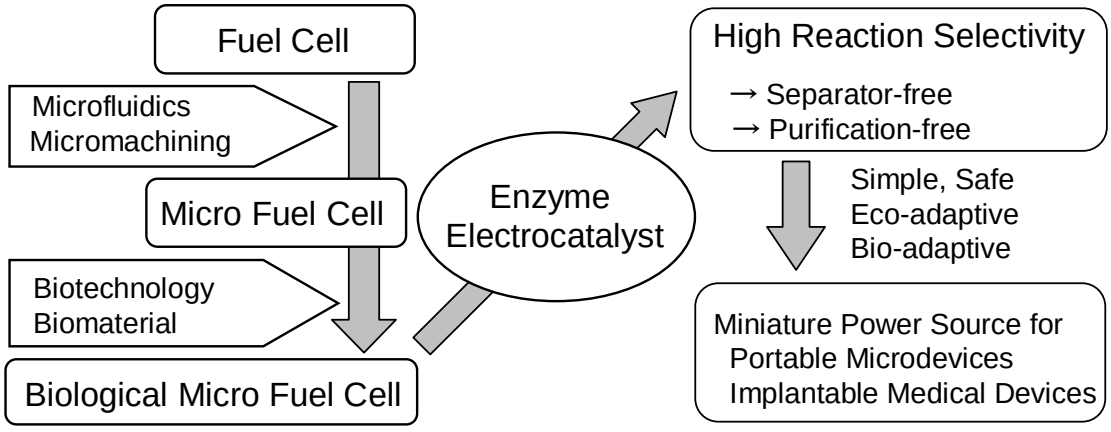

Figure 1 Enzymatic biofuel cells: relating technologies, points of merits and possible applications.

types of medical devices with different levels of invasiveness, from the low invasive skin-patch device for health monitoring and drug delivery to the highly invasive device such as cardiac pacemaker [6,7]. Each device requires stability and safety at each level, in addition to the power generation property.

In this paper, we will briefly review the trends in the research of enzymatic fuel cells, and then show our recent achievements mainly about the enzyme anode for glucose oxidation composed of a bi-layer polymer membrane, the inner layer containing diaphorase (Dp) and the outer, glucose dehydrogenase (GDH). The Dp membrane was formed from a newly synthesized 2-methyl-1,4-naphthoquinone (Vitamin K_3; VK_3)-based mediator polymer. By coupling with an oxygen-selective cathode, the power generation performance was evaluated in buffer solution, serum and blood. MEMS techniques have been potentially applied to array cells and to flow the fuel continuously.

2 ENZYME ELECTRODES

2.1 Biological Anodes

The electrocatalytic oxidation of biological fuel is based on an electrical contact between redox enzymes and electrode supports; thus, a wide variety of enzyme/mediator systems have been studied to date. An osmium complex-linked polymer has been reported to serve as an electron mediator of glucose oxidase, and has been used to construct a biofuel cell showing excellent performance under physiological conditions [8-10].

We have studied the diaphorase (Dp) / glucose dehydrogenase (GDH) double layer-coated anode for glucose oxidation, as illustrated in Figure 2 [11,12]. Dp is a flavine-enzyme which converts nicotinamide-adenine dinucleotide (NADH) to NAD^+ [13]. The latter is an important coenzyme participating in various biochemical redox reactions, and NAD^+-dependent enzymes constitute the largest group of redox enzymes including the NAD^+-dependent GDH. The electron transfer between the electrode and Dp should be mediated by inexpensive and safe mediators for medical application, and quinine derivatives would meet these demands. Then the inner Dp layer was prepared by co-immobilization with NADH and the newly synthesized 3-methyl-1, 4-naphtoquinone (vitamin K_3, VK_3)-based polymer, that has VK_3 moieties modified to 20% of amino group of the polyallylamine backbone. The cross-linker for making the Dp/VK_3(+NADH) film was Poly-(ethylene-glycol) diglycidyl ether (PEGDGE). The epoxide groups of PEGDGE reacted with the amino-groups of PAA-VK_3 to form a hydrogel film. The NADH (and also NAD^+) is anionic and thus expected to be electrostatically immobilized within the cationic hydrogel. The prepared Dp hydrogel membrane was further coated by a GDH membrane composed of GDH, poly-L-lysine (PLL), PEGDGE and NADH. The thickness of each enzyme membrane was 4 μm, as estimated by a surface texture analysis in dry state. The addition of a conductive support, Ketjenblack (KB), into the Dp/VK_3 film dramatically enhanced the generation of NAD^+, and thus, the activity of the outer membrane of GDH (a NAD^+-dependent enzyme).

