SEQUENTIAL POWER GENERATION FOR PLOLONGING THE NET LIFETIME OF A MINIATURE BIOFUEL CELL STACK

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ABSTRACT

We have developed a sequential power generation system, in which each of stacked enzyme fuel cells was designed to be exposed sequentially to fuel solution by automatically switched fuel flow. The time when the cover was opened by attraction with an external magnet, thereby activating the following cell, was adjustable from a few hours to a few weeks by controlling the weight ratio of Fe_3O_4 in the covers and the molecular weight of PLGA. By using sequential power generation in this way, the net lifetime of the stack has been extended as compared with that of a single biofuel cell.

KEYWORDS: miniature biofuel cell, lifetime, power generation, bioelectrode, glucose/O_2

INTRODUCTION

Enzyme-based biofuel cells are energy conversion devices that use biocatalysts to convert the chemical energy of a fuel to electric energy. Biofuel cells enable the direct utilization of various biological fuels such as glucose and fructose without purification, and allow operation under mild conditions (ambient temperature, atmospheric pressure and nearly neutral pH); thus enzyme-based biofuel cells are promising as mobile power sources supplying sustainable electrical energy. In recent years, the output current and power of enzymatic biofuel cells have been dramatically improved up to mA and mW level that motivates practical applications[1]. However, for further expansion of the possible applications of enzymatic biofuel cells, their stability needs to be improved. Here, we have developed a sequential power generation (SPG) system to extend the net lifetime of stacked miniature biofuel cell, in which the power of each cell decreases gradually but the net output can be maintained by automatic replacement with a newly activated cell. We describe the development of the SPG system, and demonstrate the feasibility of the system using our enzyme electrodes.

EXPERIMENTAL

Experimental Design. Figure 1 shows a schematic of the SPG system. It is composed of polydimethylsioxane (PDMS) microchannels and electrodes fabricated on three glass slides that are layered and connected through holes covered initially by magnetic chips and a poly(lactic-co-glycolic acid (PLGA) film. When a fuel solution flows into the microchannel of the top stage of the stack, the cell starts generating electric power, and at the same time the PLGA polymer used as glue starts dissolving. At a specified time interval, when the glue has sufficiently dissolved, the magnetic cover is lifted up by the neodymium magnet set in the roof of the microchannel. After opening the magnetic chip cover, the fuel solution then flows down into the second stage microchannel to activate and operate the fresh cell in the second stage. By repeating this cycle, the total power output is maintained at a stable level, and also the net lifetime of the stacked biofuel cells is extended.

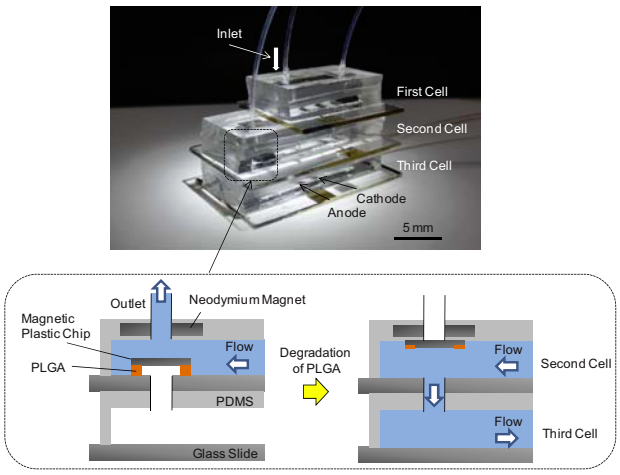

Figure 1. Photograph and schematic of the sequential electric power generation system for prolonging the net lifetime of a miniature biofuel cells stack.

