Biosensors and Bioelectronics 83 (2016) 60–67

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

An enzymatic glucose/O_2 biofuel cell operating in human blood

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ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form
4 April 2016
Accepted 5 April 2016
Available online 12 April 2016

Keywords:
Biofuel cells
Human blood
Bilirubin oxidases
Glucose dehydrogenase
Redox hydrogel

ABSTRACT

Enzymatic biofuel cells (BFCs) may power implanted medical devices and will rely on the use of glucose and O_2 available in human bodily fluids. Other than well-established experiments in aqueous buffer, little work has been performed in whole human blood because it contains numerous inhibiting molecules. Here, we tested our BFCs in 30 anonymized, random and disease-free whole human blood samples. We show that by designing our anodic and cathodic bioelectrocatalysts with osmium based redox polymers and home-made enzymes we could reach a high selectivity and biofunctionnality. After optimization, BFCs generate power densities directly proportional to the glycaemia of human blood and reached a maximum power density of 129 μW cm^{-2} at 0.38 V *vs.* Ag/AgCl at 8.22 mM glucose. This is to our knowledge the highest power density attained so far in human blood and open the way for the powering of integrated medical feedback loops.

1. Introduction

Enzymatic glucose/O_2 biofuel cells, miniature and implantable in the human body, may power in the near future implanted medical devices (Heller, 2006b; Leech et al., 2012; Rasmussen et al., 2016; Sode et al., 2016; Willner et al., 2009). They are composed of glucose oxidizing anodes made with enzymes such as glucose oxidase (GOx), glucose dehydrogenase (GDH) or cellobiose dehydrogenase (CDH) and O_2 reducing cathodes made with laccase or bilirubin oxidase (BOD) (Gao et al., 2009, 2010; Kavanagh and Leech, 2013; Le Goff et al., 2015; Ludwig et al., 2010; Mano and Edembe, 2013). Even though the power output and lifetime are not sufficient for direct applications, significant progresses in power densities have been realized during the last 5 years leading to numerous implantations in invertebrates, in mammalians (Castorena-Gonzalez et al., 2013; Cinquin et al., 2010; Halamkova et al., 2012; MacVittie et al., 2013; Miyake et al., 2011; Rasmussen et al., 2012; Sales et al., 2013; Szczupak et al., 2012; Zebda et al., 2013) and experiments in human serum. Mao's team reported a BFC combining a NAD^+ dependent GDH and a BOD from *Myrothecium verrucaria* (BOD-*Mv*) immobilized on carbon nanotubes generating 5 μW cm^{-2} at 0.4 V in presence of 10 mM NAD^+ added in diluted serum (Gao et al., 2007), later optimized to reach 35 μW cm^{-2} at 0.39 V (Li et al., 2009). Scherbahn et al. (2014) obtained 45 μW cm^{-2} at 0.39 V in unmodified serum with a vertically aligned carbon nanotubes BFC based on PQQ dependent GDH and BOD-*Mv*. By combining FAD-GDH and BOD-*Mv*, Milton et al. (2015) reached 57.5 μW cm^{-2} at 37 °C in human serum containing 5 mM glucose. Coman et al. (2010) described the first BFC operating in direct electron transfer mode by combining cellobiose dehydrogenase (CDH) and BOD-*Mv* onto graphite electrodes generating 3 μW cm^{-2} at 0.37 V. When laccase was used on the cathodic side, in combination with GOx, Ammam et al. generated 160 μW cm^{-2} at 0.21 V on glassy carbon electrodes (Ammam and Fransaer, 2010) and 69 μW cm^{-2} at 0.151 V on multi-walled carbon nanotubes (Ammam and Fransaer, 2012). With a PQQ-GDH at the anode, Castorena et al. and Ó Conghaile et al. respectively generated 70 μW cm^{-2} at 0.23 V on buckypaper (Castorena-Gonzalez et al., 2013) and 37 μW cm^{-2} at 0.26 V on graphite (Ó Conghaile et al., 2013). MacAodha et al. (2013) reached 60 μW cm^{-2} at 0.3 V in artificial plasma. Finally, Holade et al. (2014) reported a power density of 50 μW cm^{-2} at 0.16 V after adding 5.4 mM glucose in serum for an abiotic biofuel cell using platinum and gold-based catalysts. The highest power density obtained to date in human serum was reached by Kwon et al. (2014) by combining GOx and BOD-*Mv* onto biscrolled carbon nanotube yarns generating 1.02 mW cm^{-2} at 0.40 V and later 1.1 mW cm^{-2} at 0.50 V (Kwon et al., 2015).