Figure 3a shows cyclic voltammograms of the Dp/VK_3/GDH (+ NADH) double layer electrode in 37°C air-saturated buffer solution containing 0mM (·····), 5mM (- - -) and 40mM (——) glucose. The increase in catalytic current with addition of glucose indicates the successful cycling of reaction scheme in Fig. 2. Figure 3b depicts the output performance measured by using a larger Pt cathode, showing the maximum power of ca. 0.14 mW / cm^2 (at 0.45 V) in 5mM glucose buffer solution at 37 °C.

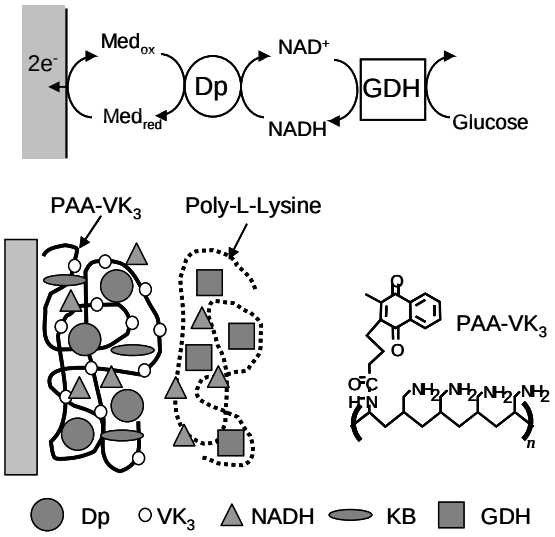

Figure 2 Schematic illustration showing structure of enzyme anode with expected reaction

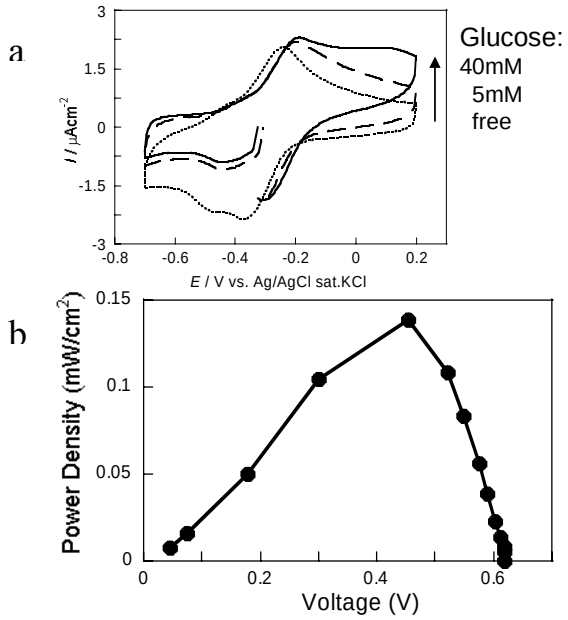

Figure 3 Cyclic voltammograms of the enzyme anode, and cell performance in 5 mM glucose buffer solution at 37 °C.

The obtained output is, just for reference, at the same level as that of commercial alkali button battery (ex. 0.15mW, LR54). The power density decreased to ca 30 % of the initial value over 4 days, and maintained this output for more than 2 weeks [12]. The initial power output decay would be due to both the deactivation of enzymes and the partial degradation of the bi-layer polymer membrane.

2.2 Biological Cathodes

The enzyme electrodes for catalytic reduction of dissolved oxygen has been developed as well, and recent reports using bilirubin oxidase (BOD) show a successful catalytic activity in physiological condition [14,15]. Such enzyme cathode is equally important in developing a totally enzymatic biofuel cell. At present, however, we are still using a conventional PDMS-coated Pt electrode as an O_2 selective cathode [11,12,16]. The PDMS coating was prepared by placing a 3 % (w/v) aqueous PDMS emulsion (Toray Dow Corning Silicone, Type DC 84 ADDITIVE) on a Pt plate electrode, and drying the coated electrode for 4 h at room temperature. The reaction of O_2 reduction was not significantly hindered by coating PDMS due to its high O_2 permeability.

