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

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A B S T R A C T
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).

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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 Na2HPO4, 2 mM KH2PO4. 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 polyactic 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 HAuCl4 and 1 M NH4Cl. 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) potentiotstat. 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 chronocamperometry 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 cm². 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®, 1 D 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
the cells and monitor the cell potential (V) with the time under closed circuit conditions. The current output (I) was calculated using Ohm’s law (I = V/R), and the power (P) was calculated using P = VI.

At least two replicates (from two different fuel cells) were performed for each test.

2.6. Iontophoresis samples

Reverse iontophoretic samples were extracted across abdominal pig skin. Abdominal skin was dermatomed (Zimmer™ Electric Dermatome, Dover, Ohio; nominal thickness 750 μm), cut into pieces of appropriate size, wrapped individually in Parafilm™, and then kept in a freezer (−20°C) until use. The skin sample was clamped between the two chambers of side-by-side diffusion cells (0.75 cm² each), consisting of an interior chamber, containing the anode, and an exterior chamber containing the cathode. Both chambers were filled with 1.7 mL of PBS (pH 7). Ag/AgCl electrodes, prepared as previously described (Green et al. 1991), were used as both the anode and the cathode. A current of 0.38 mA was applied for 330 minutes using a Yokogawa 7651 (Programmable DC source, Woodburn Green, UK) power supply. Samples (1.7 ml each) were taken from the cathodic epidermal chamber (exterior chamber) at intervals of 30, 90, 150, 210, 270 and 330 minutes. At the end of the experiment the resulting extracts were combined to create a bulk solution. Glucose was added to this bulk solution for a total concentration of 30 μM.

3. Results and discussion

3.1. GOx immobilization

We have previously reported an effective one-step immobilization procedure for GOx onto hPG that leads to the direct electro-oxidation of glucose without the use of external mediators. The immobilization process is likely to involve electrostatic attractions between the enzyme and the hPG surface. These are promoted by the development of a net charge onto the hPG surface, during six CV scans in a GOx solution, with hPG as the working electrode (du Toit and Di Lorenzo 2014a). In particular, considering that at the pH of the buffer used (7.1) GOx from Aspergillus niger has a negative charge (isoelectric point: 4.2), the hPG net charge should be positive to electrostatically attract the enzyme. The development of this positive charge could be achieved by simply applying a fixed positive potential to the electrode, with the benefit of further simplifying the immobilization procedure. To prove this hypothesis, three fixed potentials were tested for the GOx immobilization: 0.5 V, 0.6 V and 0.65 V. The performance of the resulting electrodes was compared with the GOx/hPG electrode obtained by performing a total of 6 CV scans within the range 0.05–25 mM at the potential of 0.25 V. At this potential all the GOx/hPG electrodes showed a peak in the forward scan of a CV test carried out in the presence of glucose (data not shown). This potential is related to H₂O₂ oxidation on hPG, in accordance with what we have previously reported (du Toit and Di Lorenzo 2014b). The oxidation reaction occurring onto the GOx/hPG involves in fact two steps:

\[ \text{C}_6\text{H}_12\text{O}_6 \rightarrow \text{C}_6\text{H}_10\text{O}_6 + 2e^- + 2H^+ \]

\[ 2H^+ + O_2 \rightarrow H_2O_2 \]

Firstly, glucose is oxidized by GOx to form gluconolactone and release electrons and protons. During the second step, hPG catalyzes the formation of H₂O₂ in the presence of oxygen, as previously described (du Toit and Di Lorenzo, 2014a).

