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An oxygen-independent and membrane-less glucose biobattery/supercapacitor hybrid device

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ABSTRACT

Enzymatic biofuel cells can generate electricity directly from the chemical energy of biofuels in physiological fluids, but their power density is significantly limited by the performance of the cathode which is based on oxygen reduction for in vivo applications. An oxygen-independent and membrane-less glucose biobattery was prepared that consists of a dealloyed nanoporous gold (NPG) supported glucose dehydrogenase (GDH) bioanode, immobilised with the assistance of conductive polymer/Os redox polymer composites, and a solid-state NPG/MnO_2 cathode. In a solution containing 10 mM glucose, a maximum power density of 2.3 μW cm^{-2} at 0.21 V and an open circuit voltage (OCV) of 0.49 V were registered as a biobattery. The potential of the discharged MnO_2 could be recovered, enabling a proof-of-concept biobattery/supercapacitor hybrid device. The resulting device exhibited a stable performance for 50 cycles of self-recovery and galvanostatic discharge as a supercapacitor at 0.1 mA cm^{-2} over a period of 25 h. The device could be discharged at current densities up to 2 mA cm^{-2} supplying a maximum instantaneous power density of 676 μW cm^{-2}, which is 294 times higher than that from the biobattery alone. A mechanism for the recovery of the potential of the cathode, analogous to that of RuO_2 (Electrochim. Acta 42(23), 3541–3552) is described.

1. Introduction

The use of enzymatic biofuel cells (EBFCs) is of promise in generating electricity from fuels (Leech et al., 2012; Rasmussen et al., 2015). EBFCs function at physiological temperature and pH, in comparison to traditional fuel cells utilising abiotic catalysts which generally operate in harsh environments (e.g. strongly acidic or alkaline media). Immobilisation of enzymes at the anode and cathode can eliminate the requirement for membranes that are required in conventional fuel cells to separate the anode and cathode compartments. *In vivo* EBFCs utilising oxygen and glucose are of significant interest due to potential applications as miniaturised power sources for implantable medical devices (Calabrese Barton et al., 2004) such as cardiac pacemakers (MacVittie et al., 2013) and insulin pumps. However, the successful application of autonomous biomedical devices is a significant challenge due to the requirements for high power density, biocompatibility and long lifetime (Shleev, 2017). The concentration of oxygen *in vivo* is significantly lower (0.14 mM in arterial blood and 0.08 mM in intestinal tissue (Carreau et al., 2011; Shleev, 2017)) than that of glucose (3.3 and 4.8 mM in muscle and plasma, respectively (Maggs et al., 1995)), together with possible mass transport limitation of oxygen, making oxygen reducing biocathode a significant limiting factor in the application of EBFCs. For example, the theoretical power output of an *in vivo* 1 cm long tubular glucose/oxygen EBFC is solely determined by the oxygen reduction reaction (ORR) at the cathode (Pankratov et al., 2016b). Moreover, the stability of the enzymes used, predominantly multi-copper oxidases such as laccase and bilirubin oxidase (BOx), needs to be considered. Laccase prefers a weakly acidic environment (ca. pH 4–5) and is inhibited by halide ions (Salaj-Kosla et al., 2013; Spira-Solomon et al., 1986; Vaz-Dominguez et al., 2008; Xu, 1996). In comparison to laccases, BOx is more stable under physiological conditions (pH 7.4, no inhibition in the presence of Cl^-). However, the operational stability of BOx based electrodes is limited, for example, an osmium polymer "wired" *Trachyderma tsunodae* BOx displayed a current loss of 78% after 2 h rotation at 100 rpm, a loss that was mainly ascribed to the irreversible deactivation of BOx Cu-centers in the oxidised state (Kang et al., 2006).

