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BioCapacitor—A novel category of biosensor

Takuya Hanashi[a,1], Tomohiko Yamazaki[b,1], Wakako Tsugawa[a], Stefano Ferri[a], Daisuke Nakayama[a], Masamitsu Tomiyama[c], Kazunori Ikebukuro[a], Koji Sode[a,d,*]

[a] Department of Biotechnology, Graduate School of Engineering, Tokyo University of Agriculture & Technology, 2-24-16 Nakamachi, Koganei, Tokyo 184-8588, Japan
[b] Biomaterials center/International Center for Materials Nanoarchitectonics, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
[c] Genetic Diversity Department, Applied Microbiology Laboratory, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
[d] Ultizyme International Ltd., 1-13-16 Minami, Meguro, Tokyo 152-0013, Japan

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ABSTRACT

This research reports on the development of an innovative biosensor, known as BioCapacitor, in which biological recognition elements are combined with a capacitor functioning as the transducer. The analytical procedure of the BioCapacitor is based on the following principle: a biocatalyst, acting as a biological recognition element, oxidizes or reduces the analyte to generate electric power, which is then charged into a capacitor via a charge pump circuit (switched capacitor regulator) until the capacitors attains full capacity. Since the charging rate of the capacitor depends on the biocatalytic reaction of the analyte, the analyte concentration can be determined by monitoring the time/frequency required for the charge/discharge cycle of the BioCapacitor via a charge pump circuit. As a representative model, we constructed a BioCapacitor composed of FAD-dependent glucose dehydrogenase (FADGDH) as the anodic catalyst, and attempted a glucose measurement. Charge/discharge frequency of the BioCapacitor increased with the increasing glucose concentration, exhibiting good correlation with glucose concentration. We have also constructed a wireless sensing system using the BioCapacitor combined with an infrared light emitting diode (IRLED), an IR phototransistor system. In the presence of glucose, the IRLED signal was observed due to the discharge of the BioCapacitor and detected by an IR phototransistor in a wireless receiver. Therefore, a BioCapacitor employing FADGDH as its anodic catalyst can be operated as a self-powered enzyme sensor.

1. Introduction

A biosensor is defined as an analytical system composed of a sensing element of biological origin combined with a physicochemical transducer. Sensing elements can be biocatalytic molecules, such as enzymes and microorganisms, or affinity molecules, such as aptamers and antibodies. Moreover, physicochemical transducers such as electrochemical, optical, calorimetric or piezoelectric transducers are used to convert the biological recognition event into a processable signal. While a variety of combinations are feasible for the composition of biosensors, among the most successful and practical biosensors are those that are composed of electrochemical devices such as transducers. Glass electrodes, carbon screen-printed electrodes and rare metal electrodes are employed based on the amperometric measurement principle. This particular principle can be used for measurement for the redox reaction of the redox species liberated/consumed during the enzyme reaction.

Biofuel cells utilize biocatalysts such as enzymes and microorganisms as electrocatalysts. Because of their environmental safety and sustainability, biofuel cells have been recognized as a clean energy source. These cells can be utilized not only for the conversion of biomass, but also as alternative energy sources for future implantable devices, such as implantable glucose sensors in artificial pancreas (Barton et al., 2004; Fischback et al., 2006; Mano and Heller, 2003; Mano et al., 2004). Recently, the biofuel cell configuration is also been recognized as a type of biosensor since the output voltage and current signals are dependent upon the fuel concentration (Kakehi et al., 2007; Katz et al., 2001). Instead of inorganic or organic catalysts, in biofuel cells, either enzymes or whole cell organisms are used as biocatalysts that generate the power directly from the fuel substrates such as glucose and ethanol (Bullen et al., 2006). Recently, we reported on the construction of direct electron transfer-type enzyme fuel cell that utilizes an FAD-dependent glucose dehyrogenase (FADGDH) as an anode enzyme, and its application in the construction of a continuous glucose

* Corresponding author at: Department of Biotechnology, Graduate School of Engineering, Tokyo University of Agriculture & Technology, 2-24-16 Nakamachi, Koganei, Tokyo 184-8588, Japan. Tel.: +81 42 388 7027; fax: +81 42 388 7027.
E-mail address: sode@cc.tuat.ac.jp (K. Sode).
[1] Equal contribution.

