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Continuous power generation from glucose with two different miniature flow-through enzymatic biofuel cells

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ABSTRACT

Enzymatic biofuel cells (EBFCs) can generate energy from metabolites present in physiological fluids. They represent an attractive alternative to lithium batteries to power implantable devices, as they work at body temperature, are light and easy-to-miniaturise. To be implantable in blood vessels, EBFCs should not only be made of non-toxic and biocompatible compounds but should also be able to operate in continuous flow-through mode. The EBFC devices reported so far, however, implement carbon-based materials of questionable toxicity and stability, such as carbon nanotubes, and rely on the use of external redox mediators for the electrical connection between the enzyme and the electrode. With this study, we demonstrate for the first time continuous power generation by flow through miniature enzymatic biofuel cells fed with an aerated solution of glucose and no redox mediators. Non-toxic highly porous gold was used as the electrode material and the immobilisation of the enzymes onto the electrodes surface was performed via cost-effective and easy-to-reproduce methodologies. The results presented here are a significant step towards the development of revolutionary implantable medical devices that extract the power they require from metabolites in the body.

1. Introduction

Millions of patients worldwide suffer from serious diseases such as bradycardia, fibrillation or diabetes, and consequently require active medical implants (WHO 2014). Size, weight, and reliability are fundamental characteristics of these devices, and are typically determined by the power source utilised. Active medical implants are traditionally powered by lithium batteries, which are heavy and difficult to miniaturise, leading to disproportionately large energy sources compared to the systems that they power (Bazaka and Jacob 2012). The search for alternative power sources, which are light, non-toxic, and easy-to-miniaturise, is therefore crucial.

Enzymatic biofuel cells (EBFCs) are power sources that can be employed in the human body (Barton et al. 2004). EBFCs are a specific type of fuel cell that implements redox enzymes as catalysts at the anode and cathode. They can mimic many of the metabolic pathways found within living cells (in particular the oxidation of glucose), and thus produce power from energy sources naturally found in biological fluids at body temperature (Cosnier et al. 2014). EBFCs have also the added benefit of producing the same waste products as the living organism that hosts them, and could thus use established waste metabolism routes to dispose of the by-products produced during the production of power. These features make EBFCs an attractive alternative to lithium batteries.

For the purpose of *in vivo* use, EBFCs could either be implanted in soft tissue or in blood vessels (Barton et al., 2004; Cinquin et al., 2010; Kerzenmacher et al., 2008). In the case of devices to be implanted in the soft tissue, the system is limited by lower fuel and oxidant concentrations (glucose and oxygen), and is thus reliant solely on diffusion for the supply and removal of reactants and waste products respectively. Devices designed for use in blood vessels, would instead exhibit continuous flow through operation. In this case, the EBFC would benefit by having higher concentrations of glucose and oxygen, continuously supplied by the flow of blood. The continuous flow could however lead to enzyme leaching from the electrodes, and could interfere with the electron transfer between enzymes and the electrode surface (Kerzenmacher et al., 2008).

Currently the practical application of EBFCs is prevented by several major hindrances, the most significant of which are poor stability and extremely low power yields (Wei and Liu, 2008). However, the poor stability is not necessarily caused by the deactivation of the enzymes used, but rather by a decay in the efficiency of electron transfer between the enzymes and the electrode surface. This is due to the fact that most EBFCs reported so far rely on mediated electron transfer (MET) techniques, which typically require foreign redox active particles to transfer electrons between

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the active site of the enzyme and the electrode surface (Cooney et al., 2008; Degani and Heller, 1987; Minteer et al., 2007). Over time, these free moving redox mediators can leach out from the fuel cell's electrodes causing a decay in power output.

The development of new enzyme immobilisation techniques in recent years, which achieve direct electron transfer (DET) between the enzyme and the electrode, together with major advances in material sciences allowing for much higher enzyme loading, and advances in electronics leading to ultra-low power medical devices (with a typical pacemaker now only requiring 10 μW) have opened new perspectives and reinforced the interest for EBFCs (Cosnier et al., 2014).

Power generation by EBFCs from biological fluids has been recently proven (Kim et al., 2006; Rincón et al., 2011; Sokic-Lazic and Minteer, 2009; Togo et al., 2007). Nonetheless, there is currently very little reported on fully-fledged devices capable of continuous operation under physiological conditions. The majority of devices reported so far show the use of separate enzymatic electrodes simply placed in the living organism and externally wired. In the case of continuous flow-through operation, these devices still rely on the use of mediators in the feed solution. There are no reported EBFCs that exhibit continuous flow-through operation without mediators for the potential use in line with blood vessels. Instead the few studies that have progressed to the development of devices capable of sustained operation have thus far focused on implantation in the soft tissue of animals (Castorena-Gonzalez et al., 2013; Cinquin et al., 2010; Halámková et al., 2012; MacVittie et al., 2013; Rasmussen et al., 2012; Sales et al., 2013; Szczupak et al., 2012).