Air-breathing biocathodes can be employed to circumvent limitations in the supply of oxygen, but can only be used in subcutaneous devices (Miyake et al., 2011). Recently, molecular oxygen-independent

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hybrid EBFCs or biobatteries relying on a combination of enzymatic anodes and solid-state cathodes have been proposed to address the underlying problems of enzymatic cathode based EBFCs. These abiotic cathodes utilise cheap and abundantly available materials such as Prussian Blue (PB) (Addo et al., 2011), Ag_2O/Ag (Yu et al., 2016b) and MnO_2 (Yu et al., 2016a), which can be reduced/discharged via an external circuit, resulting in rechargeable biobatteries. For example, the oxidation of PB to Berlin Green (BG) occurs at a high potential of 0.87 V vs. SCE (Neff, 1985), exceeding the redox potentials of multi-copper oxidases. Minteer et al. developed a rechargeable ethanol biobattery based on an alcohol dehydrogenase (ADH) modified bioanode and a PB paste cathode that registered an open circuit voltage (OCV) of up to 1.2 V (Addo et al., 2011). Dong et al. combined a glucose dehydrogenase (GDH) bioanode with an Ag_2O/Ag (Yu et al., 2016b) or MnO_2 cathode (Yu et al., 2016a) to fabricate oxygen-independent recycled biobatteries with reported OCVs of 0.59 V and 0.43 V, respectively. Microbial biobatteries consisting of anodes colonized by microorganisms and reoxidisable solid-state cathodes such as Ag_2O/Ag (Xie et al., 2013) and PB (Xie et al., 2015) were stable, showing no loss of capacity over 20 cycles of operation (Xie et al., 2015).

Biofuel cell (BFC)/supercapacitor hybrid devices, or self-charging biocapacitors, utilising capacitive bioelectrodes are of great interest due to their ability to generate repeated electric pulses, with an instantaneous power density that is significantly higher than that from the BFC itself (Agnes et al., 2014). Biocapacitors taking advantage of enzymes (Agnes et al., 2014; Kizling et al., 2015; Knoche et al., 2016; Pankratov et al., 2014), microbes (Santoro et al., 2016) and thylakoids (Pankratova et al., 2017) have been presented. Recently, we described a supercapacitive EBFC prepared by the immobilisation of flavin adenine dinucleotide-dependent GDH (FAD-GDH) and BOx with electrodeposited poly(3,4-ethylenedioxythiophene) (PEDOT) and the redox polymer $[Os(2,2'\text{-bipyridine})_2(polyvinylimidazole)_{10}Cl]^{+/2+}$ ($Os(bpy)_2PVI$) on dealloyed nanoporous gold (NPG) (Xiao et al., 2017). The device could operate as a pulse generator to mimic that in a cardiac pacemaker, producing 10 μA pulses for 0.5 ms at a frequency of 0.2 Hz.

In this contribution, we substitute the BOx biocathode with a non-enzymatic MnO_2 cathode to assemble an oxygen-independent glucose biobattery/supercapacitor hybrid device (Scheme 1). At neutral pH MnO_2 only shows catalytic activity towards oxygen at negative potentials (Zhang et al., 2009), outside the potential window needed in this work and is thus used as a consumed cathode. MnO_2 has been selected based on several considerations: (i) a higher pseudo-capacitance in comparison to carbon materials (Simon and Gogotsi, 2008). MnO_2 is partially charged/discharged via the intercalation/deintercalation of electrolyte cations (e.g. Na^+) and protons according to the reaction:

$$\mathrm{Mn(IV)O_2} + xNa^+ + yH^+ + (x+y)e^- \leftrightarrow Mn(III)_{(x+y)}Mn(IV)_{1-(x+y)}OONa_xH_y \quad (1)$$

where $0 < (x+y) \leq 1$. In this case, the discharged form is insoluble, avoiding issues with leakage. (ii) a moderate onset potential, resulting in a biobattery with a considerable OCV (Yu et al., 2016a). (iii) operation at neutral pH that is amenable to enzymes. (iv) inert to the oxidation of glucose, as confirmed by Dong et al. (Yu et al., 2016a), resulting in a membrane-less biobattery. A spontaneous recovery of the potential of the discharged NPG/MnO_2 was observed in open-circuit mode, similar to that reported with a pseudo-capacitive RuO_2 electrode (Liu et al., 1997). The assembled NPG/PEDOT/$Os(bpy)_2PVI$/FAD-GDH//NPG/MnO_2 biobattery/supercapacitor hybrid device delivered intermittent electric signals, with a power density much higher than that of the biobattery itself.