Fabrication of Enzyme-Modified Microelectrodes. Enzyme immobilization is carried out according to the protocol previously described [2-3]. Unless otherwise indicated, the enzyme solutions for anode modification were prepared using 50 mM phosphate buffered solution (pH 7.0). An 8 µl vitamine-K_3-modified poly-L-lysine (PLL-VK_3) solution (4.83 mM) is mixed with a 2 µl diaphorase (Dp) solution (14 µg/µl) and 1 µl of ketjenblack (KB) dispersed in water (13 mg/ml). A 2.5 µl aliquot of the mixed solution is put on a gold electrode (surface area, 0.04 cm^2) and dried in air. To create the enzymatic bilayer, the surface of a PLL-VK_3/Dp/KB electrode is coated with 2.4 µl of a solution containing a 40 µg/µl glucose dehydrogenase (GDH) solution and a 10 mg/ml PLL solution. For cathode modification, an 8 µl PLL solution (10 mg/ml) is mixed with a 2 µl bilirubin oxidase (BOD) solution (20 mg/ml) and 1 µl of KB dispersed water (13 mg/ml). A 2.5 µl aliquot of the mixed solution is put on a gold electrode two times.

Formation of Automatically-Opening Cover. The through-holes between the stacked cells are covered by magnetic chips made via the three-step process: (1) preparation of magnetic plastic chips, (2) preparation of PLGA film having a hole and (3) bonding with pressurized CO_2 gas the magnetic chip and the electrode substrates using the PLGA film. Iron oxide powder (particle diameter: 1 µm, High Purify Chemicals) is mixed with an epoxy resin (Araldite stan-

dard, NICHIBAN) at 10, 30 or 60 wt%. The mixed resin is poured into the PDMS mold (size: 4.5×4.5 mm, thickness: 1 mm), and dried at 75 ºC for 1 day to prepare a magnetic plastic chip. A PLGA5005 (Mw. 5000, WAKO), a PLGA5010 (Mw. 10000, WAKO) or a PLGA5015 (Mw. 15000, WAKO) polymer is mixed with 1,1,1,3,3,3-hexafluoro-2-propanol (WAKO) at a concentration of 0.2 g/ml. The appropriate PLGA solution is poured into the PDMS mold (outer size: 4.5×4.5 mm, hole size: 1.5×1.5 mm, thickness: 50 μm), and dried at room temperature in air for 1 day and at 40 ºC in vacuum for 2 days to form a PLGA film that is 50 μm thick. The PLGA film and the magnetic chip are superposed over a hole of the glass slide decorated with electrodes, followed by bonding in a chamber of CO_2 gas under different condition of pressure (0.5 – 4 MPa) and treatment time (1 – 3 h).

Measurement of Enzyme Activity on Electrodes. The enzyme activity after CO_2 exposure is evaluated by the oxidation current of glucose, measured by a three electrode system (BSA, 730C electrochemical analyzer) using an Ag/AgCl-ink-modified reference electrode and a gold counter electrode. The measurement is carried out in phosphate buffered solution containing 50 mM glucose and 1 mM NAD^+.

Measurement of Biofuel Cells Performance. To evaluate the performance of the biofuel cell system, we constructed microfluidic fuel cells from a PDMS microchannel (channel height: 2 mm, width: 7 mm, length: 20 mm) and glass slid including a GDH/Dp/PLL-VK_3/KB anode and BOD/KB cathode as shown in Figure 1. Although the performance of microfluidic biofuel cells is known to depend on channel structure and electrode arrangement in the flow channel, we used a simple, basic type of microfluidic biofuel cell in the present study. The system's performance is evaluated with the electrochemical analyzer in phosphate buffered solution containing 50 mM glucose and 1 mM NAD^+. The fuel solution is flowed into the microchannel at 0.8 mL/min with a peristaltic pump (SJ-1220-2, ATTO). The current and the power are evaluated from the cell voltage obtained by connecting the external resistance (100 kΩ), which is the condition for the maximum power as reported by previous work [2-3].

