

MINIATURE BIOFUEL CELLS TOWARD MEDICAL APPLICATIONS

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Abstract: This paper reports miniature biofuel cells for biomedical application. Important requirements for human-implantable and disposable biofuel cells are safeness, biocompatibility and suitable electrode structure. We developed completely organic bioelectrocatalysts consist of PLL-VK₃/Dp/PLL-NAD(H)/GDH by polymerizing electron mediators. The power performance of the electrode and biocompatibility of their surfaces were evaluated electrochemically and visually. The needle-type miniature glucose-Ag|AgCl cell produced maximum current of 110 $\mu\text{A cm}^{-2}$ in physiological condition, and of 50 $\mu\text{A cm}^{-2}$ in bovine serum.

Key words: biofuel cell, NADH, Vitamin K₃, microfluidics

1. INTRODUCTION

Various kinds of miniature power sources, which generate electricity from ambient chemicals or mechanical power, are now actively studying for powering portable electric devices. Distinctive features of biofuel cells [1-3], such as renewability, reaction selectivity and safety are provided by enzyme catalysts. Due to their high reaction selectivity and safeness, the compartmentless enzymatic biofuel cells would mostly stand out in biomedical applications [1], especially for the power generation directly from biofluids, tissue fluids and blood, containing glucose, lactate and oxygen. Because of the relatively low stability of biofuel cell, subcutaneous implantation could be the most practical application. As illustrated in fig. 1, the required performances of the biomedical biofuel cells are not only the magnitude of power output (i.e. current and voltage), but also additional three specific performances: safe bioelectrocatalysts, biocompatible surfaces and needle-shaped electrode.

Taking into account these three important points, we developed a needle-type miniature biofuel cell. At first, we studied the safe bioelectrocatalyst by constructing it only of organic substances. We have

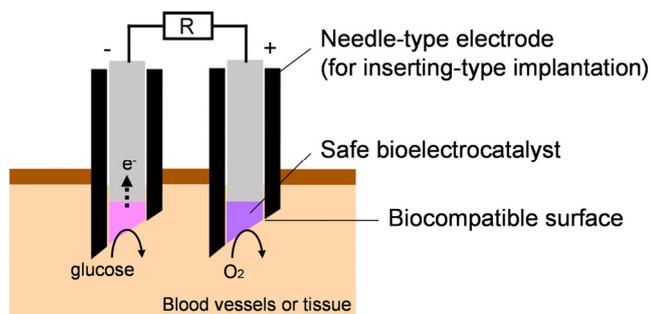


Fig. 1: Illustration of needle-type biofuel cell with requirements for medical usage

been studying the bioanode modified with bio-adaptive Vitamin K₃-pendant polymer and enzyme membranes for glucose oxidation [4, 5] (Fig. 2). In this paper, we show the immobilization methods of diffusional coenzyme, NAD, which hasn't been immobilized in previous studies. Next, biocompatibility of the electrode surface was studied. When the electrodes would be inserted into the blood vessels or tissue, our body would recognize them as foreign body and inflammation reaction would occur. And then the electrodes are covered with proteins and/or cells, which would cause not only the decay of electrodes' performances but also blood clots at the implanted site. In order to avoid that, surface treatment with several kinds of bioinert materials was applied to the electrode surface. Finally, a needle-type miniature biofuel cell was fabricated and tested its performance in a physiological buffer solution and serum.

2. EXPERIMENTAL

2.1 Preparation of electrocatalysts

For the purpose of implantable usage of the electrode, we tried to immobilize NAD on the electrode by chemical polymerization. Synthesis procedure is briefly described below. Amino groups of native NAD⁺ and poly-L-lysine (PLL) were cross-linked by glutaraldehyde (GA), and the reaction products were purified, finally we obtained polymerized NAD⁺ (PLL-NAD). A mixture solution of electron mediator polymer (PLL-VK₃), polymerized coenzyme (PLL-NAD) and enzymes (Dp and GDH) was syringed to the carbon black-modified (or smooth GC) electrode and dried over night.

2.2 Surface Modification for biocompatible surface

Electrode surface have been coated with several kinds of biomaterials. In this paper, we tested

lyotropic liquid crystal (LLC) made from lipid, MPC polymer and Nafion membrane as an overcoating membrane. Nafion dispersion solution was purchased from Wako, 2-Methacryloyloxyethyl phosphorylcholine (MPC) polymer was purchased from NOF Corporation, and LLC was made from 1-oleoyl-rac-glycerol as reported procedure [6]. Each surface modification was conducted 30 min before the measurements.