However, human serum is composed of 90% water and explains the usually good power densities and stabilities reached in this medium. One of the main challenges in developing enzymatic BFCs is to demonstrate their real use in whole human blood. Experiments in human blood have been scarce for two main reasons:

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http://dx.doi.org/10.1016/j.bios.2016.04.016
0956-5663/

1) the use of human blood requires legal and official authorizations; 2) unlike serum, human blood contains blood cells and numerous interferents making BFCs less efficient and stable. To our knowledge, the literature only reports four examples of the use of whole human blood. Nien et al. reached 25 $\mu W\,cm^{-2}$ in human blood supplemented with 0.1 M KCl by combining a GOx anode and an air breathing cathode; Pan et al. (2010) generated 5.56 $\mu W\,cm^{-2}$ at 0.07 V with a GOx/laccase based biofuel cell and Wang et al. reached 2.8 $\mu W\,cm^{-2}$ at 0.47 V in presence of 4.6 mM glucose using a CDH/BOD-*Mv* BFC. Finally, Ó Conghaile designed a BFC by assembling a cathode made with BOD-*Mv* adsorbed on a gold electrode modified with gold nanoparticles and an anode made with pyranose dehydrogenase, multiwalled carbon nanotubes and osmium based redox polymers. In whole blood containing 5.4 mM glucose, they reached 73 $\mu W\,cm^{-2}$ at 0.3 V. Among these works, only Wang and Ó Conghaile provided the glucose concentration in their blood sample.

Here, we tested our BFCs in 30 anonymized, random and disease-free whole human blood samples to allow for statistical analysis. We obtained up to 129 $\mu W\,cm^{-2}$ at 0.38 V in presence of 8.2 mM glycaemia, which is to our knowledge, the highest power density obtained in human blood. We overcome the limitation found in blood because of the high selectivity of our bioelectrocatalyst and by finely designing our cathodic electrode with a new BOD from the fungus *Magnaporthe oryzae* (Cadet et al., 2013; Durand et al., 2012).

2. Experimental section

2.1. Chemicals

All chemicals were of analytical grade or higher and purchased from Sigma (Sigma-Aldrich, Saint-Louis, MO). The cross linker (poly ethylene glycol diglycidyl ether (400) or PEGDGE) was purchased from Polysciences Inc. (Warrington, PA), conductive carbon paint (20% solid matter in isopropanol) was provided by SPI supplies, (West Chester, PA), and carbon fibers (7 μm diameter) were obtained from Goodfellow (Cambridge Science Park, England).

2.2. Enzymes and redox polymers

Glucose dehydrogenase from *Acinotebacter calcoaceticus* (GDH) and bilirubin oxidase from *Magnaporthe oryzae* (BOD-*Mo*) were produced and purified as already described (Durand et al., 2012; Durand et al., 2010). The anodic redox polymer PVP-[Os(1,1′-dimethyl-2,2′bisimidazole)$_2$−2-(6-methylpyridin-2yl)imidazole]$^{2+/3+}$was provided by Abbott Diabetes Care (Courjean et al., 2010) and the cathodic redox polymer PAA-PVI-[Os(4,4′-dichloro-2,2′-bipyridine)$_2$Cl]$^{+/2+}$was synthesized as previously reported (Cadet et al., 2013).

