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Xinxin Xiao, Peter Ó Conghaile, Dónal Leech, Roland Ludwig, EDMOND MAGNER

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10 **A symmetric supercapacitor/biofuel cell hybrid device based on enzyme-modified
nanoporous gold: an autonomous pulse generator**

Xinxin Xiao^a, Peter Ó Conghaile^b, Dónal Leech^b, Roland Ludwig^c and Edmond
Magner^{a,*}

^aDepartment of Chemical and Environmental Sciences, Bernal Institute, University of
15 Limerick, Limerick, Ireland

^bSchool of Chemistry & Ryan Institute, National University of Ireland Galway,
Galway, Ireland

^cDepartment of Food Science and Technology, BOKU-University of Natural
Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria

20 *Corresponding author: Edmond Magner, E-mail address: edmond.magner@ul.ie; Fax:
+353 61 213529; Tel: +353 61 214390

Abstract

The integration of supercapacitors with enzymatic biofuel cells (BFCs) can be used to
prepare hybrid devices in order to harvest significantly higher power output. In this
25 study, a supercapacitor/biofuel cell hybrid device was prepared by the immobilisation
of redox enzymes with electrodeposited poly(3,4-ethylenedioxythiophene) (PEDOT)
and the redox polymer $[\text{Os}(2,2'\text{-bipyridine})_2(\text{polyvinylimidazole})_{10}\text{Cl}]^{+/2+}$
(Os(bpy)₂PVI) on dealloyed nanoporous gold. The thickness of the deposition layer
can be easily controlled by tuning the deposition conditions. Once charged by the
30 internal BFC, the device can be discharged as a supercapacitor at a current density of

2 mA cm⁻² providing a maximum power density of 608.8 μW cm⁻², an increase of a factor of 468 when compared to the power output from the BFC itself. The hybrid device exhibited good operational stability for 50 charge/discharge cycles and ca. 7 hours at a discharge current density of 0.2 mA cm⁻². The device could be used as a pulse generator, mimicking a cardiac pacemaker delivering pulses of 10 μA for 0.5 ms at a frequency of 0.2 Hz.

Keywords: Biofuel cell; Supercapacitor; Hybrid device; Nanoporous gold; Osmium redox polymer; Pulse generator

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1. Introduction

Enzymatic biofuel cells (BFCs) utilizing oxidoreductases as electrocatalysts can be used to generate electricity from fuels such as sugars or alcohols in combination with dioxygen (Calabrese Barton et al. 2004; Cooney et al. 2008; Leech et al. 2012). BFCs are of interest as power sources for biosensors (Pinyou et al. 2015; Zloczewska et al. 2014), medical implants (e.g. insulin pumps, cardiac pacemakers (MacVittie et al. 2013)), and other devices (Falk et al. 2012; Ó Conghaile et al. 2016). To be able to activate commonly used microelectronic devices (such as commercial pacemakers), appropriate output voltages (minimum of 1.4 V) are required (MacVittie et al. 2013). The open circuit voltage (OCV) of glucose and oxygen BFCs is limited by the thermodynamic value of 1.179 V (Pankratov et al. 2016), and in practice by the difference between the onset redox potentials of the bioanode and biocathode (Cracknell et al. 2008). The observed OCV can be increased by using direct electron transfer (DET) or by the use of redox mediators with redox potentials closer to those of the enzyme/cofactor (Rasmussen et al. 2015). The OCV can also be increased by using multiple cells connected in series (MacVittie et al. 2013). However, due to the presence of conductive fluids within the body, implantable cell stacks suffer from the problem of short-circuits between individual cells (Andoralov et al. 2013; MacVittie et al. 2013). In such systems, isolation of the cells is essential. Another route is to couple BFCs with external electronic devices to increase the voltage. For example,

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using a charge pump and a DC-DC converter, a fluidic BFC utilizing PQQ-dependent glucose dehydrogenase and laccase with an intrinsic OCV of 0.47 V was sufficient to power a pacemaker (Southcott et al. 2013). Falk et al. presented a self-powered wireless lactose biosensing system, consisting of an energy harvesting module including a voltage amplifier and capacitor to build a power source based on a BFC using bilirubin oxidase (BOx) and cellobiose dehydrogenase (CDH) (Falk et al. 2014).