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monitoring (CGM) system, the fuel cell-type glucose sensor (Kakehi et al., 2007). Since the FADGDH showed direct electron transfer from the catalytic centre of the enzyme to the electrode via electron transfer subunit without the need for a synthetic electron mediator, the fuel cells employing FADGDH as an anodic biocatalyst did not need their own compartment. The open circuit voltages of the fuel cells were measured for checking their glucose levels since the open circuit voltage value was not correlated with the electrode area. Therefore, the fuel cell-type glucose sensor has the potential for miniaturization due to both its simple structure without compartment and its measurement principle. Furthermore, miniaturization of this system is more easily accomplished than that of the amperometric sensor system because a fuel cell-type sensor does not require potentiostat circuit.

One inherent problem that exists with respect to the principle of biofuel cells, particularly when applying biofuel cells for biosensing, is the low power of a single biofuel cell. The voltage of the biofuel cell is theoretically limited by the redox potential of cofactors and/or mediators employed in the anode and cathode. The generated voltage from a single-celled biofuel cell is very low and is not sufficient for operating any device. In order to increase power, biofuel cells are arranged in parallel to one another to maximize the surface area. Alternatively, biofuel cells can also be arranged in series to obtain high voltage. However, both these approaches are not practical when considering biofuel cell application in a biosensor. The detection of a signal generated from the single-celled fuel cell-based biosensor remains challenging.

In this paper, we report the development of an innovative biosensor, known as BioCapacitor, in which biological recognition elements are combined with a capacitor functioning as the transducer. Utilizing a capacitor as the transducer is a novel approach in the configuration of a biosensor, an advance that has not been reported previously. The analytical procedure of the BioCapacitor is based on the following principle as shown in Fig. 1(a): biological recognition elements, particularly biocatalysts, generate electric power by oxidation or reduction of the analyte, which is subsequently charged into the capacitor via a charge pump circuit until the capacitor attains maximum capacity. The rate of charging the capacitor corresponds to the function of the biocatalytic reaction of the analyte. Thus, by monitoring the time/frequency required for the charge/discharge cycle, the analyte concentration can be determined. Furthermore, we demonstrate a standard-alone and self-empowered wireless glucose-sensing system constructed with a BioCapacitor, an infrared light-emitting diode (IRLED), and IR receiver.

2. Materials and methods

2.1. Materials for BioCapacitor construction

Recombinant FADGDH complex was prepared using the expression vector pTrc99A (Pharmacia, Uppsala, Sweden), containing the structural gene for GDH complex (pTrc$\gamma\alpha\beta$), and pAYCYC184, containing the structural genes for ccm (pEC86), and was transferred into an *E. coli* strain BL21(DE3) and cultivated as described previously (Tsuya et al., 2006). Carbon cloth, SYCC18-00000-00, was provided from Toho Tenax (Tokyo, Japan). Ketjen black, ECP600JD, was purchased from Mitsubishi Chemical (Tokyo, Japan). Platinum-supported carbon (Pt/C), TEC10E50E, (Pt (wt.%) is 50) was purchased from Tanaka Kikinzoku Kogyo (Tokyo, Japan). Glutaraldehyde solution (25%, w/v) was purchased from Wako Pure Chemicals (Osaka, Japan). Nafion perfluorinated resin solution and poly(dimethyl-siloxane) (PDMS) were purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals were of reagent grade.

2.2. Construction of BioCapacitor

The Ketjen black carbon inks and Pt/C ink were made as follows: 100 mg of Ketjen black or Pt/C was mixed with 400 μL ultra pure water and 1.08 mL of 5% (w/v) nafion solution. After agitating the solutions at room temperature for 3 h using a vortex, the mixture was incubated for 3 days at 4 °C.

For construction of the anode, 240 μL of FADGDH solution (4.2 U/μL) was mixed with 60 μL of Ketjen black ink solution and 60 μL of 100 mM potassium phosphate buffer (PPB) (pH 7.0). The mixture (300 μL) was homogeneously deposited onto a 6 cm^2 of carbon cloth and air-dried at 4 °C for 3 h to allow the mixture to coat the carbon cloth. The carbon cloth electrode was immersed in 1% (w/v) glutaraldehyde solution for 30 min and then washed with 10 mM of Tris–HCl buffer (pH 7.0). The anode electrode was stored in 100 mM PPB at 4 °C until use.

