Journal of Diabetes Science and Technology
Volume 5, Issue 5, September 2011

SYMPOSIUM

BioRadioTransmitter: A Self-Powered Wireless Glucose-Sensing System

Takuya Hanashi, M.S.,[1] Tomohiko Yamazaki, Dr.Eng.,[2] Wakako Tsugawa, Dr.Eng.,[1] Kazunori Ikebukuro, Dr.Eng.,[1] and Koji Sode, Dr.Eng.[1,3]

Abstract

Background:

Although an enzyme fuel cell can be utilized as a glucose sensor, the output power generated is too low to power a device such as a currently available transmitter and operating system, and an external power source is required for operating an enzyme-fuel-cell-based biosensing system. We proposed a novel biosensor that we named BioCapacitor, in which a capacitor serves as a transducer. In this study, we constructed a new BioCapacitor-based system with an added radio-transmitter circuit and a miniaturized enzyme fuel cell.

Methods:

A miniaturized direct-electron-transfer-type compartmentless enzyme fuel cell was constructed with flavin adenine dinucleotide-dependent glucose dehydrogenase complex-based anode and a bilirubin-oxidase-based cathode. For construction of a BioRadioTransmitter wireless sensing system, a capacitor, an ultra-low-voltage charge-pump-integrated circuit, and Hartley oscillator circuit were connected to the miniaturized enzyme fuel cell. A radio-receiver circuit, comprising two field-effect transistors and a coil as an antenna, was used to amplify the signal generated from the biofuel cells.

Results:

Radio wave signals generated by the BioRadioTransmitter were received, amplified, and converted from alternate to direct current by the radio receiver. When the capacitor discharges in the presence of glucose, the BioRadioTransmitter generates a radio wave, which is monitored by a radio receiver connected wirelessly to the sensing device. Magnitude of the radio wave transmission frequency change observed at the radio receiver was correlated to glucose concentration in the fuel cells.

Conclusions:

We constructed a stand-alone, self-powered, wireless glucose-sensing system called a BioRadioTransmitter by using a radio transmitter in which the radio wave transmission frequency changes with the glucose concentration in the fuel cell. The BioRadioTransmitter is a significant advance toward construction of an implantable continuous glucose monitor.

J Diabetes Sci Technol 2011;5(5):1030-1035

Author Affiliations: [1]Department of Biotechnology, Graduate School of Engineering, Tokyo University of Agriculture and Technology, Tokyo, Japan; [2]International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS), Ibaraki, Japan; and [3]Ultizyme International Ltd., Tokyo, Japan

Abbreviations: (BOD) bilirubin oxidase, (CGM) continuous glucose monitor, (FADGDH) flavin adenine dinucleotide-dependent glucose dehydrogenase, (GDH) glucose dehydrogenase, (IC) integrated circuit, (KB) Ketjenblack, (PPB) potassium phosphate buffer, (Pt/C) platinum-supported carbon

Keywords: BioCapacitor, continuous glucose monitor, flavin adenine dinucleotide-dependent glucose dehydrogenase, glucose dehydrogenase, radio wave transmission, wireless

Corresponding Author: Koji Sode, Dr.Eng., Tokyo University of Agriculture and Technology, 2-24-16 Nakamachi, Koganei, Tokyo 184-8588, Japan; email address sode@cc.tuat.ac.jp

Introduction

Biofuel cells have received much attention as a clean energy source, because they are environmentally safe and sustainable. They can be used to supply power to devices or systems, including future implantable glucose sensors for use in an artificial pancreas. [1–5] All biofuel-cell outputs, i.e., voltage, current, and power, depend on substrate concentrations.[6–8] A system can thus function as a glucose sensor using enzymes that catalyze glucose oxidation. A biofuel-cell-type sensor is easy to miniaturize, because it does not require other devices like a potentiostat. A wireless glucose-sensing system can be used as a continuous glucose monitor (CGM) by connecting a biofuel cell and transmitter. If a cell can be made to work not only as a glucose sensor, but also as a power source for the transmitter, then a stand-alone, self-powered wireless glucose-sensing system that operates and sends signals using only the power generated in the cell without any external power source can be constructed.