In addition to low voltage outputs, BFCs are also limited by their low current/power densities, which can be improved through efficient substrate diffusion (Murata et al. 2009), enhanced rates of electron transfer between enzymes and electrodes, improving catalytic activity (Suraniti et al. 2013) and loading of enzymes (Flexer et al. 2011), as well as utilizing enzyme cascades for deep and complete oxidation pathways (Kim et al. 2013; Shao et al. 2013; Xu and Minteer 2012). The introduction of capacitors into the BFC circuit enables the accumulation of charge, resulting in output pulses of higher power. Sode et al. proposed the concept of a “BioCapacitor” with the integration of a charge pump/capacitor and a BFC that resulted in higher voltages and currents (Hanashi et al. 2009; Sode et al. 2016). Electrochemical capacitors (known as supercapacitors) (Winter and Brodd 2004) take advantage of the electrical double layer capacitance attained via ion adsorption or pseudocapacitance achieved by fast and reversible faradaic reactions, offering high specific power density and great durability. Supercapacitors externally connected to a laccase-based cathode and zinc anode based biobattery, had higher power stability than the battery itself (Skunik-Nuckowska et al. 2014). Recent progress has seen BFC assemblies with capacitive bioelectrodes (Agnes et al. 2014; González-Arribas et al. 2016; Kizling et al. 2015a; Kizling et al. 2015b; Pankratov et al. 2014b). These supercapacitor/BFC hybrids, or self-charging biocapacitors, are based on the fabrication of hybrid composite modified electrodes with integration of enzymes and capacitive materials. The main feature is their ability to generate cyclic, high power pulses from the discharge of the supercapacitor, which is recharged towards to OCV via the internal BFC in the following open-circuit mode (Agnes et al. 2014). A

supercapacitor/BFC hybrid based on wired enzymes on carbon nanotubes (CNTs) was capable of delivering discharge pulses for 5 days in the presence of glucose and O₂ (Agnes et al. 2014). The majority of supercapacitor/BFC systems have relied on the use of high-surface-area carbon nanomaterials (CNMs), such as CNT (Agnes et al. 2014; Kizling et al. 2015a; Kizling et al. 2015b; Pankratov et al. 2014b) and graphene (González-Arribas et al. 2016). However, the potential toxicity of CNMs (Magrez et al. 2006) should be taken into account for *in vivo* applications and direct exposure to CNMs should be avoided in implantable devices (Miyake et al. 2011).

Dealloyed nanoporous gold (NPG), a porous material in a self-supporting bulk form comprising three-dimensional frameworks of bicontinuous pores and ligaments (Ding et al. 2004), has been investigated as conductive and non-toxic supports for supercapacitors (Lang et al. 2011; Meng and Ding 2011) and enzyme immobilisation (Scanlon et al. 2012; Xiao and Magner 2015; Xiao et al. 2016; Xiao et al. 2014), separately. Mediators are required to enable efficient electron transfer between the cofactor of the enzyme and the NPG surface (Xiao et al. 2013). In this context, alternate potential pulses could be applied to electrodeposit osmium redox polymers with the co-immobilisation of enzymes onto electrode surface (Gao et al. 2002; Habermüller et al. 2000; Schuhmann et al. 1997). Unlike other soluble mediators that are prone to leakage (González-Arribas et al. 2016), the electrodeposited redox polymer is robust, even in hydrodynamic conditions. For example, a laccase/redox polymer composite film showed little loss in response after rotation for 24 h at 2500 rpm (Shen et al. 2013). In this contribution, we electrodeposited poly(3,4-ethylenedioxythiophene) (PEDOT) and the redox polymer [Os(2,2'-bipyridine)₂(polyvinylimidazole)₁₀Cl]⁺²⁺ (Os(bpy)₂PVI) onto NPG electrodes with the co-immobilisation of enzymes. Flavin adenine dinucleotide-dependent glucose dehydrogenase (FAD-GDH, EC 1.1.99.10, *D*-glucose: acceptor 1-oxidoreductase) was used as an oxygen-insensitive enzyme at the anode (Zafar et al. 2012), in contrast to glucose oxidase (GOx) which depletes dissolved oxygen and produces unwanted hydrogen peroxide (Milton et al. 2015). BOx was immobilised at the cathode and the properties of the cell were characterised in detail.

