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Miniature biofuel cells

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The history of electrochemical power sources shows that batteries or fuel cells were introduced only when the development of new electrical or electronic system demanded these. At this time, the already established feasibility of miniaturization of implantable sensor-transmitter systems to volumes smaller than 1 mm^3, and the demand for spatially and temporally resolved information on local temperature, flow, pressure and chemical concentrations, are likely to create a demand for a miniature, low cost glucose-O_2 biofuel cell that would power the autonomous sensor–transmitters for a few weeks. Prototypes of these cells are in hand. Their most unique feature which is their structural simplicity, is made possible by the selectivity of their "wired" enzyme catalysts: the cells consist merely of two 7 μm diameter carbon fibers, each coated with a different "wired" enzyme bioelectrocatalyst. On one, catalyzing the two-electron electrooxidation of glucose at a reducing potential, glucose oxidase is co-immobilized in and electrically connected ("wired") by an electron conducting hydrogel of a reducing redox potential. On the other, catalyzing the four electron electroreduction of O_2 to water, bilirubin oxidase is co-immobilized in and electrically "wired" by an electron conducting hydrogel of an oxidizing potential. The cells are the smallest ever built. When the volume of the fibers is 0.0026 mm^3, the current of the cell operating at 0.52 V in a physiological buffer solution at 37 °C is 8.3 μA. The 4.3 μW power output of the cell is expected to suffice for the operation of implanted sensors and for the intermittent transmission of the data collected to an external receiver.

Power sources of mobile electronic systems

Electrochemical power sources convert the free energy released upon the reaction of their oxidant and their reductant to electrical power. Even though mobile solar cells and mechanical-to-electrical power converters have been available for half a century, nearly all mobile electronic systems are powered by electrochemical sources. These dominate because their power density is higher and their cost is lower. There are two types of electrochemical power sources: Batteries, which are widely used; and fuel cells, which are widely discussed. Batteries contain the reacting chemicals. In fuel cells the reactants are fed from external reservoirs to the cells. Because fuel cells do not contain the reacting chemicals, their "theoretical", but not their true, power densities are much higher than those of batteries. Nevertheless, when the volume or weight of their external reservoirs, pumps and plumbing are added, fuel cells retain a power density advantage only in very few mobile systems. For this reason, mobile fuel cells have been historically far less successful than mobile batteries. Their most important application has been in space programs, where hydrogen, the lightest fuel, has a payload advantage.

Historical success or failure of electrochemical power sources

Although more than a thousand electrochemical cells have been described in the literature, the number of actually used cells can be counted on fingers, toes included. New cells were introduced only when a need for a new power source was created by a novel technology. (Table 1) Early in the 20th century, the introduction of hot filament-based incandescent "glow"-lamps led to portable, battery-requiring "flashlights". The $Zn/NH_4Cl/MnO_2$"dry" cell[1] answered the need. The lead-acid ($Pb/H_2SO_4/PbO_2$) battery[2] answered the need for a high power rechargeable engine-starting battery,[3] which was created by the mass manufacture of automobiles. The lead-acid battery also answered the simultaneously created need of telephony, which required a trickle-charged, reliable, long-lived power source sustaining communications during power-grid outages. In the 1930s the rechargeable Ni–Cd battery was introduced to power miners' headlamps, cordless tools and appliances.[4] The Ni–Cd battery was later replaced by the more environmentally friendly nickel hydride battery,[5] which now also powers video and other recorders. In the third quarter of the century, the shrinkage of electronic circuits made it possible to reduce the size and weight of transistor radios, quartz crystal watches, electronic cameras, military communication systems, hearing aids, pacemakers and toys, which created a need for smaller, lighter and longer-lived batteries. These needs were met by the higher energy or power density $Zn/KOH/MnO_2$,[6] $Zn/KOH/O_2$,[7] $Li/SOCl_2$,[8] Li/MnO_2,[9] Li/iodide[10] and Li/CF_x[11] batteries. In the 1990s laptop computers drove the introduction of the rechargeable $Li/LiCoO_2$[12] "lithium

Table 1 Drivers of the introduction of new electrochemical power sources

Driver	Year	Power source	Booster application
Flashlight	1900	Leclanche $Zn–MnO_2$	Military wireless
Automotive	1930	Lead acid	Telephony
IC-mobile electronics	1970	Primary lithium	Mobile electronics
Laptop	1995	Li ion	CCC electronics
Implanted medical sensors		Wired enzyme biofuel cell	Tracking

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ion'' batteries. It is now likely that the methanol–air fuel cell, studied already in the 1960s,[13] will be introduced to meet the need for instantaneous recharging of laptop computers and cellular telephones.[14–16]