However, application of biofuel cells in power biosensors and implantable devices presents some inherent problems, one of which is the low power output of the cell. Cell voltage is theoretically limited by the redox potential of cofactors and/or mediators at the anode and cathode. Voltage generated from a single-cell biofuel cell is too low to power a device such as a transmitter or other implantable components, e.g., motors and micro-processors. For increased power, either the cells can be connected in parallel or their surface area can be increased; for increased voltage, cells can be connected in series.[9] But these approaches are clearly impractical for biosensor and implantable-device applications.

These limitations inspired us to develop a novel device that generates sufficient stable power to operate biosensing devices and transducers. To increase voltage, we connected the biofuel cell to a charge pump. To gain enough power and voltage to operate an electric device, we used a capacitor to store the potential generated by the charge pump. Using a charge pump and capacitor, high voltages with sufficient temporary current to operate an electric device were generated without changing the design and construction of the biofuel cell. The frequency of the charge/discharge cycle depends on the electric power generated by the cell, which, in turn, depends on the glucose concentration in the cell. Thus a BioCapacitor using an enzyme glucose fuel cell can serve as a glucose sensor.[10]

In our previous study, we constructed a BioCapacitor-based system that uses infrared light-emitting diodes to monitor capacitor charge/discharge frequency.[10] In our current study, with a view toward future CGM applications, we constructed a BioCapacitor-based system that uses a radio-transmitter circuit to monitor glucose concentration and miniaturized the system by reducing the size of the enzyme fuel cell. We call this system a BioRadioTransmitter.

Materials and Methods

BioRadioTransmitter Materials

Recombinant flavin adenine dinucleotide-dependent glucose dehydrogenase (FADGDH) complex was prepared using the expression vector pTrc99A, containing the structural gene for glucose dehydrogenase (GDH) complex and pAYCYC184 containing the structural genes for cytochrome c maturation (pEC86) and was transferred into an *Escherichia coli* strain BL21(DE3) and cultivated as described previously.[11] Bilirubin oxidase (BOD) was purchased from Amano Enzyme. Ketjenblack (KB), ECP600JD, was purchased from Mitsubishi Chemical Corp. (Tokyo, Japan). Platinum-supported carbon (Pt/C), TEC10E50E, [Pt% (wt) = 50] was purchased from Tanaka Kikinzoku Kogyo (Tokyo, Japan). Glutaraldehyde solution (mass/volume = 25%) was purchased from Wako Pure Chemicals. NAFION perfluorinated resin solution and poly(dimethylsiloxane) were purchased from Sigma-Aldrich (St. Louis, MO). Triton X-100 was purchased from Kanto Chemical (Tokyo, Japan). All other chemicals were of reagent grade.

Electrode Construction

Ketjenblack screen-printed electrodes were constructed as follows. An underlayer electrode was formed by sputter deposition of a platinum layer on the base film. Insulation printing was performed to form an enzyme-immobilizing area (2 × 2 mm). The area was coated by screen printing with KB ink, which was made by mixing 15 mg of KB with 670 µl of ultrapure water and 30 µl of triton. The mixture was then sonicated for 20 min to disperse KB in the solution.

For constructing the anode, 3 µl of FADGDH solution (4.2 U/µl) was mixed with 2 µl of KB ink solution and 5 µl of 100 mM potassium phosphate buffer (PPB; pH 7.0). Then 4 µl of the mixture was deposited onto a KB

screen-printed electrode and air dried at 4 °C for 1 h. The electrode was cross linked in 25% (mass/volume) glutaraldehyde vapor for 30 min and washed with 10 mM of tris-HCl buffer (pH 7.0).

For construction of the cathode, Pt/C ink was made by mixing 100 mg of Pt/C with 400 µl of ultrapure water and 1.08 ml of 5% (mass/volume) NAFION solution. The mixture was agitated at room temperature for 3 h using a vortex and then incubated for 3 days at 4 °C. Then 8 µl of a mixture of Pt/C ink (3 µl) and 0.12% triton in 100 mM PPB (pH 7.0; 7 µl) were deposited onto a KB screen-printed electrode (2 × 2 mm) and air dried at 80 °C for 1 h, after which 8 µl of BOD solution (0.06 U/µl) was deposited on the electrode and air dried at 4 °C for 1 h. The electrode was cross linked in 25% (mass/volume) glutaraldehyde vapor for 30 min and washed with 10 mM of tris-HCl buffer (pH 7.0). Both anode and cathode were stored in 100 mM of PPB at 4 °C until use.

