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Enzymatic Glucose Biofuel Cell and its Application

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Abstract

Biofuel cells have received significant attention in the last few decades due to its potential application as alternative energy sources and advantages over conventional fuel cells. This review summarizes different types of glucose biofuel cells with emphasis on enzymatic glucose biofuel cells. Unlike conventional fuel cells, which use fuel such as ethanol, methanol, formic acid, etc. to generate electricity, enzymatic glucose biofuel cells convert chemical energy stored in glucose into electricity. Energy generating from complex sugar is now possible due to the most common glucose selective enzymes, Glucose Oxidase (GOx) and Pyroquinoline Quinone Glucose Dehydrogenase (PQQ-GDH). Glucose as a fuel source is cost-efficient because it is readily abundant and offers a clean source of power. The micro-power generated from the selective glucose/ O_2 redox reactions can be used to power ultra-low powered bioelectronic devices. In addition, in vivo glucose biofuel cell implantations and potential applications are highlighted.

Keywords: Enzymatic biofuel cells; Glucose oxidase; Laccase; Pyroquinoline quinone glucose dehydrogenase; Bioelectricity

Introduction: Electric energy generation

The idea of energy conversion from organic fuel sources other than fossil fuels comes from the very basic reaction of photosynthesis. In this environmental friendly energy conversion reaction, the light energy is converted into chemical energy by plants and microorganisms [1]. This stored chemical energy can then be further utilized as a fuel source for other purposes. The fundamental sugar producing reaction in photosynthesis involves the utilization of atmospheric carbon dioxide and light dependent products, Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH), to produce sugar and release oxygen back into the environment:

$$6CO_2 + 6H_2O \xrightarrow{\text{Sunlight}} C_6H_{12}O_6 + 6O_2$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

The large amount of glucose produced by the above reaction can serve as a fuel in glucose biofuel cells. Furthermore, one molecule of glucose upon complete oxidation to CO_2 during the aerobic metabolic pathway, generates 24 electrons to produce electrical energy.

Following the invention of the gas batteries by Grove [2], Neidrach and Grubb from General Electric, introduced the first conventional fuel cell for NASA [3]. This simple and efficient fuel cell is the hydrogen fuel cell, which uses hydrogen gas as the fuel (Figure 1). The cell consists of a noble metal electrocatalyst (i.e., platinum), which is used to oxidize hydrogen and reduce oxygen. In a hydrogen fuel cell, hydrogen is oxidized by the platinum electrocatalyst to produce protons and electrons, which travel through the electrolyte and the external circuitry, respectively. The flow of electrons results in the generation of electricity. At the cathode, the electrons recombine with oxygen in the presence of protons, thereby resulting in the reduction of oxygen to produce water as the only by-product. Most fuel cells employ hydrogen fuel because the platinum electrocatalyst utilized is very efficient in oxidizing hydrogen. However, its large-scale application as implantable power source is limited because platinum electrocatalyst is very expensive and nonrenewable. In addition, hydrogen fuel cell requires continuous production/ supply of hydrogen fuel in order to generate electrical power from the oxidation of hydrogen. In addition, hydrogen fuel is also susceptible to carbon contamination, which further complicates the oxidation process due to carbon monoxide poisoning of the electrocatalyst. The high cost of noble metal electrocatalyst coupled with the production of hydrogen and electrocatalyst poisoning results in the unsustainability of hydrogen fuel cells as an ideal power source for implantable bioelectronic devices.

The motivation for the extensive research into glucose biofuel cell technology is attributed to the search for an alternative sustainable 'green' fuel source that is cost-effective and can meet the increasing global energy demands and the recent advancements in microelectronics. Since glucose is an essential energy source in many living organisms, glucose biofuel cells have found applications in powering implantable bioelectronic devices used to diagnose and treat a variety of conditions ranging from metabolic disorders to neurological disorders. Conventional fuel cell assembly consists of an anode, a cathode and an electrolyte and relies on the conversion of the chemical energy of the fuel in the electrolyte, into electrical energy. Two types of reactions occur in a fuel cell: oxidation and reduction reaction also known as redox reaction. The oxidation reaction occurs at the interface of the anode and electrolyte; whereas the reduction reaction occurs at the interface of the cathode and electrolyte. Oxidation of fuel releases electrons, which travel through an external circuitry towards the cathode thus producing electricity. Glucose biofuel cell is a subclass of conventional fuel cells, wherein glucose is utilized as the fuel in the presence of oxygen and is then oxidize to form gluconolactone and releases electrons at high voltages enabling oxygen to be reduced to provide bioelectricity. The experimental set up for such energy generation is shown in (Figure 2).

Although glucose biofuel cells are classified as electrochemical power sources, they are different from batteries and can be differentiated based on the anodic catalysts used for the oxidation of the fuel. Batteries convert the chemical energy of a chemical reaction between two solid reactants (active masses) into electrical energy by

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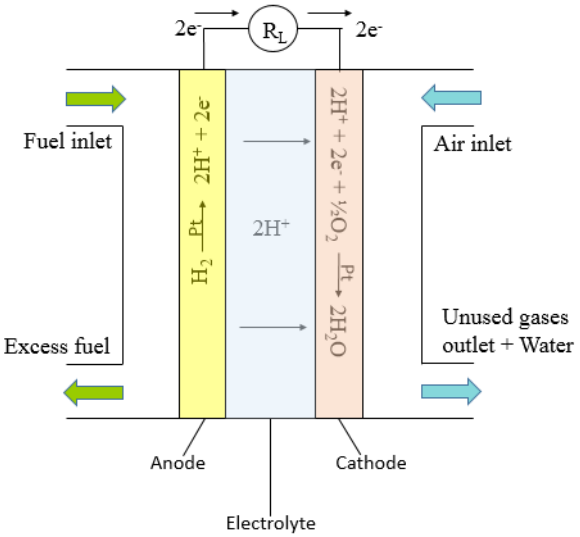

Figure 1: Schematic illustration of a hydrogen fuel cell.