In Fig. 1, the results from the chronoamperometry tests are reported in terms of steady state current versus glucose concentration. The worst performance was observed when the potential of 0.5 V was applied. At this potential, the resulting electrode produced a current approximately 2.3 times lower than the GOx/hPG electrode generated by CV scans. The use of higher potentials, such as 0.6 V and 0.65 V, led instead to performance very close to each other and to the case of the CV scans. The latter still generated the highest current, which at a concentration of 25 mM was of 6.13 (± 0.1) μA, compared to 5.33 (± 0.2) μA for the case of the 0.6 V potential. The different methodology did not cause any change in the electrode stability over the maximum testing time considered in this study (e.g. up to one week). In each case, when the electrodes were stored overnight in buffer at 4°C, a maximum of 30% activity decay was observed (for a glucose concentration of 25 mM), as previously observed (du Toit and Di Lorenzo 2014b). When the electrodes were instead kept overnight in buffer under fixed potential (0.6 V) and at room temperature, the maximum activity decay loss was of only 3%. This result supports the hypothesis that the electrostatic attractions, promoted electrochemically, play a key role in the immobilization process onto the hPG surface. To prevent any activity loss due to storage, the hPG electrodes were therefore used right after the enzyme immobilization.

3.2. Power generation from glucose in continuous flow mode

Two single-channel devices were tested and their performance compared. The first configuration, D1, was characterized by three pairs of anode and cathode, which led to a cascade of three EFCs, due to the laminar flow in the channel. The resulting fuel cells
have been indicated as: EFC1, EFC2 and EFC3. The fuel cells were operated in two different electrical configurations, as represented in Fig. 2. In one configuration (Fig. 2C) each EFC was connected independently to a fixed resistor and a voltmeter. This configuration allowed the individual analysis of the performance of each EFC. In the second configuration (Fig. 2D), the EFCs were electrically connected in parallel to a resistor and a voltmeter.

A second device, D2, was also tested for comparison. D2 was characterized by the same geometry and cross-sectional area of D1, but had a shorter channel to host only one pair of electrodes, leading to a control fuel cell, named as EFCc.

Both D1 and D2 were operated in continuous mode and fed with an aerated PBS solution of glucose (27 mM) at a flow rate of 0.35 ml min⁻¹. Fig. 3A shows the power generated by the fuel cells in the two devices over 15 hours of operation. The fluctuations observed in the graph are caused by the formation of air bubbles in the channel, which could have not been completely prevented although air release valves were used. EFCc generated the highest power, with a peak value of 0.75 (± 0.04) mW. After an initial increase in power during the first hour of operation, a gradual activity decay was observed, which is in agreement with our previous results (du Toit and Di Lorenzo 2015). In particular, the total power decay after 15 hours of operation was almost 40%. The same trend was observed for EFC1, EFC2 and EFC3 in D1, with a power decay of approximately 48%, 40% and 30% respectively. Contrary to what was initially expected, however, each fuel cell in D1 generated a much lower power output than EFCc. The best performance in the cascade was obtained with EFC2, with a peak power (0.44 ± 0.05 mW), almost three times higher than EFC1 and EFC3. This value was, however, nearly half the power generated by EFCc. EFC1 and EFC3, located respectively at the entry and exit of device D1, showed very similar performance, with an average peak power of only 0.16 (± 0.1) mW.

The polarization curves reflected the difference in the performance of the three fuel cells, as shown in Figs. 3B and C. The average open circuit voltage (OCV) was of 350 (± 20) mV for EFC2 and 200 (± 50) mV for EFC1 and EFC2, which is close to the values previously found (du Toit and Di Lorenzo 2015). The optimal value of the external resistor, corresponding to the peak power, was found to be 30 kΩ for each fuel cell.

When EFC1, EFC2 and EFC3 were electrically connected in parallel, the power output reached a peak value of 0.71 (± 0.12) mW, which, as expected, was approximately the sum of the peak power values of the three EFCs. In this configuration, the system was, however, highly unstable. After the first five hours of operation a rapid decrease in the power output was observed, reaching the value of 0.1 (± 0.2) mW after 15 hours of operation (See Fig. S2 in the Supplementary Data).

