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REVIEW PAPER

Glucose-based Biofuel Cells: Nanotechnology as a Vital Science in Biofuel Cells Performance

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ABSTRACT

Nanotechnology has opened up new opportunities for the design of nanoscale electronic devices suitable for developing high-performance biofuel cells. Glucose-based biofuel cells as green energy sources can be a powerful tool in the service of small-scale power source technology as it provides a latent potential to supply power for various implantable medical electronic devices. By using physiologically produced glucose as a fuel, the living battery can recharge for continuous production of electricity. This review article presents how nanoscience, engineering and medicine are combined to assist in the development of renewable glucose-based biofuel cell systems. Here, we review recent advances and applications in both abiotic and enzymatic glucose biofuel cells with emphasis on their "implantable" and "implanted" types. Also the challenges facing the design and application of glucose-based biofuel cells to convert them to promising replacement candidates for non-rechargeable lithium-ion batteries are discussed. Nanotechnology could make glucose-based biofuel cells cheaper, lighter and more efficient and hence it can be a part of *the solutions to these challenges*.

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INTRODUCTION

Power generation from the biological fuels, produced by the living systems, is an attractive issue in green energy technology. It is desirable to develop autonomous implantable devices that do not require any external power input instead of using non-rechargeable lithium batteries. Biofuel cells are energy conversion devices that could efficiently convert the chemical energy of diverse biofuels such as glucose, fructose, cellobiose, alcohol or hydrogen into electrical energy by catalyzing complementary electrochemical reactions. Biofuel cells which have great potential applications in supplying power for portable, implantable medical devices and biosensor systems have become an interesting research topic during the last decade. Nanotechnology has produced a number of promising nanomaterials, which could make fuel cells cheaper, lighter and more efficient and thereby it may be able to ease many of the problems facing fuel cells. Cheap bipolar materials using nanocomposites, more efficient non-platinum

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electrocatalysts, and thermally stable and more durable membranes, which are promised by nanotechnology to become available in the near future, show that the nanotechnology is a key to improve fuel cell performance.

Glucose as a main energy source in living organisms such as animals and fruits plays an important role in biofuel cell technology. This technology has a latent potential to supply power for various implantable medical electronic devices such as cardiac defibrillators/pacemakers, deep brain neurostimulators, insulin pumps, gastric stimulators, cochlear implants, spinal cord stimulator, foot drop implants and *etc*. Such devices have low power requirements and can potentially be operated through biofuel cells using glucose and oxygen present in the body fluids. Therefore, glucose biofuel cell technology has provided the possibility of progress in the bionic human-machine hybrids [1]. Also the "cyborg" creature technology based on implanted glucose biofuel cells can be employed in the future in sensing, information processing and wireless transmission systems for military, homeland security and environmental monitoring aims [2]. Despite the fact that implantable biofuel cells operating *in vivo* were suggested a long time ago [3], such bioelectronic systems are still exotic and very challenging to design. It should be noted that, there is a great difference between potentially "implantable" and really "implanted" biofuel cells. "Implantable" refers to the biofuel cells which can operate under conditions partially mimicking the natural environment and they are potentially able to be implanted in a living organism [4]. While "implanted" biofuel cells should satisfy many factors existing in a living organism under physiological conditions, hence is much more challenging to achieve. Overall, the research on implantable glucose biofuel cells operating *in vivo* shows some promising results, however requires additional investigation and optimization prior to biomedical implementation. Here, we review the latest advances in "implantable" and "implanted" abiotic and enzymatic glucose-based biofuel cells with focusing on their structural characteristics and medical applications.

GLUCOSE-BASED FUEL CELLS

Glucose as a small molecule containing high amount of energy is a suitable fuel to convert the chemical energy into electrical energy. The complete oxidation of glucose to CO_2 and H_2O can provide twenty-four electrons per molecule. Since performing such reaction is practically difficult, only the first oxidation step of glucose has been exploited which provides two electrons at high voltage for the fuel cell device.

The most common reactions in a glucose biofuel cell is the oxidation of glucose by a two electron/ two proton process to glucoconolactone at the anode and reduction of oxygen *via* a four proton/ four electron process to water at the cathode *via* electrocatalytic processes. Oxygen should not interfere with the biocatalytic oxidation of the glucose at the anode. The electrooxidation of fuel at the anode liberates electrons that conduct through an external load to the cathode. Most designed fuel cells require an ion-selective membrane between the electrodes to facilitate an unidirectional flow of protons generated at the anode, to the cathode (Fig. 1).

The use of electrocatalysts can reduce or eliminate the water. According to Ref. [5].

electrochemical reactions' overpotential. Hence, designing of an appropriate electrocatalyst is quite important. The nature of the electrocatalyst residing at the anode determines the extent of glucose oxidation and the associated oxidation products.

The type of the electrocatalysts used in glucose-based biofuel cells could be classified into three general categories: (i) abiotic glucose fuel cells, which their catalysts are abiotic, solid-state materials [6], (ii) enzymatic glucose biofuel cells, which their catalysts are isolated enzymes [7], and (iii) microbial glucose biofuel cells in which oxidation is performed by exoelectrogenic bacterial biofilms colonizing a fuel cell anode [8].

