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Continuous operation of an ultra-low-power microcontroller using glucose as the sole energy source

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ABSTRACT

An ultimate goal for those engaged in research to develop implantable medical devices is to develop mechatronic implantable artificial organs such as artificial pancreas. Such devices would comprise at least a sensor module, an actuator module, and a controller module. For the development of optimal mechatronic implantable artificial organs, these modules should be self-powered and autonomously operated. In this study, we aimed to develop a microcontroller using the BioCapacitor principle. A direct electron transfer type glucose dehydrogenase was immobilized onto mesoporous carbon, and then deposited on the surface of a miniaturized Au electrode (7 mm^2) to prepare a miniaturized enzyme anode. The enzyme fuel cell was connected with a 100 μF capacitor and a power boost converter as a charge pump. The voltage of the enzyme fuel cell was increased in a stepwise manner by the charge pump from 330 mV to 3.1 V, and the generated electricity was charged into a 100 μF capacitor. The charge pump circuit was connected to an ultra-low-power microcontroller. Thus prepared BioCapacitor based circuit was able to operate an ultra-low-power microcontroller continuously, by running a program for 17 h that turned on an LED every 60 s. Our success in operating a microcontroller using glucose as the sole energy source indicated the probability of realizing implantable self-powered autonomously operated artificial organs, such as artificial pancreas.

1. Introduction

An ultimate goal for those engaged in research to develop implantable medical devices is to develop mechatronic implantable artificial organs, such as an artificial pancreas. Such devices would comprise at least a sensor module, an actuator module, and a controller module (Fig. 1). The sensor module recognizes the marker molecules to diagnose the patient and/or to monitor pharmaceutical compounds being used as medication. The actuator module delivers pharmaceutical compounds for medication. The controller module combines the information from the sensor module with a programmed database to operate the actuator module to achieve the appropriate dose of pharmaceutical compounds for medication. One representative challenge in this field is the development of closed-loop artificial pancreas. Recent progress in continuous glucose monitoring systems and mobile device technology allows us to operate an insulin pump based on the sensing signals using a mobile device as the controller unit (e.g., artificial pancreas @ home, https://ec.europa.eu/digital-single-market/en/news/artificial-pancreas-whats-status). However, these approaches are still awaiting further investigation to realize associated medical devices.

An ideal mechatronic implantable artificial organ should be autonomously operated and be self-powered. To achieve this, substantial effort has been made on developing biofuel cell-based medical device operations, such as self-powered glucose sensors (Katz et al., 2001, Katz and Willner, 2003; Mano et al., 2004; Kakehi et al., 2007; Cinquin et al., 2010; Falk et al., 2013; Cadet et al., 2016). However, enzyme fuel cells have the inherent problem that the theoretical voltage of a single-enzyme fuel cell is low because the voltage of the enzyme fuel cell depends on the redox potential of mediators and cofactors. In addition,

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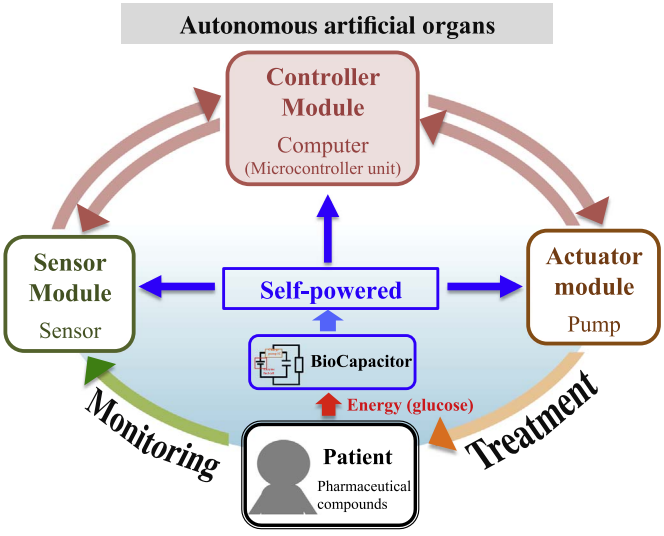

Fig. 1. Conceptual diagram of mechatronic implantable artificial organs. For the operation of self-powered sensor-based autonomous artificial organs, a controller module, i.e. a self-powered computer, is essential. The controller module combines the information from the sensor module with a programmed database to operate the actuator module to achieve the appropriate dose of pharmaceutical compounds for medication. An ideal mechatronic implantable artificial organ should be autonomously operated and be self-powered based on BioCapacitor principle.

the power of an enzyme fuel cell, which is small enough to be implantable, might not be sufficiently high due to the small electrode surface area. To increase voltage and power, enzyme fuel cells could be arranged in series and in parallel. However, these designs are difficult to apply in implantable sensing devices. These limitations inspired us to develop a novel principle called BioCapacitor (Hanashi et al., 2009). In BioCapacitor, an enzyme fuel cell is connected with a capacitor via a charge pump. The charge pump steps up the voltage, and the electricity generated from the biofuel cell is charged in a capacitor. A high voltage with sufficient temporary power can be generated by the BioCapacitor principle. This principle is currently applied for the construction of several biosensing systems using enzyme fuel cells, which can operate various devices. We have already reported that autonomous sensing devices and actuator devices can be operated using this simple and innovative principle (Hanashi et al., 2011, 2012, 2014). This means that among the three modules necessary for mechatronic implantable artificial organs, the sensor module and the actuator module can be self-powered, whereas a controller module is yet to be developed. One challenging field in which the “BioCapacitor” has yet to be exploited is the realization of the unmet technology of operation of a computer (microprocessor) using glucose as the sole energy source. In other words, the development of a self-powered computer by employing the BioCapacitor principle is needed, which is inevitable to develop programmable autonomous biodevices to operate self-powered sensor-based autonomous drug delivery systems (Sode et al., 2016).