Past objectives: Biofuel cells for an artificial heart and for a greener environment

The first enzyme-based glucose/O_2 biofuel cell operating at neutral pH was described by Yahiro *et al.* in 1964.[17] The need that was to be met by the early cells was nothing less than the powering an artificial heart.[18–23] When it was realized that the power density and operational life requirements of an artificial heart would not be met, the effort was re-oriented at implantable cells for other medical applications.[24–30] In these the glucose/O_2 biofuel cell could not compete in cardiac pacemakers with the Li/iodide battery,[10] because its operational life was much shorter. It could also not compete with the Li/$SOCl_2$ battery[8] in implanted medical systems requiring high intermittent power pulses, such as nerve-stimulators for the treatment of pain, epilepsy and Parkinson's disease, and in implanted drug-delivering pumps used in the treatment of cancer, the management of diabetes, the management of cerebral palsy and the management of chronic pain.

In the 1980s and the early 1990s an ambitious biofuel cell effort was undertaken, aimed at ''green'' sources of electrical power.[31] The intent was to supply power, initially to remote homes and villages, then to the entire grid. Because the power densities of biofuel cells were at least a thousand-fold smaller than those of conventional power generators, and because the costs of plants scale with their size, the cells could not, and did not, succeed in their intended application. The effort did result, however, in exquisitely good research. Karube *et al.* reported between 1979 and 1983 whole organism-based fuel cells.[32–35] In 1984 Turner *et al.* applied transition metal complex redox mediators in biofuel cells.[36,37] Persson and Gorton[38] reported an adsorbed redox mediator based anode in 1986. In 1999, Palmore *et al.* described a greatly improved membrane-type cell, with diffusing components in both compartments, operating at >1 V.[39] The myth that low power density biofuel cells can supply clean electrical power still persisted in 2003.[40]

Implanted miniature biofuel cells powering for a few weeks medical sensor–transmitters

Although I do not expect the expiation of human sin against the environment through biofuel cells, I do expect that the cells will help sick people. I expect that after considerable and difficult further work, simple, membraneless miniature, disposable glucose/O_2 cells will power autonomous, implanted, medical sensor–transmitters. Our cells are designed to have volumes of less than 1 mm^3, to weigh less than 100 μg and to cost less than one US dollar.[41–45] They are expected to be discarded after their first and only use. Miniature autonomous sensor–transmitter packages which would broadcast for a few weeks the local temperature of a site, indicative of local inflammation; or pressure, indicative of fluid blockage; or deviation from the normal concentration of a chemical, specific to a disease. Even without further progress in miniaturization, the volume of the autonomous sensor–transmitters will be less than 2 mm^3, and their weight less than 200 μg. The cells powering the sensor–transmitters would produce continuously a few microwatts, of which less than 1 μW will be required for the operation of the sensor; most of the power will be consumed by the transmitter. With a small ultra-capacitor storing ~10 μJ, enough for 1 ms long ~1–10 GHz bursts of 10 mW every 10 s, the transmitted information will be easily acquired outside the body.

Simple, small and disposable biofuel cells by eliminating eight of the ten components of conventional cells

In the miniature cells that we have built, eight of the ten components of conventional fuel cells are eliminated (Table 2).[41–45] In the absence of these components the size of the implantable biofuel cells, which are now in hand, is smaller by a factor of ~100 than the size of the smallest presently manufactured battery or fuel cell.

Because glucose is present in all tissues and organs, the fuel-storing reservoir, plumbing for fuel delivery and fuel pump are not needed. Because O_2 is present in all organs and tissues, the air breathing membrane of the cathode compartment is not needed. Two difficult to miniaturize components of conventional fuel cells and batteries that are eliminated are the case, usually made of stainless steel, and its seal. The case confines the strongly acidic or basic electrolyte and the gaseous, volatile or reactive reactants of fuel cells and batteries. Because our biofuel cell operates in a physiological fluid and the reactants are glucose and O_2, and because gluconate, the glucose electrooxidation product, is non-toxic, no case is needed. In the absence of the case there is no need for the case seal and for the air-breathing membrane of the cathode compartment, which is hydrophobic on the air-side (to prevent the electrolyte from oozing out by capillary action) and is wetted by the catholyte on the inside (for rapid delivery of O_2 to the catholyte). The two components which are particularly difficult to miniaturize and are eliminated are the membrane, separating their anode and cathode compartments of conventional fuel cells, and its seal.

