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A wireless transmission system powered by an enzyme biofuel cell implanted in an orange

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ABSTRACT

A biofuel cell composed of catalytic electrodes made of "buckypaper" modified with PQQ-dependent glucose dehydrogenase and FAD-dependent fructose dehydrogenase on the anode and with laccase on the cathode was used to activate a wireless information transmission system. The cathode/anode pair was implanted in orange pulp extracting power from its content (glucose and fructose in the juice). The open circuit voltage, V_{oc}, short circuit current density, j_{sc}, and maximum power produced by the biofuel cell, P_{max}, were found as ca. 0.6 V, ca. 0.33 mA·cm^{-2} and 670 μW, respectively. The voltage produced by the biofuel cell was amplified with an energy harvesting circuit and applied to a wireless transmitter. The present study continues the research line where different implantable biofuel cells are used for the activation of electronic devices. The study emphasizes the biosensor and environmental monitoring applications of implantable biofuel cells harvesting power from natural sources, rather than their biomedical use.

1. Introduction

Recent developments in the area of enzyme-based biofuel cells [1–9] resulted in the devices operating in vivo being implanted in various living animals [10,11], including insects [12], mollusks (snail and clams) [13,14], lobsters [15], rats [16–21] and rabbits [22]. From the very beginning [23], this research was directed to micro- [24,25] and even nano-size [26] devices providing power for implantable biomedical/bioelectronic systems [27], e.g., pacemakers [28]. Indeed, some preliminary results for the activation of pacemakers by implantable biofuel cells using enzyme-based biocatalytic electrodes [29] or "abiotic" electrodes based on inorganic catalytic species [30–32] have demonstrated such possibility, while operating in vitro in model systems. However, the first successful demonstrations of the pacemaker operation upon extracting electrical power from glucose in biofluids or model solutions also revealed the limitations of this approach. In order to obtain the required power for the pacemaker operation, the electrodes should have significant size (a few square centimeters), which makes their implantation in a human blood circulation problematic. More importantly, the life-time of the enzyme-based biocatalytic electrodes is incomparable to duration required for the operation of implantable medical devices. Note that a battery used for powering of a pacemaker can normally operate for about 10 years [28], while the life-time of implantable enzyme-biofuel cells is in the range of days or at very best months [1,13]. The chances that "abiotic" biocatalytic electrodes based on inorganic catalysts [30] can reach the operational time comparable with batteries, particularly operating in biofluids [33,34], are also illusive, at least at the present level of technology. While in the long perspective the biofuel cells might be improved to meet the requirements for their medical use in implanted devices, in the short run, based on the present level of technology, the biofuel cells should find other potential applications which might be practically important for much shorter time of operation. This could be the activation of biosensors for environmental monitoring, homeland security, and even military applications by extracting power from biological sources. The potential power sources might include animals or plants, while the biocatalytic electrodes could be minimally invasive [35] or even located on their surface being printed as a "tattoo" [36]. This application could be practically meaningful for the operational time comparable with the stability of the biocatalytic electrodes achievable already at the present level of technology. The first examples of the biofuel cells extracting power from the abundant "fuel" in plants were already reported using enzyme-modified electrodes implanted in a grape [37] or simply immersed in fruit juice [22,38]. On the other hand, some other experiments on non-implantable biofuel cells have demonstrated the activation of electronic sensing systems for wireless information transmission [39,40].

It should be noted that the biofuel cells utilizing abundant biofuels (e.g., glucose or fructose) and oxygen as the electron acceptor have the open circuit voltage thermodynamically limited by the potential difference between the oxidation potential of the biofuel and reduction potential of oxygen. Upon extracting current from the biofuel cell this voltage is further decreased and limited by the kinetics of the redox processes, including the mass transport, interfacial electron transfer, and

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ohmic resistance [9]. Overall, the operational voltage of biofuel cells is usually in the range of several hundred millivolts [1,9] and it is rarely overshot half a volt [41–43]. On the other side, the operational voltage of most of electronic devices, including medical implantable micropower systems [44], is in the range of several volts [45]. This voltage mismatch should be managed with electronics, which can increase the DC-voltage produced by the biofuel cell to meet the requirements of the power-consuming electronic systems [39,40]. Usually this is achieved with the use of charge pumps increasing DC-voltage at the expense of consuming more current [46]. This puts additional requirements on the current produced by the implantable biofuel cells, thus resulting in larger electrodes, unless the current density is increased upon using more efficient electrocatalysts. Overall, the question to be experimentally answered is whether the biocatalytic electrodes implanted in any source of biofuel are capable of extracting enough current to power the DC-voltage converter as well as the wireless transmitter, as an example for practically meaningful applications. The current produced by micro- [24,25] and nano-size [26] implantable biocatalytic electrodes, particularly implanted in insects, mollusks, grapes and other small biological species is too small to be practically useful [47], at least at the present level of the electrode electrocatalytic efficiency. Thus, we experimented with oranges which can provide easily available juicy fuel combined with a reasonable size for implanting electrodes of substantially large size.

The present paper reports on the first preliminary results which show the power production by enzyme biofuel cell operating in vivo in an orange and used for activation of wireless electronics.