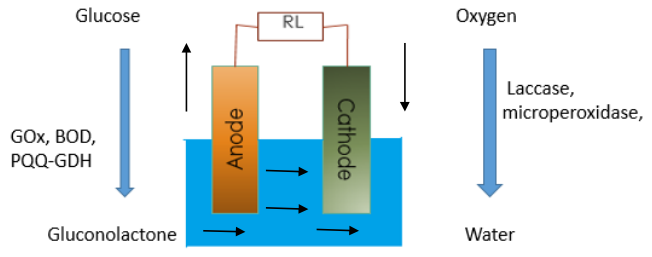

Figure 2: Schematic of an enzymatic glucose biofuel cell.

consuming one of the active masses. Once one of the active mass is fully consumed, the current-producing reaction ceases thus no current flow through the system. Moreover, batteries have limited supply of fuel and have to be recharged or replaced once all the chemical energy stored in the active mass is consumed. Meanwhile, in fuel cells (i.e., glucose biofuel cells), the chemical reaction takes place between liquid and/or gaseous reactants and electrical power is generated as long as there is a continuous supply of glucose and the enzymes remain active. A major advantage of glucose biofuel cells over conventional fuel cells is that it employs renewable enzymes that are environmentally friendly. Thereby, making enzymatic glucose biofuel cells 'green' alternative to convention fuel cells for generating power with high conversion efficiency at ambient temperatures and pH conditions. This review article presents an overview of different types of glucose biofuel cells with emphasis on enzymatic glucose biofuel cells. The challenges encountered by *in vivo* application of enzymatic glucose biofuel as implantable power sources will also be presented.

Enzymatic glucose biofuel cells

The idea of harvesting energy directly from living organisms by building from a biological model, wherein the system is designed to utilize the internal chemical mechanisms (physiological activity) of a living organism to generate electrical power is considerably more stable and reliable than physical mechanisms (i.e., mechanical motion) [4,5]. Enzymatic glucose biofuel cells enable such energy harvesting via chemical mechanisms from blood metabolites such as glucose, wherein the electrodes modified with naturally occurring glucose and oxygen selective enzymes derived from micro-organisms are used to oxidize and reduce glucose and oxygen, respectively. Glucose and oxygen are ideal fuel source and oxidant because they are readily available in all organic tissues and can be continuously replenished in biological fluids by metabolism. In addition, the purified redox enzymes utilized are renewable and less expensive compared to the precious noble metal electrocatalysts used in the development of conventional fuel cells [6]. However, the concept of the biofuel cell has been around for a very long time and was first introduced in 1911, when Porter observed that a culture of yeast and *E. coli* cells generate bioelectricity when platinum electrodes were utilized [7]. It was not until 1962, when the first glucose biofuel cell employing Glucose Oxidase (GOx) bioanode was first introduced by Davis and Yarborough [8]. Since then, the long-term goal of developing self-powered implantable bioelectronic devices was quickly abandoned due to the lack of insufficient generation of power from *in vivo* glucose to drive ultra-low powered microelectronics [9,10]. In the early 2000's, the resurgence of harvesting energy from biochemical fuel readily available in biological fluids was led by Barton el al., [6] where they employed high concentration of glucose fuel to generate sufficient power with the motivation of developing self-powered implantable biomedical devices.

In context, glucose-based biofuel cell circuit is completed with an external load to allow the conduction of electrons from the bioanode to the biocathode. Thus, the choice of the anodic and cathodic material depends on several factors including but not limited to the biocatalyst to catalyze the electrode reactions; the integration of the biocatalyst with the appropriate physicochemical transduction element for generating energy from the various concentration of glucose; effective transduction element surface area in order to increase the number of adsorption sites for the biocatalyst; ease of fabrication; and enhanced durability to ensure extended functional life time of biofuel cell devices for implantation [11-15]. Significant advancements have been made in micro-/nanostructured electrode design to enable electron transfer from the active center of the redox enzyme to the current collector and to increase the lifetime of such devices. These advancements have made it possible to employ these devices as power supplies for bioelectronic devices and microelectronics. However, there are still key challenges to overcome with employing glucose biofuel cells as implantable power sources. These challenges include low power density and short active lifetime.

In the last decade or so, various type of enzymatic biofuel cells employing enzymatic reactions at both electrodes (bioanode and biocathode) have been researched and published [12,16-25] thereby illustrating the high degree of continuous progress being made in the field of biofuel cell development. In order to successfully generate electrical power using enzyme modified electrodes, an important factor: the enzyme's ability for bioelectrocatalysis to the electrode must be considered. Enzymes are naturally occurring proteins with high molecular weights, wherein their active centers are a few angstroms deep from their surface. It is important to understand that fast electron transfer between enzymes and electrodes surface is very difficult to achieve because of the fact that the active center is buried deep in the protein shell, which naturally insulates the active center [26]. Currently, two main strategies are employed to overcome this challenge to establish efficient electrical connection between the enzyme and the current collector: Direct Electron Transfer (DET) [27,28] and Mediator Electron Transfer (MET) [29,6] as shown in (Figure 3). DET requires that the active site of the enzyme communicates directly with the

electrode surface, whereas MET employs redox couples as mediators to enable communication between both the enzyme and the electrode surface. To improve the performance of the biofuel cell, it is important to achieve DET between the enzymes and the electrode.

Previously researched biofuel cells employ DET, as well as employ MET [5,30-35]. Direct electron transfer is generally achieved with enzyme with active center located in the peripheral area of the enzyme. DET is promoted by ensuring that the electrode is placed within a 2 nm distance to the immobilized enzyme's active center and that the enzyme is properly oriented to enable direct wiring to the electroactive surface of the electrode. This enables the enzyme to effectively transfer electrons directly from its active center to the current collector. This mechanism of bioelectrocatalysis is limited to Pyrroloquinoline Quinone (PQQ)-dependent, Flavin Adenine Dinuclucleotide (FAD)-dependent and heme-containing enzymes because it is very hard to achieve DET for GOx since its active center is buried deep in its protein shell. This further leads to another limitation where the electrocatalytic current observed when employing DET is significantly lower than that of MET since the rate of electron transfer is exponentially related to the distance between the electron donor and acceptor [36]. Several attempts have been made to directly 'wire' the active center of GOx to the current collector by incorporating nanostructured materials, such as Carbon Nanotubes (CNTs) and Carbon Nanodots (CDTs) since they are closer in size to the enzymes. In 2006, Ivnitski et al., demonstrated the direct oxidation of glucose by GOx immobilized in CNT-modified porous bioanode with an open circuit potential of ~400 mV vs. Ag/AgCl and also demonstrated direct reduction of oxygen with laccase [37]. Other research groups have also achieve DET with bilirubin oxidase [38,39]. Zhao et al. made a significant contribution to the field of enzymatic glucose biofuel cells by demonstrating DET of GOx and bilirubin oxidase at Carbon Nanodots (CNDs) electrodes [40]. A high open circuit potential of 0.93 V and a maximum power density of 40.8 W/cm^2 was observed at a cell voltage of 0.41 V. The emergent of CNDs as nanomaterials for bioelectrode construction holds great promise for immobilization of enzymes and achieving DET for biofuel cell applications. However, there still remain a possibility wherein the electrode itself can inhibit access to the active center of the enzyme, thereby indirectly inactivating the enzyme [41]. Therefore, the electron transfer processes and enzyme orientation plays a very important role in DET. However, the limitations in achieving direct electron transfer can be overcome by employing redox mediators to enable the 'wiring' of the enzymes regardless of its orientation.