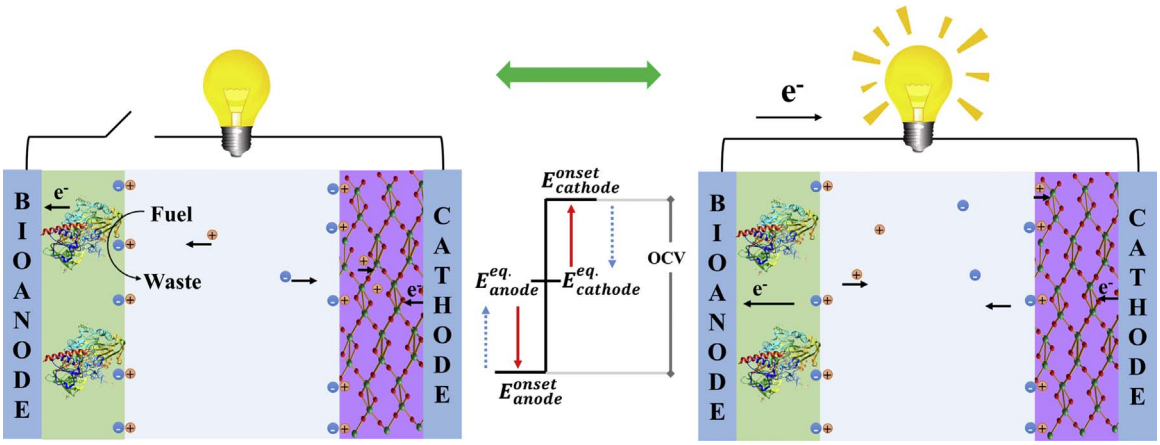
2. Experimental section

2.1. Materials and apparatus

Sodium phosphate (monobasic dehydrate ≥99% and dibasic ≥99%), sodium sulfate (≥99.99%), manganese(II) acetate tetrahydrate (99.99%), *D*-(+)-glucose (99.5%), 3,4-ethylenedioxythiophene (EDOT, 97%) were obtained from Sigma-Aldrich Ireland, Ltd. All solutions were prepared with deionised water (18.2 MΩ cm, Elga Purelab Ultra, UK). $Os(bpy)_2PVI$ was prepared according to an established procedure (Forster and Vos, 1990; Kober et al., 1988). Oxygen-insensitive, recombinant *Glomerella cingulata* FAD-GDH (EC 1.1.99.10, D-glucose: acceptor 1-oxidoreductase) was expressed in *Pichia pastoris* and purified with a specific activity of 572 U mg^{-1} (Sygmund et al., 2011).

Dealloyed NPG leaves were obtained by floating ca. 100 nm thick Au/Ag leaves (12-carat, Eytzinger, Germany) on concentrated HNO_3 (Sigma-Aldrich) for 30 min at 30 °C (Xiao and Magner, 2015; Xiao et al., 2014). And then placed on well-polished glassy carbon electrodes (GCEs, diameter: 4 mm). The NPG electrodes were cleaned by scanning the potential over the range of −0.2–1.65 V in 1 M H_2SO_4 at a scan rate of 100 mV s^{-1} for 15 cycles.

Scanning electron microscopy (SEM) images were collected using a Hitachi SU-70 microscope (operating at 15 kV), equipped with an energy dispersive X-ray spectroscopy (EDX). Transmission electron microscopy (TEM, JEOL JEM-2100, operating voltage of 200 kV) images of the electrodes were obtained on samples mounted on 300-mesh copper grids (S147-3, Agar Scientific, UK). The average pore size and layer thickness were measured with ImageJ software (National Institutes of Health, Bethesda, Maryland) (Schneider et al., 2012) using at least 30 measurement points. Raman spectra of MnO_2 deposited on



Scheme 1. Schematic diagrams of the hybrid device working at the reset (left) and galvanostatic discharging mode (right). The scheme in the middle depicts the relevant potential differences, with potential shifts caused by galvanostatic discharging (blue arrows) and on the recovery of the potential during the quiescent step (red arrows).