Table 1 summarizes the performances of both devices. The total power output generated by D1 and D2 exceed by approximately 50% the power output obtained in a similar continuous flow EFC study this group has conducted (du Toit and Di Lorenzo 2015). In this case, the electrodes were separated by a physical barrier with the aim of minimizing the effects of H₂O₂ poisoning of laccase which has widely been reported (Milton et al. 2013). The results obtained in the present study demonstrate, therefore, that by reducing the electrode spacing, the EFC performance can be enhanced while the use of laminar flow appears to be sufficient to prevent cross diffusion of H₂O₂. The differences in performance between the different EFCs in D1 could perhaps be due to the differing fluid dynamic effects throughout the channel. Reynolds numbers for D1 (based on hydraulic diameter) are calculated to be ~2.5 and ~5.6 in the channel and at the throat, respectively. The flow is consequently expected to be broadly laminar. Flow regime borders where the inertial forces are comparable to the viscous forces (typically, Re ~1). Fig. S3 shows the predicted streamline patterns to be expected in D1. The reduced performance seen for EFC1 and EFC3 is attributed to the transverse flow effects from the associated expansion and contraction. This loss manifests itself as a reduction in power generated, clearly seen on Fig. 3A. The peak performance seen from EFC2 is consistent with the one-dimensional flow found at this point in the device. It is therefore postulated that the anode–cathode pairs work best when subjected to parallel flow and exhibit power production losses when subjected to a component of velocity in cross-flow.

Contrarily to what initially expected, the total power generated in D1 is not greater than that generated by D2, especially considering that the total surface area of the electrodes is 3 times higher in D1. The higher power output generated by D2, might be caused by a greater velocity of flow in the device compared to D1, due to the different flow resistance resulting from the geometries of the two devices. The performance of single-channel EFCs can in fact be improved at higher flow velocities, as previously observed (Du Toit and Di Lorenzo 2015).

3.3. Power generation from iontophoresis extracts in batch mode

To prove the applicability of D1 more complex solutions that
better resemble real physiological fluids must be considered. Reverse iontophoresis extracts from pig skin were, therefore, tested as the feeding solution. Transdermal iontophoresis enhances molecular transport across the skin via the application of small electric currents, typically \(< 0.5\, \text{mA/cm}^2\) (Leboulanger et al. 2004). The two major transport mechanisms involved, electromigration and electroosmosis, allow the transdermal transport of charged and highly polar compounds at rates much greater than their passive permeability. Iontophoresis extracts components from both the inside the skin and the interstitial subdermal fluid, have been used to monitor glycaemia and other clinical markers of interest, such as lactate (Leboulanger et al. 2004). In particular, transdermal extracts contains several amino acids (Nixon et al. 2007; Sylvestre et al. 2010), which are likely to electrostatically interact with the hPG surface, according to the same principle through which the immobilization of GOx and LAC onto hPG is achieved. The possible interference of the constituents of the iontophoresis extraction samples with the hPG electrodes must therefore be investigated.

Although different in composition, these extracts serve as a very good approximation of sweat, since they are characterized by the same constituent compounds (Leboulanger et al. 2004). The concentration of glucose in these biological fluids ranges between 5.6 and 2,200 \(\mu\text{M}\), with a median value of 170 \(\mu\text{M}\) (Harvey et al. 2010). It is, therefore, important to demonstrate the possibility of generating power with the EFCS also from glucose concentrations up to three orders of magnitude lower than the values used in the previous experiments. In this case, considering the small volumes of the iontophoresis extracts, the system had to be operated in batch mode. Table 2 reports the results, in terms of peak power outputs, from preliminary tests run in batch mode with two different glucose concentrations in PBS: 27 mM and 30 \(\mu\text{M}\). In batch, the mass transfer processes rely merely on diffusion and the fluid dynamic effects discussed above are not applicable. As a result, the fuel cells in D1 had very similar performance, with differences that can be considered within the experimental error. For the case of 27 mM of glucose, the power generated in batch was almost three times lower than the flow-through mode. After reaching a peak within the first 2 minutes, the power output rapidly dropped with a reduction of over 80% after 1 h (data not shown). When a glucose concentration three orders of magnitude lower was used (30 \(\mu\text{M}\)), the peak power was reached right after the feeding system and decayed much more quickly, with an over 90% decay after approximately 10 min (Fig. 4). The variation between the replicates was much larger in this case, due to the sensitivity of the equipment used. For both concentrations of glucose tested, when the fuel cells in D1 were connected in parallel the power output was approximately three times higher.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Maximum absolute power ($\mu\text{W}$)</th>
<th>Maximum volumetric power density ($\mu\text{W cm}^{-1}$)</th>
<th>Maximum power density by surface area of GOx electrode(s) ($\mu\text{W cm}^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D1 (isolated EFCs)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.76</td>
<td>3.16</td>
<td>1.58</td>
</tr>
<tr>
<td>EFC1</td>
<td>0.168 ± 0.02</td>
<td>0.77</td>
<td>1.05</td>
</tr>
<tr>
<td>EFC2</td>
<td>0.44 ± 0.05</td>
<td>1.83</td>
<td>2.75</td>
</tr>
<tr>
<td>EFC3</td>
<td>0.156 ± 0.09</td>
<td>0.65</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>D1 (EFCs connected in parallel)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFC1 + EFC2 + EFC3</td>
<td>0.71 ± 0.12</td>
<td>2.95</td>
<td>1.48</td>
</tr>
<tr>
<td><strong>D2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFCc</td>
<td>0.75 ± 0.04</td>
<td>7.3</td>
<td>4.69</td>
</tr>
</tbody>
</table>