Results and Discussion

The enzymes modified on the electrode are exposed to the pressurized CO_2 during the process of PLGA bonding, the mechanism of which is based on CO_2-enhanced chain entanglement nea r the polymer surfaces. Tight adhesion of magnetic chip via the PLGA film was achieved by applying enough CO_2 pressure for a long enough exposure time (typically more than 1 h). Although CO_2 gas is inert towards biomolecules and cells in general, because the bonding is carried out under high pressure and for long time in our experiments, we first checked the enzyme activity after exposure to various CO_2 gas conditions. Figure 2 (a) shows the activity of GDH/PLL-VK3/Dp/KB electrodes for glucose oxidation after 1 h exposure to different pressure of CO_2. A gradual decrease in the enzyme activity appeared at pressures higher than 2 MPa. It was also found the stable enzyme activity at 1 MPa had been maintained even after a 3 h exposure (Figure 2 (b)). Based on these data, we typically used the condition of 1 MPa CO_2 and 1 h exposure time for the PLGA bonding. This method using CO_2 as solvent does not involve heating or immersion in organic solvent; thus deactivation of proteins and cells are prevented. After processing, CO_2 removal from the polymer is accomplished by depressurization of the system. PLGA polymers are known to adhere well to various solid surfaces such as SU-8, glass, and plastics. Therefore, we expected a PLGA film to function well as glue for packaging protein-including devices.

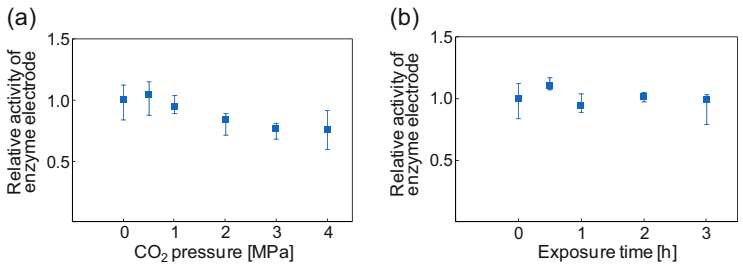

Figure 2. Relative activity of bioanodes versus (a) the CO_2 pressure applied for 1 h and (b) the time exposed to 1 MPa CO_2.

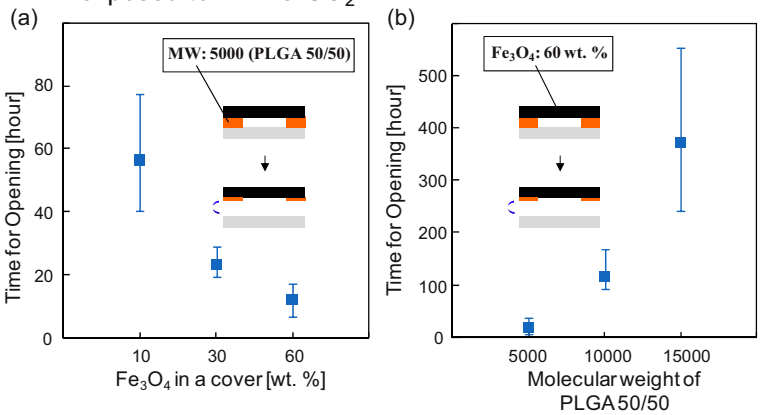

Figure 3. The time for opening of a cell with (a) the ratio of Fe_3O_4 to epoxy in the cover, and (b) the molecular weight of PLGA as parameters. The measurements were performed three times in phosphate buffer (pH 7) containing 50 mM glucose, 5 mM NAD^+ and 0.1 M NaCl.

We desire to set the time interval for the automatic opening of the cover to some specific point, for example, until the power has decreased by half. However, the lifetime of a single biofuel cell depends on the electrode structure and the construction materials, as well as the operation mode (external loads). Therefore we devised a method to control the opening interval using the ratio of Fe_3O_4 to epoxy in the cover and molecular weight of PLGA as parameters. Figure 3 shows the time required to open the magnetic chips glued by PLGA films. The magnetic chip was lifted up to the neodymium magnet when the adhesion force with the PLGA film became weaker than magnetic attraction from the magnet

as a result of degradation of the PLGA film by the fuel solution. The storage time increased with the molecular weight of the PLGA due to longer degradation time (Figure 3(a)) and decreased with for a lower ratio of Fe_3O_4 due to a weaker magnetic attraction (Figure 3(b)). These data suggest that, by controlling such conditions, the timing of the automatic opening can be roughly set from a few hours to a few weeks. In the case of our biofuel cell, the time taken for the power to halve was typically 15 hours, therefore we selected the suitable conditions for the cover opening after this delay to be 60 wt.% Fe_3O_4 and 5000 MW PLGA.