When the enzymes cannot efficiently transfer electrons to the electrode, MET is employed as the mechanism of bioelectrocatalysis. Naturally occurring oxidoreductase enzymes and coenzymes, such as Nicotinamide Adenine Dinucleotide (NAD+) dependent (i.e., glucose dehydrogenase (GDH)) are employed in glucose biofuel cell anodes. Although MET offer higher current-density over DET based biofuel cells, the major disadvantage of MET is that it requires the immobilization of additional components such as redox-mediated or redox polymers along with the enzymes. The redox mediator molecules can be directly immobilized onto the surface of the electrode, solution borne, or covalently linked to the enzyme to enable the electron generated during the biocatalysis to be shuttle to the electrode surface [42,43]. The incorporation of mediator molecules introduces a layer of complexity, which further destabilizes the system since the use of mediators can result in lower open circuit potentials and voltages [44]. Moreover, it is important that the formal potentials of the mediator and enzyme are close [6] (not too close or too far) because if it is too close, a low driving force will be observed for the enzyme/mediator cascade and if it is too far, the cell voltage will deteriorate. For this reason, biofuel cells based on DET are preferred to those based on MET.

Katz et al. [32] pave the way to tailoring biofuel cells for generating electrical power by studying the immobilization of a biocatalyst and a mediator at both the anode and cathode of a membraneless and compartmentless enzymatic glucose/O_2 biofuel cell. The anode employed a surface reconstituted GOx monolayer and the cathode employed a reconstituted cytochrome c/cytochrome oxidase couple as depicted in (Figure 4). The GOx functionalized bioanode oxidizes glucose to gluconic acid and the Cyt. c/COx electrode enable O_2 to be reduced to water. Due to the high degree of enzyme selectivity, a fully assembled compartmentless biofuel cell was realized for the first time. The biofuel cells operating in 1 mM glucose (pH 7) delivered a maximum power density of 4 μW/cm^2 at a cell potential of 40 mV. Tsujimura et al. [33] demonstrated anodic oxidation of glucose in a compartmentless glucose-oxygen fuel cell using Glucose Dehydrogenase (GDH), and the cell delivered a higher power density of 58 μW/cm^2 at a pH of 7. Another major advancement was described by Heller [10], where an

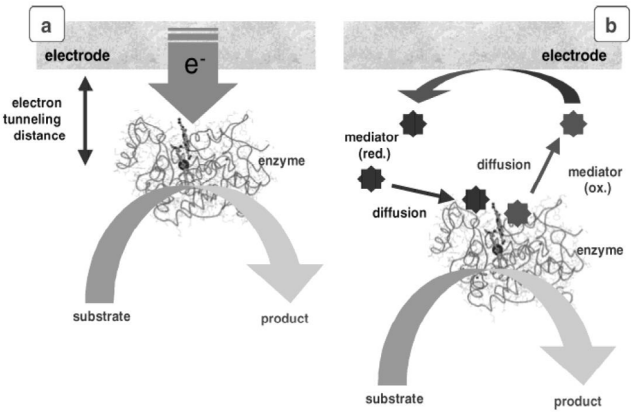

Figure 3: Alternative electron-transfer mechanisms. (a) Direct electron transfer from electrode surface to the active site of an enzyme. (b) Electron transfer via redox mediator. (Barton et al., 2004).

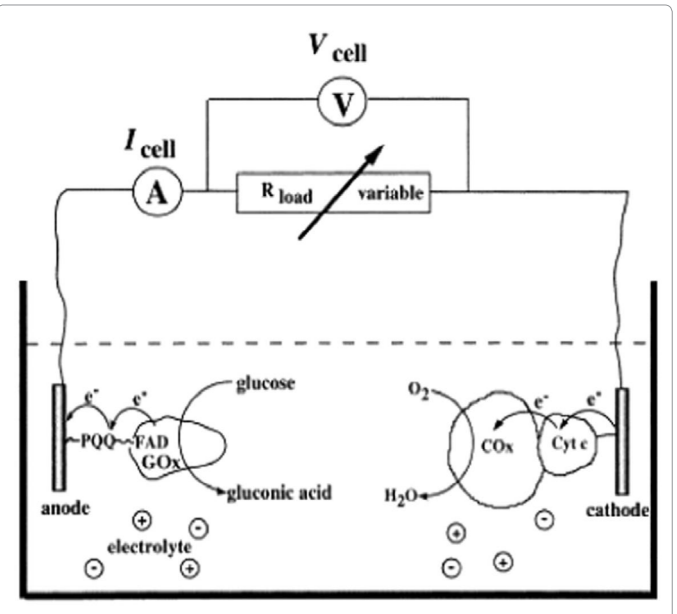

Figure 4: First compartmentless enzymatic biofuel cell (Katz etal.,1999).

osmium-based redox hydrogel was employed to immobilize both GOx and copper oxidase on the bioanode and biocathode, respectively. This enabled the communication between the enzyme and the current collector and resulted in a high open circuit voltage of 0.8 V. Moreover, the development of stable and continuous enzymatic biofuel cells designed to catalyze the oxidation of fuel continues to be limited by low power densities [10,29,45,46]. Ivnitski et al. [45] employed Single-Walled Carbon Nanotube (SWCNT) anodic substrate modified with Pyroloquinoline Quinone Glucose Dehydrogenase (PQQ-GDH) in the implementation to enhance the power densities generated. The use of SWCNT as the bioanode substrate material in half-cell electrochemistry, for the first time, demonstrated DET between the active sites of PQQ-GDH and SWNT as characterized by cyclic voltammetry. Campbell reported on the development of a membraneless enzymatic glucose biofuel cell based on graphene and SWCNT cogel to enable high enzyme loading and DET between the enzyme and the current collector. GOx and bilirubin oxidase were physically adsorbed onto the electrode surface and it delivered a maximum power density of 0.19 mW/cm^2. An interesting feature of the developed bioelectrodes is that they can be easily washed and reloaded for subsequent use.