Cell Construction

Anode and cathode electrodes were attached to a 10 ml water-jacket cell. In the cell, 100 mM of PPB (pH 7.0) was stirred at 250 rpm with a magnetic stirrer at 37 °C. Glucose was added to the cell for measurements. In the presence of 20 mM glucose, voltage generated by the cell between the electrodes was measured by applying an external variable-load resistance (model 278620; Yokogawa Electric Corporation, Tokyo, Japan) and the HZ3000 electrochemical analyzer (Hokuto Denko, Tokyo, Japan).

BioRadioTransmitter Operation

Figure 1 shows a block diagram of the BioRadioTransmitter circuit. A 4.7 µF capacitor, an ultra-low-voltage charge-pump integrated circuit (IC; S-882Z18-M5T1G, Seiko Instruments, Chiba, Japan) and Hartley oscillator circuit were connected to an enzyme fuel cell. The Hartley oscillator circuit was formed from a field-effect transistor (2sk241, Toshiba, Tokyo, Japan), 10 nF capacitor, and coil. A radio-receiver circuit, comprising two field-effect transistors and a coil as an antenna was used to amplify the signal generated from the biofuel cells. The distance of coils between the BioRadioTransmitter and the radio receiver was 25 mm. Resonance frequency was measured by an oscilloscope (ek-50, Matsusada precision, Shiga, Japan). Radio wave signals generated by the BioRadioTransmitter were received, amplified, and converted from alternate to direct current by the radio receiver. The frequency of the received signal was calculated from the change in voltage across the resistance in the radio receiver circuit, which was measured using an HZ3000 electrochemical analyzer. Glucose concentration in the cell was increased by adding a stock solution of glucose.

Results and Discussion

We constructed a miniaturized direct-electron-transfer-type compartmentless enzyme fuel cell with FADGDH complex-based anode and a BOD-based cathode, which is also capable of direct electron transfer (**Figure 2**). We investigated the dependence of current and power

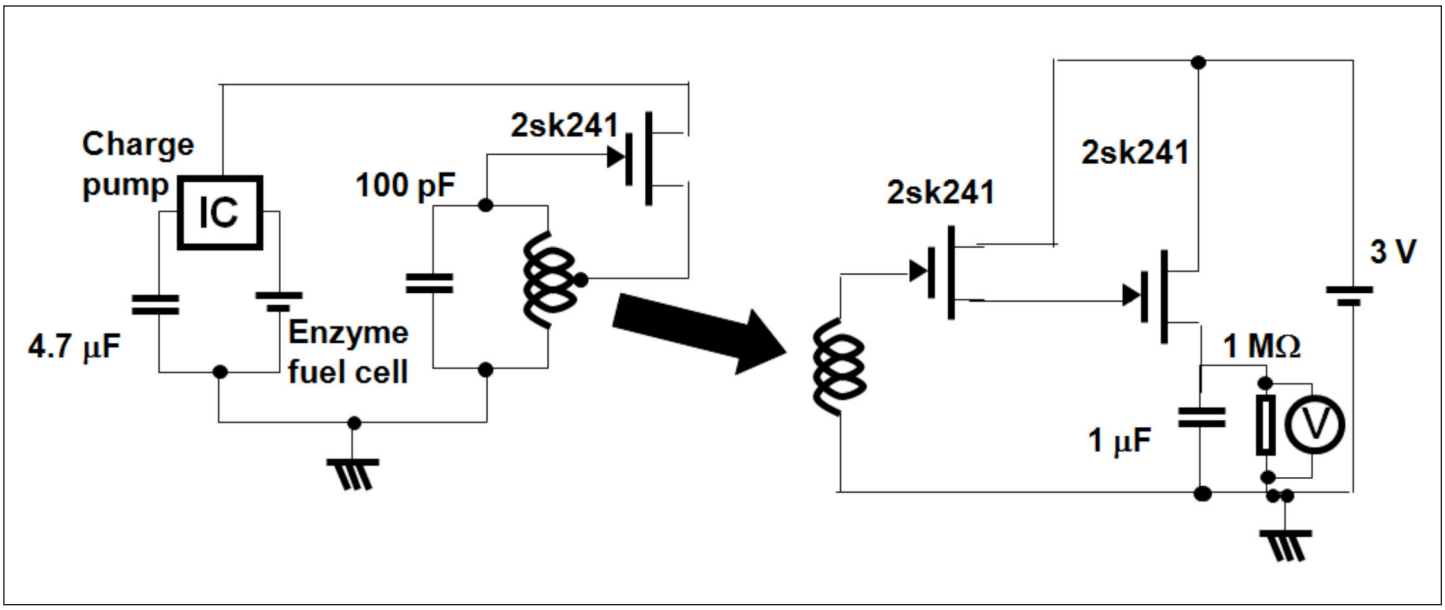

Figure 1. Block diagram of the BioRadioTransmitter circuit and its use in a wireless sensing system.