Figure 4 (a) - (c) shows photographs of a SPG system, in which three cells are stacked and connected in parallel with 100 kΩ load, at (a) 0 h, (b) 20 h, and 30 h after introducing fuel solution into the first stage of the stack. During the initial 15 hours, since the hole between the first and the second stage was covered by a magnetic chip, the fuel solution flows out from the outlet of the first stage (Figure 4 (a)). After the cover opens, the magnetic chip moved up to the neodymium magnet at the roof of the channel and closed the outlet. Then the solution flowed down into the second stage and out from the second outlet (Figure 4 (b)). The next switching of the flow to the third stage proceeded in the same manner (Figure 4 (c)). Figure 4(d) shows the time course of the power produced during these switches of the fuel solution (blue curve). The magnetic chips were automatically moved at a time when the power halved (about 15 hours), and the subsequent power generation by the freshly fueled cell was superimposed on the decay of the old cells at this interval. In general, an electrical cell cannot be connected in parallel to a cell of different voltage because of reverse reaction at each electrodes. In contrast, since enzyme-modified electrodes have intrinsic reaction selectivity and show resistance to reverse reaction, enzyme-based biofuel cells enable parallel connection between different voltage cells. For comparison, the dotted black curve in Figure 4 (d) shows the power decay observed by a single biofuel cell without the stack. The similar profile of power decay was shown by also the stacked cells without the magnetic covers (3 times larger electrodes). In contrasts, in the case of our SPG system, a power level of 2 μW ± 1 μW was maintained for long periods (blue curve in Figure 4 (d)), about 3 fold compared with single power generation.

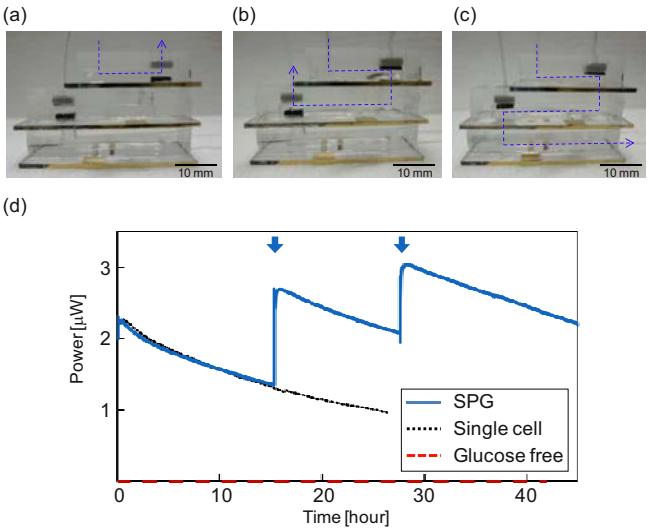

Figure 4. Photographs of the biofuel cells stack at (a) 0 h, (b) 20 h, (c) 30 h after introducing fuel solution into the first stage of the stack. (d) Time course of the power measured in 50 mM glucose, 5 mM NAD^+ and 0.1 M NaCl-containing phosphate buffer solution (pH 7). The cells were connected in parallel with a 100 kΩ load. The lifetime of SPG system (blue line) is prolonged as compared with a single biofuel cell (dashed-line).

CONCLUSION

We have developed a sequential power generation system for prolonging the net lifetime of a miniature biofuel cell stack, and demonstrated the expected performance using our bioelectrodes as an example. Since the time interval for the switching of fuel flow can be adjusted from a few hours to a few weeks by the Fe_3O_4 content in the cover chip and the molecular weight of PLGA sealant, our system can be applicable to other biofuel cells, the lifetime of which are different from the preset case. This concept of prolonging the net lifetime by stepwise activation would be effective to various other kinds of biodevices, including enzyme-based biosensors.

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