A compartment-separating membrane and its seal were needed when the anode or cathode electrocatalyst contains a platinum group metal, or if the cell contained a diffusing redox mediator in either its anode or in its cathode compartment. The anode of fuel cells, where the fuel is electrooxidized, is poised at a reducing potential; and the cathode, where O_2 is electroreduced, at an oxidizing potential. The more reducing the anode potential and the more oxidizing the cathode potential, the higher the operating voltage of the cell. When the anode or cathode catalyst of a fuel cell is a platinum group metal, which catalyzes not only the desired oxidation of a fuel (like H_2 or methanol) but also the undesired reduction of O_2, access of the fuel to the cathode and

Table 2 Components of a conventional fuel cell and of an enzyme-wiring based implanted biofuel cell

Conventional cells	Enzyme-''wiring'' based biofuel cells
Case[a]	
Case seal [a]	
Membrane[a]	
Membrane seal[a]	
Ion conducting electrolyte, *e.g.* phosphoric acid	
Anode	Anode
Cathode	Cathode
Plumbing to the anode compartment[a]	
Plumbing to the cathode compartment[a]	

[a] Difficult to reduce to <1 mm^2 footprint; <0.1 mm^3 volume.

access of O_2 to the anode is prevented by a compartment-separating membrane. Because anions are consumed at the anode and cations are consumed at the cathode, this membrane must conduct ions and block the passage of either the fuel or O_2. Most of the earlier glucose-electrooxidizing cells also required a membrane, because their O_2 cathodes contained platinum group metal catalysts, which were fouled by glucose electrooxidation products.

Unlike the platinum group metal catalysts, enzyme-based catalysts of biofuel cells can be selective, allowing the anode and the cathode to operate in the same compartment. The glucose electrooxidation products, which poison the platinum group metal-containing catalysts, do not poison the enzyme based cathode-catalysts. Therefore, the cathode compartment can contain glucose. Because the electrical connection between electrodes and the redox centers of most enzymes is poor, diffusing redox mediators were earlier applied to shuttle electrons from the glucose-reduced cathodic enzyme to the anode, or from the cathode to the O_2-oxidized cathodic enzyme.[39,46–48] In the absence of a membrane, the anodic redox mediator, reduced by the anodic enzyme, would have mixed with and would have reduced the cathodic redox mediator oxidized by the cathodic enzyme. In addition, if the anodic mediator were allowed to diffuse to the cathode, where it would have been oxidized, or the cathodic mediator to the anode, where it would have been reduced, the power output would have dropped to nil.

The way to membrane-less biofuel cells was through immobilized, highly substrate-specific bioelectrocatalysts, their respective enzymes electrically connected (''wired'') to the electrodes through electron conducting hydrogels. Unlike platinum group metal catalysts, the ''wired'' enzymes selectively catalyze either glucose electrooxidation at the anode, or only O_2 electroreduction at the cathode, but not O_2 electroreduction at the anode or glucose electrooxidation at the cathode. It was Katz, Willner and their colleagues who first reported a membrane-less biofuel cell, based on two enzymes connected to two electrodes.[49] The minuscule operating voltage and the very low power density of their cell, however, did not allow miniaturization. The cells operated at 60 mV, and their power density was $\sim$4 μW cm^{-2}. The intended sensor–transmitters, consuming a few microwatts, would have required a cell with an area of about 1 cm^2. Such large cells would have been incompatible with the size of the rest of the system and with the intended medical applications.

In 2001 we increased the operating voltage of a membrane-less biofuel cell to 0.4 V and the power density to 140 μW cm^{-2}, allowing us to build the first miniature biofuel cells.[41] The cell consisted of nothing but two bioelectrocatalyst coated 7 μm diameter carbon fibers. It was the first cell suggesting that the goal of a miniature autonomous sensor–transmitter system was realistic. We based the cell on a redox-polymer ''wired'' laccase O_2-cathode, on which glucose was not electrooxidized,[50–52] and on a glucose electrooxidizing redox polymer ''wired'' glucose oxidase anode.[53,54] Because electrons were transported between the enzymes and the electrodes not by diffusing mediators, but by redox polymers, we were able to eliminate the membranes. Furthermore, because the ''wires'' connected not a monolayer, but multiple layers, of enzymes to electrodes, the current densities exceeded 1 mA cm^{-2} for the anode and 5 mA cm^{-2} for the cathode. Because our first miniature cell was laccase-based, and because laccase loses its activity at neutral pH and in 0.1 M chloride, the cell was operated in a pH 5, chloride-free, citrate buffer.[50–52]

In 2002, Tsujimura *et al.* introduced a membrane-less biofuel cell, which operated at neutral pH in a chloride containing solution at 0.19 V with a power density of 58 μW cm^{-2}. Their study showed that a membrane-less biofuel cell can operate in a physiological buffer solution.[55]

Tailoring of substrate-selective ''wired'' enzyme electrocatalysts for biofuel cells