For construction of the cathode, Pt/C was used as a cathode catalyst. The mixture of Pt/C ink (60 μL) and 100 mM PPB (pH 7.0) (300 μL) was homogeneously deposited onto a 6 cm^2 of carbon cloth and air-dried at 4 °C for 3 h. Then, 300 μL of 3.0% (w/v) PDMS was homogeneously deposited on the carbon cloth.

As shown in Fig. 1(b), the BioCapacitor circuit was designed. The capacitor and ultra-low voltage operation charge pump IC (S-882Z18-M5T1G Seiko Instruments, Chiba, Japan) and yellow LED (OSYL5161A-QR, OptoSupply, Hong Kong, China) were connected to the enzyme fuel cells. For the construction of a wireless sensor based on a BioCapacitor circuit, the IRLED (TLN103A, Toshiba, Tokyo, Japan) was connected to the BioCapacitor circuit instead of a yellow LED and the receiver circuit with an IR phototransistor (SPS-135C, Sanyo, Osaka, Japan) was designed as illustrated in Fig. 2. The distance between the IRLED and the IR phototransistor was 5 mm.

2.3. Operational condition of BioCapacitor

Glucose was added into the fuel cell for measurements and the fuel cell was then exposed to the open air. The anode and the cathode electrodes were attached to a 10-mL water-jacket cell (model VC-2; Bioanalytical Systems, West Lafayette, IN). In the enzyme fuel cell, 100 mM PPB (pH 7.0) was stirred at 250 rpm with a magnetic stirrer at 25 °C. In the presence of glucose, both the closed-circuit voltage generated by the fuel cell and the output of the capacitor was measured by the HZ3000 electrochemical analyzer (Hokuto Denko, Tokyo, Japan). The frequency of the charge/discharge cycles was calculated from the electric power change in the circuit.

3. Results

3.1. Construction and evaluation of the BioCapacitor

As a representative configuration, we constructed a BioCapacitor composed of FADGDH as the biological molecule and attempted a glucose measurement. Fig. 1(a) shows the basic configuration of the BioCapacitor. It is composed of an enzyme fuel cell connected to the capacitor via a charge pump circuit. In order to increase the voltage, the biofuel cell was connected to a charge pump circuit. Additionally, to gain enough power and signals to operate the electrical devices, the generated electricity from the biofuel cell was charged into the capacitor.

Charge/discharge switching is operated by the charge pump circuit (Fig. 1(b)). In this study, we used a charge pump IC, model S-882Z18-M5T1G to enhance the potential. The charge pump IC needed 250 mV to drive its oscillation circuit. Therefore, the minimum input potential required to drive the BioCapacitor system was 0.3 V. In the charge pump circuit, when a power is input from

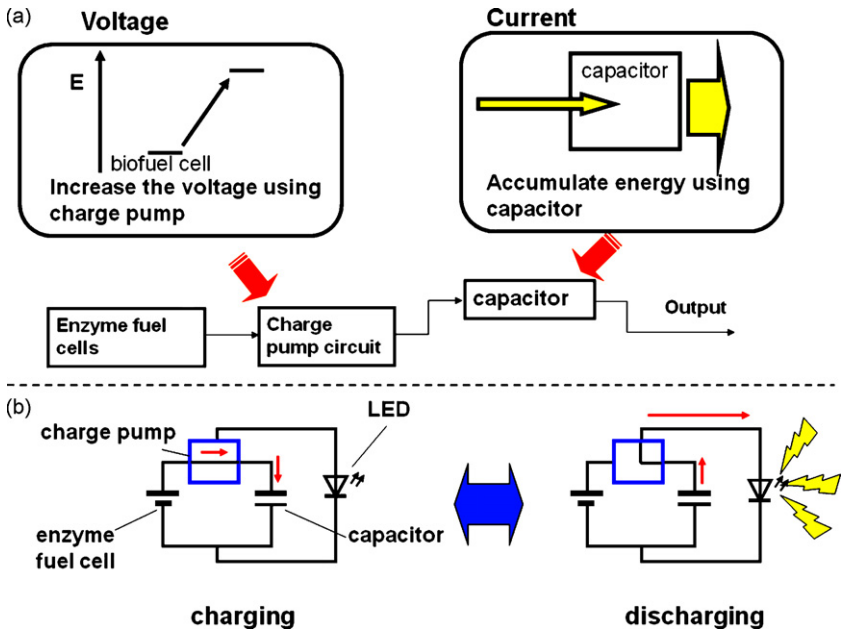

Fig. 1. Schematic diagram of the BioCapacitor (a) and its analytical operation principle (b). Resulting from the driven charge pump circuit, the closed-circuit voltage was converted into stepped-up electric power and charged into the capacitor until its potential reached 1.8 V. Then, the charge pump circuit switch discharged the capacitor and the current flowed and encountered resistance.