Abiotic implantable fuel cells take the abiotic catalytic electrodes' advantages such as simple sterilization and reasonable operational lifetime (typically higher than enzyme-based electrodes). But abiotic glucose fuel cells are non-selective towards oxygen or glucose and represent the least

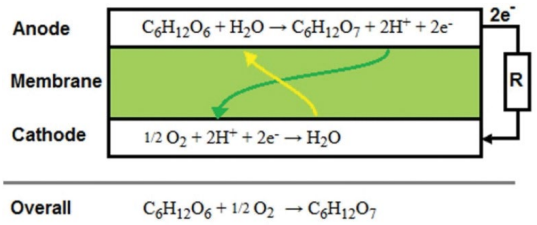

Fig. 1. The most common reaction studied for glucose biofuel cells is the oxidation of glucose to gluconolactone and the reduction of oxygen to water. According to Ref. [5]

catalytic efficiency among other glucose fuel cells at neutral pH.

Enzymatic glucose biofuel cells have high catalytic efficiency, which together with their small size results in high volumetric power density [7]. This type of biofuel cells with superior biocompatibility is bioimplantable. The drawback of enzymatic glucose biofuel cells is the limitation of their lifetimes by the tendency of enzymes to degrade and ultimately degenerate with time.

In microbial glucose biofuel cells that living microorganisms are used to catalyze the anodic reaction, the complete oxidation of glucose occurs and liberates twenty-four electrons per molecule of glucose consumed. Hence, this type of biofuel cells has the highest catalytic efficiency among the others. In contrast with enzymatic glucose biofuel cells, microbial types with inherent ability of self-regenerating are long-lifetime power sources. They use a fraction of input biomass to power and supply molecular substrates for maintenance functions such as resynthesis of degraded enzymes [8]. It should be noted that the electrodes' materials used in the microbial biofuel cells must be biocompatible and chemically stable in the presence of the microbes. Microbes interestingly produce their own nanowires (pilli) to increase their surface area and then the electron transfer capacity of fuel cells [8]. However, from the viewpoint of safety and biocompatibility, present microbial biofuel cells play only a minor role for the application in implantable glucose biofuel cells [9]. They are usually constructed as large-scale biological reactors. Nanotechnology can play an important role in the field of glucose-based biofuel cells. In the abiotic glucose-based biofuel cells, nanocatalysts with the reduction of catalyst efficiency, amount and cost, could be suitable alternatives. In addition, nanotechnology by improvement of enzyme stability could overcome short lifetime of enzymatic glucose-based biofuel cells.

The performance of a glucose biofuel cell is defined by its maximum power density (P_{max}), maximum current density, and open circuit voltage (V_{oc}). Also their operational stability under continuous or discontinuous discharge is an important issue in designing glucose fuel cells. Also it should be noted that for realistic characterization of biofuel cells the formation of a tissue capsule around implanted foreign device [10] which could impose the additional diffusion resistance has to be considered.

ABIOTIC GLUCOSE FUEL CELLS

The platinum or other noble metals are attractive abiotic catalysts for the glucose fuel cells intended to supply long-term medical implants. Kerzenmacher *et al.* in their review have covered the "state of the art" in implanted and implantable abiotic glucose fuel cells and their developments from the 1960s to 2008 [6]. The use of platinum nanoparticles or nanoparticles of other expensive noble metals could reduce the amount and subsequently the cost of abiotic catalysts.

Despite the long-term stability of this type of fuel cells, their non-selectivity towards oxygen or glucose that are simultaneously present in body fluids, and also their low electrocatalytic activity at neutral pH will result in the formation of mixed electrode potentials and consequently a drastic decrease in cell voltage and power output. The performance enhancement in abiotic fuel cells could achieve by developing more selective anodic catalysts [6] or by increasing the specific surface area of the electrodes.

Three general constructional designs are suggested in literature for abiotic glucose fuel cells: (i) "stacked assembly" proposed by Drake *et al.*, at which the reactants diffuse into the fuel cell from two sides and a hydrophobic membrane is placed in front of the cathode that blocks the glucose to pass through and then prevents its interference during the reduction of oxygen, also a thick porous noble metal anode is situated on the opposite side (Fig. 2a) [1]; (ii) "depletion design" suggested by Rao *et al.*, with a selective oxygen consuming cathode made from a glucose-insensitive catalyst for oxygen reduction mounted in front of the anode to create anoxic conditions there (*e.g.* activated carbon [11]) (Fig. 2b) [12,13]; (iii) "single layer design" recently introduced by Kloke *et al.*, at which electrodes are placed side by side without requiring the elaborate stacking of electrode layers with a lateral framework which requires an anode with sufficient tolerance towards the presence of oxygen in body fluids (*e.g.* Raney-platinum film anodes [14]) (Fig. 2c) [15].

The disadvantage of "stacked assembly" design of glucose fuel cells is a direct reaction of oxygen with glucose on the surface of the noble metal as anodic catalyst. This reaction quickly consumes all incoming oxygen and results a mixed potential in the outmost regions of the anodic catalyst. While the "depletion design" with an oxygen-consuming cathode creates anoxic conditions at the anode

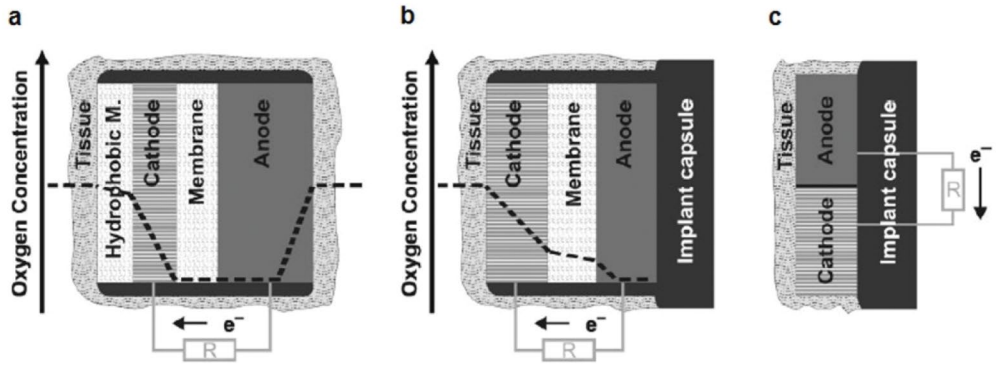

Fig. 2. Abiotic glucose fuel cell designs: (a) "stacked assembly" [1], (b) "depletion design" [12,13]; (c) "single layer design" [15]. The dashed line qualitatively indicates the progression of the oxygen concentration inside the abiotic glucose fuel cell.

