ENZYME-BASED BIOFUEL CELL DESIGNED FOR DIRECT POWER GENERATION FROM BIOFLUIDS IN LIVING ORGANISMS

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ABSTRACT

Enzymatic biofuel cells have attracted much attention for their potential to directly use biochemical energy sources in living organisms such as animals, fruits, etc. However, generally natural organisms have a skin, and the oxygen concentration in the organisms is lower than that of biofuels like sugars. Here, we fabricated a miniature assembly that consists of a needle bioanode for accessing biofuels in organisms through their skins and a gas-diffusion biocathode for utilizing abundant oxygen in air, for the first time. The performance of the biocathode was fourfold improved by optimizing its hydrophobicity. The assembled device with a needle anode for fructose oxidation was inserted into a raw grape, producing a maximum power of 26.5 μW (115 μW cm^{-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 the tip of the needle anode with an anti-biofouling agent was effective to stabilize output power.

KEYWORDS: miniature biofuel cell, power generation, bioelectrode

INTRODUCTION

An enzymatic biofuel cell is a type of fuel cell where enzymatic catalysts are used to convert the chemical energy of biological fuels into electricity, instead of the metallic catalysts commonly used in fuel cells. The high reaction selectivity of enzymes results in unique advantages, including the possible power generation from biofluids such as juices and bloods without purification. For example, Dong et al. have reported the straight energy-harvesting from natural fruit juices. Furthermore, Mano et al. have demonstrated the direct power generation in a grape berry using a glucose oxidase (GOD)-modified fine anode and a bilirubin oxidase (BOD)-modified fine cathode; both are inserted into a peeled grape.

In the present investigation, we attempt to develop a miniature insertion device for energy-harvesting from living organisms without any pretreatments [1]. Such device entails the following considerations. (1) Natural organisms are generally covered by a skin. (2) Oxygen in organism is limited to a lower concentration than sugars. (3) Biofluids contain reaction inhibitors for cathodic enzymes, such as ascorbic acid and urate. (4) Blood will form a clot on the surface of inserts. In order to address these considerations, our present device has been designed as shown in Figure 1, which consists of a needle bioanode for oxidation of fructose or glucose inside living organisms (Figure 1b), and a carbon paper-based gas-diffusion biocathode for reduction of the abundant oxygen in the ambient air (Figure 1c). This anode and cathode are assembled using an ion-conducting agarose hydrogel as the inner matrix. This novel structural design allows insertion into the organisms even through tough skins, and protects the cathode from reaction inhibitors present in the biofluids.
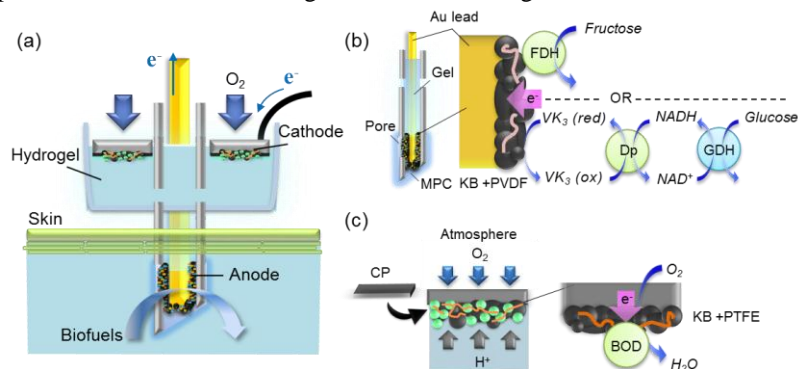

Fig. 1 (a) Biofuel cell structure. (b) needle bioanode. (c) gas-diffusion biocathode.

Results and Discussion
Performance of gas-diffusion biocathodes

Figure 2a shows cyclic voltammograms of the gas-diffusion cathode at 10 mV/s. The carbon paper-based biocathodes were put on an oxygenic pH 7.0 buffer solution so as to contact with the solution by the BOD-modified face (thin solid plot). The reduction current density reaches -0.5 mA cm^{-2} at 0 V, a value of which is twice that of a biocathode that was entirely immersed in the solution (broken plot). Such superior performance of a gas-diffusion cathode originates in the efficient supply of oxygen from the ambient air through the carbon paper. The oxygen solubility in water is limited to ~0.3 mM and its diffusion coefficient is also small (2×10^{-5} cm^2 s^{-1}). In contrast, these values in air are orders of magni-
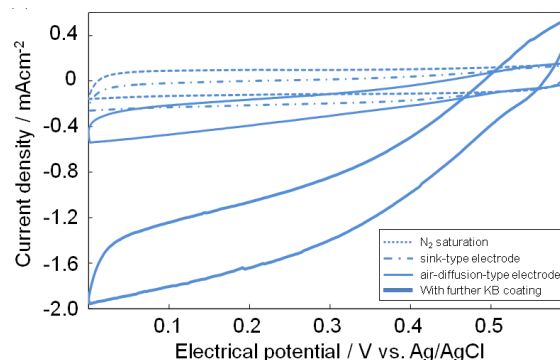