The ''wires'' that allowed the elimination of the membranes and the operation of biofuel cells at current densities and voltages much higher than earlier possible were crosslinked redox polymers, which swelled in water to form redox hydrogels. Like all hydrogels, the redox hydrogels conducted ions and allowed the diffusion of water soluble reactants like glucose and products like gluconolactone. But unlike other hydrogels, they also conducted electrons.[56–61] Although the non-swollen redox hydrogels were poor electron conductors, the apparent electron diffusivity of the swollen ''wires'' reached 6×10^{-6} cm^2 s^{-1},[62] a value matching the diffusivity of ions in water. The hydrogels conduct electrons through electron-transferring collisions from their reduced to their oxidized mobile redox centers which are tethered to the backbone of the polymers. (Fig. 1)[57–59] Long and flexible tethers greatly increase the frequency of electron transferring collisions and, thereby, the apparent diffusivity of electrons.[62]

It is the unique selectivity of the ''wired'' enzymes for their substrate that prevents oxidation of glucose at the cathode and the reduction of O_2 at the anode, even when the operating potential of the cathode is oxidizing with respect to the anode by as much as 0.78 V.[43] The reaction centers of glucose oxidase are connected to the anode through an anodic ''wire'', and the reaction centers of laccase or bilirubin oxidase are connected to the cathode through a cathodic ''wire''. Because of their very low entropy of mixing, a mixture of two macromolecules will phase-separate unless their two components are designed to bind with each other. For this reason the ''wires'' are polycations, forming electrostatic adducts with the enzymes, which are polyanions at physiological pH.[63]

To maximize the operating voltage, which is the difference between the operating potentials of the anode and the cathode, four electron transport-driving potential differences, each of which is a loss in operating voltage, are kept to a minimum. To drive the electrons from the glucose reduced enzyme to its ''wire'', the redox potential of the anodic ''wire'' is tailored to be just positive of (slightly oxidizing *versus*) the redox potential of the $FADH_2$/FAD centers of glucose oxidase. To drive the electrons from the anodic ''wire'' to the anode, *i.e.* to

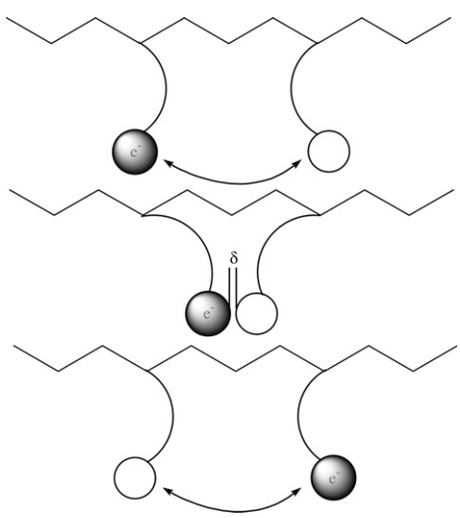

Fig. 1 Electron diffusion derives of collisions between redox centers, tethered to the backbone, in the electron conducting redox hydrogels that ''wire'' reaction centers of enzymes to electrodes. Long and flexible tethers allow the redox centers to sweep large volume elements, providing for high electron diffusion coefficients. Unless the polymers are crosslinked, they are water soluble. When crosslinked, they swell to form electron conducting hydrogels that are permeable to water-soluble molecules and ions.

electrooxidize the anodic "wire", the operating anode is poised at a potential just slightly positive of the redox potential of the anodic "wire". To drive the electrons from the electroreduced cathodic "wire" to the O_2-oxidized cathodic enzyme, the redox potential of the cathodic "wire" is tailored to be just negative of (slightly reducing *versus*) the redox potential of laccase or bilirubin oxidase. To drive electrons from the operating cathode to the cathodic "wire", *i.e.* to electroreduce the cathodic "wire", the operating cathode is poised at a potential just slightly positive of the redox potential of the cathodic wire. Furthermore, to minimize the internal resistance of the "wired" enzyme films, their redox polymers are designed for maximal electron diffusivity and the weight fraction of the insulating enzyme is minimized.

Thermodynamic and operating cell voltages

The calculated thermodynamic voltage for the reversible reaction

$$\text{glucose} + \tfrac{1}{2}O_2 \leftrightarrow \text{gluconolactone} + H_2O$$

is 1.0 V at 25 °C. This is the actual open circuit voltage of some of the miniature cells, such as the two-fiber glucose/O_2 cell operating at pH 5 in citrate buffer. Because protons are neither consumed nor released in the cell reaction, both the calculated and measured open circuit voltages are pH independent.