the biofuel cells, the charge pump circuit is driven. The power is then converted to a stepped-up electric power and charged to the capacitor until it attains a potential of 1.8 V. When the potential of the capacitor reaches 1.8 V, the charge pump circuit switch discharges the capacitor until the potential is reduced to 1.4 V and the power is charged to the capacitor again. Therefore, electrical devices operated below 1.8 V generated signals that were observed in correlation with the charge/discharge frequency of the capacitor. Throughout the utilization of the charge pump circuit and capacitor, the generation of high voltage along with high current sufficient enough to operate electrical devices was achieved without changing the design and construction of the biofuel cell.

Fig. 3(a) shows the dependence of the closed-circuit voltage on the enzyme fuel cells used in the BioCapacitor. Outputs of the biofuel cell, such as potential, current, and power are dependent on fuel concentration. Therefore, the biofuel cell system can function as a glucose sensor using an enzyme that catalyzes glucose oxidation (Kakehi et al., 2007; Katz et al., 2001). Increased glucose concentration in the enzyme fuel cell resulted in an increased closed-circuit voltage, since the glucose oxidation catalyzed by glucose dehydrogenase immobilized in the anode increased in glucose concentration-dependent manner. The generated electric power correlates to the closed-circuit voltage, which in turn is dependent on glucose concentration. Therefore, the charge/discharge frequency of the capacitor via a charge pump circuit is dependent on both the capacitance of the capacitor and the electric power generated by the enzyme fuel cell, which in turn is dependent on glucose concentration.

We observed the time required for the charge/discharge cycle of the capacitors with differing capacities utilized in the BioCapacitor circuit, as shown in Fig. 4. The charge/discharge cycle was observed 5 times/s with 5 Hz frequency using a 0.47 μF capacitor with 20 mM glucose in the enzyme fuel cell. The frequencies of the charge/discharge cycle decreased with increasing capacity of capacitors. The frequencies of the charge/discharge cycle using 1, 10 and 100 μF capacitors were 2.4, 0.27 and 0.028 Hz, respectively. These results demonstrated that the charge/discharge frequency was a function of the capacitor's capacitance.

We investigated the effect of glucose concentration on charge/discharge frequency in the BioCapacitor circuit, as shown in Fig. 5. The time required to charge the capacitor with 0.1 mM of glucose was 30 s, corresponding to a charge/discharge frequency of 0.033 Hz. The time required to charge the capacitor with 0.43, 1.1 and 7.4 mM glucose were 6.4, 4.6, and 3.6 s, corresponding to a charge/discharge frequency of 0.16, 0.21, and 0.27 Hz, respectively. The frequency of the charge/discharge cycle increased with the increase in glucose concentration. The result indicated that a BioCapacitor utilizing enzyme glucose fuel cells can serve as a glucose sensor.

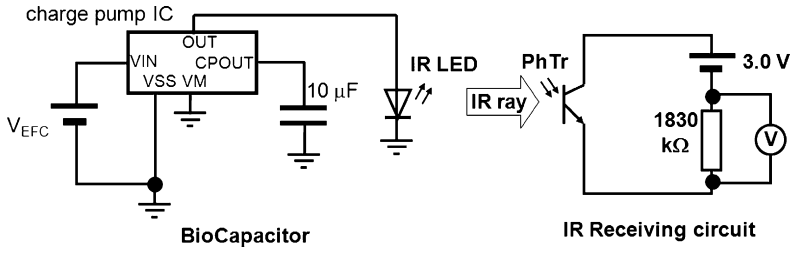

Fig. 2. Circuit block diagram of the BioCapacitor circuit and its application in wireless sensing system.