on cell voltage (**Figure 3**). The open-circuit cell voltage was 460 mV, and the maximum power was 6 μW (150 μW/cm^2) at V_{cell} = 240 mV. The cell generates electric power as high as previously reported GDH-based systems that use electron mediators.[6]

To investigate the possibility of using BioCapacitor-based CGM systems for patients with diabetes, we constructed a new system, a BioRadioTransmitter wireless sensing system as shown in **Figure 1**. The analytical procedure of the BioCapacitor, which is reported in our previous paper,[10] is based on the following principle: 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, analyte concentration can be determined. Oscilloscope measurements show that system voltage oscillates when the capacitor discharges (**Figure 4A**). Transforming the derived waveform into its frequency spectrum shows that the resonance frequency of the oscillator is 88 kHz (**Figure 4B**). Therefore, the BioRadioTransmitter generates enough power to operate an oscillator, generates a radio wave (with a resonance frequency of 88 kHz) intermittently without any external electricity source, and operates only by the electricity generated and accumulated electricity in the capacitor as a result of glucose oxidation in the sample. For system operation, closed-circuit voltage of the enzyme fuel cell must exceed 250 mV.[7] Even the miniaturized fuel cell can generate sufficient voltage to operate the BioRadioTransmitter.

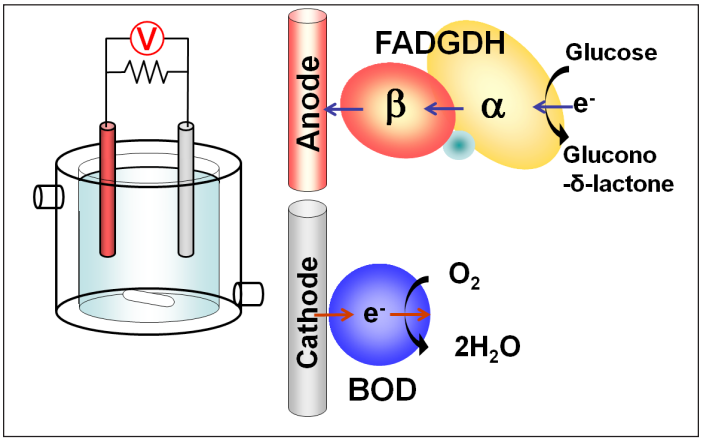

Figure 2. Schematic diagram of a direct-electron-transfer-type enzyme fuel cell employing a GDH-complex-immobilized electrode (anode) and BOD-immobilized electrode (cathode).

When the capacitor discharges in the presence of glucose, the BioRadioTransmitter generates a radio wave, which is monitored by a radio receiver connected wirelessly to the sensing device. We investigated the effect of glucose concentration on the transmission frequency of the radio wave of the BioRadioTransmitter as observed by

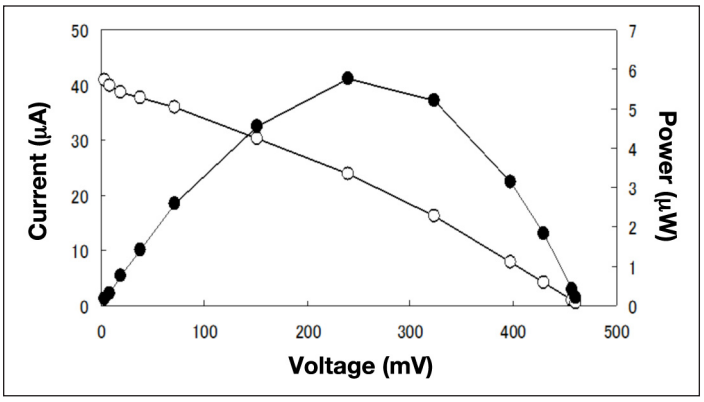

Figure 3. Electrochemical character of a direct-electron-transfer-type enzyme fuel cell with a GDH-complex-immobilized anode and BOD-immobilized cathode. The cell contains 20 mM glucose at 37 °C, evaluated in 100 mM PPB (pH 7.0). Empty circles show the dependence of current on cell voltage; filled circles show the dependence of power output on cell voltage.

