Improvements in the design of catalytic carbon nanotube matrices for glucose (bio)fuel cells

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ABSTRACT

Ambient energy sources present in the human environment like electromagnetic radiation, temperature, chemical, or motive energy are generally renewable and / or "quasi-free". Even when some of such energies are limited, they may be sufficient for various applications. Glucose, present in sugar, is stable, non-toxic, and easy to handle, in the sense that there is no risk of injury or poisoning. Furthermore, glucose is present in many living organisms and represents an ambient energy source within such organisms like plants or mammals. The most likely application of glucose fuel cells in future is therefore the power supply of implanted medical devices like pacemakers, sensors, actuators, or artificial organs.
In this context, a vast number of milestones have to be passed before this technology becomes competitive with up to date dominating Lithium batteries. Here, we present our recent results about the optimization of individual bioelectrodes and biofuel cell performances.

Keywords: Biofuel cells, bioelectrodes, glucose, enzyme wiring, carbon nanotubes

1 INTRODUCTION

A common strategy for glucose fuel cells development is to use enzymatic catalysts – e.g. glucose oxidase at the anodic side and laccase or bilirubin oxidase at the cathodic side as presented in Figure 1. The overpotential and the catalytic activity of these biological catalysts are much more interesting than noble metals in aerated glucose solutions at physiological pH values. However, enzymes have a limited stability, depending on the environment in which they are used (temperature, pH, inhibitors...). Furthermore, their active centers are often deeply embedded in the protein structure which makes the electron transfer difficult. Carbon nanotubes (CNTs) became the material of choice since they provide a highly conductive matrix and a high specific surface leading to high catalyst densities at reduced volume (Figure 1). Due to their shape, CNTs also enable close contact to the redox centers of enzymes and bio-inspired catalysts as well, improving therefore the electron transfer. In this context, we recently developed a mediator less high-power glucose biofuel cells based on compressed carbon nanotube-enzyme electrodes [1]. A revised deign of this setup could be implanted in a rat scavenging sufficient electric energy from a rat's body fluid to light a LED and a digital thermometer [2] using one individual biofuel cell. However, in spite of this encouraging result, there are still some issues to be resolved before enzymatic biofuel cells become competitive in practical applications. Two critical obstacles are the short lifetime and still poor power densities, where both are related to enzyme stability, electron transfer rate, and enzyme loading. Clear improvements of biofuel cell performances could be obtained using electron shuttles, called redox mediators, for efficient wiring of the biocatalysts [3]. Significantly enhanced direct electron transfer was achieved by targeted orientation of laccase, a biocatalyst for fuel cell cathodes [4, 5].

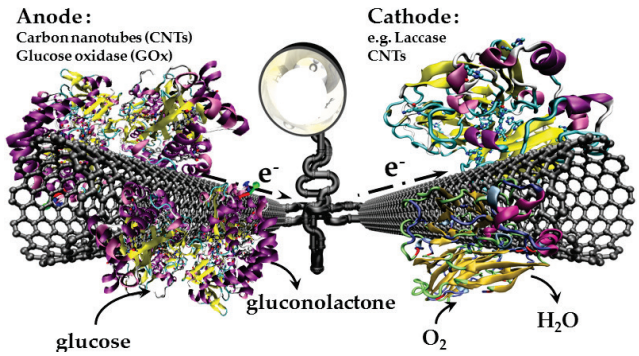

Figure 1: Schematic presentation of an enzymatic glucose biofuel cell.

These and further alternative bioelectrode designs for improved biofuel cell lifetimes and performances are presented.

2 IMPROVED ENZYME WIRING

2.1 Bioanode

The copression of CNTs with glucose oxidase and laccase enables direct electron transfer (DET) between the enzymes and the CNT matrix thus shutteling the involved electrons

of the oxidation of glucose and the reduction of oxygen to an external cicuit at optimal open circuit voltage (~1 V). Even when the glucose concentration was markedly higher than that of O_2, the glucose biofuel cell performance is limited by the bioanode indicating a clearly reduced yield of wired glucose oxidases. The electrical wiring of laccase was actually more efficient than that of glucose oxidase which has its redox active prosthetic groups deeply inside the protein shell. Only few CNTs could get in sufficient close contact to this center by this compression method to regerate the enzyme by collecting the two electrons obtained from glucose oxidation.