Most biofuel cells reported in literature employ mediated electron transfer rather than the desired direct electron transfer because direct electron transfer is difficult to achieve and produces lower current densities when compared to biofuel cells based on mediated electron transfer. A biofuel cell device based on glucose oxidase and laccase both mediated by osmium-based redox hydrogels 7 μm diameter carbon fibers have been shown to achieve power density of 137 $\mu W/cm^2$ operating in 15 mM glucose at a temperature of 37°C and a pH of 5 [46]. Kim et al. [34] fabricated a membraneless biofuel cell comprising of anodic and cathodic enzymes mediated by a redox polymer, which generated a power density of 50 $\mu W/cm^2$ at 0.50 V (37°C). The power density, however, dropped from 50 $\mu W/cm^2$ to 30 $\mu W/cm^2$ over a period of 48 hours. To enhance the bioelectrocatalytic capability of cathode, Farneth et al. [48] employed laccase mediated by 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) using a three dimensional porous electrodes instead of a planar electrode. Although these studies focused on anodes and cathodes composed of different enzyme systems under a common pH, temperature and electrolyte simultaneously, the low operating pH (pH 5), which is optimal for the laccase cathode but not optimal for the current-limiting glucose oxidase anode have significant impact on the power densities of the biofuel cell devices. Due to the low operating pH of laccase, Lopez et al. [49] immobilized bilirubin oxidase and its functional analogue, 2, 5-dimethyl-1-phenyl-1H-pyrrole-3-carbaldehyde, onto Multi-Walled Carbon Nanotube (MWCNT) via 1-PyreneButanoic acid, Succinimidyl Ester (PBSE) crosslinker. This cathodic modification resulted in an increased current density, which was found to be 20 times higher than that of unmodified bilirubin oxidase cathode. Moreover, the performance of the enzyme is optimized in pH neutral buffers, thereby making them more attractive for powering ultra-low power consuming bio-implantable devices.

Consequently, the stability of enzymatic glucose biofuel cells is critical and have been evaluated over a period of 24 h [17,49,50] to a few months [18,49,51]. The modification of the bioelectrodes with micro-/nanostructured materials have been reported to enhance the performance of the enzyme bioelectrodes [52,53]. A major advancement in biofuel cells is the achievement of the greatest lifetime of immobilized enzymes (1 year) due to the encapsulation of the enzyme in micellar polymers [54]. Most recently, Reuillard et al. [55] demonstrated one year stability of their MWCNT-based glucose biofuel cell under continuous operation using 5 mM glucose (pH 7, 37°C). The power output rapidly decrease by 25% after 1 h of operation due to the deactivation of laccase at neutral pH. Using an intermittent reactivation/ discharge cycle, wherein the biocathode is reactivated in phosphate buffer (pH 5), the maximum power density was restored to 22% of its initial value. Kwon et al. [56] reported a high surface area yarn biofuel cell that retained over 70% of its initial power output (1 mW/cm^2) after continuous operation in human blood serum over a 20 days period. Recently, El Ichi et al. [57] reported the best stability for laccase biocathode by employing chitosan-MWCNT as a biocompatible matrix to increase the stability of laccase under both storage and discharge conditions, where laccase was entrapped in a conductive nanostructured matrix. The open circuit potential observed with the chitosan-MWCNT-laccase biocathode was 0.55 V vs. SCE, thereby demonstrating good electrical communication between laccase and the conductive chitosan matrix. A stable current of -0.19 mA/ mL was observed for a period of two months under continuous discharge. Although the stability of the chitosan-MWCNT-laccase biocathode is the best reported thus far, stability of biofuel cells is a major challenge that still remains to be addressed if the end goal is to supply sufficient power for implantable bioelectronic devices.

Furthermore, several studies have been focused on miniaturized membraneless enzymatic biofuel cells operating on glucose and oxygen [17,20,34,35,47,58,59-61] where power densities ranging from a few $\mu W/cm^2$ to nearly 2 mW/cm^2 have been reported [17]. For the first time, Miyake et al. [17] prepared a complete miniaturized enzymatic biofuel cells consisting of a needle bioanode for accessing the fuels in organisms through their skins and a gas-diffusion biocathode to reduce the oxygen available in air. The assembled device with a needle anode for glucose oxidation achieve a power density of 130 $\mu W/cm^2$ at a cell voltage of 560 mV. Miyake et al. [20] developed a triple-layer stack of carbon nanotubes-decorated carbon fiber biofuel cells composed of fructose dehydrogenase bioanode for fructose oxidation and O_2 diffusion bilirubin oxidase biocathode for oxygen reduction, with hydrogel sheets containing electrolyte and fructose fuel in between the bioelectrodes to excite the relative low voltage of a single cell construct. With a total size of $5 \times 5 \times 3.3$ mm^3 (Figure 5), the miniaturized assembled biofuel cell system resulted in an open-circuit voltage of 2.09 V and maximum power of 2.55 mW/cm^2 at a cell voltage of 1.21 V. Renaud et al. [62,63] also demonstrated a compact 2-D multi-level microfluidic enzymatic glucose biofuel cells consisting of an array of microchannels with gold bioelectrodes in series or in parallel configuration to excite the voltage or current, respectively. The biofuel cell delivered a maximum power of 12.62 μW using a network of three microchannel biofuel cells in series at a total flow rate of 300 μL/min. When a parallel configuration was employed, a maximum power of 13.37 μW was observed. These advancement in the development of biofuel cell miniaturization paves the route to the realization of enzymatic glucose biofuel cells as implantable power sources by deriving power from the ambient fuels and oxidants in plants, fruits, and eventually physiological fluids. Despite all these achievements, the low power densities, short lifetimes, and operation at neutral pH still impede the generation of power with sufficient drive strength to supply implantable biomedical devices for over a long time periods.

In vivo implantations of enzymatic biofuel cell

The most promising and challenging application for enzymatic glucose biofuel cell is its integration into implantable bioelectronics. Therefore, dependable power harvesting technologies are required for sustaining implantable bioelectronics operation *in vivo* for the lifetime of the patient without the need of continuous battery replacements [4].

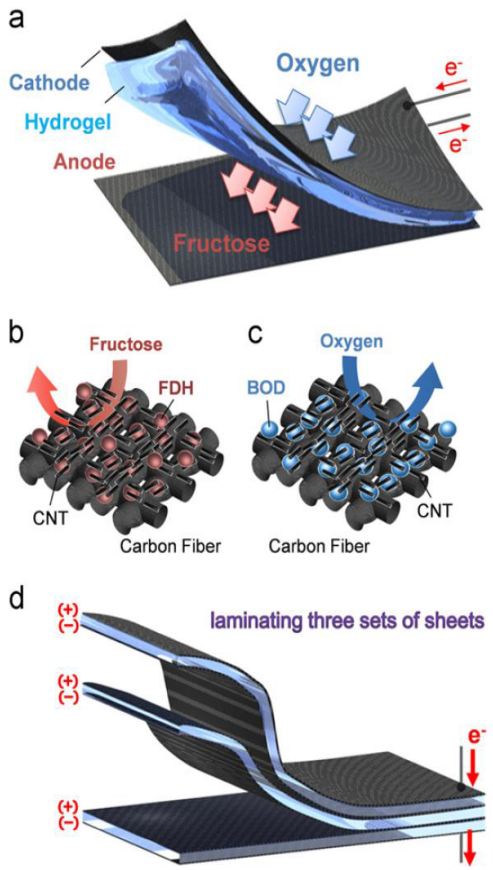

Figure 5: (a) Illustration of a triple-layer biofuel cell constructed by laminating enzyme-modified carbon fabric with a hydrogel film as electrolyte and fructose as fuel. (b) Fructose oxidation at the anode.(c) Oxygen reduction at the gas-diffusion cathode.(d) Illustration of multi-lamination for boosting power (Miyake et al., 2013).