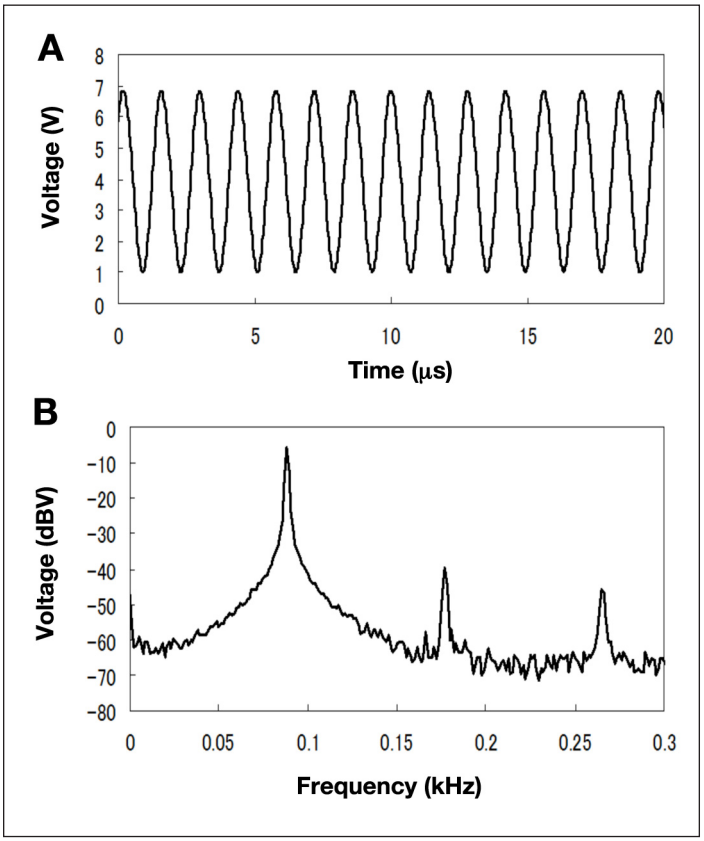

Figure 4. Oscillation of the BioRadioTransmitter observed at regular intervals in the presence of constant (20 mM) glucose concentration: **(A)** waveform and **(B)** frequency spectrum.

the radio receiver (**Figure 5**). For 1.7 mM glucose, the time between two sequential signals corresponding to 0.1 Hz is 9.9 s. Signal frequency (0.12, 0.18, and 0.23 Hz, respectively) increases with increasing glucose concentration (1.9, 2.9, and 6.6 mM, respectively). We also investigated the dependence of radio wave transmission frequency on glucose concentration (**Figure 6**). Transmission frequency increases with increasing glucose concentration. Thus the BioRadioTransmitter can be used for wireless monitoring of glucose. The minimum detectable glucose concentration is approximately 1.5 mM, and the transmission frequency ceases to increase at approximately 6.6 mM. Within this range, the system can monitor glucose concentration. The transmission frequency was saturated at glucose concentrations higher than 6.6 mM, because the closed-circuit voltage of the enzyme fuel cell was saturated at this concentration due to the enzymatic reaction. Therefore, the dynamic range of the BioRadioTransmitter can be improved with optimization of the BioCapacitor components, such as the amount of enzyme used, cathodic area, electrode material affecting diffusion barrier, and electron transfer rate efficiency from catalytic center of enzyme to electrode .