Biocompatibilities of the polymer-coated electrodes were judged from amount of adsorbed protein. Each electrode was soaked in a PBS solution of fluorescence-labeled albumin (Albumin, Fluorescein isothiocyanate conjugate bovine serum, SIGMA) for 1, 3, 6, 12 hours and washed with buffer solution before measurements. Fluorescent micrographs were recorded using the LAS-3000mini, a compact Luminescent Image analyzer (Fuji film). Exposure times were 10 s. The images were analyzed using Multi Gauge software (version 3.0, Fuji film).

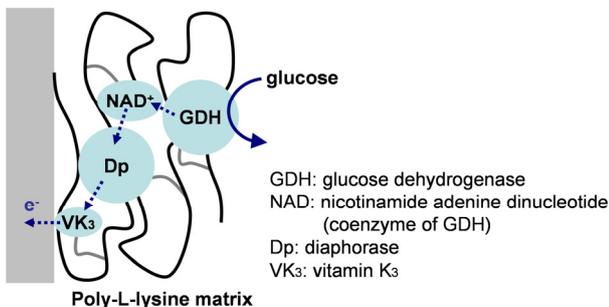


Fig. 2: Schematic of electron relay at the bioanode

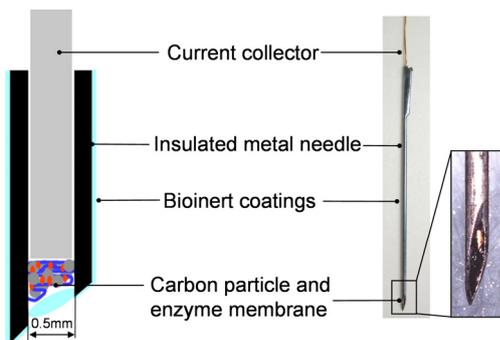


Fig. 3: Schematic illustration (left) and photograph (right) of the prototype of needle-type biofuel cell.

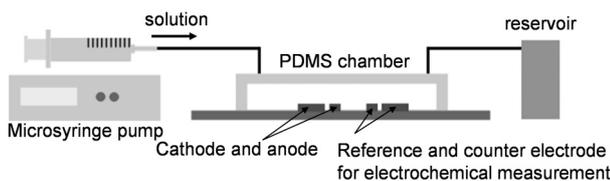


Fig. 4: Illustration of the microfluidic-type cell.

2.3 Fabrication of a needle-type electrode

A prototype of needle type biofuel cell (for inserting into subcutaneous or blood vessels) was fabricated. As shown in fig. 3, in order to avoid the peel-off of an enzymatic catalyst, we tried to immobilize an enzyme within a needle. At first, a disposable injection needle (inner diameter was 0.5 mm) was insulated by electrodeposition of an insulating paint. A gold wire, which used as a current collector, was inserted in the insulated needle. Then, carbon particle and enzyme membrane were packed in order. Finally, the electrode surfaces were coated by biocompatible film.

2.4 Electrochemical measurement

The performances of the electrodes were evaluated by electrochemical measurement using three electrode systems. Except for the cyclic voltammety shown in 3.1, all measurements were conducted using the flow-cell shown in fig. 4. Gold electrodes were patterned onto glass slide by conventional photolithography and sputtering. Anode catalysts were modified as described 2.1, and cathode catalysts were modified as reported before [7]. Flow channel (3 mm wide and 1 mm height) was made from PDMS. The flow was regulated by microsyringe pump and set to 0.3 mL min⁻¹ in all measurements.

3. RESULTS AND DISCUSSIONS

3.1 Performances of NAD-immobilized anodes

Fig. 5 shows the cyclic voltammograms (CVs) of the PLL-VK₃/PLL-NAD/Dp/GDH-immobilized anodes in a N₂-saturated phosphate buffered solution (pH 7). The peak couples around -0.3 V shows the redox activity of VK₃ on the electrode. The oxidation currents were clearly increased and reduction currents were almost disappeared in the presence of glucose, which indicated that the successful working of the polymeric NAD. Unfortunately, the catalytic activity of glucose anode was smaller than the case of dissolved NAD [4], which probably due to the low flexibility of the polymeric NAD. Utilization of longer linker would improve the flexibility of NAD [2, 4]. However, the catalytic current gradually decreased with the several time of scan, probably due to the dissolution of PLL-NAD. In order for avoid the dissolution of the PLL-NAD and improve the stability, the electrode surfaces were overcoated with bio-adopted membranes: Nafion membrane, MPC polymer and LLC membrane. Among these three membrane-coated electrode, LLC-coated one showed the highest stability (data not shown).