and hence reduces the mixed potential there. The "depletion design" has another advantage over the "stacked assembly" design that is diffusion of the reactants into the fuel cell from only one side which enables the glucose fuel cell to directly mount on top of an impermeable implant capsule. These two types of constructional designs require a large volume due to the thickness of each stacked electrodes and need for a lateral framework to complicated fabrication and assembly protocols. Kloke *et al.* claimed that the "single layer design" is desirable in terms of simplified fabrication, reduced thickness and facilitated implementation of the fuel cells in implantable devices [15].

Implantable Abiotic Glucose Fuel Cells

A membrane less stacked glucose biofuel cell with small volume, low ohmic resistance and good glucose diffusion to the anode was developed by Oncesc *et al.* [16]. They used a porous carbon paper as a conductive support for the anodic catalyst layer which increases the electrode surface area and then reduces the amount of platinum or other noble metal catalysts over 100 times compared with previous mesh-free designs [17]. Such design makes it a relatively low-cost power source with a high power density that is enough for powering implantable devices. The peak power output of this fuel cell is approximately 2 μW cm^{-2} and has a sustainable power density of 1.5 μW cm^{-2} at 10 μA cm^{-2}. The performance of platinum on carbon paper supported electrodes applied by Oncesc *et al.* [16] is much better than that on many types of electrode supported materials [18,19].

Carbon nanotubes with the large accessible surface areas, unique electronic properties, antifouling ability, and high stability have become the ideal candidates for the electrodes and catalyst supports [20,21]. Furthermore, the synergistic effect of metal/carbon nanotubes can lead to improved reaction promotion and process stabilization [22,23]. Zhao *et al.* applied three-dimensional flowerlike Pt nanoparticle clusters electrodeposited onto the multi-walled carbon nanotubes as electrodes in an implantable abiotic glucose fuel cell [24]. Such unique three-dimensional morphology exhibits significantly higher electrocatalytic activity and better stability than the dispersive morphology for each of oxidation and reduction reactions. It is because the surface density of Pt nanoparticles in dispersed morphology is limited by the surface density of the broken carbon nanotubes' bonding, which deteriorates the electrical conductivity and mechanical strength [25]. A Nafion membrane was placed between the electrodes and then they were sandwiched between a polycarbonate sealed frame. Such electrodes exhibited a high power density of 2.3 μW cm^{-2} and an V_{oc} of 0.70 V in physiological environment and therefore have a great potential to be optimized for *in vivo* applications.

In another work, Kerzenmacher *et al.* developed an abiotic glucose fuel cell benefited from Raney-platinum in both electrodes that completely obviates the necessity of polymeric hydrogel binders in electrode fabrication [19]. The Raney-platinum cathode was constructed from the dealloying of thermally formed Pt-Al alloy deposited on a silicon substrate with micro-fabricated feedholes for glucose permeability. This cathode was placed in front of a Raney-platinum film anode constructed from the dealloying of thermally formed Pt-Zn alloy (Fig. 3b).

This approach could overcome the limited stability against hydrolytic and oxidative attack

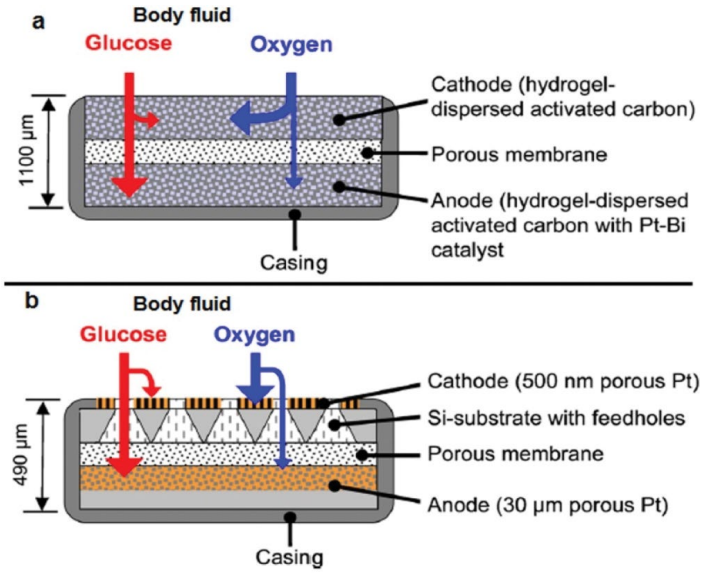

Fig. 3. (a) State-of-the-art approach for abiotic glucose fuel cells with hydrogel-dispersed activated carbon electrodes [18]. (b) Binder-less electrodes in abiotic glucose fuel cells [19].

observed in state-of-the-art approach in fabrication of hydrogel-dispersed activated carbon electrodes (Fig. 3a) [18].

The authors indicated that the high catalytic activity of platinum cathodes and also the high oxygen tolerance of the Raney-platinum anodes result in a relatively high power density of up to 4.4 ± 0.2 µW cm^{-2} at 7.0% oxygen saturation.