A proof-of-concept pulse generator for a pacemaker was demonstrated, which was able to deliver a 10 μ A pulse at a frequency of 0.2 Hz.

2. Experimental section

5 2.1. Materials

Potassium phosphate monobasic ($\geq 99\%$) and dibasic ($\geq 98\%$), D-(+)-glucose (99.5%), 3,4-ethylenedioxythiophene (EDOT, 97%) were obtained from Sigma-Aldrich Ireland, Ltd. Potassium chloride (KCl, $\geq 99\%$) was purchased from Fisher Scientific Ireland, Ltd. All solutions were prepared with deionised water
10 (resistivity of 18.2 M Ω cm) from an Elgastat maxima-HPLC (Elga, UK). Os(bpy)₂PVI was synthesised using a published procedure (Jenkins et al. 2009). BOx from *Myrothecium verrucaria* (EC 1.3.3.5, 2.63 U mg⁻¹) was purchased from Amano Enzyme Inc., Japan. Recombinant, (in *Pichia pastoris*) expressed *Glomerella cingulata* GDH (EC 1.1.99.10) with a specific activity of 572 U mg⁻¹ was prepared
15 according to a published route (Sygmund et al. 2011).

NPG leaves were fabricated by dealloying ca. 100 nm thick Au/Ag leaf alloy (12-carat, Eytzinger, Germany) in concentrated HNO₃ (Sigma-Aldrich) for 30 min at 30 °C. The NPG films were then attached onto pre-polished glassy carbon electrodes (GCEs) with a diameter of 4 mm. Prior to use, cyclic voltammetry (CV) of NPG in
20 1 M H₂SO₄ were carried out to create clean surfaces and left to dry naturally.

2.2. Enzyme immobilisation procedures

The electrodeposition solutions contained 0.1 M pH 7.0 phosphate buffer solution (PBS) with 2 mM polyethylene glycol 3400 (PEG3400), 20 mM EDOT, 0.5 mg ml⁻¹
25 Os(bpy)₂PVI and either 0.5 mg ml⁻¹ of FAD-GDH or BOx. The presence of PEG enabled the dispersion of EDOT in aqueous media and increased the hydrophilicity of the polymer (Reiter et al. 2001; S. Fabiano et al. 2002). A pulse sequence of 0.9 V (2 s) and -0.4 V (3 s) was used for deposition. The electrodes were then gently rinsed with PBS. For comparison purposes, films were also deposited onto polycrystalline planar
30 Au electrodes.

2.3. Morphology characterisation

Scanning electron microscopy (SEM) images were recorded using a Hitachi SU-70 microscope operated at 15 kV. Transmission electron microscopy (TEM) images were
5 obtained using a JEOL JEM-2100 instrument at an acceleration voltage of 200 kV. The average pore size and deposition layer thickness were obtained by performing at least 30 different measurements with ImageJ software (National Institutes of Health, Bethesda, Maryland) (Schneider et al. 2012).

2.4. Electrochemical measurements

Generally, electrochemical studies were performed using a CHI802 potentiostat (CH Instruments, Austin, Texas) in a standard three-electrode electrochemical cell containing 0.1 M pH 7.0 PBS and 0.1 M KCl. Enzyme-modified electrodes, a platinum wire and saturated calomel electrode (SCE) were used as the working,
15 counter and reference electrodes, respectively. The polarisation and power curves of the assembled biofuel cells were measured using the bioanode as working electrode and the biocathode as a combined counter/reference electrode. The potential was scanned at a scan rate of 1 mV s^{-1} in the presence of O_2 -bubbled 20 mM glucose, while recording the current in the circuit. All experiments were carried out at room
20 temperature ($20 \pm 2 \text{ }^\circ\text{C}$). The current densities or power densities were calculated using the geometric surface area of the working electrode or bioanode unless stated otherwise.

The specific capacitance of the individual electrode in three-electrode system was calculated from the cyclic voltammograms in the region where no faradaic processes
25 were occurring (Eq. S1). The specific capacitance of the assembled supercapacitor was obtained from the galvanostatic discharge curves (Eq. S2).

The charge/discharge performance of the hybrid devices in air-equilibrated buffer solution containing 20 mM glucose was examined with an Autolab PGSTAT100 potentiostat (Eco Chimie, Netherlands) using the biocathode as working electrode and
30 the bioanode as a combined counter/reference electrode. Testing of the devices

involved the test sequence: (i) charging at open-circuit mode using the BFC component and (ii) galvanostatic discharge of the capacitor at various current densities (Fig. 4C).