Fig. 2 CV of O2 reduction at BOD/KB (sink- and diffusion-type) and KB/BOD/KB (diffusion-type) electrode at 10 mV S^{-1}.

tude larger, ca. 10 mM and 2×10^{-1} cm^2 s^{-1}. The performance of an O$_2$-diffusion biocathode can be further improved by modifying the three-phase interface, consisting of the current collecting solid phase (enzyme-modified electrode), the electrolyte solution liquid phase, and the gas phase for oxygen supply. For example, an additional coating of hydrophobic KB onto the BOD-modified face of the biocathode was effective in controlling excess penetration of liquid, which led to the fourfold performance up to ca. -2 mA cm^{-2} at 0 V (bold plot in Fig. 2a). This improved cathode will contribute to the miniaturization of the biofuel cell assembly as described later. Because the BOD enzyme shows activity over a wide pH range (pH 4 ~ pH 7), cathode performance of the same quality was observed even under acidic pH 5.0 conditions, as shown in Supplementary Figure S2. Therefore, the present O$_2$-diffusion BOD cathode is applicable to experiments both of a grape with pH 5.0 gel and of a rabbit vein with pH 7.0 gel.

Power generation from raw grapes

A biofuel cell device for fruits was constructed using the O$_2$-diffusion BOD cathode and the needle bioanode modified with FDH for fructose oxidation. These electrodes were mounted in a PDMS chamber filled with agarose hydrogel prepared with 750 mM McIlvaine buffer solution (pH 5.0). The device was inserted to a grape as shown in Figure 3a, and at first the performance of needle anode was evaluated using an externally inserted Ag/AgCl reference electrode (Figure 3b). The needle anode without the side pore shows oxidation current density of 0.35 mA cm^{-2} (current: 20.1 μA , geometric electrode area: 0.057 cm^2) at 0.6 V by oxidation of fructose that penetrated though the needle aperture. By opening the side pores, the anodic performance in a grape was enhanced up to 1.53 mA cm^{-2} (current: 87.6 μA) at 0.6 V.

Figure 3c shows the cell performance using a raw grape at room temperature. The upper panel shows the cell voltage, the anode potential and the cathode potential as functions of the current density, while lower panel shows the cell power density. The device generated 111 μW/cm^{-2} of electrical power (6.3 μW) with a current density of 442 μA/cm^{-2} at 0.25 V (0.23 V anodic potential and 0.48 V cathodic potential vs. Ag/AgCl). The cell performance was strongly dependent on the concentration of the buffer in the agarose gel. For example, the device using 30 mM buffered gel produced only 39 μW/cm^{-2}. Importantly, the total performance could be amplified by connecting an array of needle anodes in parallel; the device with the array of four needle anodes had a fourfold output power, ca. 26.5 μW (Figure 3d).