As mentioned previously, Kloke *et al.* introduced a "single layer design" for assembling implantable abiotic glucose fuel cells [15]. They applied Raney-platinum film electrodes and indicated that by increasing the cathode to anode area proportion, the oxygen mass transfer to the cathode is limited. They found that the optimum cathode to anode area proportion of 2.4 yields a P_{max} of 2.9 µW cm^{-2} which is only 34% lower than that of the conventional "depletion design". Their designed fuel cells still mandate the mounting of separately fabricated electrodes onto an implant capsule.

Oncesc *et al.* developed a novel abiotic glucose fuel cell assembled in "single layer design" and demonstrated a cost-efficiently manufacture protocol to reduce mixed potential effects when used them directly as a coating layer on large implantable devices [26]. Oncesc *et al.* applied nickel as the non-noble metal at the anode and aluminum at the cathode. The Ni/Pt alloy employed as the anodic catalyst exhibited greater selectivity towards glucose than other abiotic catalysts [6]. Such electrodes used only 100 nm of evaporated platinum which is significantly less than that in other works that used from zinc or nickel electroplated on 50 µm thick platinum foils [14,15]. These abiotic single layer fuel cells exhibit up to 2.01 µW cm^{-2} peak power output in oxygen and glucose physiological concentration. Also, the authors easily stacked these single layer glucose fuel cells to produce high power density fuel cell units. In such stacked single layer glucose fuel cells only a small volume of interstitial fluid can diffuse through the sides and between the glucose fuel cell layers which cause the reduction of the amount of oxygen reaching to the anode. While the "depletion design" fuel cells (wherein the cathode is placed on top of the anode), are unsuitable for stacking and low volume applications. They indicated that a stacked fuel cell unit of 12 single layer glucose fuel cells can provide a volumetric power density of 16 µW cm^{-3} (Fig. 4).

In designing the glucose fuel cells it should be noted that both nonselective electrodes are exposed to the tissue fluid which simultaneously contains glucose and oxygen. It can lead to the formation of mixed electrode potentials and then a drastic decrease in cell voltage. A solution for this problem reported in past works is the use of platinum glucose fuel cells [19].

electrodes with different specific surface areas. Such electrodes have been fabricated by dealloying of thermally alloyed bilayers of platinum and different alloying partners such as zinc, aluminum, and nickel [27,28,14]. In this regard, Kloke *et al.*

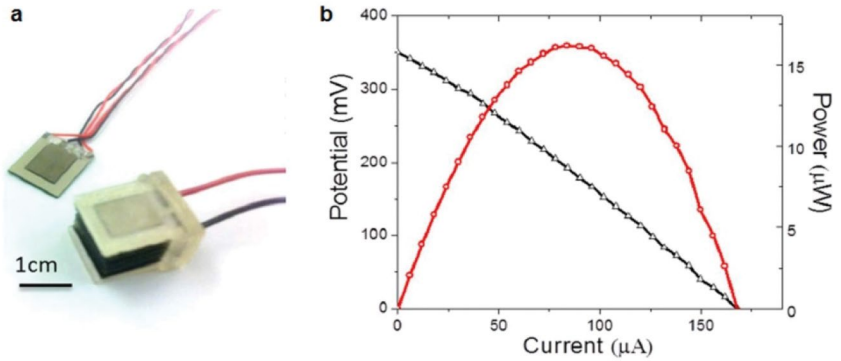

Fig. 4. (a) Picture of one double sided single layer glucose fuel cell along with a stack of 12 single layer glucose fuel cells (b) Performance of the 12 single layer glucose fuel cells stack connected in parallel [26].

introduced cyclic electrodeposition of Pt-Cu alloy as a facile method for the fabrication of porous platinum electrodes wherein the specific surface area is adjustable with adjusting the number of deposition cycles [29]. They indicated that this process is advantageous due to shorter fabrication times, lower temperatures, and the requirement of only one process for the fabrication of both electrodes. They used a module consisting of six "depletion design" fuel cells connected in parallel for fuel cell experiments. Such fuel cells were assembled from 5 deposition cycle cathodes and 500 deposition cycle anodes and showed a power density of 5.1 μW cm^{-2}, which would be sufficient to power a cardiac pacemaker (about 16 cm^2 surface area, 5-10 μW [30,31]). Also, they expected that their fabrication process directly applicable to any conductive surface, such as titanium capsule of medical implants *e.g.* cardiac pacemakers.

Rapoport *et al.* described a novel design and manufacturing of an abiotic glucose fuel cell using semiconductor fabrication techniques that could in principle permit manufacture together with integrated circuits on a single silicon wafer [32]. Their designed cell was configured in half-open geometry that shields the anode while exposing the cathode. This fuel cell used a solid-state platinum anode catalyst with roughen surface to increase its catalytic capacity. Also, a self-assembled network of single-walled carbon nanotubes embedded in a Nafion film formed the cathode electrod. The catalytic electrodes were separated by a Nafion membrane. This abiotic glucose fuel cell could produce 3.4 μW cm^{-2} steady-state power and up to 180 μW cm^{-2} peak powers. An innovation in Rapoport and coworkers' work was the use of cerebrospinal fluid as a physiologic niche for an implantable power source. They have claimed that the cerebrospinal fluid around the human brain which is under minimal immune surveillance provides a promising environment with no adverse physiologic effects for implantation of glucose fuel cells. It is because of that the cerebrospinal fluid is virtually a cellular and its protein content is hundred-fold lower than blood and other tissues then it has less prone to induce biofouling of implanted devices. Also, this fluid is suitable in terms of glucose and oxygen concentrations for implanted glucose fuel cells [33]. This investigation reinforced the possibility of powered or recharged the implanted units of low-power brain-machine interfaces by glucose fuel cells.