5 3. Results and discussion

3.1 Electrochemical characterisation of the capacitive bioelectrodes

Cyclic voltammograms (CVs) displayed an onset potential of 0.7 V (vs. SCE) for the growth of PEDOT on the NPG electrode in an aqueous solution (Xiao et al. 2013). The potentiostatic pulse comprised an anodic potential of 0.9 V (2 s) to generate the
10 radical cation and a cathodic potential of -0.4 V (3 s) to enable the EDOT concentration in the proximity of the electrode surface to return to that in the bulk state, thus allowing polymer formation on the electrode surface (Schuhmann et al. 1997). In the presence of Os poly(N-vinylimidazole) redox polymer, the weakly coordinated chloride ions exchanged with more strongly coordinating pyridine or
15 imidazole groups on proximal chains when Os^{3+} was reduced to Os^{2+} during the cathodic pulse (Gao et al. 2002). This crosslinking effect led to irreversible polymer precipitation onto the electrode. The resting period at the anodic potential enabled the reestablishment of the bulk concentration of precursor at the electrode surface. Overall, the potential sequence led to the alternate deposition of PEDOT and
20 $\text{Os}(\text{bpy})_2\text{PVI}$, which was confirmed by electrochemical studies (Fig. 1A). Enzymes in the deposition solution were physically and/or coordinately entrapped into the resulting films.

CVs of various modified electrodes (deposition time of 300 s) in PBS at 100 mV s^{-1} were compared to confirm the successful electrodeposition of the polymers (Fig.
25 1A). CVs of NPG/PEDOT/ $\text{Os}(\text{bpy})_2\text{PVI}$ /FAD-GDH electrodes showed the faradaic redox reaction of $\text{Os}^{2+/3+}$ (ΔE_p of 76 mV) superimposed on the charge/discharge capacitive currents. The Os polymer modified NPG electrode without PEDOT displayed a pair of reversible redox peaks with a peak separation of 20 mV. NPG/PEDOT exhibited a rectangular charge/discharge curve without any redox peaks.
30 Table S1 compares CV derived specific capacitances that are normalised with respect

to the projected surface area. Bare NPG showed a 9.6-fold higher capacitance than that possible with the bare planar gold electrode, consistent with the surface roughness factor (the ratio between the electrochemically addressable and geometric surface areas) obtained from the outermost layer of Au oxide stripping (a specific charge of $390 \mu\text{C cm}^{-2}$ is required for gold oxide reduction (S. Trasatti and O. A. Petrii 1991)). NPG/PEDOT and NPG/PEDOT/Os(bpy)₂PVI had 3.2 and 4.4 times higher capacitance than that of bare NPG.

The amount of deposited hybrid polymer, with the associated increase in the capacitance, and the enzyme loading increased with potential cycling before levelling off after a number of cycles (200 cycles for the case of (Gao et al. 2002)). On increasing the deposition time, the resulting film tended to block the pores (Fig. S3) of the NPG electrode (Fig. S1). For the FAD-GDH modified electrode, a deposition time of 300 s exhibited the optimal response to 10 mM glucose (Fig. S4A), attributed to a compromise between loading of biocatalyst and mass transport of substrate through the film. For the BOx modified electrode, a shorter pulse duration of 150 s afforded the highest electrocatalytic response to oxygen, with relatively low capacitance (Fig. S4B). A pulse of 300 s duration was chosen as a compromise between the electrochemical response and capacitance.

Both bioelectrodes were separately studied in detail at a scan rate of 5 mV s^{-1} (Fig. 1B and C). As can be seen from Fig. 1B, NPG/PEDOT/Os(bpy)₂PVI/FAD-GDH displayed a pair of redox peaks with a midpoint potential, E_m , of +191 mV (vs. SCE), in agreement with the reduction-oxidation of the Os^{2+/3+} couple. The ratio of the integrated area of the anodic to cathodic peak was ca. 1.1. The variation of peak current with scan rate was linear, indicative of a surface controlled process (Fig. S5). In the presence of 10 mM glucose, a sigmoidal catalytic wave with an onset potential of $-18 \pm 9 \text{ mV}$ vs. SCE and a background-corrected limiting current density of $59.7 \pm 2.4 \mu\text{A cm}^{-2}$ was observed. These results were indicative of the successful immobilisation of FAD-GDH with high activity. The apparent Michaelis-Menten constant, K_M^{app} , of the enzyme modified electrode was 7.9 mM (Fig. S6), lower than the value of 17.4 mM obtained from the same enzyme when chemically crosslinked onto graphite