Advances in implantable bioelectronics such as cardio-stimulators, drug delivery, and glucose biosensors make feasible the concept of using glucose biofuel cells to power low-powered biomedical devices [2,13,64]. It shows that conceptually a biofuel cell can harvest electrical power from cerebrospinal fluid when implanted within the subarachnoid space as illustrated in (Figure 6). This integration has the potential to restore proper function to damage organs without the need for external power supply. Currently, high performance glucose biofuel cells mostly employ Carbon Nanotubes (CNTs) as the electrode materials due to their high surface area mediated electron transfer. The development of these nanostructured electrode materials have been show to greatly improve the electroactive area for enzyme immobilization [18,65,66]. Several reports also demonstrated the transition from *in vitro* to *in vivo* characterization of enzymatic glucose biofuel cells [13,15,24,25,67,68].

For *in vivo* energy conversion, Mano et al. [34,35] implanted a glucose biofuel cell composed of a GOx and laccase electrically connected to the bioanode and biocathode, respectively via osmium-based redox hydrogel in a grape and it delivered a stable but very low power of 0.47 $\mu W/cm^2$. Once the glucose biofuel cell was relocated to be in close proximity to the skin of the grape, the biofuel cell delivered a power of 2.4 $\mu W/cm^2$ at a cell voltage of 0.54 V. This increase in power density is attributed to the higher oxygen concentration in the sap closer to the skin of the fruit, thereby suggesting that the power density produced by cell is oxygen-transport dependent. To decrease the complexity of biofuel implantation, Rasmussen et al. [12] partially implanted a two-compartment biofuel cell (bienzyme anode consisting of trehalose and GOx and bilirubin oxidase cathode) in the abdomen section of a cockroach. In this work, only the bioanode was implanted in the cockroach and the bilirubin oxidase cathode was used as an open-air cathode and maintained outside the cockroach in order to avoid low oxygen levels in the body. A maximum power and current densities of 55 $\mu W/cm^2$ at 0.2 V and 460 $\mu A/cm^2$ were obtained. Thus, Rasmussen et al. demonstrated that bioelectricity can be produced on a micro-scale from trehalose within an insect and the oxygen from air. MacVittie et al. [69] implanted a biofuel cell based on buckypaper modified with PQQ-GDH and FAD dependent fructose dehydrogenase at the anode and laccase at the cathode inside an orange. Such assembly produced an open circuit voltage and a short circuit current of 0.6 V and 0.33 mA/cm^2, respectively and a peak power of 670 µW. Another research group implemented a trehalose/O_2 biofuel cell in *Blaberus discoidalis* (tropical cockroach) species, where the chemical energy stored in haemolymph was used to deliver a maximum power output of 0.12 µW at a cell voltage of 0.1 V [70].

A diverse group of organisms have been employed in the characterization of enzymatic glucose biofuel cells. Invertebrates such as cockroaches and mollusks have open circulatory blood system meaning, their blood flow is not confined to distinct blood vessels but flows freely throughout the animal's body thus, supplying nutrients to different parts of their body. Similarly, snail has an open circulatory system in which haemocyanin is the main blood (haemolymph) component that carries oxygen. (Figure 7) depicts the implantations of glucose biofuel cells in a rat, rabbit and snail models. Cinquin et al. [11] reported on the development of a mediated GOx bioanode implanted in the retroperitoneal space of a rat, where the biofuel cell generated a maximum power density of 24.4 $\mu W/cm^3$ at a cell voltage of 130 mV. Miyake et al. [17] partially implanted a needle bioanode into a vein of a rabbit's ear, while using an air-breathing biocathode to reduce the abundant O_2 in air. These contributions to the biofuel cell field pave the way for subcutaneous implantation of enzymatic glucose biofuel cells. Katz's group also reported a biofuel cell comprising of buckypaper electrodes modified with PQQ-GDH at anode and laccase at cathode, which was implanted between the body wall and internal organ of the snail. Such assembly produced an open circuit voltage of 0.53 V and a maximum current of 42.5 µA. The peak power obtained was 7.45 µW

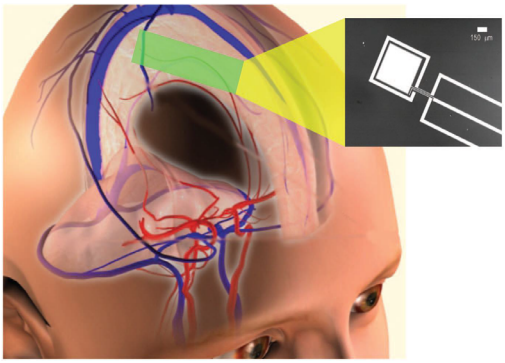

Figure 6: Conceptual schematic of energy harvesting from cerebrospinal fluid by an implantable glucose fuel cell. The inset illustrates a micrograph of one prototype, showing the metal layers of the anode (central electrode) and cathode contact (outer ring) patterned on a silicon wafer (Rapoport et al. 2012).