Implanted Abiotic Glucose Fuel Cells

The point that should be noted in designing the implanted abiotic glucose fuel cellsis that their activity decrease under amino acids and small organic molecules present in body fluids. The performance loss of abiotic glucose fuel cells mainly originates from catalyst poisoning by those materials at the anode. Köhler *et al.* recently reported for the first time a detailed investigation of the electrical performance of platinum-based abiotic glucose fuel cells in realistically approached simulated body fluids [34]. They conducted their investigations on the realistically simulated tissue and cerebrospinal fluids and indicated that the loss of the overall cell voltage for their investigated platinum-based abiotic glucose fuel cells were 92% and 80%, respectively. In simulated tissue fluid the maximum lifetime of abiotic glucose fuel cells is about 19 h and in simulated cerebrospinal fluid it is 37 h which is in agreement with the lower amino acid concentration in simulated cerebrospinal fluid.

Hence the importance of further investigation on the catalyst poisoning at the anode becomes clear.

The considerable researches in the implanted abiotic glucose fuel cell field was developed since the early 1970s [6]. First *in vivo* experiment, this field was performed by the implantation of a platinum-based glucose fuel cell in the flank of a dog [1]. This abiotic glucose fuel cell could operate for a period of 30 days but delivered low power of 2.2 μW cm^{-2} with V_{oc} of ~0.5 V. Another abiotic glucose fuel cell which was implanted in a vein of a sheep delivered power of 40 μW cm^{-2} but its performance dropped rapidly within an hour [35,36]. Weidlich *et al.* developed a double chamber abiotic glucose fuel cell containing high purity aluminum as anode and activated carbon as cathodes (Fig. 5) [37]. Their designed fuel cell was implanted subcutaneously in a dog and delivered a continuous power of 4 μW cm^{-2} at 575 mV over 150 days which so far would be sufficient to power a cardiac pacemaker that situated on the implant's casing [9].

Since an important problem with abiotic glucose fuel cells is the lack of an abiotic catalyst that can selectively oxidize glucose, hence the importance of the separator membrane used between the electrodes becomes clear. Various weakly ionic polymer membranes [37,18,38] and hydrophilic membranes [37,18,24] have been developed to facilitate glucose diffusion and separate the electrodes. Some of the deficiencies of these membranes are the high variation in their physical properties (pore sizes, pore distribution, and porosity) and their swelling and delamination tendency that could be problematic when implanted *in vivo* [37,12]. Sharma *et al.* with focusing on the creation of high-power abiotic glucose fuel cells used inorganic mesoporous (nanoporous) silica membranes (270 nm) to construction of highly efficient ultra-thin abiotic glucose fuel cells for a blood vessel implantable device [39]. This membrane provides well controlled physico-chemical properties of the nanopores for enhanced glucose diffusion and drastically reduces the thickness of the direct glucose fuel cell. Thin membrane reduces the path-length of the glucose molecules in the abiotic glucose fuel cell and thereby enhances its efficiency [40]. Also the hydrophilic nature of the mesoporous silica membrane leads to the decrease in crossing of oxygen to the anode and then decreasing of its interference with glucose oxidation. In this fuel cell a platinum thin-film deposited on silicon substrate serves as the anode, and graphene pressed on a stainless steel mesh serves as the cathode (Fig. 6). This research group with implantation of this abiotic glucose fuel cell into the right ventricular chamber of a live pig demonstrated power density as high as 10 μW cm^{-2} over the period of 50 min. Such high power density output could extend the lifetime of a pacemaker from 7-12 years. The continuous performance of this implanted abiotic glucose fuel cell over time was limited by neo-intimal tissue formation which creates great barriers to glucose diffusion. Thus, further investigations on the chronic performance of intra-vascular implanted abiotic glucose fuel cells seem to be critical.

The introduction of powerful lithium-iodine batteries [41,42], leads to a significant improvement of pacemaker battery lifetime. Hence, presumably

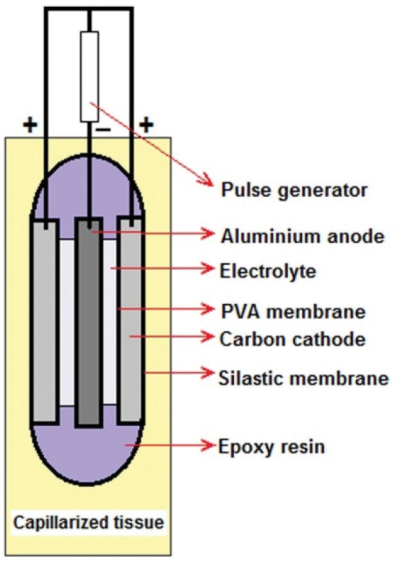

Fig. 5. The construction of an implanted abiotic glucose fuel cell [37].

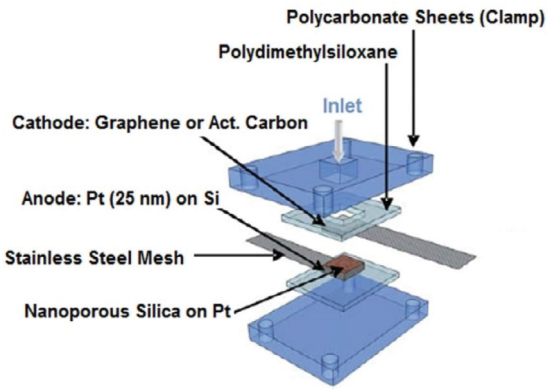

Fig. 6. Schematic for abiotic glucose fuel cells using mesoporous silica as the membrane [39].

the strong involvement of industry causes that the studies in technology of the implanted abiotic glucose fuel cell remains unfulfilled and unproductive.

