A Miniature Membrane-less Biofuel Cell Operating at +0.60 V under Physiological Conditions

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The engineering objective of our work is the design of a power source enabling further miniaturization of autonomous implanted electronic devices, the size of which is usually limited by their battery. As the size of microelectronic circuits and of sensors shrinks, the size of the low-power sensor-transmitter package (of potential value in physiological research and in medicine) becomes increasingly dependent on the size of its power source. If stabilized for operation in vivo, a miniature glucose/O_2 biofuel cell could power an implanted sensor-transmitter that would broadcast, for a few weeks, the local glucose concentration, relevant to diabetes management; or the local temperature, indicative of infection after surgery; or a pressure difference, indicating blockage of the flow of fluid in the central nervous system.[1]

Contrary to conventional cells, which contain at least nine components (anode, cathode, case, case seal, membrane, membrane seal, ion-conducting electrolyte, plumbing to the anode compartment, and plumbing to the cathode compartment), the miniature biofuel cell that we are developing contains only two, an anode and a cathode.[1–3] These consist only of two 7 μm diameter "wired" enzyme bioelectrocatalyst-coated carbon fibers.

Underlying the simplicity and the unprecedented miniaturization of the cells are the selectivities of the thick, immobilized, glucose-electro-oxidation and O_2-electroreduction catalyzing "wired" enzyme coatings of the carbon-fiber electrodes. Their immobilization and selectivity enables the operation of a single compartment cell, containing both glucose and O_2 in the same compartment. Because the anode is selective for glucose, oxygen is not rapidly electroreduced on it, even though the anode is poised at a highly reducing potential. Similarly, because the cathode is selective for O_2, glucose is not electro-oxidized on it, even though the cathode is poised at a highly oxidizing potential. In classical fuel cells, H_2/O_2 or methanol/O_2, H_2 or methanol would be oxidized at the cathode and O_2 would be reduced at the anode if the reactants were allowed to mix. As a result, the power output would approach nil.

As illustrated in Figure 1, in the compartment-less biofuel cell, glucose electrons reduce glucose oxidase (GOx), glucose being electro-oxidized to δ-glucono-1,5-lactone [Eq. (1)]. The electrons are collected and transported to the anode by an

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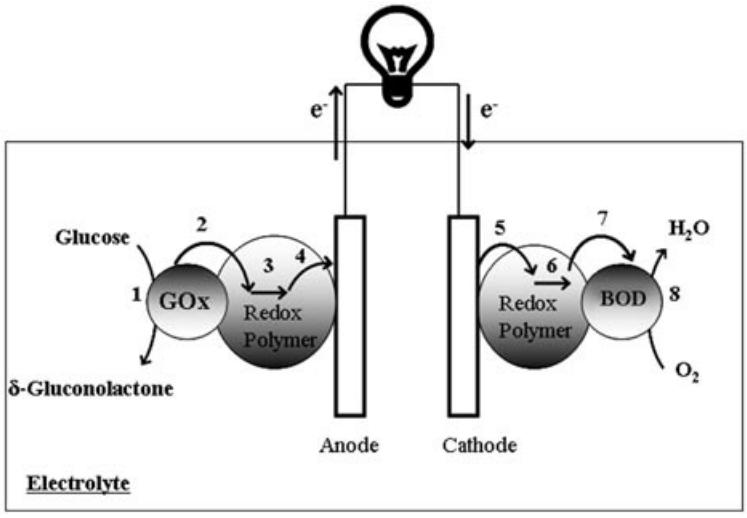

Figure 1. *Electron-transfer steps in the compartment-less biofuel cell. At the anode, electrons are transferred from glucose to the enzyme (1), from the enzyme to the polymer (2), through the polymer (3), and from the polymer to the electrode (4). The electrons pass then from the anode to the cathode through an external load. At the cathode, electrons are transferred from the electrode to the polymer (5), through the polymer (6), from the polymer to the enzyme (7,) and from the enzyme to the O_2 (8).*

anodic redox polymer. The electrons pass then from the anode to the cathode through the external load. They are then transported from the cathode through the cathodic redox polymer to O_2-oxidized bilirubin oxidase, catalyzing its electroreduction of O_2 to water [Eq. (2)]. Equation (3) represents the overall cell reaction.