3 PERFORMANCE IN BIOFLUIDS

The performance of the cell, with the enzyme-based anode and the PDMS-coated Pt cathode, was preliminarily evaluated in 37 ^{o}C biofluids: bovine serum and human venous blood (Figure 4). Note these measurements were previous experiments conducted without the co-immobilization of NADH, and therefore the exact value of output is low, compared with the recent results shown in Fig. 3b. It is worthwhile to note that the fuel cell performance in serum (●) is comparable with that in buffer solution (○), although serum contains proteins, lipids, redox active vitamin C and so on. The reaction selectivity of enzyme anode to glucose and the PDMS cathode to O_2 ensures these performances. On the other hand, the performance in blood was significantly unstable. The data in Fig. 4, taken after 2hs' incubation of electrodes in biofluids, was less than the half of that in serum. The output voltage was decreased, suggesting a breakdown of the reaction selectivity of the electrodes. Biofouling was observed especially on the PDMS cathode. In order to get higher stable power from blood, biological stability, associated with the natural immune response to foreign materials, should be ensured. We have preliminarily examined that anti-biofouling coatings such as MPC polymer significantly blocked the biofouling on electrode surfaces without hindering the electrode reaction itself.

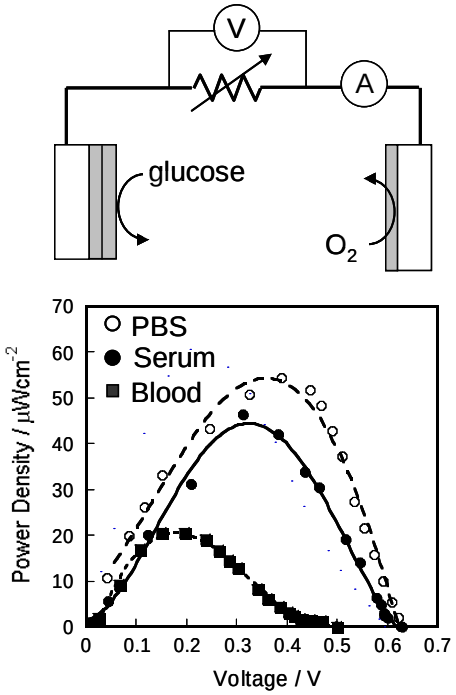

Figure 4 Performance of the cell in PBS, FBS and human venous blood at 37 ^{o}C.
0.5 mM NADH was added.

4 MICROFABRICATIONS

4.1 Fuel Cell Array on a Chip

The output voltage of a biofuel cell is less than 1V, thus the connection of cells would be required depending on applications. The stacking structure of separator-free enzyme-based fuel cells can be designed flexibly. Figure 5 shows the series-connected six cells on a chip as a simple example. Each cell is composed of the

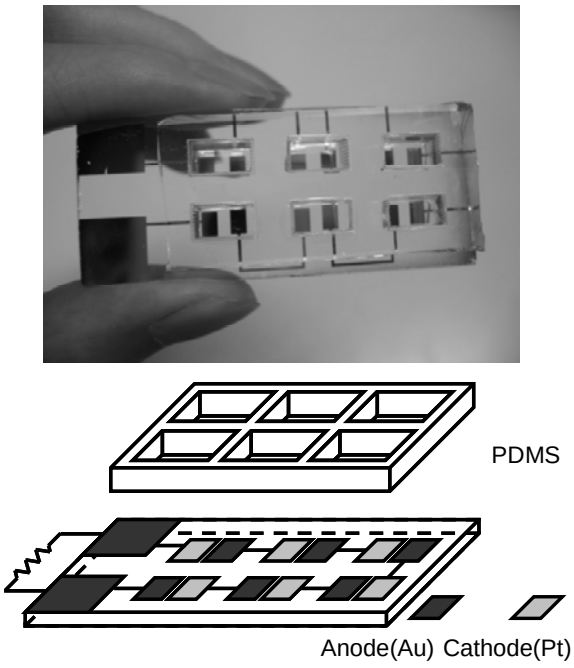

Figure 5 Biofuel cells stacked on a chip.

Dp/VK_3/GDH(+NADH) double-layer-coated Au anode and the PDMS-coated Pt cathode. The cells were connected electrically by a printed circuit, but ionically separated by an arrayed camber made of PDMS. The resulting assembly output amplified power as to drive an electronic device as demonstrated in Figure 6a. The total performance of the assembly measured in a glucose (5 mM)-containing buffer solution is shown in Figure 6b. Output voltage was increased as expected, while the flowing current is one-order lower as compared with the case using larger cathode (Fig. 3b), suggesting that the total performance of the present cell was kinetically determined by cathode. Thus, we are now hurriedly focusing on development of a comparatively active enzyme cathode.