To minimize the potential difference driving electrons from the glucose-reduced glucose oxidase to the anodic "wire" and driving electrons from the electroreduced cathodic "wire" to O_2–oxidized laccase or to bilirubin oxidase, the difference between the redox potentials of the enzymes and their "wires" is set to be 50 mV by tailoring the redox centers of the "wires". With about 50 mV driving each of the two anodic (enzyme to "wire", "wire" to anode) and the two cathodic (cathode to "wire", "wire" to enzyme) electron transferring steps, the expected voltage loss in the optimally operating cell, which is the cell operating at its maximal power point, is about 200 mV. Because the thermodynamic potential of the glucose/O_2 cell is 1.0 V, the expected optimal operating voltage of the cell is about 0.8 V, near the observed maximum power point voltage of 0.78 V in our operating voltage cell.[43] This operating voltage exceeds half of the 1.1 eV band gap of silicon and can, therefore, be up-converted with available silicon integrated circuits to 3 V, the standard operating voltage of integrated circuits.

Unlike the cell voltage, which is independent of pH, the half cell potentials of the anode and the cathode are pH dependent: Protons are released at the anode and are consumed at the cathode. In contrast, protons are neither consumed nor released in the electroreduction and the electrooxidation of the "wires". The redox potentials of the "wires" are, therefore, pH independent. As a consequence, the difference between the redox potentials of the enzymes and their "wires" is pH dependent. For this reason, a "wire" tailored to provide the optimal 50 mV potential difference at one pH will not provide it at another pH. When the pH is changed, a different pair of wires is required. At neutral pH the half cell potential of glucose electrooxidation,

$$\text{glucose} \rightarrow \text{gluconolactone} + 2H^+ + 2e^-$$

is −0.25 V *vs.* Ag/AgCl. The half cell potential for the electro-reduction of O_2 at the cathode at neutral pH,

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O,$$

is +0.75 V *vs.* Ag/AgCl. Because we have not tailored as yet "wires" for optimal performance at physiological pH, the 0.78 V operating voltage realized through tailoring the redox potentials of the "wires" for operation at pH 5, is yet to be achieved in the cell operating at the physiological pH of 7.2. The optimal operating voltage of our best performing miniature physiological pH 7.2 cell is presently 0.52 V. At 0.55 V, half the band gap of silicon, the power output is ~90% of its maximum.

Glucose-selective wired glucose oxidase based anode electrocatalysts

The best glucose electrooxidizing anode was made by wiring glucose oxidase with a redox hydrogel having an apparent electron diffusion coefficient of 6×10^{-6} cm^2 s^{-1}, greater by an order of magnitude than that of other redox hydrogels. The high apparent electron diffusion coefficient results from the tethering of redox centers to the backbone of the cross-linked redox polymer backbone through 13 atom spacer arms. The long and flexible tethers allow the redox centers to sweep electrons from large volume elements and to collect electrons of glucose oxidase efficiently. The structure of the "wire" is shown in Fig. 2.[62] The spacer arms make the collection of electrons from glucose oxidase so efficient, that glucose is electrooxidized already at −0.36 V *vs.* Ag/AgCl, the reversible potential of the redox potential of the FAD/$FADH_2$ centers of the enzyme at pH 7.2. The kinetic limit of the operating current density for glucose electrooxidation at the "wired" glucose oxidase anode is 1.1 mA cm^{-2} at 37°. It is reached already at a potential as reducing as −0.1 V *vs.* Ag/AgCl.[45] The polymer redox center is a tris-dialkylated *N*,*N*′-biimidazole $Os^{2+/3+}$ complex.[62] Its redox potential, −0.195 V *vs.* Ag/AgCl, is 0.8 V reducing relative to that of $Os(bpy)^{2+/3+}$, its 2,2′-bipyridine analog. The anode is stable for a week in serum at 37° when operating at current density of $> 10^{-3}$A cm^{-2}.[45]

Fig. 3 shows the dependence of the current density on the potential of the "wired" glucose electrooxidizing anode.

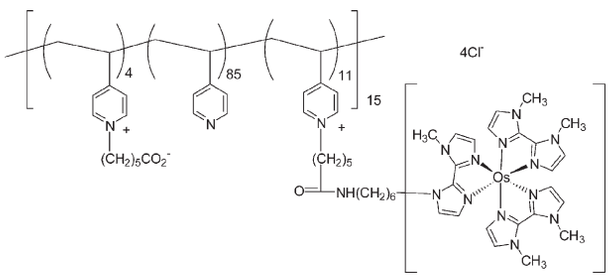

Fig. 2 Structure of the anodic "wire". The wire is built with 13-atom long flexible tethers between the backbone and the [Os(*N*,*N*′-dialkylated-2,2′bi-imidazole)$_3$]$^{2+/3}$ redox centers, the redox potential of which is −0.195 V *vs.* Ag/AgCl. The long tethers enable effective collisional electron-exchange, increasing the apparent electron diffusion coefficient to 6×10^{-6} cm^{-2} s^{-1}, the highest for a hydrogel. The quaternized pyridines are randomly distributed.[62]