$$\beta\text{-D-glucose} \rightarrow \delta\text{-glucono-1,5-lactone} + 2\,H^+ + 2\,e^- \quad (1)$$

$$O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O \quad (2)$$

$$2\beta\text{-D-glucose} + O_2 \rightarrow 2\delta\text{-glucono-1,5-lactone} + H_2O \quad (3)$$

By eliminating the membrane, the case, and the seal, we were able to build a 0.88 mm^2 biofuel cell, the smallest ever, which we implanted and operated in a grape. The power output of the in vivo cell, made of a pair of 0.44 mm^2 area smooth nonporous carbon fibers, was 2.4 μW at +0.52 V/AgAgCl, translating to a power density of ~5.5 $\mu W\,mm^{-2}$, and ~4.4 $\mu W\,mm^{-2}$ at +0.6 V/AgAgCl.[2] Because this potential exceeded half the band gap of silicon, it could be converted by a conventional silicon voltage converter to the standard 3 V operating voltage of ICs. The cell followed a series of earlier compartment-less glucose/O_2 biofuel cells, the first of which, reported in 1999 by Katz et al., had a footprint of 1 cm^2, operated at +0.06 V and produced only 3.5 $nW\,mm^{-2}$.[4] Tsujimura et al. reported a membrane-less glucose-O_2 biofuel cell operating in O_2-saturated 30 mM MOPS buffer in the presence of 50 mM glucose, producing 580 $nW\,mm^{-2}$ at an operating potential of +0.19 V.[5] The cells were later miniaturized, and their operating voltages and power densities were raised; specifically, membrane-less and case-less cells, consisting only of two 7 μm-diameter, 2 cm-long carbon fibers, were built and operated either under physiological conditions (pH 7.2, 20 mM phosphate, 0.14 M NaCl)[2] or in a 0.2 M citrate buffer at pH 5,[3,6,7] depending of the cathodic enzyme used: bilirubin oxidase or laccase, respectively.

To build a more efficient biofuel cell, (i.e., increasing the operating cell voltage and the current density), we "wired" glucose oxidase (GOx) with poly(4-vinylpyridine)-[Os(*N*,*N*′-dimethyl-2,2′-biimidazole)$_3$]$^{2+/3+}$, comprising 10.7 wt% of osmium (Polymer **I**).[10] We showed that binding of fast redox centers to the backbone of this water-soluble polymer through 13-atom-long flexible tethers and its immobilization by cross-linking provided the basis for electrocatalytic hydrogels in which electrons, water soluble reactants, and products diffused rapidly ($D_{app} = 5.8 \times 10^{-6}\ cm^2\,s^{-1}$). When the redox hydrogel connected the reaction centers of glucose oxidase to a carbon-fiber electrode, the limiting current density of glucose electro-oxidation (11.5 $\mu A\,mm^{-2}$) was reached at a potential only 0.26 V to the positive of the redox potential of the FAD/$FADH_2$ centers of glucose oxidase at pH 7.2.[9] As the Blauch and Saveant[10,11] bounded-diffusion model predicted that D_{app} should scale in redox hydrogels with the concentration of the redox species C_{RT}, we designed a new "redox wire" for GOx, with the osmium loading increased from 10.7 wt% (polymer I)[8] to 12.8 wt% osmium (polymer II).[12] As illustrated in Figure 2, under quiescent solution, a current density of 13 $\mu A\,mm^{-2}$ for polymer II and 9.6 $\mu A\,mm^{-2}$ for polymer I was reached at a polarization as low as 0.3 V/AgAgCl under physio-