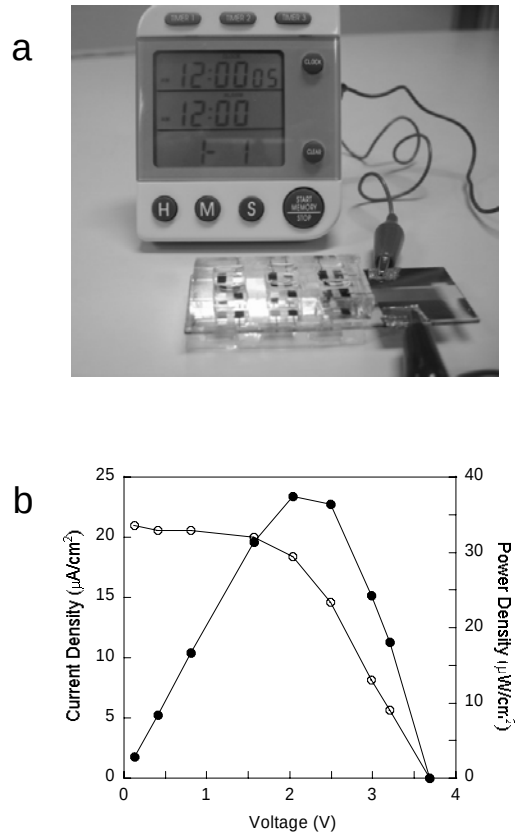

Figure 6
(a) The demonstration powering digital timer.
(b) Performance of the arrayed cells on a chip, evaluated in air-saturated buffer solution containing 5mM glucose, by changing the load (10k ohm to 3M ohm).

4.2 Microfluidic Design

The assembly shown in Fig. 5 is one of the batch-type reactors, and thus the output power degrades with the depletion of fuels or dissolved oxygen like in a battery. Rather, the longer term power generation by continuous fuel supply is the typical mode of fuel cell. Figure 7a is a simple fluidic chip composed of a couple of electrodes and a PDMS microchannel. As shown in Figure 7b, for the operation with stationary fuel (□), the output current decreased gradually due to the depletion of reactants. We can reproduce the same trace with the electrode after recharging the solution, indicating that this profile is not corresponding to the electrode degradation. When the solution was flowed at 0.01 ml/min (○) and 0.1 ml/min (●), the output shows stable higher current at higher flow rate. The dependency of the fuel cell performance on the flow rate is now studied in detail. The techniques for controlling fluid, progressing in the field of micro-TAS, will be a powerful means to study optimum operating conditions.

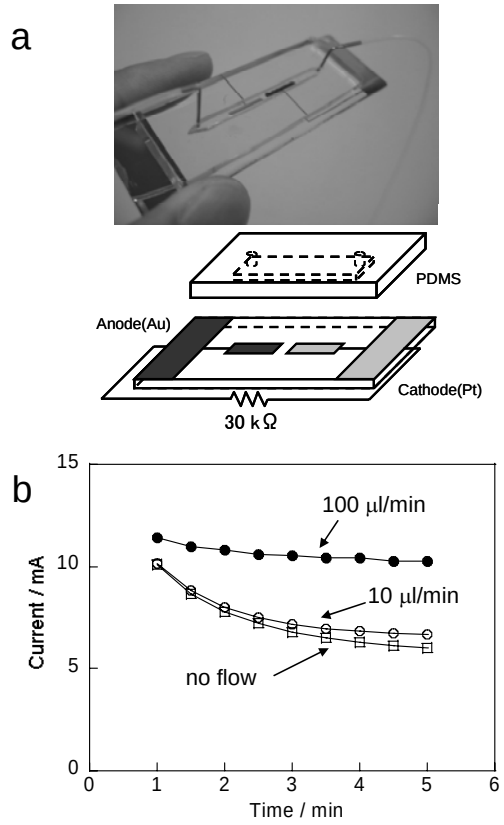

Figure 7
(a) Biofuel cell on a microfluidic chip.
(b) Cell current behaviors as a function of time at various flow rates.