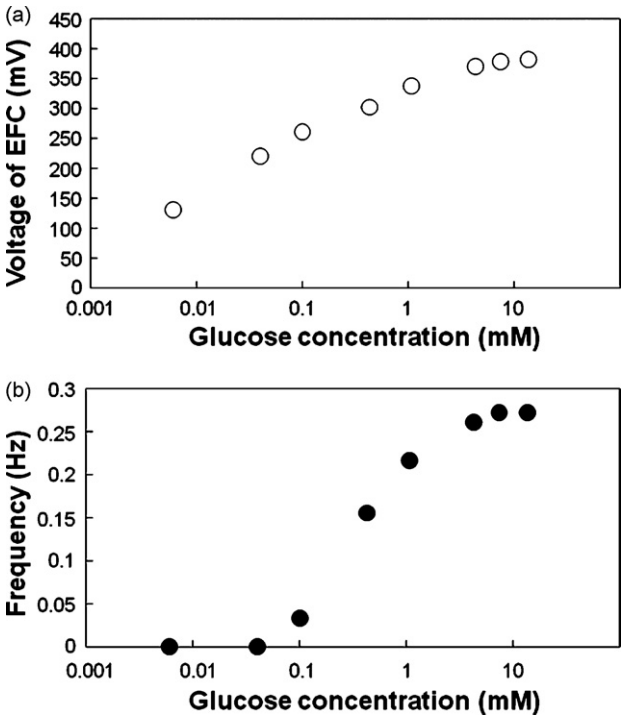

Fig. 3. Dependence of the closed-circuit voltage and the frequency of charge/discharge cycles on glucose concentration. (a) The closed-circuit voltage was measured using a digital multimeter connected in parallel to the enzyme fuel cell (EFC). (b) The potential change in the 10 μF capacitor was measured using the digital multimeter that was connected to the yellow LED in parallel. Then, the frequency of the charge/discharge cycles was calculated.

Based on the observed the frequency of the charge/discharge cycle of BioCapacitor, a calibration curves was plotted. Fig. 3(b) illustrates the correlation between glucose concentration and frequency of the charge/discharge cycle of the BioCapacitor circuit using a 10 μF capacitor. The frequency of the charge/discharge cycle was saturated at glucose concentrations above 7.4 mM. The minimum required glucose level for detection in the BioCapacitor circuit was 0.1 mM glucose. Since the charge pump circuit was not energized when voltage was below 250 mV, frequency was not observed when glucose concentration was below 0.1 mM. The coefficient of variations of sensor signals corresponding to glucose concentration, which were measured with the same BioCapacitor repeatedly, was below 10%.

3.2. BioCapacitor combined with an IRLED and its receiver

Future applications of the system aim to serve, as a device of CGM that will be utilized for the glycemic control of diabetic patients. We attempted to construct a wireless sensing system by combining an IRLED with its receiver, as shown in Fig. 2. The BioCapacitor is able to generate enough power to operate an IRLED intermittently without the supply of any external electricity source and is operated solely by the generated and accumulated electricity in the capacitor as the result of the oxidation of glucose in the sample. The signal generated from the BioCapacitor can be monitored by the IR receiver, which is wirelessly connected to the sensing device.

This system can operate at a potential above 1.4 V. As shown in Fig. 2, an IRLED was connected to the BioCapacitor circuit as a transmitter. In the presence of glucose, the IRLED signal was observed due to the discharge of the BioCapacitor. The IR phototransistor, used as an electronic switch in the receiver circuit, received an IRLED signal and electrical current flowed in the receiver circuit. Input signal of the IR phototransistor coincided with the charge/discharge cycle of a capacitor in the BioCapacitor circuit. Fig. 6 illustrates the correlation between glucose concentration in the sample and the frequency of the IRLED emittance observed either directly by the digital multimeter or at the IR receiver. The frequency of the charge/discharge cycle of BioCapacitor increased with increasing glucose concentration. The minimum detectable glucose