ENZYMATIC GLUCOSE BIOFUEL CELLS

Enzymatic glucose biofuel cells, with employing enzymes as electrocatalysts, benefit from specificity for oxygen reduction and glucose oxidation reactions, however they are limited by low loading and poor stability of enzymes that leads to low power density and short lifetime. The choice of the enzymes for anode and cathode reactions is an important issue in designing enzymatic biofuel cell. For the biocathode multicopper enzymes such as bilirubin oxidase (BOD) [43], laccases (LAC) [44] and ascorbate oxidases [45] and for bioanodes, different oxidizing enzymes such as glucose oxidase (GOx), nicotinamide adenine dinucleotide and pyrroloquinoline quinine dependent glucose dehydrogenase ((PQQ)GDH), fructose dehydrogenase, cellobiose dehydrogenase and hydrogenases are suited for this purpose [46,47].

There are two ways for electron transfer between the enzyme and the electrode, which include direct electron transfer (DET) and mediated electron transfer (MET) approaches (Fig. 7).

Only 100 out of the 1300 existing oxidoreductases enzymes can be directly immobilized on solid substrates (electrodes) and then transfer electrons from their active site to the electrode surface [48]. These enzymes utilizing a stable redox species should be able to convert a chemical signal into an electric signal *via* charge transfer [49]. This capability depends on the enzyme structure, the redox center location, the enzyme orientation on the electrode surface, and the distance of the electron transfer [50].

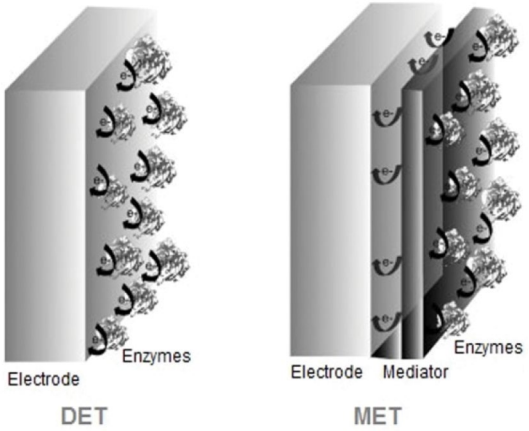

Fig. 7. Two ways for electron transfer between the enzyme and the electrode in enzymatic biofuel cells [51].

One great advantage of DET is the elimination of the mediator species which leads to miniaturization of the enzymatic biofuels [49]. But due to the limitations of DET approach it is essential to use mediator molecules in designing the biofuel call. The MET approach can generate higher output power than the direct mechanism.

Implantable Enzymatic Glucose Biofuel Cells

The power density of enzymatic glucose biofuel cells is closely related to the enzyme loading capacity of electrodes, particularly on the anode. Hence, various nanostructures such as mesoporous media [52-58], nanoparticles [57,59], nanofibers [60,61] and nanocomposites [62,63] with large surfaces have been used to increase enzymes loading. On the other hand, the improvement of electron transfer efficiency is another important issue in designing enzymatic glucose biofuel cells. In this regard, first small molecules have been used as mediators (*e.g.,* osmium or ferrocene containing complexes, 2,2′-azino-bis(3-ethylbenzthi-azoline)-6-sulfonic acid, *etc.* [64-69]). Then, electrode materials with large surface area which could closely communicate with the enzyme active site, such as metal nanoparticles, graphene or carbon nanotubes were used to enhance electron transfer from enzyme to electrode and increase enzyme immobilization [63,64,70,71].

One special feature of carbon nanotubes is that they bear chemically inert and hydrophobic graphene sidewalls with a dense π-π stacking which make them well suited as a support for the redox mediators [72-74].

The first single-walled carbon nanotube-based glucose biofuel cells were demonstrated at 2006 by Yan *et al.* [75]. They applied NAD^+-dependant glucose dehydrogenase supported onto the poly methylene blue-single-walled carbon nanotube as a bioanode and LAC supported onto the single-walled carbon nanotube as a biocathode. The V_{oc} of this biofuel cell was 0.80 V and the power density was 9.5 μW cm^{-2} at 0.52 V in phosphate solution at pH 6.0.

Despite the high surface area of the electrodes consisting entirely of carbon nanotubes (carbon nanotube paper), they have typically weak mechanical strength. Won *et al.* [76] applied cellulose in combined with carbon nanotube as electrodes for enzymatic glucose biofuel cells

because of its inherent biocompatibility and nontoxic properties [77]. They immobilized GOx on the cellulose-carbon nanotube composite paper as an ideal bioanode and indicated the DET between the GOx and the composite electrode. Also, they investigated the dependence of DET and the type of carbon nanotube and indicated that it is not observed with the single-walled carbon nanotubes.

In other work, Kim and Yoo developed a GOx nanotube-based glucose biofuel cell with an improved power density and stability [68]. The authors deposited GOx nanotubes on polypyrrole-carbon nanotubes-GOx layers surface and applied it as an anode. Previously this research group constructed polypyrrole nanowire-based glucose biofuel cells, as GOx and 8-hydroxyquinoline-5-sulfonic acid (as a mediator), were co-immobilized in polypyrrole nanowires [78]. They indicated that the power density in their newly designed enzymatic biofuel cell is improved presumably due to an increase in enzyme loading of GOx nanotubes and also the improved electrochemical properties of the polypyrrole-carbon nanotubes-GOx layers. In this glucose biofuel cell the cathode was made by co-immobilization of LAC and 2,2′-azinobis(3-ethylbenzothi-azoline-6-sulfonate) diammonium salt (ABTS) as a mediator, in polypyrrole films. Such glucose biofuel cell delivered the P_{max} of 1.39 mW cm^{-2} and the volumetric power of 231.7 mW ml^{-1}. Kim and Yoo indicated that the stability of this biofuel cell is related to the leakage of mediator from the cathode. When the leakage of ABTS was suppressed, the power density of biofuel cells was nearly unchanged over 8 days under physiological conditions.