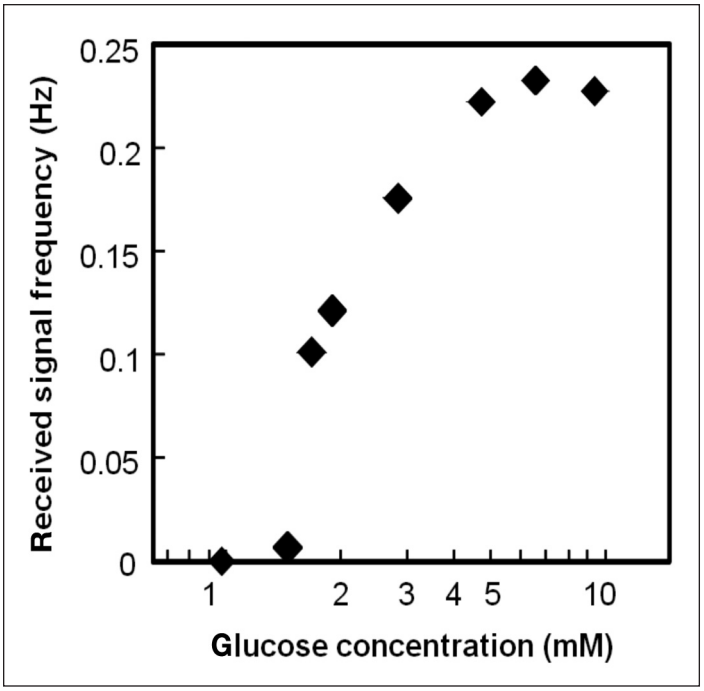

Figure 6. Dependence of radio wave transmission frequency observed at the radio receiver on glucose concentration in the fuel cell. Frequency was calculated from the voltage change in the circuit.