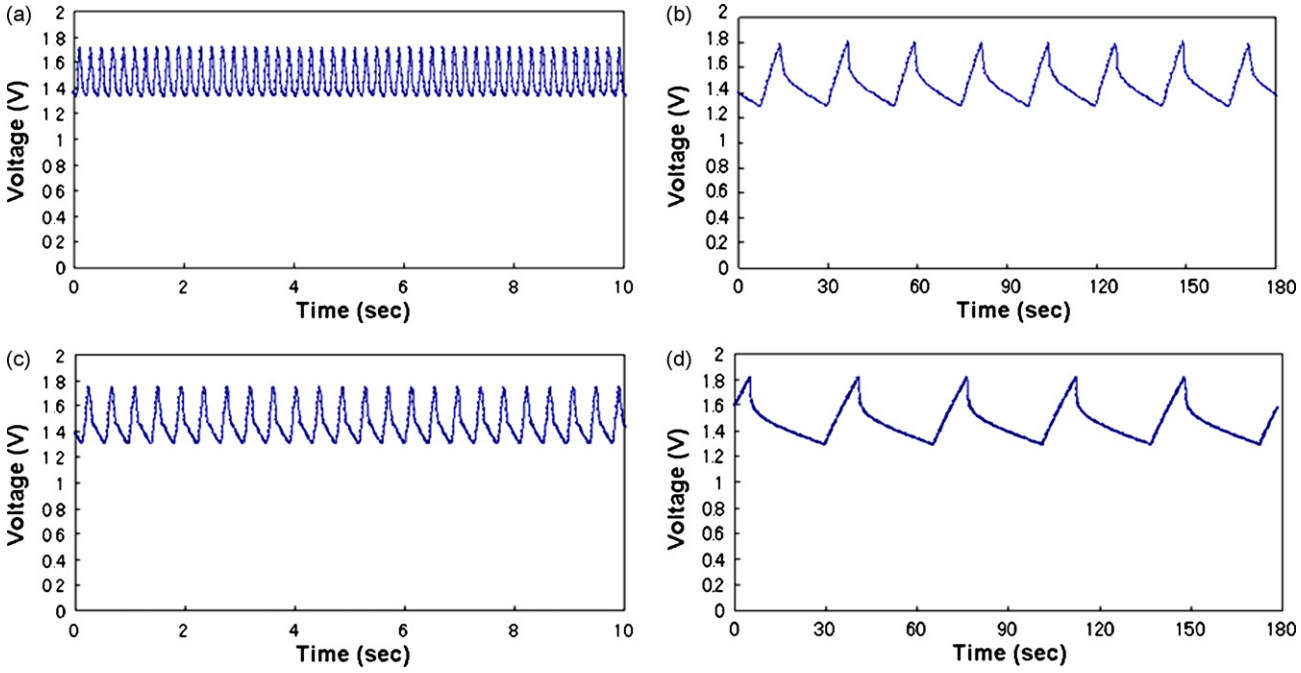

Fig. 4. Effect of the capacitor's capacitance on charging and discharging cycles of the BioCapacitor. The BioCapacitor circuit was operated in the presence of 20 mM glucose in the enzyme fuel cell. (a) 0.47 μF, (b) 1 μF, (c) 10 μF and (d) 100 μF capacitors were connected to a charge pump and the respective potential change of the circuit were measured.