2. Experimental

2.1. Chemicals and materials

PQQ-dependent glucose dehydrogenase (GDH; E.C. 1.1.5.2, from microorganism — not specified by the company) was purchased from Toyobo Co., Japan, and used as supplied. Laccase (E.C.1.10.3.2, from *Trametes versicolor*) was obtained from Sigma-Aldrich and used in experiments after the purification procedure described elsewhere [48]. 1-pyrenebutanoic acid succinimidyl ester (PBSE) was purchased from AnaSpec Inc. FAD-dependent D-fructose dehydrogenase (FDH; E. C. 1.1.99.11, from *Gluconobacter industrius*), D-(+)-glucose (≥99.5%), D-(−)-fructose (≥99%), 3-(*N*-morpholino)propanesulfonic acid (MOPS-buffer) and other standard chemicals were purchased from Sigma-Aldrich and used as supplied without any further purification. Water used in all of the experiments was ultrapure (18.2 MΩ·cm) from a NANOpure Diamond (Barnstead) source. Washington Navel Oranges (http://www.citrusvariety.ucr.edu/citrus/washington.html) were purchased from Walmart Supermarket (containing 1.98–2.05 g/100 mL fructose and 1.82–1.83 g/100 mL glucose in the juice) [49]. The average orange size was 8 ± 1.5 cm diameter.

2.2. Electrode preparation

Buckypaper composed of compressed multi-walled carbon nanotubes (Buckeye Composites; NanoTechLabs, Yadkinville, NC) was used as the electrode material. Electrodes were washed with isopropyl alcohol with moderate shaking for 15 min at room temperature prior to their modification. The electrodes were incubated with PBSE, 10 mM, in ethanol with moderate shaking for 1 h at room temperature, subsequently rinsed with ethanol to remove excess of PBSE and then with MOPS-buffer (50 mM, pH 7.0) to remove ethanol. The biocatalytic anodes were prepared by immobilization of GDH and FDH. The PBSE-functionalized electrode was incubated for 1 h in the solution of GDH (2.4 mg·mL^{-1}) and FDH (0.5 mg · mL^{-1}) in MOPS-buffer (50 mM, pH 7.0) containing Na_2SO_4 (100 mM) and 1 mM $CaCl_2$ (1 mM). The biocatalytic cathode was prepared by immobilization of laccase. The PBSE-functionalized electrode was incubated for 1 h in the solution of laccase (1.5 mg·mL^{-1}) in potassium phosphate buffer (10 mM, pH 7.0). The immobilization reactions proceeded at room temperature with moderate shaking. Then the enzyme-modified electrodes were stored (4 °C) in the same buffer until implanted in oranges.

2.3. Electrochemical characterization of the modified electrodes

Cyclic voltammetry measurements were carried out using a conventional three-electrode cell at room temperature (22 ± 2 °C) with an ECO Chemie Autolab PASTAT 10 electrochemical analyzer using the GPES 4.9 (General Purpose Electrochemical System) software package. The working electrode was made of buckypaper and modified with the enzymes as described above. The geometrical surface area exposed to the solution was 2 cm^2, accounting for both sides of the modified electrodes, but not taking into account the internal surface of the 3D-electrodes. A slab of glassy carbon (6.48 cm^2 geometrical surface area) was used as a counter electrode and a Metrohm Ag|AgCl|KCl, 3 M, electrode served as a reference electrode. Cyclic voltammograms were recorded at a scan rate of 2 mV·s^{-1} in a phosphate buffered (0.2 M, pH 7.4) electrolyte solution. For experiments without oxygen, the solution was deoxygenated by bubbling argon prior to the cyclic voltammetry measurements. The cathodic electrocatalytic reduction of oxygen was studied in the solution containing soluble oxygen, being in equilibrium with air (ca. 0.25 mM O_2 under these experimental conditions) [50]. The anodic electrocatalytic oxidation of biomolecular "fuels" was studied in the solutions containing 20 mM glucose or 20 mM fructose.

2.4. Biofuel cell polarization curve measurements

The performance of the biofuel cell was analyzed with the biocatalytic electrodes implanted in orange. The peel was cut and partially removed together with a small segment inside, providing the necessary space for the electrodes. Then the catalytic anode–cathode pair (each electrode with the geometrical surface area of ca. 7.5 cm^2 accounting for both electrode sides) was inserted into the pulp with the distance between the electrodes of ca. 1 cm, Fig. 1, inset. Note that the cut resulted in destroying the juice vesicles and the electrodes were allowed to contact their juicy content. A polarization function was obtained when the cell was connected to an external variable resistance load (varied from 0 to 900 kΩ) and the voltage/current produced on the load was measured by a high impedance multimeter (Meterman 37XR). The polarization measurements were performed ex situ in the orange explanted from a tree. The experiments were repeated with 5 oranges from the same batch.

2.5. The energy harvesting circuit design and wireless transmitting system

A charge pump was used to amplify the low voltage produced by the biofuel cell to 2.3 V, a level required by the wireless transmitting system. The used charge pump included two main components. A commercially available EH4295 unit (Advanced Linear Devices, Inc., CA) first harvested the low voltage electrical power from the biofuel cell; then the electrical power was sent to the EH300 companion unit. The latter was applied to charge the onboard 1 mF capacitor. This onboard capacitor was configured in parallel with an external 6.8 mF super-capacitor. The final super-capacitor was chosen for its high storage capacity, small size, and low current leakage. This was then connected to the wireless transmission device. The radio transmission module consists of 2 main components: a UHF OOK radio transmitter combined with an ATTiny 85 micro-controller. The radio transmitter sent data at 9600 baud. The data sent by this transmitter was read by a companion receiver circuit connected to a standard desktop PC. Reception of the transmitted data is provided by an integrated radio receiver module coupled with a Cortex M4 micro controller. This receiver module is paired with the transmitter by the manufacturer. The micro-controller's role is to filter the raw received data so only data from our transmitter is displayed on the computer.