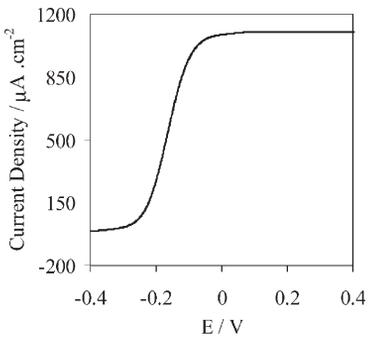

Fig. 3 Polarization of the 7 μm diameter, 2 cm long glucose electro-oxidizing carbon fiber anode, made with the electrocatalyst formed by crosslinking the electrostatic adduct of glucose oxidase and the "wire" of Fig. 2. Quiescent solution, under air, 37.5 °C, PBS buffer, 15 mM glucose, 1 mV s^{-1} scan rate.[45]

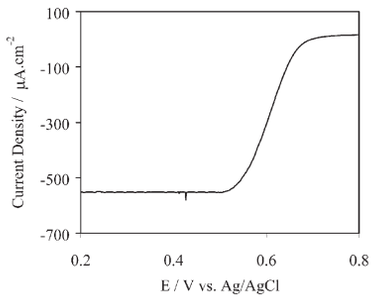

Fig. 5 Polarization of the 7 μm diameter, 2 cm long, O_2-electroreducing carbon fiber cathode, made with the electrocatalyst formed by crosslinking the electrostatic adduct of laccase from *Coriolus hirsutus* and the "wire" of Fig. 4. Quiescent solution, under air, 37 °C, pH 5 20 mM citrate buffer, 1 mV s^{-1} scan rate. Because the solution was stagnant and the dissolved O_2 concentration is only about 0.2 mM, the current was O_2-transport limited.[43]

O_2-selective wired laccase and bilirubin oxidase based cathode electrocatalysts

The enzyme-based catalysts of four electron O_2–electroreduction to water are most substantially superior to platinum, even the classical platinum in 0.5 M H_2SO_4. At −0.3 V of the reversible potential of the four electron half cell reaction $O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O$ the kinetically limited current density of the "wired" bilirubin oxidase cathode is 16 times higher than that for platinum.[42] On smooth vitreous carbon electrodes coated with "wired" bilirubin oxidase[64] or laccase,[51,52] O_2 is electroreduced to water at true current densities as high as 10^{-2} A cm^{-2}.

Laccases are most active near pH 5 and are inhibited by chloride. Their activity in a pH 5.0 chloride-free citrate buffer is higher by more than an order of magnitude than their activity in a pH 7.2, 0.14 M NaCl physiological buffer solution. The redox potential of the O_2/H_2O half cell at pH 5 is 0.71 V *vs.* Ag/AgCl. A well performing cathode has been made with the 0.55 V *vs.* Ag/AgCl redox potential "wire" poly(*N*-vinylimidazole)–[Os-2,2′,6′,2″-terpyridine-4,4′-dimethyl-2,2′-bipyridine)$_2$Cl]$^{2+/3+}$ the structure of which is seen in Fig. 4.[51,52] The polarization curve of the cathode made with laccase "wired" by this polymer is seen in Fig. 5.[51,52]

The enzyme of choice for operation in the physiological pH 7.2, 0.14 M NaCl solution is bilirubin oxidase,[55,65] preferably from *Trachyderma tsunodae*.[66,67] Unlike laccases, the bilirubin oxidases are active at neutral pH and are not inhibited by chloride. At pH 7.2 the potential of the reversible O_2/H_2O half cell is 0.58 V *vs.* Ag/AgCl. Because the potential difference of 30 mV is too small to drive electrons from the 0.55 V *vs.* Ag/AgCl wire of Fig. 4 to bilirubin oxidase, this wire can not be used. Instead, we are using the 0.35 V *vs.* Ag/AgCl redox polymer, shown in Fig. 6.

