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Coupling of an enzymatic biofuel cell to an electrochemical cell for self-powered glucose sensing with optical readout☆

Piyanut Pinyou [a,1], Felipe Conzuelo [a,1], Kirill Sliozberg [a], Jeevanthi Vivekananthan [a], Andrea Contin [a], Sascha Pöller [a], Nicolas Plumeré [b], Wolfgang Schuhmann [a,*]

[a] Analytical Chemistry — Center for Electrochemical Sciences (CES), Ruhr-Universität Bochum, D-44780 Bochum, Germany
[b] Center for Electrochemical Sciences (CES) — Molecular Nanostructures, Ruhr-Universität Bochum, D-44780 Bochum, Germany

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ABSTRACT

A miniaturized biofuel cell (BFC) is powering an electrolyser invoking a glucose concentration dependent formation of a dye which can be determined spectrophotometrically. This strategy enables instrument free analyte detection using the analyte-dependent BFC current for triggering an optical read-out system. A screen-printed electrode (SPE) was used for the immobilization of the enzymes glucose dehydrogenase (GDH) and bilirubin oxidase (BOD) for the biocatalytic oxidation of glucose and reduction of molecular oxygen, respectively. The miniaturized BFC was switched-on using small sample volumes (ca. 60 μL) leading to an open-circuit voltage of 567 mV and a maximal power density of (6.8 ± 0.6) μW cm^{-2}. The BFC power was proportional to the glucose concentration in a range from 0.1 to 1.0 mM ($R^2 = 0.991$). In order to verify the potential instrument-free analyte detection the BFC was directly connected to an electrochemical cell comprised of an optically-transparent SPE modified with methylene green (MG). The reduction of the electrochromic reporter compound invoked by the voltage and current flow applied by the BFC let to MG discoloration, thus allowing the detection of glucose.

1. Introduction

The development of simple, affordable and inexpensive sensors for medical diagnosis and other sensing applications is of great interest since it may provide several benefits in remote communities and developing countries [1], as well as allowing simple and rapid determinations in the everyday analysis. To date, the development of analytical devices has been mainly directed towards rather complex systems, most of them requiring extra powered devices for the readout of the sensor's response. In contrast, self-powered sensors are of increased attention because of their capability for detection without requiring external power sources [2]. Evidently, one major aim is towards instrument-free analysis in which also the read-out process does not require any external power.

Enzymatic biofuel cells provide the option for a self-powered device, combining the efficiency of biocatalysis with that of electrochemical energy conversion [3]. The use of biofuel cells (BFCs) as self-powered devices has recently renewed the interest in the development of BFCs since they allow the fabrication of simple and miniaturized detection devices [3–7], where the sensor itself is able to provide the power for sensing of the analyte, for instance, by extracting electrical power useful from a biocatalytic reaction sequence in stoichiometric dependence from the analyte concentration [4]. By using different enzymes, diverse species have been used as fuel/analyte with the power output depending on the fuel concentration.

Although the potential capability of a BFC to power a readout device has been suggested since the early 2000s [8], only recently has a truly self-powered biodevice avoiding any externally powered multimeter or potentiostat for the readout of the resulting signal been demonstrated. Former attempts [9,10] provided sensing devices for fructose and glucose using a self-powered capacitor as the transducer coupled to a light-emitting diode (LED). The charging rate of the capacitor was proportional to fuel concentration and determined the blink interval of the LED. Alternatively, paper-based sensing platforms containing an electrodeposited Prussian blue spot were used to change color in the presence of the analyte [11] using a metal/air battery-powered electrochemical sensor. Although no quantitative response was obtained, the presence of the analyte (glucose or hydrogen peroxide) initiates the color change of the Prussian blue spot, thus not requiring other devices for sensing or signal read-out. Recently, a self-powered electrochromic sensor for the determination of ascorbic acid using an ascorbic acid/O_2 BFC was described [6]. An electrochromic display was modified with Prussian blue and the chromogen was reduced when the anode of the BFC was connected to the electrochromic display, with the rate of change from blue to transparent depending on the concentration of the analyte, thus allowing the quantification of ascorbic acid. The Prussian blue-display was regenerated by connecting it to the BFC cathode,

☆ Dedicated to Prof. Hanns-Ludwig Schmidt on the occasion of his 85th birthday.
* Corresponding author.
E-mail address: wolfgang.schuhmann@rub.de (W. Schuhmann).
[1] These authors contributed equally to this work.

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allowing at least 20 regeneration cycles. A camera was used to take pictures of the electrochromic display measuring the rate of discoloration.

Here we present a miniaturized BFC for glucose sensing, based on screen-printed electrodes (SPE) modified with the enzymes bilirubin oxidase (BOD) and glucose dehydrogenase (GDH). Fabrication of screen-printed electrodes is well advanced, allowing a commercially available inexpensive high volume production with many possible designs [1,12] allowing the development of miniaturized and portable devices [13]. Quantification of glucose becomes possible through the variable power supplied after the oxidation of the fuel which is transduced into an optical read-out in a connected secondary electrochemical cell.

2. Materials and methods

2.1. Chemicals and materials

Graphitized carbon nanofibers (CNFs)-modified screen-printed carbon electrodes consisting of a CNF-modified working electrode and a carbon counter electrode (110CNF) and optically transparent screen-printed electrodes (P10) were provided by DropSens (Spain). Pyrroloquinoline quinone (PQQ) and methylene green (MG) were purchased from Fluka (Switzerland). Soluble glucose dehydrogenase (sGDH) was a gift from Roche Diagnostics (Germany). Bilirubin oxidase from *Myrothecium verrucaria* (*Mv*BOD), 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (PBSE) and 2,2′-(ethylenedioxy)diethanethiol were from Sigma-Aldrich (Germany). All solutions were prepared using deionized water (Millipore, USA). All experiments were carried out at room temperature.