* calculated on the basis on the total volume of D1.
containing 30 mM of glucose; b) fuel cells electrically connected in parallel fed with PBS containing 30 mM of glucose; c) average power generated by EFC1, EFC2 and EFC3 with independent electrical connection fed with the reverse iontophoresis sample containing 30 mM of glucose (maximum variance 6.3%); d) average power generated by EFC1, EFC2 and EFC3 with independent electrical connection fed with PBS containing 30 mM of glucose (maximum variance 1.6%).

Table 2
Summary of peak power outputs obtained in batch mode for two glucose concentrations. The data is the average of two replicates.

<table>
<thead>
<tr>
<th>Glucose concentration in PBS</th>
<th>Peak power</th>
<th>Peak power density by surface area of GOx electrode(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D1 (isolated EFCs)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27 mM</td>
<td>0.47 μW</td>
</tr>
<tr>
<td></td>
<td>30 μM</td>
<td>2.2 nW</td>
</tr>
<tr>
<td>EFC1</td>
<td>27 mM</td>
<td>0.16 ± 0.01 μW</td>
</tr>
<tr>
<td></td>
<td>30 μM</td>
<td>0.89 ± 0.3 nW</td>
</tr>
<tr>
<td>EFC2</td>
<td>27 mM</td>
<td>0.17 ± 0.05 μW</td>
</tr>
<tr>
<td></td>
<td>30 μM</td>
<td>0.70 ± 0.4 nW</td>
</tr>
<tr>
<td>EFC3</td>
<td>27 mM</td>
<td>0.14 ± 0.07 μW</td>
</tr>
<tr>
<td></td>
<td>30 μM</td>
<td>0.70 ± 0.5 nW</td>
</tr>
<tr>
<td><strong>D1 (EFCs connected in parallel)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFC1 + EFC2 + EFC3</td>
<td>27 mM</td>
<td>0.40 ± 0.07 μW</td>
</tr>
<tr>
<td></td>
<td>30 μM</td>
<td>3.20 ± 1 nW</td>
</tr>
<tr>
<td><strong>D2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFCc</td>
<td>27 mM</td>
<td>0.17 ± 0.3 μW</td>
</tr>
<tr>
<td></td>
<td>30 μM</td>
<td>0.60 ± 0.7 nW</td>
</tr>
</tbody>
</table>

Fig. 4. Power generated from the reverse iontophoresis sample. a) fuel cells electrically connected in parallel fed with the reverse iontophoresis sample containing 30 μM of glucose; b) fuel cells electrically connected in parallel fed with PBS containing 30 μM of glucose; c) average power generated by EFC1, EFC2 and EFC3 with independent electrical connection fed with the reverse iontophoresis sample containing 30 μM of glucose (maximum variance 6.3%); d) average power generated by EFC1, EFC2 and EFC3 with independent electrical connection fed with PBS containing 30 μM of glucose (maximum variance 1.6%).

than the individual fuel cells.