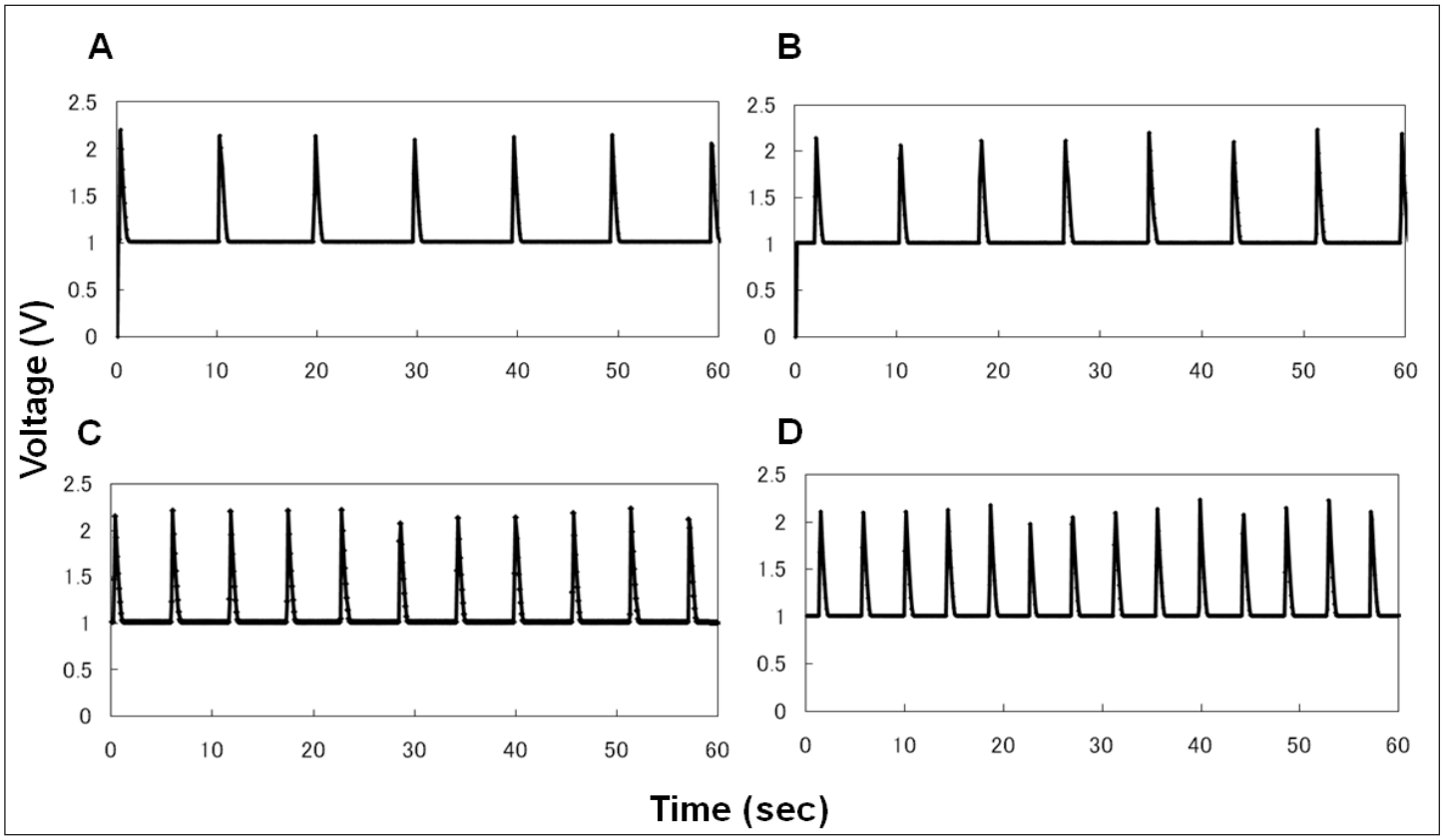

Figure 5. Effect of glucose concentration in the fuel cell on the transmission frequency of the radio wave signal observed at the radio receiver, from which the transmission frequency can be determined by calculating the time between two sequential signals, measured for the following glucose concentrations: **(A)** 1.7 mM, **(B)** 1.9 mM, **(C)** 2.9 mM, and **(D)** 6.6 mM.

In order to utilize the enzyme fuel cells for the implanted power supplies, miniaturization of the fuel cell is required. By miniaturization of fuel cells, the output power of enzyme fuel cells is too low to power a device such as a currently available transmitter and operating system, and an external power source is required for operating a biosensing system. In contrast, the BioCapacitor is a stand-alone, self-powered, wireless glucose-sensing system that generates a high voltage and sufficient current to operate an electrical device by means of a charge-pump circuit and a capacitor.[10] The simplicity of BioRadioTransmitter's structure suggests that an implantable cell is feasible. The anode and cathode electrode size is 2 × 2 mm^2. Both the charge pump IC and the oscillator circuit are less than several millimeters. All components could be packaged together in a small device. The BioCapacitor-based BioRadioTransmitter operating on radio waves and used as an implantable CGM may be possible with further optimization of the electrode structure and sensor component, which would be significantly advantageous.

Conclusion

We constructed a stand-alone, self-powered, wireless glucose-sensing system called a BioRadioTransmitter by using a radio transmitter in which the radio wave transmission frequency changes with the glucose concentration in the fuel cell. We can similarly transduce electric signals to different types of signal to power not only biosensing systems, but also to power a wide variety of biological devices.

References:

1. Barton SC, Gallaway J, Atanassov P. Enzymatic biofuel cells for implantable and microscale devices. Chem Rev. 2004;104(10):4867–86.
2. Fischback MB, Youn JK, Zhao XY, Wang P, Park HG, Chang HN, Kim J, Ha S. Miniature biofuel cells with improved stability under continuous operation. Electroanalysis. 2006;18(19-20):2016–22.
3. Heller A. Miniature biofuel cells. Phys Chem Chem Phys. 2004;6:209–16.
4. Mano N, Heller A. A miniature membraneless biofuel cell operating at 0.36 V under physiological conditions. J Electrochem Soc. 2003;150(8):A1136–8.
5. Mano N, Mao F, Heller A. A miniature membrane-less biofuel cell operating at +0.60 V under physiological conditions. Chembiochem. 2004;5(12):1703–5.
6. Kakehi N, Yamazaki T, Tsugawa W, Sode K. A novel wireless glucose sensor employing direct electron transfer principle based enzyme fuel cell. Biosens Bioelectron. 2007;22(9-10):2250–5.
7. Katz E, Bückmann AF, Willner I. Self-powered enzyme-based biosensors. J Am Chem Soc. 2001;123(43):10752–3.
8. Katz E, Willner I. A biofuel cell with electrochemically switchable and tunable power output. J Am Chem Soc. 2003;125(22):6803–13
9. Desriani, Hanashi T, Yamazaki T, Tsugawa W, Sode K. Enzyme fuel cell for cellulolytic sugar conversion employing FAD glucose dehydrogenase and carbon cloth electrode based on direct electron transfer principle. Open Electrochem J. 2010;2(5):6–10.
10. Hanashi T, Yamazaki T, Tsugawa W, Ferri S, Nakayama D, Tomiyama M, Ikebukuro K, Sode K. BioCapacitor--a novel category of biosensor. Biosens Bioelectron. 2009;24(7):1837–42.
11. Tsuya T, Ferri S, Fujikawa M, Yamaoka H, Sode K. Cloning and functional expression of glucose dehydrogenase complex of *Burkholderia cepacia* in *Escherichia coli*. J Biotechnol. 2006;123(2):127–36.