As the final experiment using grapes, we demonstrate the possibility of monitoring sugar levels in the fruit. A FDH-based needle biofuel cell was combined with an LED device consisting of a charge pump IC, a 1 μF ceramic capacitor and a red LED. As we reported previously [2], the blink interval of the LED is inversely proportional to the power of the biofuel cell, which is roughly proportional to the concentration of the biofuel. In practice, the LED blinks at a higher frequency with an increase in the fructose concentration (Figure 3e), and the concentration of fructose within the grape was estimated to be roughly 20-40 mM. In fact, the power curve in the 35 mM fructose experimental solution was almost identical with that in the grape, as shown in Supplementary Figure S5.
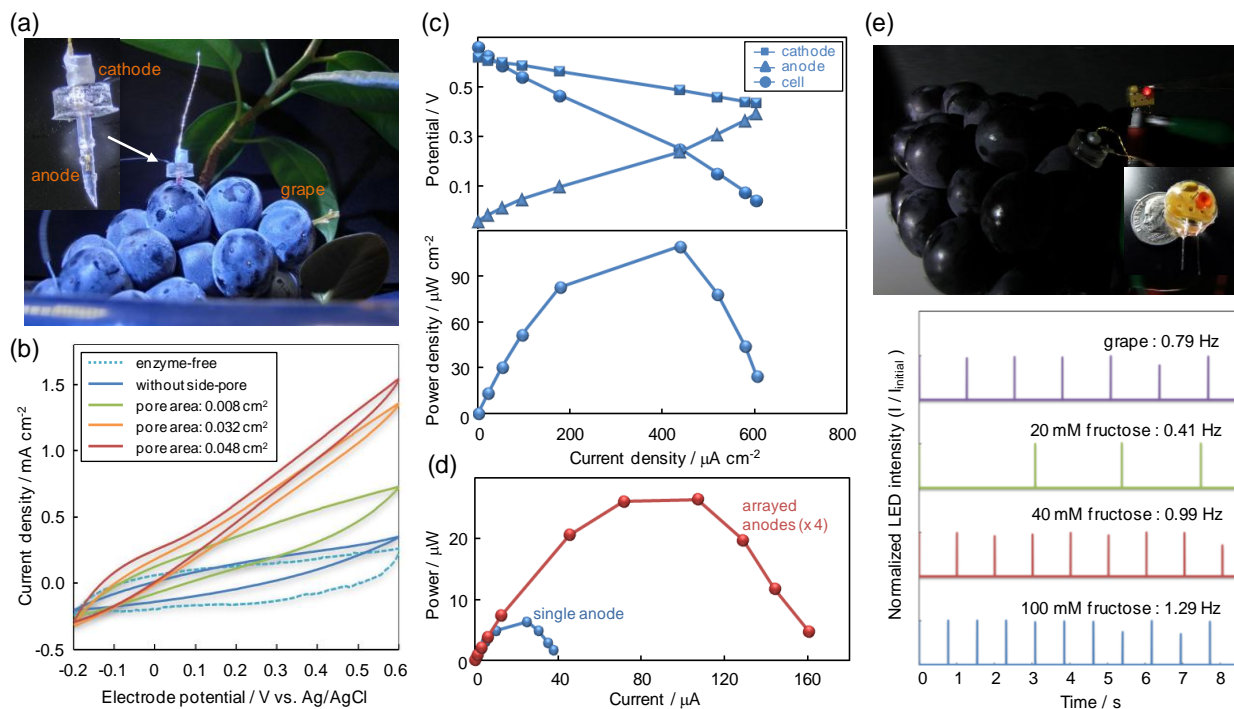

Fig. 3 (a) Photograph of the assembled biofuel cell inserted into a grape. (b) Cyclic voltammograms of FDH-modified needle anodes at 10 mV s^{-1} in a raw grape. Side-pores were drilled in the wall of the needle (pore area: 0.008, 0.032 and 0.048 cm^2). (c) (upper panel) Polarization curve of the biofuel cell in the grape at room temperature. The cell voltage (●), the cathode potential vs. Ag/AgCl (■) and the anode potential vs. Ag/AgCl (▲) are plotted as function of the current density. (lower panel) Variation of the power density with the current density, normalized by the geometric area of anode. (d) Power output of the biofuel cell using single anode and arrayed anodes (× 4). (e) Monitoring of sugar level in a raw grape with a self-powered fructose-sensing devices. The device consists of the biofuel cell and an LED system, whose blink interval is correlated with the fructose concentration.

Power generation from a rabbit ear vein

Power generation from blood requires biocompatibility in order to prevent the formation of a blood clot on the electrode surface. To make the electrode biocompatible, a coating with 2-methacryloyloxyethyl phosphoryl-choline (MPC)-polymer is effective. In fact, as shown in Figure 4a, an MPC-treated substrate resisted blood clotting even after immersion in blood for 2 hours. Figure 4b shows cyclic voltammograms of the MPC-coated or uncoated GDH/PLL-NAD+/Dp/PLL-VK$_3$ needle anodes in PBS solution containing 10 mM glucose. Both reached to current density of 1.5 mA cm^{-2} (current: 5.0 μA, geometric electrode area: 0.0032 cm^2) at 0.5 V with a CV shape corresponding to glucose oxidation at GDH/PLL-NAD+/Dp/PLL-VK$_3$ electrode [3]. These results indicate that the MPC coating serves as a bioinert layer without significant disturbance for the glucose transport to the enzyme electrodes inside the needle.