Therefore, in this study, we demonstrate a self-powered microcontroller using glucose as the sole energy source. A microcontroller is a small computer including a central processing unit (CPU), random access memory (RAM), read only memory (ROM), input/output (I/O), and a timer. In a microcontroller, the memory stores the instructions (program) in advance and the CPU executes these instructions. Subsequently, the CPU of the microcontroller processes input data in accordance with the instructions and enables the output of data in different forms. When power is supplied to the microcontroller, this series of processes is automatic. Therefore, if the microcontroller can be operated using glucose as the sole energy source, we can construct a self-powered, stand-alone, implantable, and autonomous biodevice operated by the program. To realize this idea, an enzyme fuel cell consisting of a direct electron transfer type glucose dehydrogenase immobilized on a micro-electrode, a capacitor connected with a power boost converter, and an ultra-low-power microcontroller were combined, and the operation of microcontroller by glucose as the sole energy source was achieved.

2. Materials and methods

2.1. Materials

In this study, bacterial FAD-dependent glucose dehydrogenase (FADGDH) was used, which is capable of direct electron transfer. FADGDH comprises three subunits, namely the catalytic subunit, the cytochrome *c* subunit, and the small subunit. A recombinant FADGDH complex was prepared using the expression vectors pTrc99A, containing the structural gene for the FADGDH complex, and pACYC184, containing the structural genes for cytochrome *c* maturation (pEC86); the vectors were transformed into *Escherichia coli* strain BL21 (DE3) and cultivated as previously described (Tsuya et al., 2006). Bilirubin oxidase (BOD) was kindly donated by Amano Enzyme Inc. (Aichi, Japan). Ketjen black, ECP600JD, was purchased from Mitsubishi Chemical Corporation (Tokyo, Japan). Mesoporous carbon (Cnovel) was donated by Toyo Tanso Co., Ltd. (Osaka, Japan). 1-Pyrenebutyric acid N-hydroxysuccinimide ester was purchased from Sigma-Aldrich (St. Louis, MO). Au rod electrode, Pt mesh, and Ag/AgCl reference electrode were purchased from BAS Inc. (Tokyo, Japan). Further, 25% (w/v) glutaraldehyde solution was purchased from Wako Pure Chemical Industries, Ltd. (Oosaka, Japan), and Triton X-100 was purchased from Kanto Chemical (Tokyo, Japan).

2.2. Electrode preparation

For electrode preparation, 10 mM 1-pyrenebutyric acid N-hydroxysuccinimide ester solution was prepared using acetone as a solvent. Ketjen black carbon ink was prepared by mixing 15 mg of Ketjen black with 670 μl of ultrapure water and 30 μl of Triton X-100.

The enzyme anode was prepared using FADGDH complex-immobilized mesoporous carbon, as follows. Fifty-six micrograms of mesoporous carbon particles were incubated with 10 mM 1-pyrenebutyric acid N-hydroxysuccinimide ester for 1 h. The mixed solution was dried for 1 h and then supplemented with 7.9 mg/ml FADGDH complex and 50 mM HEPES buffer (pH 8.0). Next, 2% sucrose was added to the mixed enzyme solution containing 1-pyrenebutyric acid N-hydroxysuccinimide ester and FADGDH complex. A total of 7 μl of each enzyme solution was dropped onto the miniaturized Au electrode (7 mm^2). The electrode was cross-linked in 25% glutaraldehyde vapor for 1 h and washed with 10 mM Tris-HCl buffer (pH 7.0). The anode was then stored in 100 mM potassium phosphate buffer (PPB; pH 7.0, electrolyte composition; KH_2PO_4+K_2HPO_4) until use.

The enzyme cathode was prepared using BOD-immobilized Ketjen black, as follows. Two hundred microliters of Ketjen black ink, 300 μl of 20 mg/ml BOD solution, and 500 μl of 100 mM PPB (pH 7.0) were mixed, and then 200 μl of this solution was dropped onto a Pt mesh (80 mesh). The electrode was cross-linked in 25% glutaraldehyde vapor for 1 h and washed with 10 mM Tris-HCl buffer (pH 7.0). The cathode was then stored in 100 mM PPB (pH 7.0) until use.

2.3. Electrochemical characterization of the enzyme anode

Chronoamperometric evaluation of the enzyme anode (+0.4 V vs. Ag/AgCl) was conducted using a 10-ml water jacket cell. The constructed anode, Ag/AgCl, and Pt wire were used as a working electrode, a reference electrode, and a counter electrode, respectively. All electrochemical characterizations were performed at 37 °C in 100 mM PPB (pH 7.0). Chronoamperometric evaluation was carried out in triplicate, using independently prepared three enzyme anodes.