Fig. 6 shows the cell performances using the LLC-coated bioanode evaluated in the microfluidic cell.

Circle plots show the cell performance using Ag|AgCl as a cathode and triangle plots show the cell performance using birirubin oxidase (BOD)-adsorbed carbon black electrode as a biocathode. The maximum current of both of two cells was about $170 \mu\text{A cm}^{-2}$. The maximum voltage was 0.45 V higher in the glucose- O_2 cell because of the positive reaction potential of BOD cathode. The obtained maximum power densities of $80 \mu\text{W cm}^{-2}$ and $20 \mu\text{W cm}^{-2}$ were thought to be sufficient for powering the low power CMOS circuit. Therefore the cell can be used as the batteries of the low power-required electronic devices such as sensors. The time courses of the biofuel cells were plotted in fig.6 bottom. The output currents were almost stable during 6 hour operation.

3.2 Biocompatibility of electrode surfaces

Fig. 7 shows the time courses of the back ground-collected fluorescent intensity on the untreated (\bullet), MPC-coated (\blacktriangle), Nafion-coated (\times) and LLC-coated (\blacklozenge) bioanodes. We evaluated the biocompatibility of the electrode surface by observing the amount of adsorbed albumin, because it is the major protein in body fluids. As shown in fig. 7, the amount of albumin was gradually increased with time. LLC-coated anode showed relatively higher albumin adsorption among the four electrodes. Not only the MPC-coated electrode surface, which is well known to biologically-inert surface due to its lipid bilayer-like structure, the surfaces of the bare and Nafion-coated electrode showed lower albumin adsorption, which probably because their negatively charged surfaces electrostatically inhibited the albumin adsorption.

The relation between protein adsorption and electrode activity was evaluated. Albumin-adsorbed electrodes showed somewhat lower glucose oxidation activity except for the LLC-coated electrodes. Interestingly, the activities of LLC-coated electrodes stored in albumin solution were gradually increased with time in spite of higher protein adsorption to the surface (data not shown).

The protein blocking ability of the membrane-coated electrode surfaces is important because the protective response of our body against a foreign body starts with protein adsorption to the surface. The results obtained in fig. 7 suggested that the addition of negatively charged lipids would improve the bio-inactivity and the stability of the LLC-coated bioanode in body fluids.

3.3 The performances of needle-type electrodes

The performance of the enzyme-modified needle-type electrodes inserted in a silicone tube (1 mm in inner diameter) was shown in fig. 5. The surface of the enzyme membrane was coated with LLC and

whole electrode surface was further coated with MPC polymer. An Ag|AgCl electrode was used as a cathode. The maximum voltage of the needle-type cell were slightly smaller than that obtained in 3.2 (Fig 6), which probably caused by nonuniform coatings of the enzyme membrane at needle-type miniature electrode; some area of the carbon black surface might not be covered with enzyme membrane. On the other hand, the maximum current density of a $110 \mu\text{Acm}^{-2}$ could be obtained.

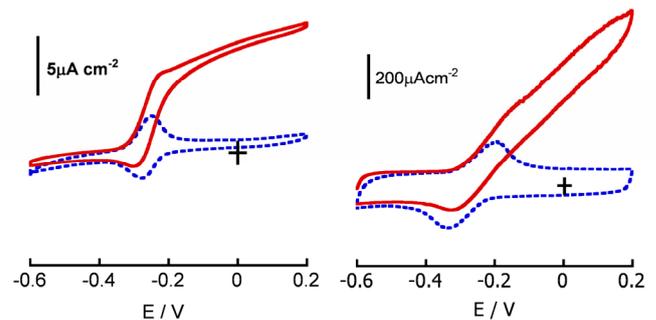


Fig. 5: CVs of the bioanode on a smooth GC electrode (left) and a CB-modified GC electrode (right) in absence of (dotted lines) and presence of (solid lines) 200 mM glucose. Scan rate was 5 mV s^{-1} .