electrode (Zafar et al. 2012). This decrease may arise from improved substrate transport through the thin immobilizing layer (Fig. 2). BOx based cathodes also showed a pair of redox peaks in N₂ bubbled PBS (Fig. 1C). The ratio of the integrated area of the anodic to cathodic peak was however less than 1, due to competition with residual O₂ for the oxidation of BOx. The E_m was +202 mV, a slight increase in comparison to that of FAD-GDH modified electrodes. In O₂ bubbled solution, electrocatalytic reduction commenced at 387±13 mV and reached a maximum net catalytic current density of 65.2±4.5 $\mu\text{A cm}^{-2}$.

3.2 Morphology characterisation

NPG and NPG/PEDOT/Os(bpy)₂PVI/FAD-GDH electrodes were examined by SEM (Fig. S2A and B). Typical porous structures with bicontinuous pores/ligaments of NPG were observed. The average pore size was 30.6±4.7 nm for the bare NPG (Fig. S1 and Fig. S2). The deposited layer uniformly grew along the pore surfaces, making the pores smaller and ligaments thicker, but not plugging the pores. The core-shell structure was clearly observed by TEM (Fig. 2), with the contrast between the modified film and the gold support clearly visible. The spatially homogeneous film was 7.4±1.4 nm in thickness, a size sufficient to encapsulate the enzyme.

3.3 Hybrid device testing

NPG/PEDOT/Os(bpy)₂PVI/FAD-GDH and NPG/PEDOT/Os(bpy)₂PVI/BOx electrodes were subsequently assembled into a dual-functioning device comprising a BFC and a capacitor. This type of device can perform as a glucose/O₂ BFC when connected to a load in an external circuit (Fig. 3A). The polarisation curve of the BFC was obtained with linear sweep voltammetry at a scan rate of 1 mV s⁻¹, with the power curve calculated accordingly (Fig. 3B). The BFC registered an OCV of 459.6±9.5 mV, a maximum current density of 28.9 $\mu\text{A cm}^{-2}$, and a maximum power density of 1.3 $\mu\text{W cm}^{-2}$ at a potential of 0.09 V in O₂ bubbled PBS containing 20 mM glucose. The assembled cell can also act as a supercapacitor, whose performance was examined by galvanostatic charge/discharge at a given external current density of 10

$\mu\text{A cm}^{-2}$ (Fig. S7). A specific capacitance of $391.9 \pm 2.1 \mu\text{F cm}^{-2}$ was obtained (Eq. S2). The total capacitance of the supercapacitor is determined by the series connection of the two capacitive electrodes (Eq. S3) (Khomenko et al. 2005), leading to a lower overall capacitance compared with those of individual electrodes.

5 Recent reports described the underlying mechanism of a hybrid supercapacitor/microbial fuel cell (Pankratov et al. 2014a; Santoro et al. 2016). The integration of a BFC with a capacitor enables the hybrid device to work as a self-powered capacitor, without the requirement for external input. In rest conditions, i.e. in open-circuit, the cell voltage tended to the equilibrium potential, i.e. OCV of the BFC. The existing
10 potential difference between the two electrodes polarised the anode and cathode, leading the NPG backbones to be negatively or positively charged, respectively, and triggering the *p*-dopable PEDOT film to insert/deinsert anions (Fig. 4A). In other words, the capacitive cell was electrostatically charged at the thermodynamically induced potential difference, driving its voltage profile close to the value of OCV. As
15 shown in Fig. 4C, the voltage increased with time initially rising rapidly before levelling off with time.