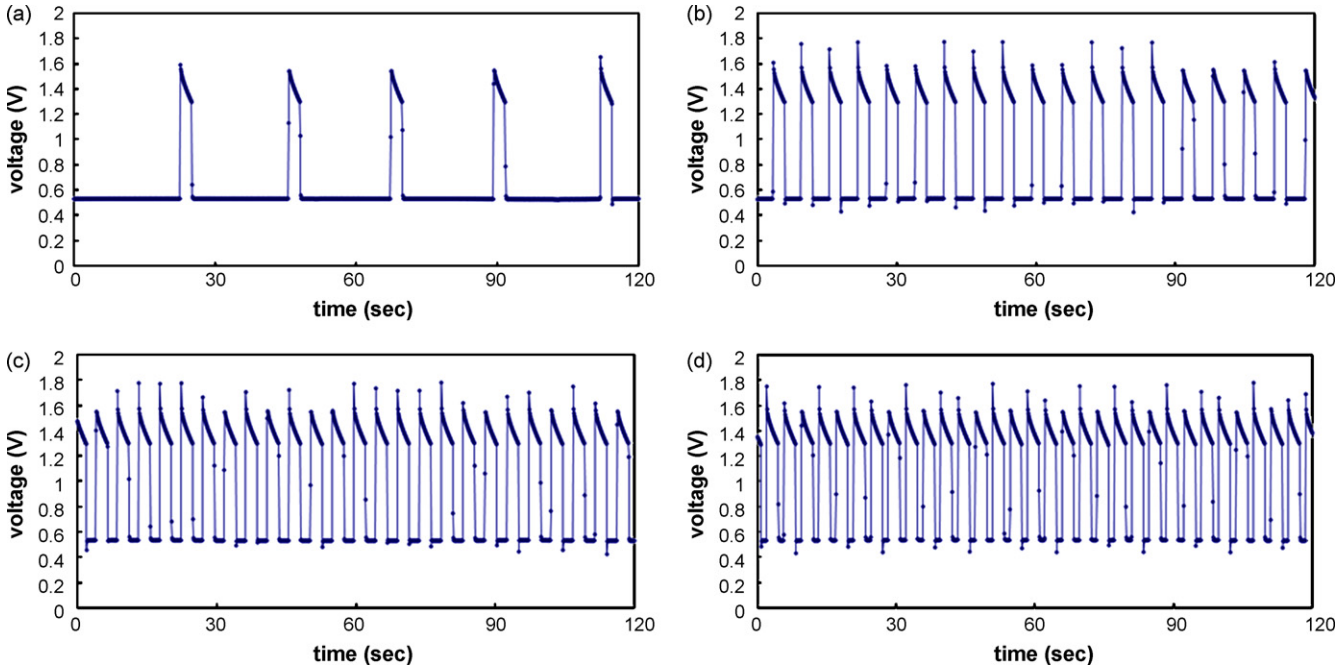

Fig. 5. Effect of glucose concentration in the fuel cell on charging and discharging cycles of the BioCapacitor. (a) 0.10 mM, (b) 0.43 mM, (c) 1.1 mM and (d) 7.4 mM glucose was added in the biofuel cells and the respective potential change in the circuit was measured. Ten microfarad capacitor was connected in the BioCapacitor circuit.

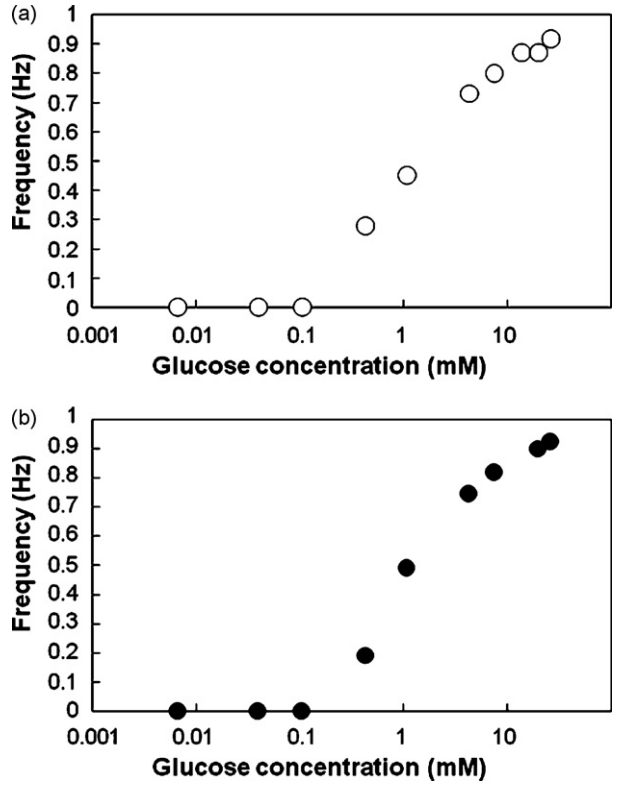

Fig. 6. Dependence of the frequency of charge/discharge cycles on the glucose concentration, as measured in the BioCapacitor circuit (a) or in the receiver circuit (b). The frequency of the charge/discharge cycles of the BioCapacitor was calculated from the electric power change in the circuit. In contrast, the frequency in the receiver circuit was calculated from the voltage change with regard to the 1830 kΩ resistance.

concentration in this system was about 0.2 mM and the increase in the frequency saturated at a glucose concentration of approximately 20 mM. The frequency observed in the receiver circuit also showed a good correlation with glucose concentration similar to the frequency of the charge/discharge cycle of the BioCapacitor circuit. Therefore, the system, combined with the BioCapacitor and an IRLED, can be applied to a wireless glucose-sensing system.