In spite of the reduced amount of wired glucose oxidase, this glucose biofuel cell setup delivered a maximum power density of 1.25 mW cm^{-2} (1.66 mW mL^{-1}, 1.85 mW g^{-1}) and 0.14 mW h cm^{-2} under continuous discharge in a 50 $mmolL^{-1}$ glucose solution. By simple addition of naphthoquinone as redox mediator to the glucose oxidase/CNT mixture before compression. This redox mediator serves as electron shuttle whis is, being a small molecule, capable to collect the electrons from the glucose oxidase and to transfer them to the CNT matrix (Figure 2). By using the same biocathode, the power density of the completed biofuel cell increased to 1.54 $mWcm^{-2}$ (1.92 $mWmL^{-1}$, 2.67 mWg^{-1}) [3]. This biofuel cell setup with the modified bioanode is also able to constantly deliver 0.56 mW h cm^{-2} under long-term discharge.

In this case, mediated electron transfer (MET) represents a real advantage compared to DET for high yield wiring of glucose oxidase. This bioanode realized a 7-fold increase in catalytic current densities, despite the increase in the open circuit potential (OCP) from -0.45 V to -0.2 V vs Ag/AgCl.

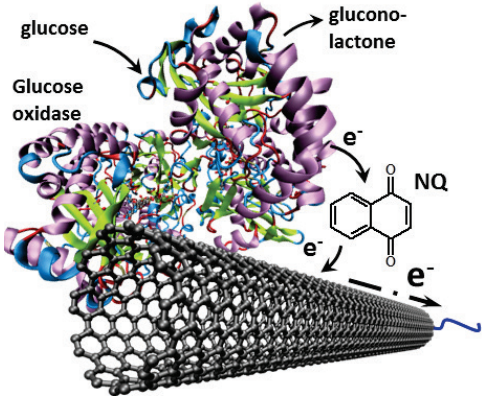

Figure 2: Schematic presentation of mediated electron transfer between glucose oxidase and carbon nanotubes using naphthoquinone as electron shuttle.

2.2 Biocathodes

Within the context of optimizing DET with enzymes, the possibility to orientate during immobilization clearly increases the electrron transfer rated and therefore, the catalytic current. For optimized wiring of laccase, the F. A. Armstrong group proposed few years ago an alternative by elegantly taking advantage of the presence of the hydrophobic pocket near the laccase T1 centre to orientate and immobilize the enzyme on anthracene modified surfaces [6] (Figure 3). The T1 center of the multicopper enzyme has the task to supply the T2/T3 copper centers with electrons in order to reducer oxygen. These electrons are usually obtained via oxidation of phenolic compouns, the natural substrate of laccase. When correctly oriented, this T1 center can also get these electrons from an electrode, wiring in this case the laccase.

Several examples report the efficient immobilization, orientation, and wiring of laccase using polyaromatic hydrocarbons such as anthracene [7], or naphthalene [8]. We have shown that other polyaromatic hydrocarbons are very efficient to interact with a hydrophobic domain of laccase leading to an orientated immobilization of this enzyme and to clearly enhanced direct electron transfer after oxygen reduction. The capacity of anthraquinone to immobilize and orientate laccase was optimized using a pyrene [5] or anthraquinone [4] derivatives (Figure 3) leading to a high performance biocathode with a catalytic current density of up to 1.85mA cm^{-2} in oxygen-saturated solution. These electodes showed also excellent stabilities under continuous discharge.

Further interest was invested for a new design of biocathodes. In fact, multi copper enzymes suffer from fast deactivation in physiological conditions by inhibitors like chloride ions. One goal was therefore to evaluate alternative and more resistant enzymes like horseradish peroxidase.

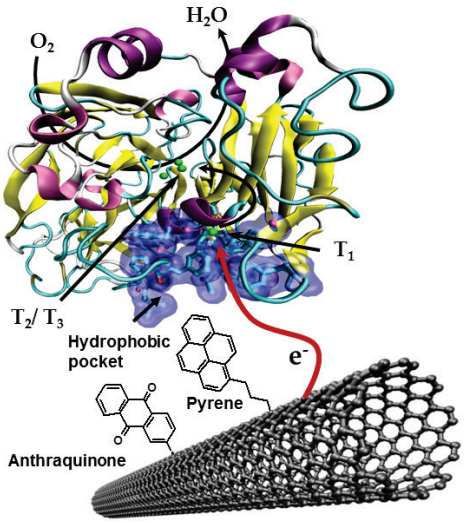

Figure 3: Schematic presentation of oriented immobilization and wiring of laccase on CNTs using pyrene and anthraquinone derivatives.