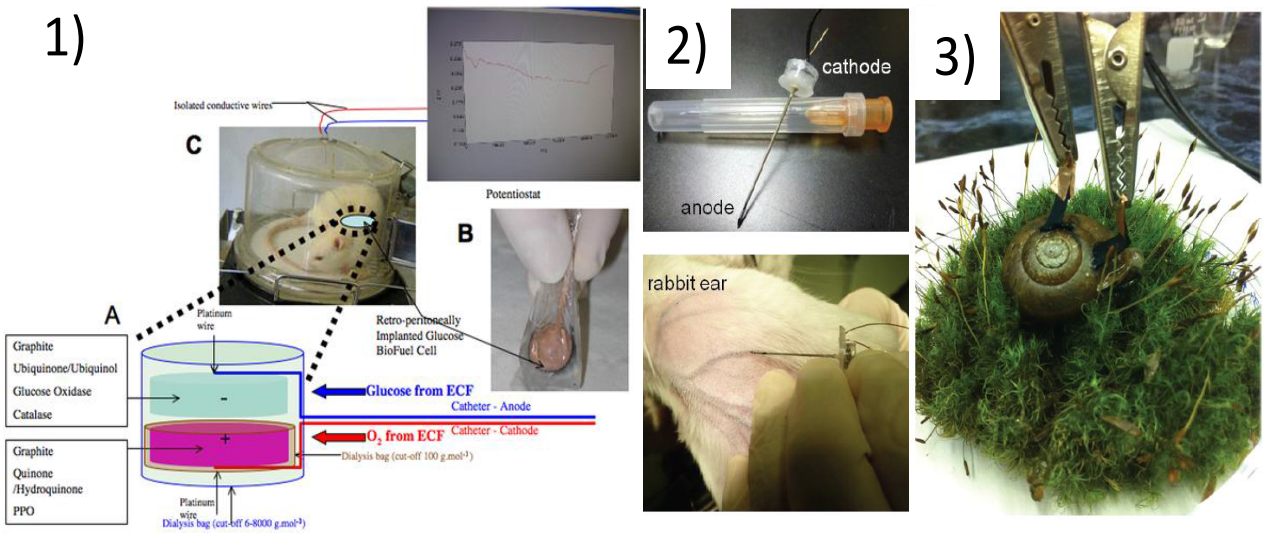

Figure 7: (1) Schematic diagram of the principle, preparation, implantation and operation of an implantable "Quinone-Ubiquinone Glucose BioFuel Cell" (2) Photographs of an assembled biofuel cell for power generation from a rabbit vein. (3) Photograph of a snail with implanted biocatalytic electrodes.

at an optimum resistance of 20 kΩ. The open circuit potential observed in this work is much higher than those previously reported [11,17]. Interestingly, enzymatic biofuel cells have gain some attention as power sources for electronic contact lenses. Falk et al. [16,19] demonstrated a biofuel cell consisting of cellobiose dehydrogenase at the bioanode and bilirubin oxidase at the biocathode in human lachrymal liquid and achieved an open circuit potential of 0.57 V and a power density of 1 μW.

To achieve higher voltages *in vivo*, Katz's group demonstrated that multiple enzymatic glucose biofuel cells can be connected in series or in parallel to deliver sufficient power to power an electric motor and a pacemaker [7]. They connected enzymatic glucose biofuel cells in series using two lobsters each implanted with a biofuel cell (Figure 8). This arrangement resulted in an enhanced potential of 1.2 V as opposed to 0.54 V for a single cell. Szczupak et al. [62] implanted a similar biofuel cell based on buckypaper modified with PQQ-GDH and laccase and achieved an open circuit voltage, short circuit current and maximum power of 0.8 V, 25 μA and 5.2 μW, respectively for series combination and 0.36 V, 300 μA and 37 μW for parallel combination. Thus, series or parallel combination of the enzymatic biofuel cells can be implemented based on the desired electrical parameter. Using the same buckypaper electrode platform, Castorena-Gonzalez et al. [24] placed their biofuel cell in direct contact with the exposed tissue of a rat without having to implant the electrodes. The active surface area of each of the electrode was 2 cm^2. The tissue exposed biofuel cell produced a current density of 5 μA/cm^2 and exhibited an open circuit voltage and short circuit current of 140 mV and 10 μA, respectively. Although the electrical parameters were low, it provided a promising path for the *in vivo* implantations. Zebda et al. [65,66] implanted a glucose biofuel cell based on CNTs inside the abdominal cavity of the rat. The *in vivo* biofuel cell delivered a stable power of 38.7 μW, which corresponds to a peak power density of 193.5 μW/cm^2 at a cell voltage of 0.57 V. This implanted biofuel cell assembly was capable of powering a digital thermometer for several minutes. Zebda et al. [65,66] also reported on the implantation of a novel glucose biofuel cell based on compressing MWCNT with enzyme to create bioelectrodes that were implanted inside a rat. *In vitro* demonstration showed DET was achieved between the MWCNT and enzymes to result in an open circuit voltage of 0.95 V and a high power density of 1.3 mW/cm^{-2}. These bioelectrodes were further characterized *in vivo*, where great care was taken to ensure that the electrodes were encapsulated in a biocompatible Dracon® bag to enhance the biocompatibility of the implant. The biofuel cell was implanted in the retroperitoneal space of a rat and was capable of generating enough power to power a LED and a digital thermometer for a few minutes. Nevertheless, the DET enabled by the use of nanomaterials such as CNTs has paved the way for lower overpotentials of the oxidation and reduction of glucose and oxygen, respectively. It is now possible to achieve an open circuit potential of approximately 1 V using GOx and laccase, which can be used to power ultra-low powered electronic devices via a charge pump circuit for power management.

With the overarching goal of replacing external power sources, these enzymatic glucose biofuel cells can serve as micro-power generators for micro-/nanosize implantable bioelectronic devices [4] such as cardiac pacemakers and implantable biosensors for monitoring physiological state [71]. The advantage of using this chemical approach to harvest energy is that the fuel source, glucose is continuously replenish in living organisms [6]. It is important to note that a cardiac pacemaker circuit requires approximately 10 μW of power and has a lifetime of approximately 10 years [72]. In order to power such device, it is important that the enzymatic glucose biofuel cell be capable of generating enough energy and the size of the biofuel cell must remain as small as possible. Thus using multiple biofuel cells connected in series to enhance the power output will only increase the bulkiness and the complexity of the biofuel cell circuit. To overcome these inherent complexity associated with the biofuel cell system, a significant degree of innovation is required although lower power densities (few μW) are currently generated from enzymatic glucose biofuel cells. A few research groups have reported the generation of sufficient energy from living organisms to power low-powered implantable devices and microelectronics.

