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Generating power from transdermal extracts using a multi-electrode miniature enzymatic fuel cell

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ABSTRACT

The development of self-powered wearable biodevices is highly attractive for a number of applications, such as health monitoring and drug delivery. Enzymatic fuel cells (EFCs) hold great potential as power sources for such devices, since they can generate power from physiological fluids and operate at body temperature.

In this study, we present a cascade of three EFCs embedded in a compact and handy single channel device and we demonstrate for the first time power generation from iontophoresis extracts obtained from pig skin. The EFCs implement non-toxic highly-porous gold electrodes; an easy-to-reproduce procedure is adopted for the immobilization of glucose oxidase and laccase at the anode and cathode respectively; no external mediators are used; and the system design can easily be further miniaturized.

When electrically connected in parallel, the EFCs generated a power output close to the sum of the power generated by each unit, with peak values of 0.7 μW (flow-through mode) and 0.4 μW (batch mode), at a glucose concentration of 27 mM. When the device was fed with transdermal extracts, containing only 30 μM of glucose, the average peak power was proportionally lower (0.004 μW).

1. Introduction

The recent enhancements in electronic technologies and nanotechnology open up exciting prospects in the field of wearable healthcare devices for a diverse range of applications, including non-invasive detection of biomarkers (Bandodkar and Wang, 2014), transdermal drug delivery (Riahi et al., 2015) and fitness monitoring (Kim et al., 2015). These new technologies aim at painless point-of-care testing, which could help with early prevention, optimum disease management, reduce healthcare costs, and improve patients' lifestyles (Chan et al., 2012). Wearable devices can also be coupled with wireless transmission systems for rapid and remote data processing, thus heading to the new era of 'virtual healthcare' (Alemdar and Ersoy 2010; Sultan, 2015). Generally, these devices require a power source to function, which is typically provided either by lithium batteries or by wireless power transmission *via* radio frequency induction (Wang et al., 2015; Yao et al., 2012). In alternative, enzymatic fuel cells (EFCs) could lead to self-powered healthcare devices that harvest the energy required to function from physiological fluids. EFCs are particular types of fuel cells that implement redox enzymes as catalysts to convert organic substrates, such as carbohydrates, into useful electricity. Recently, a fully self-sustained biodevice with wireless radio signal transmission, powered by an EFC was reported (Falk et al., 2014a). The high specificity of the enzymes, the capability to operate at body temperature and pressure, and the possibility to develop membrane-less devices that are easy-to-miniaturize, make EFCs the ideal candidate for implantable power sources over other types of fuel cells. In the past few years, EFCs have been successfully implanted in rats (Castorena-Gonzalez et al., 2013), snails (Halámková et al., 2012), lobsters (MacVittie et al., 2013) and cockroaches (Rasmussen et al., 2012). Electricity generation from human physiological fluids *via* EFCs has also been reported, which proved the possibility of harvesting energy from tears (Reid et al., 2015), blood and serum (Wang et al., 2012) and saliva (Falk et al., 2014b). The short-term stability of the enzymes implemented, however, seriously compromises the use of EFCs for implantable devices (Cosnier et al., 2014). Non-invasive applications of EFCs, such as in attachable and adhesive devices, appear instead to be

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much more feasible. A recent practical example is given by the EFC tattoo that harvests energy from lactate in sweat (Jia et al., 2013).

The majority of the EFCs reported implement external electron mediators, either in solution or co-immobilized with the enzyme onto the electrode surface, to improve the electron transfer to the anode. The use of these mediators, however, limits practical applications of EFCs, due to their potential toxicity and/or risk of leaching out from the electrode. Moreover, these devices usually implement electrode materials, such as carbon nanotubes or carbon nanoparticles, which would not be suitable for wearable applications, given their potential toxicity.

We have recently reported continuous power generation from two miniature flow-through glucose/oxygen enzymatic fuel cells (du Toit and Di Lorenzo, 2015). The EFCs used highly-porous gold (hPG) electrodes, as an alternative to carbon-based electrodes. hPG is characterized by a very high specific surface area, with a pore size distribution ranging from the micro to the nano scale (du Toit and Di Lorenzo, 2014a). This property, in combination with high conductivity and biocompatibility, makes hPG electrodes an ideal support for enzyme immobilization and allows for a good electrical communication between the electrode and the redox center of the enzyme (du Toit and Di Lorenzo. 2014b).

In this study, we report the effect of combining three pairs of anodes and cathodes in a flow-through single channel EFC device. Due to laminar flow, the resulting device can be considered as a cascade of three enzymatic fuel cells. Its performance is analyzed when the EFCs are electrically independent from each other and when they are electrically connected in parallel. A second device, characterized by the same cross-sectional area but hosting only one pair of electrodes, is also considered for comparison. The incidence of fluid dynamic effects on the fuel cells' performance is analyzed and supported by a comparison with the case of batch mode operation. Finally, we test the use of transdermal fluid, obtained by reverse iontophoresis from pig skin, as potential biological fuel for these EFCs, and we prove the stability of the hPG electrodes towards the typical impurities of biological fluids, and in particular of transdermally extracted fluids (i.e. amino acids, lactate, small proteins) (Bouissou et al., 2009).