4. Discussion

Since blood glucose levels constantly fluctuate, frequent monitoring of blood glucose levels would ensure more accurate control of glycemia. With the currently available self-blood glucose monitoring devices that measure blood glucose levels intermittently, there is a risk of overlooking hyperglycemia and hypoglycemia. Therefore, CGM is expected to be an ideal sensing system for monitoring glycemic levels in diabetic patients. We reported a glucose biosensing system utilized a direct electron transfer principle-based enzyme sensor (Nakazawa et al., 2003; Okuda et al., 2007; Yamazaki et al., in press). As a result of the use of the novel FADGDH complex (Sode et al., 1996; Yamazaki et al., 1999), which is composed of a catalytic subunit containing FAD, the cytochrome *c* subunit that harbors heme c as the electron transfer subunit, and a chaperone-like subunit, the compartment-less enzyme-based direct electron transfer-type fuel cell was developed (Kakehi et al., 2007). Together with a wireless transmitter system, a simple and miniaturized fuel cell-type CGM system was constructed (Kakehi et al., 2007). However, output voltage and current signals limited the power supply required to operate a transmitter. The detection of signals obtained from the fuel cell-type biosensor remains challenging.

In order to increase the voltage, the biofuel cell will be connected to a charge pump circuit. Alternatively, to gain enough power and signals to operate the electrical devices, the generated electricity from the biofuel cell will be charged in a capacitor. Throughout the utilization of the charge pump circuit and capacitor, the generation of high voltage with high current sufficient to operate an

electrical device will be achieved without changing the design and construction of the biofuel cell. This is our idea of the 'BioCapacitor'.

The charge/discharge frequency correlates with the capacity, e.g., the period required for charge increased and frequency decreased with increasing capacity. Similarly, with increasing glucose concentration, the frequency of charge/discharge increased at a constant capacity. We have indicated that a BioCapacitor utilizing enzyme glucose fuel cells can serve as a glucose sensor. The frequency of the charge/discharge cycle of the BioCapacitor is dependent on the electric power generated by the enzyme fuel cell, which in turn is dependent on the glucose concentration. The detection limit of glucose in the BioCapacitor critically depends on the enzyme FADGDH property and the operation of input voltage of the charge pump IC. The charge pump IC used in this study needed 250 mV to drive its oscillation circuit. The closed-circuit voltage of the enzyme fuel cell exceed 250 mV at the glucose concentration of 0.1 mM, as shown in Fig. 1. Therefore, the detection limit of the BioCapacitor constructed in this study is 0.1 mM. Using a lower voltage-operation charge pump IC increased the detection limit. For example, using a charge pump IC whose minimum operation voltage is 100 mV, the detection limit can be reduced to less than 0.01 mM. On the other hand, the frequency of the charge/discharge cycle was also saturated at glucose concentrations above 7.4 mM, because the closed-circuit voltage of the enzyme fuel cell was saturated at glucose concentrations above 7.4 mM. Additionally, the optimization of the BioCapacitor components, such as the amount of enzyme used, cathodic area, electrode material, and electron transfer rate efficiency form catalytic center of enzyme to electrode are necessary in future.

We have constructed a wireless glucose-sensing system combined with a BioCapacitor and an IRLED. In the presence of glucose, IRLED signal was observed due to the discharge of BioCapacitor, and the frequency was in good correlation with glucose concentration. Therefore, BioCapacitor can be operated as a self-empowered enzyme sensor. Enzyme fuel cell can also act as a glucose sensor. However, the output power generated is very low, and is not capable of powering devices such as a transmitter or an operating system. Therefore, an external power source is required for operating the biosensing system. In contrast, the BioCapacitor does not need any external power source to either operate or transmit data, because it generates a high voltage along with sufficiently high current to operate electrical devices via a charge pump circuit and a capacitor. BioCapacitor behaves a stand-alone, self-empowered, wireless glucose-sensing system, which operates and sends signals only by the power generated in the enzyme fuel cell without requiring an external power source. Using the BioCapacitor as an implantable CGM system would be significant advantage. Further miniaturization and optimization of the electrode structure and sensor component will produce an implantable BioCapacitor principle based CGM system. Furthermore, the principle of the BioCapacitor will be widely applied in future for the construction of several biosensing systems, as well as novel power sources of the devices expected to be operated *in vivo*.

5. Conclusions

In summary, we developed an innovative biosensor, known as BioCapacitor, in which biological recognition elements are combined with a capacitor functioning as the transducer. As a representative model, we constructed a BioCapacitor composed of FAD-dependent GDH as the anodic catalyst, and attempted a glucose measurement. Furthermore, we demonstrated a self-empowered wireless glucose-sensing system combined with the BioCapacitor and an IRLED.

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