On one hand, these results highlight the relevance that flow-through operations have over batch processes for optimal energy conversion. In batch, the electrochemical processes are diffusion rate limited, while in continuous mode operation, the mass transport phenomena are improved, thus enhancing the power output. On the other hand, these results confirm that the fluid dynamic effects in D1 have a marked incidence on the performance of each fuel cell. This is also the case for D2, since in batch mode EFCc generated a power output comparable to that of the fuel cells in D1. As such, important guidelines on how to design an effective multi-electrode device with reduced fluid dynamic effects when operating in continuous mode could be extracted. Possible improvements to the design could include the use of baffles or flow guides to shallower diffusion gradients. A multi-electrode design can lead to higher power outputs by increasing the electrode surface area while decreasing the thickness of the diffusion layer along the electrode length in the direction of the flow, as previously suggested (Lim and Palmore 2007). Moreover, it allows exploring the possibility of achieving deep fuel oxidation by strategically patterning different enzymes along the device channel (Kjeang et al. 2006; Moehlenbrock et al. 2015).

D1 was subsequently fed with the iontophoresis extracts. Fig. 4 reports the average power generated with the time by the individual fuel cells and by the fuel cells stack electrically connected in parallel. The power generated from glucose in PBS is also shown for comparison.

As reported, the iontophoresis extracts lead to higher power outputs. This result could be due to the presence of extra glucose in the pig skin that leads to an overall concentration higher than 30 μM. It is well known that the skin holds a reservoir of glucose and amino acids that are easily extracted with iontophoresis (Rao et al. 1993). Based on the reverse iontopheretic work done with pig skin in conditions very similar to our experiments (Bouissou et al. 2009), we estimate, however, that the extraction of skin glucose would have increased the glucose concentration in the extracts tested only of a maximum of 1 μM. As a consequence, the glucose concentration would shift from 30 μM to 31 μM as a maximum. It can also be observed that the fuel cells generate a power far more stable than the case of a PBS-based feeding solution. In particular, when the EFCs are operated individually the power output was stable at an average value of 1.2 nW during 40 min. The reasons behind this result are not clear. It should be noted that the iontophoresis extracts were characterised by the same conductivity of the PBS sample. Other constituents of the biological fluid might, therefore, contribute to the power generation, or improve electron transfer efficiency, though further investigation is necessary. For the case of connection in parallel the observed power is still higher than the individual cells. Overall, these results demonstrate the applicability of the hPG biocatalysts, and more in general of D1, also for the case of biological fluids.

It is difficult to perform a direct comparison of these results with the few other studies on miniature EFCs tested with physiological fluids. This difficulty is not only due to the different device designs and catalysts used but also to the different operating conditions and mainly to the diverse fluids in which such devices have been tested. Nonetheless, few close studies show very similar performance in terms of power output. Falk et al. have recently reported a peak power by polarization of 0.26 μW cm⁻² for a glucose concentration of approximately 0.5 mM with a miniature EFC fed with sweat, using gold nanoparticles-based electrodes and cellulose dehydrogenase and bilirubin oxidase as respectively the anode and the cathode biocatalyst.
Another close example is a hybrid fuel cell consisting of a bilirubin oxidase cathode and a abiotic (conductive organic complex) anode contact lens recently reported, which was able to produce up to 9 nW from tears (containing approximately 50 μM of glucose) (Falk et al. 2013).

**Concluding remarks**

Continuous electricity generation from biological fluids by enzymatic fuel cells opens up an attractive perspective in the field of self-powered wearable healthcare devices.

In this study, we report the development of a cascade of three EFCs embedded in a small-scale device, which is aimed at scaling-up the power generated by a single fuel cell in an innovative, compact and handy device. We also demonstrate power generation from reverse iontophoresis extracts obtained from pig skin. The device implements non-toxic electrodes, based on highly porous gold, and a very cost-effective and an easy-to-reproduce on an industrial-scale enzyme immobilization procedure was adopted for the bioelectrodes fabrication.

Future research will focus on the development of effective designs for flow-through operations that minimize fluid dynamic effects while enhancing the mass transport of reagents/products. Further miniaturization of the system and integration with a reverse iontophoresis set-up will also be considered for practical applications.

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**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.11.074.

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