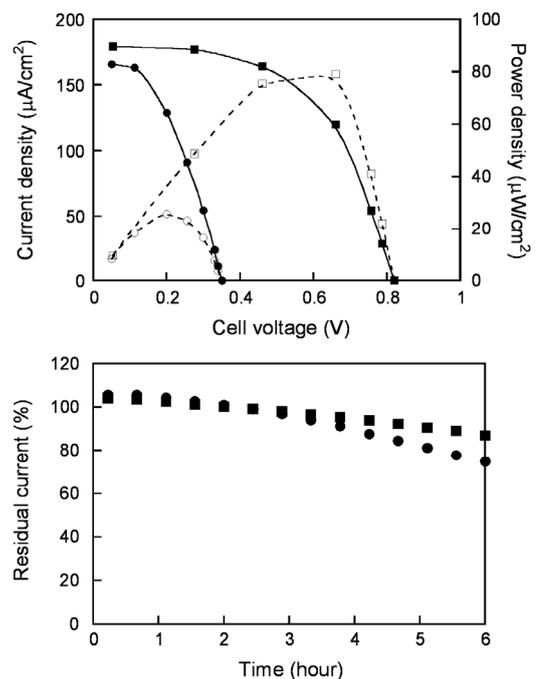


Fig. 6: Power performances (top) and stability (bottom) ($R=1 \text{ M}\Omega$) of the glucose-Ag|AgCl cell (\bullet) and glucose- O_2 cell (\blacksquare). The cells were working under 0.1 M glucose and 0.1 M NaCl-contained phosphate buffer (pH 7). Flow rate: 0.3 mL min^{-1}

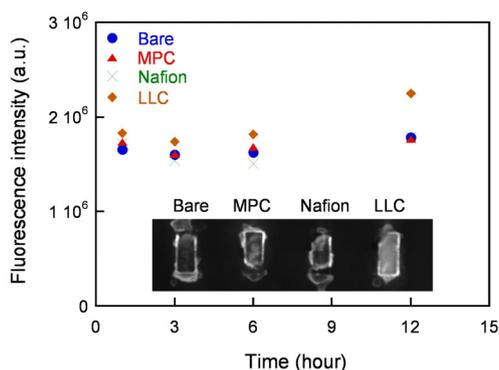


Fig. 7: Time course fluorescence intensity of the untreated (●) MPC-coated (▲) Nafion-coated (×) LLC-coated (◆) bioanode. Inset shows fluorescence micrograph of each electrode.

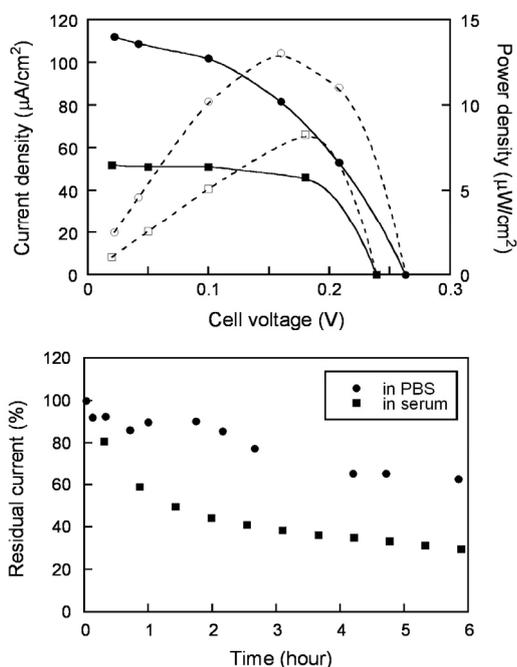


Fig. 8: Power output (top) and stability (bottom) ($R = 2 \text{ M}\Omega$) of the needle-type biofuel cells operating under 5 mM glucose-contained phosphate buffer ($\text{pH } 7$) (●) and bovine serum (■). Flow rate: 0.3 mL min^{-1}

The performance of the needle-type biofuel cell in a bovine serum was evaluated. The output maximum current was a half of that in a buffer solution, which also due to the uncovered carbon black electrode, so the various kinds of electroactive species might be reacted on the electrode surface. In a buffer solution, needle-type biofuel cell maintained its output current over 60 % of the initial during 6 hours of operation. The value 60 % was close to the result obtained in fig. 6. On the other hand, residual activity of biofuel cell in a bovine serum at 6 hour was smaller than that in

buffer solution. Output current was rapidly decreased for first 1 hour, and then the current was almost stable, which probably due to the protein adsorption to the surface and/or degradation of the LLC-membrane was caused at early stage. Uniform coatings of enzyme catalysts and development of a stable overcoat membrane would improve the stability of needle-type biofuel cell.

4. CONCLUSION

The prototype miniature biofuel cells composed of needle-type electrode immobilized with organic and harmless electrocatalysts produced ca. $110 \mu\text{A cm}^{-2}$ of current density and maintain the current over 60 % of the initial during 6 hours of operation. To stabilize the power output in serum, bioinert coating, fabrication method and structure of miniature electrode should be optimized. Future work will also be focused in making a miniature polymer-needle array by microfabrication techniques for developing the painless and easily disposable implantable miniature biofuel cell.

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