 The energy stored in the biocapacitor could be subsequently discharged at desired currents by releasing ions (Fig. 4B). As can be seen in Fig. 3C and 3D, a galvanostatic discharging current density of 0.2 mA cm^{-2} , almost 7 times higher than the $28.9 \mu\text{A cm}^{-2}$
20 possible with the BFC mode, resulted in a rapid release of power. In the following cycle, the rest step at open-circuit mode without any external load enabled the recovery of the cell potential to OCV (0.45 V) of the BFC. The following cycles almost overlapped, indicative of the excellent stability (Fig. 4C). On a closer examination of the first discharging segment (Fig. 4D), a capacitance of $357 \mu\text{F cm}^{-2}$
25 was calculated by dividing the given current density, j_{pulse} , by the absolute value of the slope of the discharging curve (Eq. S2). A j_{pulse} dependent voltage drop of 11 mV was observed due to the internal resistance (Eq. S4), probably assigned to the ohmic resistance of electrode, mass/charge transfer resistance, and/or low intrinsic biocatalytic activity (Liang et al. 2007). The resistance was predominantly attributed
30 to the internal resistance from the capacitor, instead of the biocatalytic processes, as

the cell also showed a voltage drop when used only as a capacitor (Fig. S7).

The long-term operation (7 hours, 50 cycles) of the hybrid device was tested by recording the potential at the open-circuit with a cutoff at 0.4 V, followed by discharge at 0.2 mA cm^{-2} for 0.5 s (test sequence is shown as the red line of Fig. 4C and D). For a period of 7 hours (50 cycles) (Fig. 5A), the discharge finishing potentials remained constant at 0.07 V, demonstrating the stable capacitance of the supercapacitor for each discharge cycle. The reset time did increase, e.g. ca. 300 and 800 s for the first and final cycles, respectively. After ca. 7 hours of operation, the device exhibited a loss of 70% in maximum power density ($0.38 \text{ } \mu\text{W cm}^{-2}$) (Fig. S8A) when tested as a BFC. Cyclic voltammograms of PEDOT/Os(bpy)₂PVI showed little change, indicative of a stable modification layer (Fig. S8B). Thus, decreased enzymatic activity, in particular of the FAD-GDH based bioanode (data not shown), was responsible for the extended self-charge time.

The discharge capability of the biocapacitor at various current densities was examined (Fig. 5B), with a current density up to 2 mA cm^{-2} . Generally, a larger discharge current density provided a larger power density (Eq. S8), as well as a longer recovery time. Table S2 compares the instant maximum power densities that can be delivered. For example, power pulses of 352 and 609 $\mu\text{W cm}^{-2}$ at 1 and 2 mA cm^{-2} were achieved, 271 and 468 times higher than that obtained from a traditional BFC configuration ($1.3 \text{ } \mu\text{W cm}^{-2}$). The maximum voltage output, V_{max} , decreased with higher pulse current densities due to the potential loss caused by the equivalent series resistance (ESR) (Santoro et al. 2016) (Eq. s7, Table S2). As a result, doubling the current density did not result in the same increase in the maximum power density. Decreasing the ESR could improve the maximum power density (Santoro et al. 2016).

To highlight the important role of the NPG substrate, a planar Au based hybrid electrode system was constructed using the same conditions. Au based BFC displayed a poor performance with an OCV of 365 mV, a maximum current density of $1.5 \text{ } \mu\text{A cm}^{-2}$, and a maximum power density of $0.08 \text{ } \mu\text{W cm}^{-2}$ at a potential of 0.11 V in O₂ bubbled PBS containing 20 mM glucose (Fig. S9A). The internal resistance was larger, leading to a voltage drop of 84 mV (Fig. S9B). A specific capacitance of $31.6 \text{ } \mu\text{F cm}^{-2}$,

11 times lower than that reported on the NPG based device, was estimated.