2. Experimental section

2.1. Materials

All the chemicals used were of analytical grade and were purchased from Sigma Aldrich. *Glucose oxidase* (GOx) from *Aspergillus niger* and *laccase* (LAC) from *Rhus vernicifera* were purchased from Sigma Aldrich. A Saturated Calomel Electrode (SCE) was used as a reference electrode and was 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 aqueous solutions used were prepared using reverse osmosis purified water. The phosphate buffered saline (PBS) solution was prepared on a weekly basis and consisted of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4. The pH of the resulting solution was adjusted to a value of 7.1 with the drop-wise addition of HCl or NaOH.

All potentiostatically-controlled electrochemical processes were performed using the Autolab PGSTAT128 N (Metrohm, UK) potentiostat. The applied resistance across the fuel cells was varied using a Cropico variable resistance box and the potential difference across the cell was recorded using a PicoLog ADC-24 multichannel data logger. The molds for the PDMS structures were 3D printed in polylactic acid using a Makerbot Replicator.

2.2. Electrode fabrication

The electrodes were made of platinum wire (diameter: 0.5 mm) coated by a film of highly porous gold (hPG) fabricated via a hydrogen bubbling template as previously described (du Toit and Di Lorenzo, 2014a). Briefly, the platinum wires were immersed in an electrolyte consisting of 0.1 M $HAuCl_4$ and 1 M NH_4Cl. Gold was deposited by gradually stepping down the working potential to −4.0 V (vs. SCE) over a period 10 s using the Autolab PGSTAT128N (Metrohm, UK) potentiostat. This potential was maintained for a further 10 s.

2.3. Enzyme immobilization

GOx and LAC were used as catalysts at the anode and cathode respectively. A PBS solution (pH 7.1) containing 15 mg ml^{-1} of GOx was prepared and placed in a three-electrode set-up with the Pt/hPG electrode used as a working electrode and platinum wire (diameter: 1 mm) and SCE used as a counter and a reference electrode respectively. The GOx immobilization was achieved either by performing a CV scan as previously reported (du Toit and Di Lorenzo 2014b), or, to further simplify the immobilization protocol, by applying a fixed potential, in the range of 0.425–0.6 V vs SCE, for one hour. The performance of the GOx/hPG electrode at increasing concentration of glucose was tested by chronoamperometry in a three electrode set up, with Pt and SCE as a counter and a reference electrode respectively. The same principle was adopted for the immobilization of LAC. A LAC solution of 2.5 mg ml^{-1} in PBS was prepared and, considering the isoelectric point of LAC from *Rhus vernicifera* (6.8 to 7.4), to develop a negative charge onto the hPG electrode surface, a fixed potential of −0.5 V vs SCE in a three-electrode set up was applied for a total of 1 hour.

2.4. Device fabrication

Two single-chamber EFC designs were developed. The first device (D1) consisted of a single chamber of 0.6 cm x 0.1 cm x 4 cm, hosting three pairs of anodes and cathodes. The second device (D2) was made of a shorter chamber, 0.6 cm x 0.1 cm x 1.7 cm, hosting a single pair of electrodes. A mold with the negative of the EFC device design was fabricated by 3D printing. A PDMS cast reproducing the channel design was then obtained, which was fastened between two acrylic plates, as shown in Fig. S1 in the Supplementary Data. In both devices, the anodes and cathodes consisted of Pt/hPG electrodes, with a total surface area exposed to the channel of 0.16 cm^2. The electrodes were placed in the channels, parallel to each other following the direction of flow.

2.5. Fuel cells operation

The inlet and outlet streams of D1 and D2 were connected to a programmable multichannel peristaltic pump (Masterflex®, Cole Parmer), equipped with 2-stopped pump tubing (Masterflex®, ID 1 mm). The system set-up was as previously described (du Toit and Di Lorenzo 2015). Briefly, the devices were placed inside an incubator at 37 °C and, in the case of continuous mode operations, were continuously fed with an aerated PBS solution containing 27 mM glucose at a rate of 0.35 ml min^{-1}. The feed solution was pre-heated with a tubing coil to reach the temperature of 37 °C and prior to entering the devices, it passed through a drip to remove any gas bubbles. During batch mode operation, the feed solution was aerated and pre-heated prior to being manually introduced into the device with a syringe.

The anode and cathode of the fuel cells were connected through a voltmeter and a fixed resistor (R) of 30 kΩ, to polarize