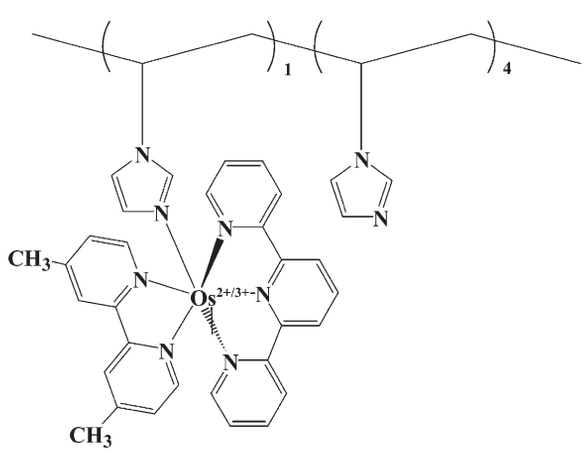

Fig. 4 Structure of the laccase "wire" poly(*N*-vinylimidazole)–[Os-2,2′,6′,2″-terpyridine-4,4′-dimethyl-2,2′-bipyridine)$_2$Cl]$^{2+/3+}$. The distribution of complexed imidazoles in the polymer is random.

Characteristics of the miniature cell and its operation in a living plant

Because the "wired" enzyme electrocatalysts are fast, the current densities are limited by O_2 mass-transport, not by the kinetics of the catalysts. The concentration of O_2 in air-equilibrated water at 37 °C is only 0.2 mM. The fine carbon fiber electrodes were chosen to increase the diffusional flux of the reactants, the flux being cylindrical rather than planar to the fine fibers. In a 15 mM glucose air-equilibrated physiological buffer solution, the measured diffusion-limited steady-state current densities are of ∼0.8 mA cm^{-2} at 37 °C when the solution is stagnant. (Fig. 7)[42,45]

The diameter of the coated bioelectrocatalyst coated fibers is about 13 μm. When the fibers are 2 cm long, the area of each electrode is about 0.8 mm^2, its footprint about 0.26 mm^2 and its volume about 0.0026 mm^3. The current of the cell operating at 0.52 V in a physiological buffer solution at 37 °C is 8.3 μA and the power output of the cell is 4.3 μW. The dependence of the power output on the cell voltage is shown in Fig. 8 for the cell operating in the pH 7.2 physiological buffer solution.

The power declines daily in the first six days of operation by about 5%. In its 6 days of operation the cell produces 0.9 J, while 0.016 C pass through the cell. If a caseless zinc–air cell

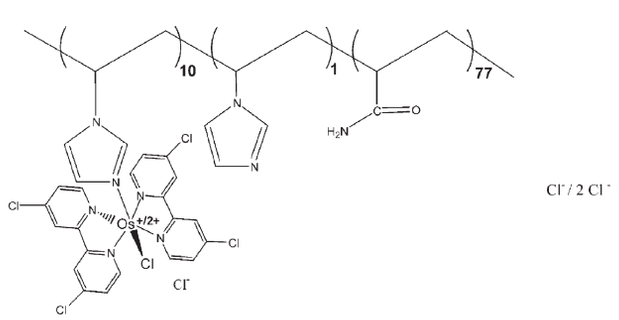

Fig. 6 Structure of the "wire" of bilirubin oxidase. The three polymer constituents are randomly distributed.

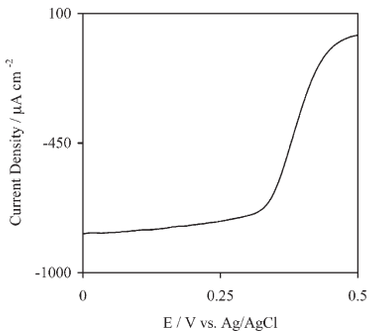

Fig. 7 Polarization of the 7 μm diameter, 2 cm long O_2 electroreducing carbon fiber cathode, made with the electrocatalyst formed by crosslinking the electrostatic adduct of bilirubin oxidase from *Trachyderma tsunodae* and the "wire" of Fig. 6. Quiescent solution, under air, 37 °C, pH 7 physiological buffer solution with 10 mM phosphate, 1 mV s^{-1} scan rate. Because the solution was stagnant and the dissolved O_2 concentration is only about 0.2 mM, the current was O_2-transport limited.[45,66]

existed (it does not), if it had no alkaline electrolyte (it always does) and if the zinc utilization efficiency in this cell could have reached the unattainable value of 100%, a zinc anode with a volume of 0.028 mm^3 would have been required to produce the 0.9 J. This volume is ten times the volume of the glucose electrooxidizing anode. In reality, the volume of the zinc–air cell producing the 0.9 J would have been at least 2 mm^3, about 1000 times larger than the volume of the two fibers. The diameter of the two smallest miniature silver oxide batteries now manufactured is 4.8 mm and its height is 1.65 mm. Its footprint is 18 mm^2 and its volume 30 mm^3, reflecting the difficulty of miniaturizing battery cases and seals. Thus the footprint of the biofuel cell is 1/30th, and the volume of its components is about 1/10 000th of the volume of the smallest presently manufactured silver oxide cell. When the cell consisting of two "wired" enzyme coated carbon fibers was formed in a grape, by implanting the two fibers, the cell produced 2.3 μW at 25 °C. The grape was chosen for the >30 mM glucose concentration in its sap.

