Biofuel Cells

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From In Vitro to In Vivo—Biofuel Cells Are Maturing

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Biofuel cells, also referred to as bioelectrochemical fuel cells, are hybrid systems that combine the efficiency of electrochemical energy conversion with that of biocatalysis. In this way they are suitable for the room-temperature conversion of compounds for which no chemical electrocatalyst exists. According to the nature of the biocatalyst, the two complementary classes of biofuel cells are enzymatic and microbial fuel cells. Microbial fuel cells exploit redox enzymes in living microbial cells. The simplicity of electrode preparation (electroactive bacteria actively and voluntarily colonize an electrode surface), the robustness and longevity of the electrode (the bacteria reproduce and regenerate), the access to a virtually unlimited number of even complex substrates, and the possibility of complete oxidation of the substrate are the major features of microbial fuel cells. These features predestine their use in large-scale applications such as energy generation from wastewater. In enzymatic fuel cells, on the other hand, redox enzymes are used in their isolated forms and directly applied in the electrochemical system. Without the protective cell membrane the enzymes are much more exposed to degradation, yet their reaction specificity prevents cross-reactions, which usually affect conventional catalysts as well as microbial catalysts. Based on this specificity, even the physical separation of the anode and cathode compartments generally required in fuel cells can become unnecessary, making major simplification and miniaturization of these devices possible.[1,2] Based on miniaturization, the concept of implantable fuel cells, which harness energy from blood or a comparable body fluid to power implanted devices such as pacemakers, was born.[3]

After years of progress in biofuel cell research, enzymatic fuel cells are still called "implantable", although there are only very few examples of actually implanted systems. The same applies to microbial fuel cells, which are still described as "scalable" despite only scarce examples of actually upscaled systems.[4] So, what makes the transition from fundamental biofuel cell research to applied research so challenging?

The scaling issue: Fundamental research on biofuel cells is usually performed with medium-sized systems (electrodes usually with areas on the square-centimeter scale). The transition towards application thus requires scaling up the electrodes of microbial fuel cells to the square-meter scale and scaling down enzymatic fuel cells to micro- and micrometer dimensions. This scaling is not just a matter of the size of the electrode; changes in the fluid dynamics and increasing internal resistances for large and miniature systems, respectively, require completely different electrode and cell designs.

The "real-world" issue: Systematic fundamental research requires experimental work to be performed under reproducible and comparable conditions. For biofuel cells this means that whereas the respective target substrates of microbial fuel cells and enzymatic fuel cells may be wastewater and blood, laboratory systems are usually operated using artificial, strongly simplified substrate solutions. These solutions consist of a given substrate (fuel) and a buffer/electrolyte (e.g. phosphate or carbonate) to adjust the pH and increase the ionic conductivity. The basic physical-chemical properties of such solutions may indeed mimic those of the target environment, but they do not approach the same level of chemical and biological complexity. Thus, in the case of a miniaturized enzymatic biofuel cell the electrochemical performance and longevity of an in vitro tested fuel cell may not necessarily match that of an in vivo system.

The ethics barrier: Inevitable in the development of an implantable fuel cell (or any other implantable device) is the point of transition from in vitro to in vivo tests, in other words, animal experiments. Depending on the life form (invertebrate, vertebrate, mammal, ape) the ethics barrier for the performance of such experiments can be considerable.

What are the actual requirements for the (sustainable) operation of an implantable biofuel cell? First of all, the anode and cathode redox enzymes have to be confined to or ideally immobilized on the respective electrode. The specificity of the anode and cathode reactions should be high enough to avoid cross-reactions. The use of soluble redox mediators and cofactors should be avoided. The implanted electrodes must be biocompatible in order to prevent, for example, the formation of blood clots.

The first biofuel cell actually implanted in an animal was described by Cinquin et al. in 2010.[5] In this work a glucose oxidase (anode)/polyphenol oxidase (cathode) biofuel cell was implanted in the abdomen of a rat. To simplify the setup and maximize the flexibility of the enzyme selection, the authors relied on the simple mechanical confinement of the redox enzymes and mediators in dialysis bags. The use of size-selective dialysis membranes assured the glucose and oxygen supply and avoided enzyme/mediator losses and the cross-

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reaction between anode and cathode components. This concept may represent a possibility for the powering of implants such as pacemakers. Despite of the potential of this concept it does not exploit the full potential of enzyme electrodes; the possibilities for miniaturization in particular are rather limited.

Recently, decisive progress was demonstrated in independent studies by two research groups. In these studies miniaturization was achieved by using redox enzymes fully confined on the electrodes. Despite the similarity of the overall approach, the two groups follow different philosophies and concepts concerning the mode of implantation and enzyme immobilization. The concepts were demonstrated in vivo with cockroaches and mollusks. These invertebrates possess an open circulatory system that is common amongst many invertebrates such as mollusks, spiders, crustaceans, and some insects. Here, the blood equivalent, the so-called hemolymph, is not confined to distinct blood vessels but flows freely through the animal's body and supplies the organs with nutrients and oxygen. This open system allows comparatively straightforward implantation of the biofuel cell components without causing severe damage to the animal.