This enzyme reduces hydrogen peroxide to water by a catalytic heme unit which also favors direct electron transfer with the CNT matrix. In order to produce locally the needed hydrogen peroxide, glucose oxidase was added. Contrary to the functioning in bioanodes, glucose oxidase serves here exclusively as H_2O_2 producer (Figure 4). In presence of glucose (5 mM) and oxygen, the polarization curve for the biocathode with the co-immobilized

horseradish peroxidase and glucose oxidase exhibits a maximum current density reaching 115 µA cm^{-2}. The complete biofuel cell using indirectly wired glucose oxidase at the bioanode (as presented in section 2.1) exhibits an OCV of 450 mV and a maximum power output of 30 µW cm^{-2} at 0.3 V in 5 mM glucose and 0.14 M NaCl at 37 °C [9]. This represents a novel alternative to the use of copper oxidases in conventional glucose/O_2 biofuel cells.

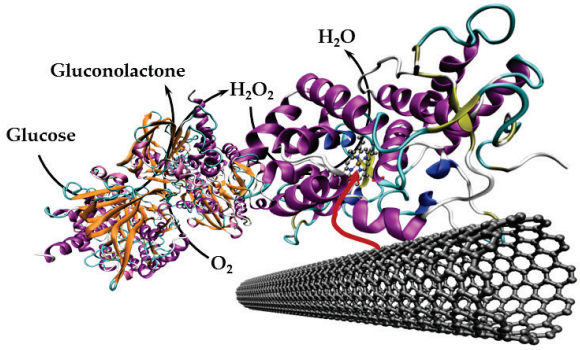

Figure 4: Illstration of the bisenzymatic reduction of oxygen in a biocathode setup using glucose oxidase and HRP.

3 ABIOTIC GLUCOSE FUEL CELLS

A promising alternative to enzymes is the use of bio-inspired catalysts, which generally have improved stability over time and better electron transfer rates.

The first abiotic glucose fuel cell was developed in the 1970s and used noble metals, alloys, or activated carbon as catalysts for both, oxygen reduction and glucose oxidation. Although platinum shows the best catalytic performances towards glucose oxidation and oxygen reduction, the latter is inappropriate because both substrates are present as mixture in body fluids. In this context, other catalysts were proposed for in vivo conversion of energy. Concerning the electrocatalytic reduction of oxygen, beside palladium, gold and their alloys, activated carbon [10] or molecular catalyst based on phthalocyanines with copper, cobalt or iron as active centers are promising alternatives because of their insensitivity towards glucose [11]. On the other hand, a series of different noble metals and alloys, such as platinum–ruthenium alloys, rhodium, and iridium were proposed for the electro-oxidation of glucose in neutral media. However, all these catalysts were evaluated under deaerated conditions [12]

Facing the difficulties in designing selective abiotic catalysts towards glucose oxidation and oxygen reduction, Kerzenmacher et al [13] reviewed different approaches relying on selective membranes which allow the diffusion of oxygen and hamper the diffusion of glucose [14]. One of the most relevant approaches was proposed by von Sturm et al [15] in which the anode is separated from physiological fluid by the cathode itself. This design leads to depletion of oxygen through its flowing through the cathode enabling then glucose to be selectively oxidized at the anode.

Despite the proposed strategies and achievements, the persistent non selectivity towards oxygen or glucose and the low electrocatalytic activity of metal-based catalysts at neutral pH still prevents the envisioned power supply of electronic devices for neither *in vitro* nor *in vivo* conditions.

In tis context, a rhodium porphyrin catalyst, incorporated in a CNT/Nafion® matrix was studied for the oxidation of glucose at different pH values. These studies revealed a particular behavior of this catalyst. Electrochemical investigations showed that rhodium porphyrin is stable at intermediate pH and shows a predominance of a two electrons redox system at low and high pH. This two-electron system is particularly involved in the electrocatalytic oxidation of sugars such as glucose. The catalytic oxidation shows oxidative deactivation which is reactivated during the reductive scan. Such a phenomenum was previously observed for redox enzymes, but not yet for a molecular catalyst. The CNT / rhodium porphyrin electrode was finally integrated in a new concept of an alkaline (pH 14) glucose/O_2 fuel cell with a catalytic electrode using cobalt phthalocyanin / CNT for catalyzing the reduction of oxygen. With was the first glucose fuel cell based on purely nonenzymatic molecular catalysts (Figure 5). A power density of 0.182 mW.cm $^{-2}$ at 0.22 V and an open circuit voltage of 0.6 V could be reached [16].