El Ichi *et al.* developed a biocathode with fibrous microstructure combined of chitosan-carbonnanotube-enzyme that creates a protective microenvironment to prevent the loss of the electrocatalytic activity of the enzyme, and provide good oxygen diffusion [79]. The multi-walled carbon nanotubes in such biocathode act as conducting fillers between the chitosan nanoporous fibers. Chitosan with a high degree of deacetylation (DD > 85%) provides optimal properties such as biocompatibility, stability and antimicrobial activity [80] that are desirable properties in designing the implanted glucose biofuel cells. Such properties help in minimizing the risk of infections if any contamination occurs during the implantation process. Then, El Ichi *et al.* used chitosan to increase the longevity of a LAC-based biocathode, both under storage conditions and continuous discharge. The presence of chitosan during the compression of the multi-walled carbon nanotubes with the LAC, results in stability enhancement of the biocathode. Chronoamperometry measurement which was used to evaluate the stability of this biocathode under continuous discharge, at neutral pH in PBS buffer, at ambient temperature and without O_2 saturation showed a stable current response during 2 months (I~ -0.19 mA ml^{-1}). Also, the assaying of such biocathode operation in sheep serum at 37 °C results in -0.17 mA ml^{-1} current density during 48 h.

Park *et al.* fabricated a bioanode by sequentially coating of multi-walled carbon nanotubes, ferrocene, and GOx on a glassy carbon electrode and entrapping them by chitosan membrane without using any complicated linking processes [81]. Ferrocene and its derivatives with good electrochemical reversibility and stability at low potential have been reported as the most efficient electron mediators [82]. They also utilized multi-walled carbon nanotubes to further improve electron transfer [83-87]. The carbon nanotube nanowires create electrical bridges to facilitate electron transfer between the immobilized GOx, mediator, and electrode. Then, they integrated their designed bioanode with a BOD immobilized biocathode and indicated that this glucose biofue cell showed the P_{max} of 13 μW cm^{-2} at a cell voltage of 0.19 V when the glucose concentration was 10 mM in pH 7.0 phosphate buffer at room temperature under ambient conditions.

Wen *et al.* introduced the first membraneless glucose biofuel cell using Pd-based aerogels as electrode materials [71]. They used β-cyclodextrin-modified Pd aerogel as the host to immobilize ferrocenecarboxylic acid as an anodic mediator. Such three-dimensional porous support coimmobilized the GOx and simultaneously mediated the bioelectrocatalytic oxidation of glucose. Also a biocathode was fabricated by the BOD encapsulation into a Pd-Pt alloy which promoted the direct electrocatalytic reduction of O_2. This membraneless glucose biofuel cell indicated a P_{max} of 20 μW cm^{-2} at 0.25 V in pH 7.0 PBS.

Devadas *et al.* combined multi-walled carbon nanotube and electrochemically reduced graphene oxide *via* non-covalent π-π stacking interactions to harvest their excellent electrochemical properties

[88]. They modified a glassy carbon electrode by that composite and employed it to immobilize the GOx, then applied it as a bioanode. Authors employed such bioanode and graphene-Pt modified glassy carbon electrode as a cathode to construct a membraneless glucose biofuel cell that achieved the P_{max} of 46 μW cm^{-2} in PBS (pH 7) containing hydroquinone (HQ) as an external mediator (Fig. 8).

The synergistic integration between two carbon isotopes (graphene and carbon nanotubes) was also investigated by Prasad *et al.* [64]. They used single-walled carbon nanotubes decorated three-dimension graphene as both anode and cathode in a novel enzymatic glucose biofuel cell designing (Fig. 9a). The GOx was successfully immobilized on the electrode surface and observed good electrical coupling (DET) between the enzymes and the anode. Also, the LAC was immobilized on the graphene/single-walled carbon nanotubes to fabricate the cathode. In order to obtain a suitable cathodic open circuit potential, they applied ABTS as a mediator. The authors demonstrated the electron transfer pathways from the active centers of LAC (T1-T3) to the cathode (Fig. 9b). Although the T2 center of LAC is in close approximation to the cathode surface to allow DET [89,90], the T1 center is crucial to achieve efficient O_2 reduction and high open circuit potential [91]. On the other hand, the redox potential of ABTS matches with that of the T1 center [58,92], thus the diffusive small ABTS molecules can assist to transfer electrons to the T1 center of LAC that is distant to the electrode surface [93,94], whereby electrons are intramolecularly passed to the T2/T3 cluster

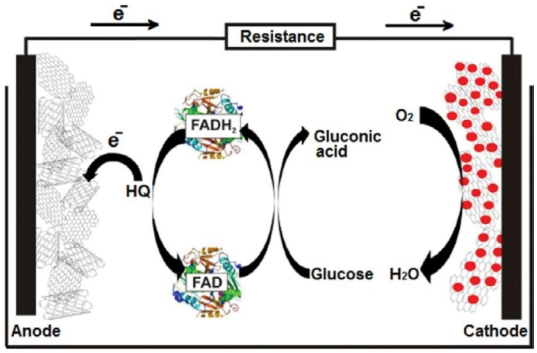

Fig. 8. Schematic representation of the glucose biofuel cell with graphene-multi-walled carbon nanotube/GOx/nafion as ioanode and glassy carbon electrode/ Graphene-Pt composite as cathode [88].