3.4 A proof-of-concept pulse generator

A cardiac pacemaker possesses dual-function of sensing and pacing the heart (Sanders 2008). To be able to pace the heart, an electric stimulus generated by the pulse generator with a fixed pulse potential and width (i.e. threshold, the minimum voltage from the pacemaker to initiate a heartbeat) is required. Previous attempts proposed the possibility of using BFCs as power sources to replace lithium based batteries currently used in pacemakers (MacVittie et al. 2013; Southcott et al. 2013). To increase the voltage to the required value, three individual cells were connected in series, giving a potential of ca. 1.24 V for 2 hours in the presence of 20 mM glucose. A test sequence of 5 s reset (i.e. a frequency of 0.2 Hz) and 0.5 ms discharge at 10 μ A that matched typical pacemaker working characteristic was applied (Mallela et al. 2004). As shown in Fig. 5A, the voltage dropped steadily to ca. 0.8 V in the initial 200-250 cycles, as 5 s was not long enough for voltage recovery (inset of Fig. 6A), and then maintained at ca. 0.74 V in the following long-term testing cycles. Such an output voltage stabilised at 0.74 V is enough to exceed the mean pacing threshold (e.g. 0.51 ± 0.22 V reported previously (Ritter et al. 2015)). Upon refilling the solution, the series connected cells recovered to a potential of ca. 1.21 V after a 2-hour incubation period, followed by 1300 cycles of charge/discharge (Fig. 6B). The output voltage gradually decreased for the initial 200 cycles due to the relatively short reset time of 5 s and attained a stable value of 0.7 V after the 220th cycle. The results demonstrate that three cells connected in series can mimic a pacemaker generating 0.2 Hz pulses (10 μ A, 0.5 ms) with a stable output potential of 0.7 V.

4. Conclusions

A supercapacitor/enzymatic biofuel cell hybrid device was prepared by a facile, one-step electrodeposition of PEDOT/Os polymer/enzyme onto dealloyed nanoporous gold electrodes. The dual-function properties of this hybrid device allowed the energy yielded by the biofuel cell to be stored in the supercapacitor and delivered at a

significantly high power pulse. For instance, it permitted a pulse current density of 2 mA cm⁻², with an instant maximum power density of 609 μW cm⁻², 468 times higher than that of the BFC. The modification layer showed reasonable stability without visible leakage of the redox mediators after 50 cycles operation at 0.2 mA cm⁻² for approximately 7 hours. In contrast to the planar Au based system, nanoporous gold electrodes improved the performance in terms of lower resistance, higher bioelectrochemical signal and capacitance. A proof-of-concept pulse generator (0.2 Hz pulse at 10 μA for 0.5 ms) to mimic a pacemaker was demonstrated using electrodes connected in series.

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Figure Captions

Fig. 1. (A) Cyclic voltammograms (CVs) of various electrodes (deposition time: 300 s). CVs of (B) NPG/PEDOT/Os(bpy)₂PVI/FAD-GDH and (C) NPG/PEDOT/Os(bpy)₂PVI/BOx electrodes at a scan rate of 5 mV s⁻¹.

5 **Fig. 2.** TEM image of NPG/PEDOT/Os(bpy)₂PVI/FAD-GDH (300 s deposition).

Fig. 3. (A) Schematic diagram of the BFC. (B) Polarisation and power curve for the BFC consisting of NPG/PEDOT/Os(bpy)₂PVI/FAD-GDH bioanode and NPG/PEDOT/Os(bpy)₂PVI/BOx biocathode.

10 **Fig. 4.** Schematic diagrams of the hybrid device working at the self-charging (A) and galvanostatic discharging mode (B) (with simplified charge-discharge description on the capacitive NPG/PEDOT hybrid). (C) Charge/discharge curves of the as-assembled biocapacitor (black line); Experimental setup: reset at open-circuit and cutoff at 0.4 V, followed by discharging at 0.2 mA cm⁻² for 0.5 s (red line). (D) Magnified image the first discharge segment.

15 **Fig. 5.** (A) Charge/discharge curves of the biocapacitor for 50 cycles; Experimental setup: reset at open-circuit and cutoff at 0.4 V, followed by discharging at 0.2 mA cm⁻² for 0.5 s. (B) Charge/discharge curves of the biocapacitor upon various discharging current densities; Experimental setup: reset at open-circuit and cutoff at 0.4 V, followed by discharging at 0.005 (a), 0.01 (b), 0.02 (c), 0.05 (d), 0.1 (e), 0.2 (f), 0.5 (g), 1 (h), 2 (i) mA cm⁻² for 0.2 s.

20 **Fig. 6.** Charge/discharge curves of the series connection of three biofuel cells (see Fig. S9); Experimental setup: the connected cells were allowed to reset at open-circuit for 2 hours, followed by discharging at 10 μA for 0.5 ms every 5 s reset. (A) is the first measurement of 1500 discharging pulses; (B) is for the measurement of 1300 discharging pulses upon refilling of fresh solutions; insets show zooms at the specific cycles.