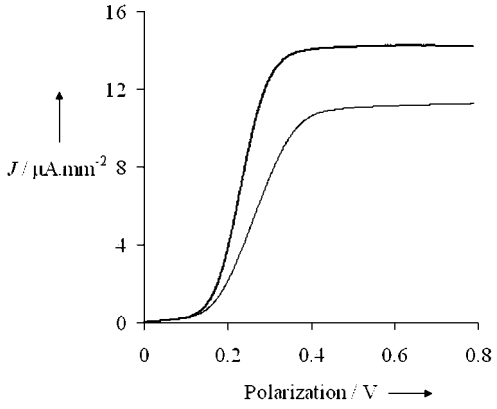

Figure 2. *Polarization of the 7 μm-diameter, 2 cm-long carbon-fiber anodes modified with "wired" GOx and polymer II (top curve) and polymer I (bottom curve). Top curve: 45 wt% glucose oxidase/54 wt% polymer II/1 wt% poly(ethylene glycol) diglycidyl ether (PEGDGE). Bottom curve: 39.5 wt% glucose oxidase/59.5 wt% polymer I/1 wt% PEDGE. Quiescent solution under N_2, pH 7.2, 0.1 M NaCl, 20 mM phosphate, 15 mM glucose, 37.5 °C, 1 $mV\,s^{-1}$.*

logical conditions. The glucose transport-limited current density was 15 $\mu A\,mm^{-2}$ for polymer II versus 11.5 $\mu A\,mm^{-2}$ for polymer I at a polarization of 0.46 V/AgAgCl. By increasing the amount of osmium by 1.3 wt%, full coverage of the anodic carbon fiber by the tougher polymer increased the glucose-flux-limited current density by 20% and significantly decreased the overpotential for glucose electro-oxidation.

Using the novel anode, we built a miniature compartmentless biofuel cell, consisting of two 7 μm-diameter, 2 cm-long carbon fibers. The bioelectrocatalyst of the anode consisted of glucose oxidase from *Aspergillus niger* (GOx) electrically "wired" by polymer II, having a redox potential of −0.19 V versus Ag/AgCl. The bioelectrocatalyst of the cathode, which was superior to platinum, was reported earlier.[13] It consisted of purified bilirubin oxidase from *Trachyderma tsunodae* (BOD) "wired" by PAA-PVI-[Os(4,4′-dichloro-2,2′-bipyridine)$_2$Cl]$^{+/2+}$ and had a redox potential of +0.36 V versus Ag/AgCl. As illustrated in Figure 3, the power of the cell peaked at ~+0.60 V/AgAgCl, where it reached 2.1 μW. The power density was 4.8 $\mu W\,mm^{-2}$,

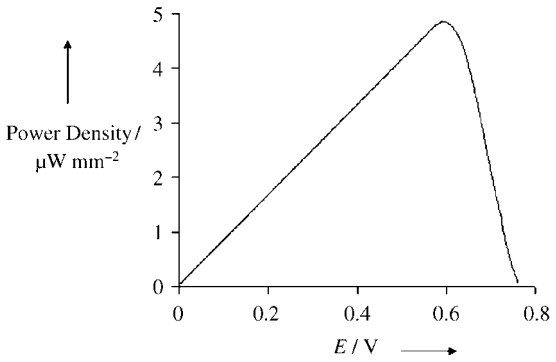

Figure 3. *Dependence of the power density on the cell voltage for the cell made with a 7 μm-diameter, 2 cm-long carbon-fiber anode coated with "wired" glucose oxidase and with the novel polymer II. 15 mM glucose, other conditions as in Figure 2.*

above the 4.4 $\mu W\,mm^{-2}$ power density of the +0.52 V cell previously described.[2,14] This value represents the highest operating voltage in a miniature membrane-less biofuel cell operating in a physiological buffer solution. Because, the redox potential of *A. niger* GOx at pH 7.2 is −0.35 versus Ag/AgCl[9] and that of bilirubin oxidase from *Trachyderma tsunodae* is about +0.36 versus Ag/AgCl,[15] the operating potential is just 0.11 V less than the difference between the redox potentials of the two enzymes. The cell lost ~8% of its power per day when operating at +0.6 V/AgAgCl and at 37 °C in a physiological buffer solution.

In summary, increasing the density of the polymer-bound redox sites that electrically connect the reaction centers of GOx to the anode increased the glucose flux limited current density by 20%, and decreased by 50 mV the overpotential for glucose electro-oxidation. A miniature, membrane-less glucose-O_2 biofuel cell built with the novel anode operated at the highest voltage to date, +0.60 V while producing 4.8 $\mu W\,mm^{-2}$ in a physiological buffer at 37.5 °C.

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