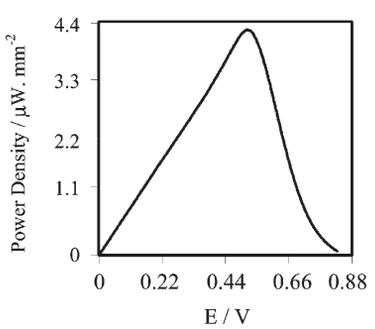

Fig. 8 Dependence of the power density of the miniature glucose–air cell on its operating voltage. 15 mM glucose-containing stagnant, air-equilibrated, physiological buffer solution at 37 °C.[42]

Causes of instability and improvements needed before implantation in animals

When it operated in the grape, the cell was less stable than in the physiological buffer solution. In the buffer solution the power output dropped by less than 5% per day; in the grape it dropped to half of its initial value in about 20 h; in calf serum it dropped to half of the initial value after about 2 h. The voltammograms of the electrodes revealed that the performance of the glucose anode remained intact and that it was the oxygen cathode that was damaged. The voltammograms also showed severe loss of electron conduction by the redox hydrogel "wiring" bilirubin oxidase, the cathodic enzyme, resulting in drastic reduction of the voltammetric peak heights in the absence of O_2. A similar reduction of peak heights was observed in our early glucose anodes, and was shown to be caused by the electropolymerization of serum urate.[68] When the polyanion formed by urate electropolymerization binds with the polycationic "wire", the segmental mobility, necessary for the collision of the tethered redox functions underlying the electron diffusion, is drastically reduced. The rate of urate electropolymerization increases with the square or higher power of the urate concentration in the redox polymer, which, in turn, increases with the density of the cationic sites. Thus we hope that by reducing the density of cations in the "wire" we shall extend the life of the biofuel cell in serum.

In subcutaneously implanted, miniature, glucose electrooxidizing anodes for the management of diabetes, our practice has been to reduce fouling and immune response by overcoating the anodes with a crosslinked hydrogel of polyethylene oxide also known as polyethylene glycol.[69–72] This hydrogel does not impede the flux of glucose substantially, and is unlikely to impede the flux of O_2. It is, therefore, likely to be used also in the implanted biofuel cells.

Conclusion

As proposed in Table 3, the characteristics, and therefore the applications, of the miniature glucose–O_2 biofuel cell will differ vastly from those of other fuel cells, exemplified by the methanol/O_2 cell. The glucose–O_2 cell, consisting only of a pair of 7 μm in diameter 2 cm long carbon fibers, with a footprint of 0.26 mm^2 and a volume of 0.0026 mm^3, is now in hand. Because of the extreme chemical selectivity of its "wired" enzyme electrodes and their functioning under physiological conditions, the cell is structurally simpler and far smaller than any battery or fuel cell. The earlier complex fuel cells were reduced to contain only two of the ten components.

In a physiological, glucose-enriched, buffer solution (pH 7.2, 0.14 M NaCl, 20 mM phosphate, 30 mM glucose), the cell continuously generates 4.4 μW at 37 °C. When implanted in a grape, it generates 2.4 μW at 25 °C. In 6 days of operation in the physiological buffer solution, the cell generates about a thousand times more energy than a zinc–air cell would have generated, were it possible to make such a small zinc–air cell. The simplicity of its structure suggests that an implantable,

Table 3 Comparison of projected characteristics of methanol–O_2 (introduced in 1972 by James J. Auborn and the author of this article. This battery is used today) and glucose–O_2 cells

Cell	Current density	Life	pH	Temp °C	Volume	Footprint	Cost
Methanol/O_2	>100 mA cm^2	Years	0	>70	>1 cm^3	>1 cm^2	>$100
Glucose/O_2	~1 mA cm^2	Weeks	7	37	0.01 mm^3	<1 mm^2	<$ 1

disposable (1 USD or lower cost) cell is feasible. The 1–10 μW continuous output of the cell implanted in any glucose and O_2containing tissue of the body could suffice for powering an autonomous biosensor–transmitter, which would be smaller than 1 mm^3 and would report local conditions in a small volume element of a tissue or organ. The biofuel cell-powered sensor–transmitter would broadcast for a few weeks after its implantation the local glucose concentration, relevant to diabetes management; the local temperature, indicative of infection of an internal wound after surgery or microsurgery; local flow, indicative of blockage of a duct, such as the bile duct; or a pressure difference in the central nervous system, indicative of partial blockage of the flow of the cerebrospinal fluid.

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