Katz et al. [73] implemented a pacemaker circuit using a single glucose biofuel cell and boost convertors consisting of a charge pump

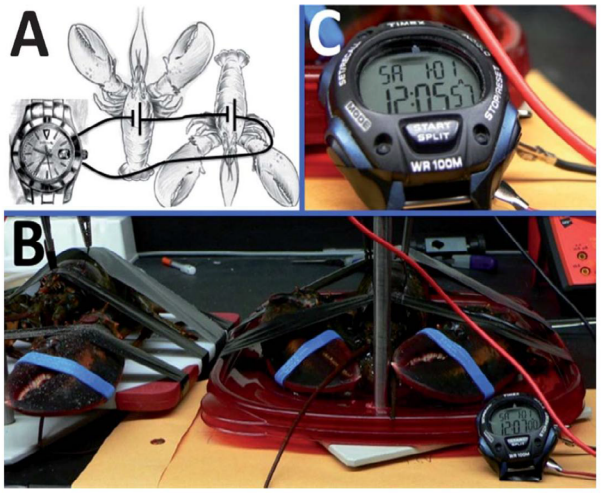

Figure 8: Glucose biofuel cell implantation inside lobster. A) Pictorial representation of two biofuel cells connected in series. B) Experimental set up of implanted glucose biofuel cell C) A stopwatch powered by glucose biofuel cell implanted inside the lobster. (MacVittie et al., 2013).

and a DC-DC converter to generate the 3 V to drive the pacemaker circuit. In this work, the charge pump assembly was used to excite the input voltage (0.3 – 0.5 V) to 2 V and the DC-DC converter was used to amplify the 2V supply to result in a stable 3V power supply used to drive the pacemaker circuit. Thus, a single implanted biofuel cell inside a lobster exhibited an open circuit voltage of 0.47 V and a short circuit current density of 0.83 mA/cm^2 and delivered sufficient energy to power the cardiac pacemaker circuit. In addition, Southcott et al. [25,68] connected an implantable biofuel cell operating under conditions mimicking the blood circulatory system, to a charge pump and a DC-DC converter circuit to amplify and stabilize the power produced by the single biofuel cell to power a pacemaker circuit. Satisfactory operation of the pacemaker circuit was observed (Figure 9). In 2015, Falk et al. reported a wireless self-powered bioelectronic devices, which employed self-sustained carbohydrate and oxygen sensitive biofuel cell system. The self-sustained wireless system consists of a micropotentiostat for biosensing, an energy harvesting module for amplifying the input voltage, a radio transmitter for data transmission and separate sensing bioelectrodes for carbohydrate and oxygen sensing. The enzymatic biofuel cell was operated in a carbohydrate and oxygen containing buffer and was shown to supply sufficient drive strength (at least 44 mA and 0.57 V) to power the wireless units interfaced to it. Bench-top prototype was demonstrated using various concentrations of the carbohydrates and oxygen to wirelessly monitored real-time changes in analyte concentrations, using the enzymatic biofuel cell as the power supply. Desmaele et al. [62] fabricated a multilevel membraneless enzymatic glucose biofuel cell using thin polyester films as flexible electrode substrates. The overall setup comprised of four anodes and cathodes having an individual geometric surface area of 25 mm^2 and thickness of 425 µm. The parallel arrangement resulted into maximum power of 12.5 µW and the circuit comprising of voltage booster produced an output voltage of 3.1 V, which is sufficient for powering the wireless sensor circuit and transmitting temperature measurements to a remote desktop. This important advancement in the development of self-contained biosensing paves the way to realize real-time physiological monitoring of blood metabolites of interest, such as glucose, lactate, neurotransmitters, etc.

Conclusion and Future Outlook Application of *in vivo* glucose biofuel cells

The development of implantable glucose biofuel cells from well-established biological materials for the generation of electrical power have a much higher chance of being biocompatible, and surviving long term implantation in living organisms. The energy converted from glucose in the body using glucose biofuel cells finds potential application in powering bioelectronic devices and portable electronic devices, such as cardiac pacemaker, neurophysiological monitors, Continuous Glucose Monitors (CGMs) and glucose sensing contact lenses. A cardiac pacemaker is currently powered by lithium batteries and is used to treat arrhythmias or abnormal rhythm of the heart-beat. Usually these batteries last anywhere between 5-10 years and needs to be replaced once worn out [74]. The battery consumption depends on a number of factors including but not limited to the age of the pacemaker, the pacemaker work load and power settings. Typically the pacemaker consumes 10 – 30 µW [75] and lithium battery replacement requires an open heart surgery, which results in significant surgical cost and risk to the patient. Thus, miniaturized glucose biofuel cells implanted inside the heart will utilize the glucose present in the blood stream to produce bioelectricity to power the pacemaker circuit. Since, glucose is abundant in the body, the glucose biofuel cell will continuously generate electricity as long as there is a continuous supply of glucose with the assumption that the enzymes does not loss its activity over time. Thus, numerous efforts have been made to developed lightweight glucose biofuel cells that can power ultra-low power devices over a long period of time without the need for surgical replacements.

Although enzymatic glucose biofuel cells have limitless applications *in vivo*, potential applications of these biofuel cells are in the area of continuous monitoring neurophysiological activities and blood metabolites. Recently, Andoralov et al. [76,77] reported on the fabrication of a glucose/O_2 biofuel cell based 3D nanostructured gold bioanode and biocathoide modified with Corynascus thermophiles cellobiose dehydrogenase and Myrothecium verrucaria bilirubin oxidase, respectively. Power densities of 7 W/cm^2 and 2 W/cm^2 at a cell

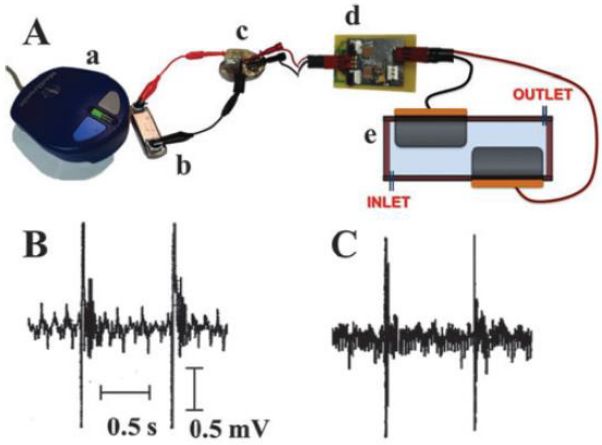

Figure 9: (A) Experimental setup depicting (a) a sensor, (b) an implantable loop recorder, (c) a pacemaker, (d) the charge pump–DC–DC interface circuit, and (e) the biofuel flow cell (B) Registered pulses generated by the pacemaker when it is powered by the standard battery. (C) Registered pulses generated by the pacemaker when it is powered by the biofuel cell (Southcott et al., 2013).