All of these reported studies rely on the use of carbon-based electrodes. In particular, carbon nanotube (CNT) aggregates have been widely implemented, since they allow DET with glucose oxidase (GOx), which is the most prevalent enzyme used for glucose oxidation at the anode of EBFCs (Anthony et al., 2002; Ivnitski et al., 2006; Liu et al., 2005). However, the long term toxicity and stability of CNTs is still unknown. CNTs have been reported to cause the destruction of T lymphocyte cells in mammals (Bottini et al., 2006). Direct contact between the CNTs electrode and physiological fluids should therefore be carefully prevented in implantable applications. Some researchers have tackled this obstacle by enclosing their devices in semi-permeable membranes which would prevent direct contact between the electrodes and white blood cells (Cinquin et al., 2010). This may, however, not be possible when designing a device for implantation in or around blood vessels, since the device would have to be small enough to cause minimal disruption to normal blood flow. The search for electrode materials, as an alternative to carbon-based systems is therefore critical for implantable EBFCs. In this context, highly porous gold (hPG) electrodes are a promising alternative. These non-toxic electrodes have remarkable properties, such as high conductivity, large surface area, three-dimensional open porosity, and biocompatibility. Moreover, their large surface area and foam-like morphology make hPG electrodes the ideal support for enzyme immobilisation at high loadings as previously demonstrated by this group and others (Chen et al., 2012; du Toit and Di Lorenzo, 2014b; Hakamada et al., 2012; Xiao et al., 2013).

In this study, we demonstrate for the first time sustained power production from continuous flow-through enzymatic biofuel cells (CFEBFCs) for periods of up to one month without the use of external redox mediators. We also demonstrate a simple and low cost methodology for fast prototyping of CFEBFCs using 3D printed moulds, as well as the electrochemical production of hPG electrodes without the need for potentiostatic control. Moreover, GOx, implemented at the anode, is electrostatically immobilisation onto the hPG surface *via* the simple and easy-to-reproduce method that we have previously reported (du Toit and Di Lorenzo, 2014b).

2. Experimental

2.1. Materials

GOx from *Aspergillus niger*, laccase (LAC) from *Rhus vernicifera*, and all other reagents used were of analytical grade and purchased from Sigma-Aldrich. Unless stated otherwise, all the aqueous solutions used were prepared with reverse osmosis purified water. Saturated calomel electrodes (SCE) were purchased from IJCambria Ltd. Platinum wire was purchased from Cookson Precious Metals Ltd. Polydimethylsiloxane (PDMS, Dow Corning Sylgard 184) was purchased from Ellsworth Adhesives.

All analytical experiments were performed in phosphate buffered saline (PBS). This was prepared with the following constituents: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4. The pH of this solution was then adjusted to 7 with the drop wise addition of 1 M solutions of HCl and NaOH.

All potentiostatically-controlled electrochemical processes were conducted using the Autolab PGSTAT128 N (Metrohm, UK) potentiostat. The load on the fuel cells was controlled using a Cropico variable resistance box (RS Components) and the potential difference was monitored and recorded using a PicoLog ADC-24 multichannel data logger. The moulds for the PDMS replicas were 3D printed in polylactic acid using a Makerbot Replicator.

2.2. Deposition of hPG onto platinum wires

The hPG was fabricated with a two-stage process similar to the process previously described (du Toit and Di Lorenzo, 2014a). Briefly, controlled lengths of platinum wire electrodes were immersed in an electrolyte consisting of 0.1 M $HAuCl_4$ and 1 M NH_4Cl. Gold was deposited in two steps. For platinum wire lengths of 1 cm or less, the working potential was first gradually stepped down to −4.0 V (vs. SCE) over a period 10 s using the Autolab PGSTAT128N (Metrohm, UK) potentiostat. This potential was then maintained for a further 10 s. For platinum wire lengths greater than 1 cm (where the required deposition current exceeded the capabilities of the potentiostat), a simple two-electrode system was used with a second platinum wire as the counter electrode. This time the potential applied across the two electrodes was gradually stepped down to −10 V and then maintained at this potential for 10 s using the Basetech BT-305 variable bench-top power supply unit. This was done in accordance with the actual potential applied across the working electrode and counter electrode when using a potentiostat with a three-electrode setup.

The morphology of the resulting electrodes was characterised using a Hitachi S-4300 field emission scanning electron microscope (FESEM).

2.3. Enzyme immobilisation onto hPG electrodes

GOx and LAC were immobilised onto the hPG anode and cathode respectively to facilitate the oxidation of glucose at the anode and the reduction of oxygen at the cathode according to the following reactions:

$$\underbrace{C_6H_{12}O_6}_{glucose} \xrightarrow{GOx} \underbrace{C_6H_{10}O_6}_{D-glucono-1,5-lactone} + 2e^- + 2H^+$$

$$\frac{1}{2}O_2 + 2e^- + 2H^+ \xrightarrow{LAC} H_2O$$

GOx was electrochemically adsorbed onto the prepared hPG wire electrodes using a process based on the method described previously (du Toit and Di Lorenzo, 2014b). Simply, 6 CV scans were conducted between 0.42 V and 0.60 V (vs. SCE) at a scan rate