2.3. Biofuel cells preparation

Biofuel cells were made of carbon fibers of 2 cm long and 7 μm diameter fixed onto glass slides and connected to electrical cables using carbon paste. The connections were insulated by Araldite glue deposited on top. Prior to modification, biofuel cells were made hydrophilic by O_2 plasma surface treatment. The anode was prepared by depositing 2000 $\mu g\,cm^{-2}$ of hydrogel composed of 72 wt% of redox polymer, 22 wt% of GDH and 6 wt% of PEGDGE (Flexer and Mano, 2014). The cathode was made with 62.6 wt% of redox polymer, 30 wt% of BOD*Mo* and 7.4 wt% of PEGDGE for a total loading of 2000 $\mu g\,cm^{-2}$. Electrodes were allowed to dry 18 h at 4 °C before testing (Cadet et al., 2013). For the stability measurements in blood a cellulose dialysis bag (10 kDa pore size) was used as protective membrane.

2.4. Human blood samples

All experiments were approved by the French Ministry of National Education, Research and Technology according to the agreement number AC-2013–2025. 30 anonymized, random and disease-free human blood samples were obtained from the French Blood Institution (*Etablissement Français du Sang*) according to the convention number DC 2014–2203. Each sample was provided in form of a bag of 50 mL. Manipulation of human blood was performed using required precautions including the use of gloves, glasses, mask and labcoat. All contaminated consumables were eliminated by incineration. Glycaemia and O_2 concentration of each sample were respectively measured prior to electrochemical testing using the glycaemia monitor One Touch® Vita® from LifeScan Company and a Clark electrode (microelectrode 1302 from Harvard Apparatus). For each blood sample, experiments were repeated at least four times with four different BFCs.

2.5. Electrochemical measurements

The measurements were performed using two bipotentiostats (CH-Instruments, model CHI 842B and 760C, Austin, TX, USA) with dedicated computers. Platinum spiral wires were used as counter electrodes and all potentials were referred to a Ag/AgCl (3 M NaCl) electrode (BAS, West Lafayette, IN). Power and current densities have been normalized with the geometric surface of the electrodes. All electrochemical measurements were performed in a water-jacket electrochemical cell. The temperature was maintained at 37.5 °C by using an isothermal circulator (Lab Companion, FR). For experiments with a dialysis bag as protective membrane, the biofuel cell was simply inserted in the bag which was rinsed with deionized water beforehand. Power densities were measured by performing linear polarization at 1 $mV\,s^{-1}$ between the OCP and 0 V. In these experiments, convection was added to produce shear stress and mass transport conditions similar to those in blood vessel flowing at 5 $cm\,s^{-1}$ which would be the case for implanted BFCs in vessels (Binyamin and Heller, 1999; Wan et al., 1999). The loss in enzymatic activity was evaluated by subtracting the loss in catalytic current density and the loss in redox polymer measured at 5 $mV\,s^{-1}$.

3. Results

3.1. Characterization of the biofuel cells in buffer and in human blood

30 anonymized, random and disease free whole human blood samples (composed of 55% of plasma and 45% of blood cells) were used in this study and the set-up is seen in Fig. 1. 7 μm diameter and 2 cm long carbon fibers were modified with their respective bioelectrocalalyst for the O_2 reduction and glucose oxidation. (Fig. 1C) It consisted of electrostatic adducts formed between polyanionic enzymes; GDH at the anode and BOD-*Mo* at the cathode; cross linker and two different polycationic osmium redox polymers finely tuned to suit to the redox potential of the enzymes (Heller, 1990). The formation of such hydrogel allow for the diffusion of water soluble substrates, ions, and products while offering a high selectivity. Electron conduction within the adduct results from the collisional electron transfer between oxidized and reduced osmium complexes (Heller, 2006a). The anodic, cathodic and overall cell reactions are respectively represented by Eqs (1), (2) and (3).

$$\beta\text{-D-glucose} \rightarrow \delta\text{-gluconolactone} + 2H^+ + 2e^- \quad (1)$$

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (2)$$