voltage of 0.4 V were observed for the cell operating in cerebrospinal fluid and in the brain of a rat, respectively. Furthermore, they observed inductive loops in the impedance spectra of the biofuel cell implanted in the brain and this is attributed to inductive properties of neuronal membranes. The inductive coupling between the bioelectrodes and neurons is of great promise, especially for monitoring neurophysiological activities in the brain. Furthermore, continuous monitoring of blood glucose levels in people suffering from diabetes is essential since diabetes is the 7th leading cause of death in the United States [78,79]. Current CGM technologies require the patient to periodically prick his/ her finger using a test-strip in order to maintain tight control over their blood glucose level. In addition, CGM uses potentiostatic circuit to acquire blood glucose concentration and this technique requires the use of an external power source such as a battery. The current approach for monitoring blood glucose is very cumbersome and is associated with a degree of discomfort to the patient especially when one has to prick multiple times to maintain normal blood glucose level. Thus, using self-powered glucose biosensors implanted *in vivo* will help patients monitor blood glucose levels without using any invasive technique. One such attempt in fabricating a self-powered glucose sensor (SPGSs) comprising of Glucose oxidase (GOx) anode and a Pt/C cathode was achieved by Liu et al. [15]. The biosensor exhibited a linear dynamic range of 2-30 mM *in vitro* at relatively low O_2 concentrations. A stability of 2 months was achieved under continuous operation at 37°C in 30 mM glucose. In addition, Pinyou et al. [79] reported a miniaturized glucose biofuel cell based on screen printed electrode modified with glucose dehydrogenase and bilirubin oxidase. The power generated from the oxidation of fuel is used to power an electrolyser and transduced into an optical read-out. An open circuit voltage of 567 mV and a maximum power of 6.8 μW/cm² was achieved. Since the power generated by the biofuel cell is proportional to the concentration of glucose, a linear dynamic range of 0.1 mM to 1 mM was reported. Yoshino et al. [80] also reported on a free standing enzyme/mediator/electrode consisting of polyvinylimidazole-[Os(bipyridine) 2 Cl] (PVI-[Os(bpy) 2 Cl]) composites and glucose oxidase (GOD) inside a 1 mm x 1 mm film of carbon nanotubes wound on one of the lead of a light emitting diode (LED) device to serve as the anode and an air breathing cathode (carbon fabric) composed of bilirubin oxidase was employed as the cathode and connected to the other lead of the LED. The assembled biofuel cell serve as a self-powered glucose monitor inside a grape, where the blinking of the LED is inversely proportional to the power of the biofuel cell. The presence of glucose/sugar in the fruit resulted in the blinking of the LED (Figure 10) and this has potential applications in blood glucose monitoring, if the exact concentration of glucose/sugar can be deduced.

Falk et al. [76,77] reported the development of a miniaturized biofuel cell as a power source for a glucose sensing contact lenses by harvesting the biochemical energy stored in ascorbate, which is naturally present in tears. The anode was constructed from 3-D nanostructured gold electrodes (gold nanowires and gold nanoparticles conductive complex) and cathode employed Myrothecium verrucaria bilirubin oxidase. The biofuel cell was operated in human lachrymal liquid and an open circuit voltage of 0.54 V, a maximal power density of 3.1 μW/cm² at cell voltage of 0.25 V was reported. A stable current density of 0.55 μA/cm² at a cell voltage of 0.4 V was observed for 6 h of continuous operation. Although the biofuel cell was not directly used to power glucose biosensing contact lenses, it provides a proof-concept that the reported findings that electrical power can be generated from an ascorbate/O_2 biofuel cell and used as power source for glucose-sensing contact lenses for continuous monitoring of blood glucose. Another

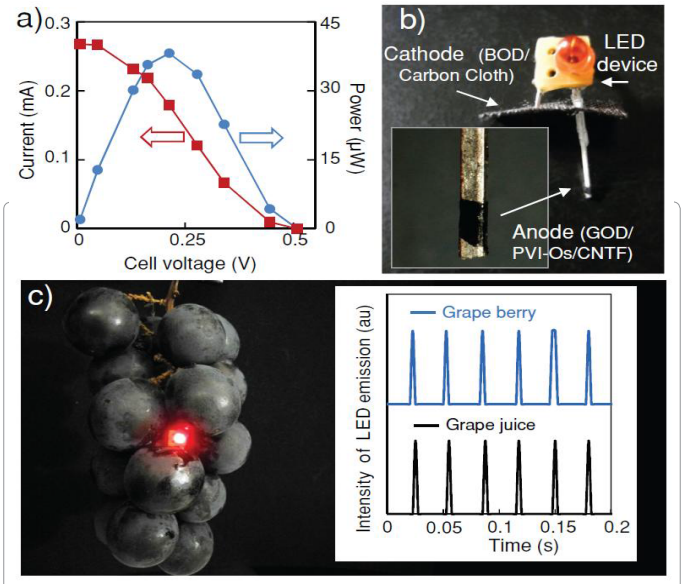

Figure 10: a) Performance of a biofuel cell composed of an anode of GOD/PVI-[Os(bby) 2 Cl]/CNTF film (20 μ m thickness) and a cathode of BOD-modifi ed carbon cloth (1 cm × 1 cm), b) Photograph of the LED-based self-powered sugar indicator, c) Biofuel cell implanted in a grape and the time course of LED emission (inset) (Yoshino et al., 2013).

research group, also investigated the development of contact lenses powered by glucose biofuel cell. The glucose biofuel cell employed buckypaper modified anode with poly(methylene green) and hydrogel matrix containing lactate dehydrogenase and NAD^+. The cathode was modified with 1-pyrenemethyl anthracene-2-carboxylate and bilirubin oxidase immobilized [81-84]. The open circuit voltage achieved was 0.413 V and maximum current and power density of 61.3 μA/cm² and 8.01 μW/cm² in a synthetic tear solution at 35°C. However, the output current decreased rapidly within the first 4 hours. Afterward the current remained stabled for 13 hours. This is a major contribution towards the development of self-powered electronic contact lenses.

Although enzymatic glucose biofuel cells are still plague by low power densities and short lifetimes, considerable amount of research in biofuel cells development have been conducted in the field since 1911 to enable the realization of enzymatic biofuel cells as power sources for implantable bioelectronic devices thus decreasing our reliance on batteries. Improving the stability of immobilized enzymes on transducer surfaces still present a great challenge in the quest of developing biofuel cells with a lifetime greater than 3 months. Nanostructured materials, hydrogels and polymers have been incorporated to improve the performance, stability and longevity of the enzymatic biofuel cells. Future advancement in energy conversion approaches using glucose biofuel cells may move to achieving direct electron transfer between the enzyme and current collector using bioelectrodes constructed from conductive nanostructured materials [85,86]. This approach will continue to be driven by the ever increasing need to improve biofuel cell stability and performance in terms of open circuit potentials and operational lifetime in order to meet the energy demand of renewable source of energy that are cost-effective, environment friendly and readily available to drive pacemakers, implanted biosensors and neurophysiological monitors.

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