Under the leadership of Evgeny Katz an international team from Clarkson University (USA) and Ben-Gurion University of the Negev (Israel) demonstrated in two consecutive studies the operation of biofuel cells fully implanted in snails (*Neohelix albolabris*, a North American land snail)[6] and clams (*Mercenaria mercenaria*, a saltwater clam of the family of Venus clams).[7] In both studies the authors used electrodes based on the direct electron transfer (DET) between the redox enzyme and the electrode. For this purpose a pyrroloquinoline quinone (PQQ) dependent glucose dehydrogenase (anode biocatalyst) and an oxygen-reducing laccase (cathode catalyst) were covalently bound to compressed carbon nanotubes through a heterobifunctional cross-linker (1-pyrenebutanoic acid succinimidyl ester) to form the respective enzyme electrode and to allow efficient unmediated electron transfer. The implanted cells were operated for more than two weeks without showing noticeable signs of enzyme deactivation.

A competing team from Case Western Reserve University (USA) led by Daniel Scherson took a different path.[8] First of all, the team chose to implant their biofuel cell in the abdomen of a cockroach (*Blaberus discoidalis*). The main blood sugar of cockroaches and other insects is trehalose, a disaccharide based on glucose. Since there is no enzyme that can directly oxidize this disaccharide, the authors used a bi-enzyme electrode consisting of trehalase (a hydrolase that splits trehalose into its glucose units) and glucose oxidase. This combination of a hydrolase and an oxidoreductase is very important since it is thus possible to adapt the anode catalyst to the nature of the dominating storage carbohydrates. In contrast to the previously discussed studies the electron transfer between the enzyme and the electrode was accomplished by mediated electron transfer (MET) through immobilized bipyridine osmium complexes. In this study the cathode, a bilirubin oxidase cathode, was not implanted in the animal. Apparently, here the authors aim to develop a half-implanted biofuel cell system that uses a non-implanted, open-air cathode. A similar concept has already been demonstrated by Miyake et al.[9] Their aim is to circumvent the problem of low oxygen levels in body liquids by placing the cathode outside the body, on the skin surface. Certainly, this is an elegant concept which, however, requires an unimpeded proton flux from anode to cathode.

Where does this research lead (Figure 1)? The press coverage on these studies ("Researchers Create Cyborgs") has been dominated by military applications—the development of biopowered monitoring systems for the detection of explosives and chemical weapons, and biological drones. Yet, the potential of biofuel cells extends far beyond. For example, environmental monitoring and pollution control may become important applications. Last but not least these studies lay the cornerstone for the development of self-powered biomedical devices.

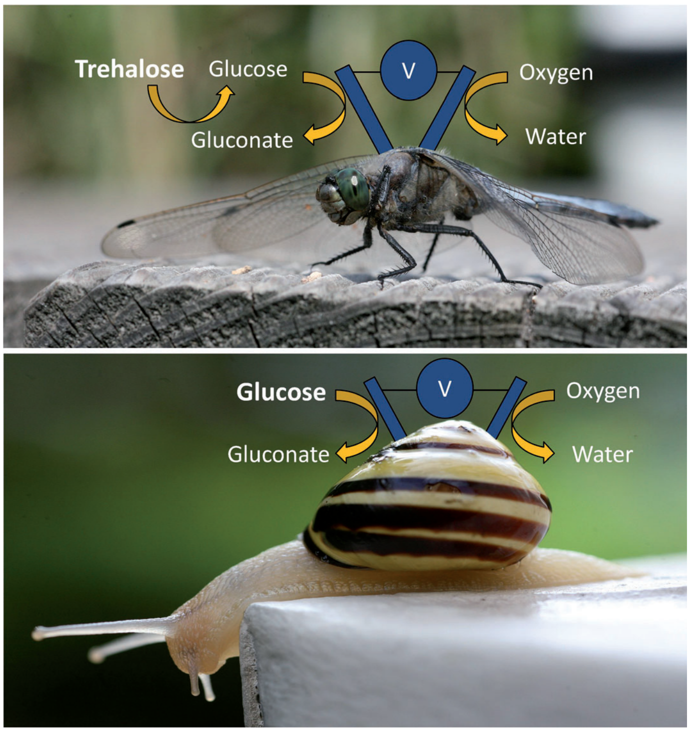

Figure 1. Autonomous biomonitors or bioelectronic spies of the future? Implanted biofuel cells, adapted to the energy source (blood sugar) of the host organism, may offer new fields of application.

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