2.2. Enzymatic biofuel cell preparation

PQQ-sGDH holo-enzyme was prepared by dissolving 1.08 mg of apo-sGDH in 30 μL of a 500 μM PQQ solution containing 150 mM $CaCl_2$. The mixture was incubated at least 30 min at 4 °C prior to electrode preparation. The lyophilized *Mv*BOD powder was dissolved in 100 mM phosphate buffer, pH 7.4, to attain a protein concentration of 20 mg mL^{-1}. The BOD solution was stored at −20 °C.

2.2.1. Biocathode

Following a recently described procedure [14] the working electrode of the CNF-modified SPEs was modified by non-covalent functionalization using 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (PBSE), allowing direct electrical communication between BOD and the electrode through an oriented immobilization of the enzyme [14]. The working electrode was immersed in 10 mM PBSE in dimethyl formamide (DMF) for 1 h. After that, the working electrode was rinsed with DMF to remove excess pyrene. The electrode was subsequently rinsed with n-hexane to remove DMF. Finally, the electrode was thoroughly rinsed with phosphate buffer pH 7.4. The electrode was dried at room temperature before adding 3 μL of BOD solution on the electrode surface. The electrode was left to dry and stored at 4 °C. The weakly adsorbed components were removed from the electrode surface by rinsing with PBS buffer.

2.2.2. Bioanode

For the BFC anode the carbon counter electrode of the SPE was used. The mixture for the modification was prepared by mixing 1.5 μL of the Os-complex modified polymer (P024-P195; 10 mg mL^{-1}), 0.5 μL of the reconstituted PQQ-sGDH (36 mg mL^{-1}) and 1.0 μL of 2,2′-(ethylenedioxy)diethanethiol (21.3 mg mL^{-1} in phosphate buffer, pH 7.4). 2 μL of the resulting mixture was carefully drop-casted exclusively on the surface of the SPE counter electrode in order to prevent contamination of the BOD-modified working electrode. The modified anode was allowed to dry at room temperature and subsequently rinsed with PBS buffer prior to measurement.

2.3. Electrochemical measurements

Electrochemical measurements were performed using an Autolab potentiostat (PGSTAT, Eco Chemie, The Netherlands). The three electrode set-up consisted of a Pt wire as a counter electrode, a Ag/AgCl/3 M KCl reference electrode and the modified cathode or anode on the SPE as the working electrode. Cathode characterization was carried out by cyclic voltammetry in phosphate-buffered saline (PBS) buffer (12 mM phosphate, 137 mM NaCl, 2.7 mM KCl), pH 7.4, under argon- and air-saturated solutions. Anode characterization was performed by cyclic voltammetry in the absence and presence of glucose. The catalytic current from glucose oxidation was monitored by chronoamperometry at a constant applied potential of +50 mV vs. Ag/AgCl/3 M KCl.

2.4. Biofuel cell measurement

Biofuel cell tests were performed by chronoamperometry using an Autolab PGSTAT potentiostat. The anode was chosen to be the limiting electrode, thus the power of the biofuel cell was determined by the glucose concentration in the electrolyte solution. The solution for the evaluation of the BFC consisted of 60 μL of air-saturated PBS buffer pH 7.4, containing 10 mM glucose as a fuel for the anode. The power output curve of the developed BFC was evaluated by applying successive potential pulses ranging from 0 to 0.55 V against the obtained open-circuit voltage (OCV) and recording the equilibrium steady-state current at each potential interval.

2.5. Self-powered detection of glucose

The capability of the developed BFC for the self-powered detection of glucose was evaluated using the BFC as the power source for the reduction of an electrochromic reporter compound. For this, the BFC was connected to a second SPE consisting of an optically-transparent poly(3,4-ethylenedioxythiophene) (PEDOT) working electrode modified with methylene green (MG). MG was deposited on the surface of the P10-SPE working electrode by electrochemical deposition in a three electrode cell, where a Pt wire and Ag/AgCl/3 M KCl were used respectively as counter and reference electrodes and the PEDOT surface of the SPE constituting the working electrode. The adsorption of MG was performed by cyclic voltammetry at a scan rate of 5 mV s^{-1} between −0.5 and 0.4 V (vs. Ag/AgCl/3 M KCl) in a solution containing 0.5 mM MG and 0.1 M KNO_3 dissolved in phosphate buffer pH 7.0 [15].

The modified P10-SPE was placed in a home-made methacrylate cell filled with a small volume (ca. 100 μL) of 0.1 M KCl solution. For the optical measurements, a UV–Vis DT-MINI-2-GS light source and a USB2000 spectrometer (Ocean Optics, USA) were used. Two P400-2 optical fibers (Ocean Optics, USA) were used to route the light from the light source to the electrode setup and the transmitted light through the MG-modified PEDOT SPE to the spectrometer. The spectra were recorded in the wavelength range from 240 to 800 nm every 10 s. The color change was followed at a wavelength of 650 nm in transmission mode. The potential was recorded using a DT-9939 digital multimeter (Reichelt Elektronik, Germany). A scheme of the setup used for the self-powered detection of glucose with the developed BFC is shown in Scheme 2. The anode (A) and cathode (C) of the BFC were connected to the voltmeter in order to measure the open-circuit voltage (OCV) of the cell. After the addition of electrolyte solution to the miniaturized BFC and once the recorded OCV was stable, the MG-modified electrode was connected to the BFC by switching S2. Switch 1 allowed inverting the polarity of the cell for the oxidation or reduction of the electrochromic reporter.

3. Results and discussion

The developed miniaturized BFC was intended to be able to work with small volumes of electrolyte solution containing the analyte. In