5. ACKNOWLEDGEMENT

Author express appreciation to Dr. Kosuge and Dr. Fukasaku (Daiichi Pure Chemicals Co., Ltd.) for synthesis of mediator polymers. This work was partly supported by Health and Labor Sciences Research Grant for Research on medical devices for analyzing, supporting and substituting the function of human body from the Ministry of Health, Labor and Welfare of Japan.

REFERENCES

[1] S. C. Barton, J. Gallaway, P. Atanassov, "Enzymatic Biofuel Cells for Implantable and Microscale Devices", Chem. Rev., **104**, pp 4867-4886, 2004.

[2] A. Heller, "Miniature Biofuel Cells", *Phys. Chem. Chem. Phys*., **6**, pp209-216, 2004.

[3]I. Willner, E. Katz, "Integration of Layered Redox Proteins and Conductive Supports for Bioelectronic Applications", *Angew. Chem. Int. Ed*., **39**, pp1180-1218, 2000.

[4]T. Ikeda, K. Kano, "An Electrochemical Approach to the Studies of Biological Redox Reactions and Their Applications to Biosensors, Bioreactors, and Biofuel Cells", *J. Biosci. Bioeng*., **92**, pp9-18, 2001.

[5] C. M. Moor, S. D. Minteer, R. S. Martin, "Microchip-based Ethanol / Oxygen Biofuel Cell", *Lab Chip*, **5**, pp218-225, 2005.

[6] A. Heller, "Integrated Medical Feedback Systems for Drug Delivery", *AIChE J.*, **51**, pp1054-1066, 2005.

[7] C. F. Holmes, "Electrochemical Power Sources and the Treatment of Human Illness", *Electrochem. Soc. Interface*, Fall, pp26-29, 2003.

[8] V. Soukharev, N. Mano, A. Heller, "A four-electron O_2 electroreduction biocatalyst superior to platinum and a biofuel cell operating at 0.88 V", *J. Am. chem. Soc.,* **126**, pp. 8368-8369, 2004.

[9] N. Mano, F. Mao, A. Heller, "Characteristics of a miniature compartment-less glucose-O_2 biofuel cell and its operation in a living plant", *J. Am. chem. Soc.,* **125**, pp. 6588-6594, 2003.

[10] S. Tsujimura, K. Kano, T. Ikeda, "Electrochemical Oxidation of NADH Catalyzed by Diaphorase Conjugated with Os-complexs", *Chem. Lett.*, pp1022-1023, 2002.

[11] M. Nishizawa, J. Sato, T. Ohashi, M. K. Islam, T. Yasukawa, and T. Matsue, " Diaphorase-Based Enzyme Electrodes for Fuel Cell Applications ", *Proc. 204th Meeting of Electrochem. Soc*., Orlando, 2003.

[12]F. Sato, M. Togo, M. Islam, T. Matsue, J. Kosuge, N. Fukasaku, S. Kurosawa, M. Nishizawa, "Enzyme-based Glucose Fuel Cells using Vitamin K_3-immobilized Polymer as an Electron Mediator", *Electrochem. Commun*., **7**, pp643-647, 2005.

[13] (a) Y. Ogino, K. Takagi, K. Kano, T. Ikeda, "Reactions between diaphorase and quinine compounds in bio-electrocatalytic redox reactions of NADH and NAD^+", *J. Electroanal. Chem.*, **396**, pp. 517-524, 1996.

(b) K. Takagi, K. Kano, T. Ikeda, "Mediated bioelectro- analysis based on NAD-related enzymes with reversible characteristics", *J. Electroanal. Chem.*, **445**, pp. 211-219, 1998.

[14]S. Tsujimura, H. Tatsumi, J. Ogawa, S. Shimizu, k. Kano, T. Ikeda, "Bioelectrocatalytic reduction of dioxygen to water at neutral pH using bilirubin oxidase as an enzyme and 2,2′-azinobis (3-ethylbenzothiazolin-6-sulfonate) as an electron transfer mediator", *J. Electroanal. Chem*., **496**, pp69-75, 2001.

[15]T. Tsujimura, K. Kano, T. Ikeda, "Glucose/O_2 biofuel cell operating at physiological conditions", *Electrochemistry*, **70**, pp940-942, 2002.

[16]F. Mizutani, Y. Sato, Y. Hirata, S. Iijima, "Interference-free, amperometric measurement of urea in biological samples using an electrode coated with tri-enzyme/polydimethylsiloxane-bilayer membrane ", *Anal. Chim. Acta*, **441**, pp. 175-181, 2001.