By assembling this needle anode with the O$_2$-diffusion BOD cathode, the fuel cell performance was evaluated by inserting it into a vein of a rabbit ear (Figure 4c). Figure 4d shows the typical current-voltage and current-power curves obtained by changing the external resistance (10 kΩ~2 MΩ). The open-circuit voltage of the cell was 0.81 V, which is similar to the difference between the potentials at which glucose oxidation and oxygen reduction start to occur in cyclic voltammograms (0.55 V in Fig. 2a and -0.30 V in Fig. 4b, respectively). The performance of the anode is reflected in the maximum current density of the cell, 466 μA cm^{-2}. The power density for this cell reached 130 μW cm^{-2} at 0.56 V, while the device without a MPC coating showed ~40% loss in power. Since the MPC coating has no effect on the electrode process (Fig. 4b), the lowered performance would be due to the formation of blood clots. In fact, after the insertion experiment, the formation of some biofilms were observed only on the needle anodes without the MPC coating. Probably, the blood clots would interfere the transport of glucose into the needle, while also the effects on the cell resistance and kinetics are not improbable.
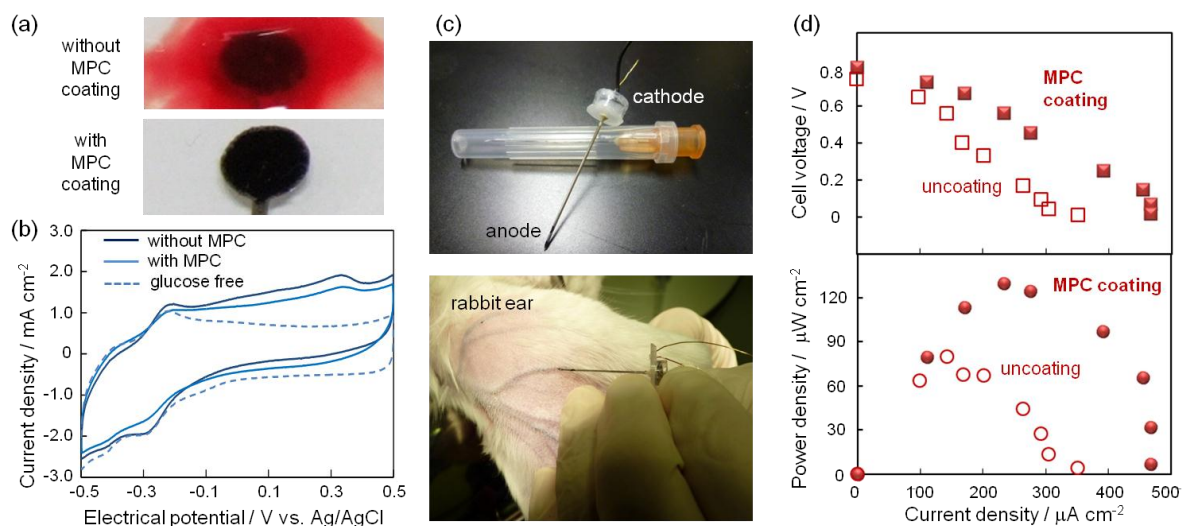

Fig. 4 (a) Photographs of electrode substrates with (and without) a MPC-coating, taken after soaking in withdrawn blood for 2 hours. (b) Cyclic voltammograms of bare and MPC-coated needle bioanodes at 10 mV s^{-1} in phosphate buffer containing 10 mM glucose. The control experiment (glucose-free) is also shown. (c) Photographs of the assembled biofuel cell for power generation from a rabbit vein. (d) Performance of the biofuel cells using a needle anode, with and without MPC-coating, taken by inserting the needle anodes into a rabbit vein. The current density and power density were estimated by geometric area of anode.

CONCLUSION

We assembled a biofuel cell with a needle anode and gas-diffusion cathode, as a practical form of cell for direct power generation from natural organisms with skins. The results presented here include techniques to improve the performance of the devices. (1) A gas-diffusion cathode treated to be hydrophobic showed higher activity. (2) Incorporating side-pores into the needles effectively enhanced the supply of biofluids to the inner anodes. (3) Modification of the needle tip with MPC polymer was required to obtain comparatively stable power from bloods. We also demonstrated that an array of needle anodes led to an increase in the output power (Fig. 3d). In the near future, a finer array of microscopic needle anodes will be fabricated by advanced micro-/nano-techniques for realizing a minimally-invasive patchable biofuel cell system.

REFERENCES

[1] Miyake, T., Haneda, K., Nagai, N., Yatagawa, Y., Onami, H., Yoshino, S., Abe, T., Nishizawa, M. "Enzymatic biofuel cells designed for direct power generation from biofluids in living organisms." *Energy. Environ. Sci.* **4**, 5008 (2011).
[2] Miyake, T., Yoshino, S., Yamada, T., Hata, K., Nishizawa, M. "Self-regulating enzyme-nanotube ensemble films and their application as flexible electrodes for biofuel cells." *J. Am. Chem. Soc.* **133**(13), 5129-34 (2011).
[3] Miyake, T., Oike, M., Yoshino, S., Yatagawa, Y., Haneda, K., Nishizawa, M. "Automatic, sequential power generation for prolonging the net lifetime of a miniature biofuel cell stack." ***Lab. chip,*** 10(19), 2574-8 (2010).
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