These studies were essential to understand the mechanisms of the oxidation of glucose using molecular catalyst. The need for such high pH values is , inter alia, due to an initial deprotonaton step of glucose which is followed by its oxidation. The gained knowledge led to the design of a synthetic molecular anode catalyst using just a small amount of a noble metal.

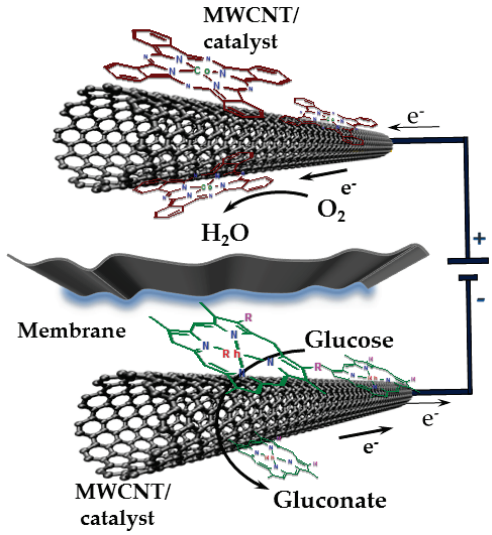

Figure 5: Sketch of an abiotic glucose fuel cell design using a rhodium porphyrin as molecular anodic catalyst and a cobalt phthalocyanin as molecular cathodic catalyst.

With the aim to optimize the performance of the alkaline abiotic fuel cell, a series of membranes were studied.

The mostly used membranes are generally organic polymers with sulfonic acid (-SO_3H) as Nafion®, carboxylic acid (-COOH) groups for the condution of cations, or quaternary ammonium (-NH_3^+) groups to provide improved anionic conductivity. Such cationic and anionic exchange membranes were tested using a breathing cobalt phthalocyanin / CNT cathode and a carbon / Pt anode. The simle use of an anionic membrane led to a 2.5 fold increse of the power denity of the glucose fuel cell setup and finally delivered at ambient conditions a maximum power density of 0.92 mW cm^{-2} at a cell voltage of 0.9 V in KOH solution (pH 13) containing 0.5M glucose [17].

4 CATALYTIC BUCKYPAPER ELECTRODES

Carbon Nanotubes (CNTs) can form flexible and high conductive sheets called buckypapers. The formation of such sheets strongly depends on the purity and the graphitization of the CNTs. Commercial mass produced multiwalled carbon nanotubes generally have highly defective outer walls which prevent the formation of buckypapers. To overcome these issues, buckypaper electrodes with enhanced mechanic stability were formed using a classical filtration technique of a carbon nanotube (CNT) suspension in presence of a bis-pyrene crosslinker containing the redox mediator 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) [18].

These redox mediator electrodes showed particular interesting performances when laccase was in solution compared to immobilized laccase (Figure 6). The developed mediator electrodes provided catalytic current densities of up to 2 $mAcm^{-2}$ and an showed excellent operational stability since the biocatalyst solution was simply exchanged when the enzyme actifity decreased. Such redox electrodes represent a great opportunity for an alternative biofuel cell design.

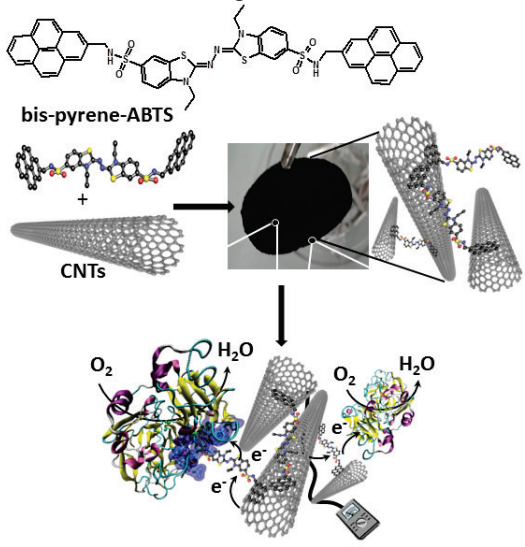

Figure 6: Redox active buckypaper formation using as crosslinker bis-pyrene-ABTS for the wiring of laccase in solution.

In conclusion, in spite of the impressing reults aout the energy harvesting using glucose as fuel, further progresses are necessary that this technology becomes competitive with Lithium batteries, currently used for implantable electronic devices. For instance, state of the art glucose fuel cells, considering all types of catalysts and electrode materials, deliver generally several tenth µW up to few mW at around 0.5V [19]. This is not sufficient to power such electronic devices without step-up converters and sophisticated power management.

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