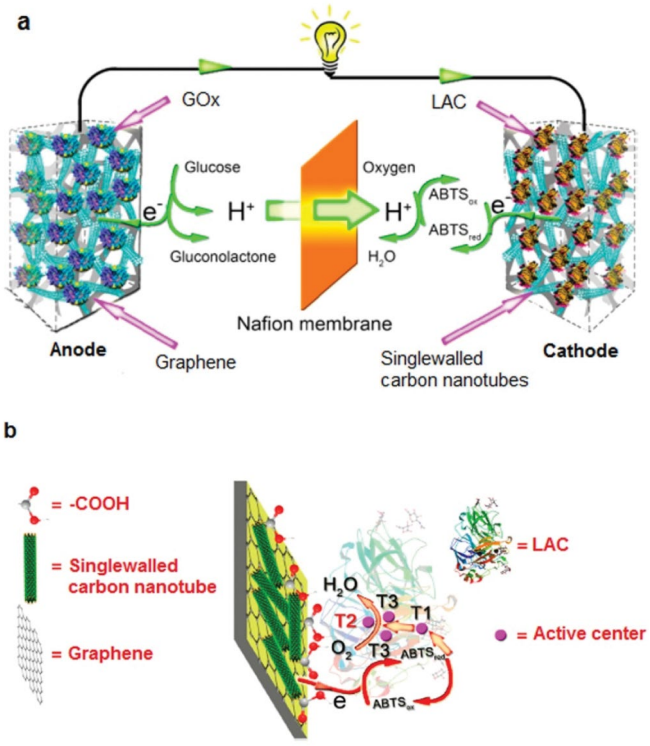

Fig. 9. (a) Illustration of the enzymatic biofuel cell equipped with 3-dimansion graphene-single-walled carbon nanotube hybrid electrodes; (b) Illustration of electron transfer pathways [64].

(the oxygen reduction site) [95]. This enzymatic glucose biofuel cell exhibited a V_{oc} nearly reaching the theoretical limit (~1.2 V), a high power output density (2.27 ± 0.11 mW cm^{-2}), and good long-term stability (only ~20% drop of V_{oc} after 30 days).

Recently Campbell *et al.* developed the first membrane/mediator-free rechargeable enzymatic glucose biofuel cell utilizing free standing co-gels of graphene and single-walled carbon nanotubes as electrodes (Fig. 10) [96]. These simple prepared co-gels with high specific surface area (~800 m^2 g^{-1}), porosity and electrical conductivity (~0.2 S cm^{-1}) allowed high enzyme loading, unhindered glucose transport to the enzymes and efficient charge collection from enzymes. GOx and BOD were physically adsorbed onto these electrodes to form anodes and cathodes, respectively, and the power densities up to 0.19 mW cm^{-2} with an V_{oc} of 0.61 V were obtained in pH 7.0 sodium phosphate buffer. They indicated more than 20% of available co-gel surface area in anode occupied by electroactive GOx. This loading capacity was almost two orders of magnitude higher than similar three-dimensional structures comprised of Pd aerogels [71] or graphene foams decorated with single-walled carbon nanotubes [64]. The authors believed that the robustness and simple enzyme loading procedure in their designed enzymatic biofuel cell could lead to rejuvenate of power output by cyclic removal of degraded enzyme with acid and replenishing the system with fresh, active enzyme which allowed for the reloading and reuse of such enzymatic biofuel cells.

(PQQ)GDH now becomes a promising enzyme for the development of high-performance glucose biofuel cells. This enzyme is independent to O_2 unlike GOx and also keeps its stability at physiological pH and temperature. Durand and coworkers with utilizing molecular modelling suggested that the pyrroloquinoline quinine (PQQ) as a cofactor can well interact with enzyme which leads to a good electron transfer in the catalytic site during the redox reaction and consequently more activity toward glucose compared to the parental wild-type enzyme [97].

In this regard Scherbahn *et al.* developed membraneless and mediatorless, glucose biofuel cells based on (PQQ)GDH attached on poly(3-aminobenzoic acid-co-2-methoxyaniline-5-sulfonic acid) /carbon nanotube as anode and BOD covalently bound to a PQQ/carbon nanotube electrode as cathode [98]. Two types of carbon nanotube electrodes include buckypaper and vertically aligned carbon nanotubes were applied in structure of the electrodes. The vertically aligned carbon nanotube-based fuel cells showed a better power exhibition and an enhanced stability as they exhibited a maximal power density of 41 μW cm^{-2} (390 mV) in human serum samples.

Kim *et al.* fabricated enzymatic glucose biofuel cells with utilizing of GOx and LAC as anode and cathode enzymes on polyaniline nanofibers (PANFs), respectively [99]. The PANFs as a supporting material compared to other nanostructured materials such as electrospun nanofibers, nanoparticles, carbon nanotubes and mesoporous materials can be easily and economically synthesized. They have a large surface area and high electron conductive property [100] that has the potential to immobilize and stabilize various enzymes [101-103]. Also, in order to further improve the power density output, they applied 1,4-benzoquinone and ABTS as electron transfer mediators on the enzyme anode and cathode, respectively. The authors employed three different approaches to immobilize enzymes on PANFs including: enzyme adsorption, enzyme adsorption and crosslinking and enzyme adsorption,

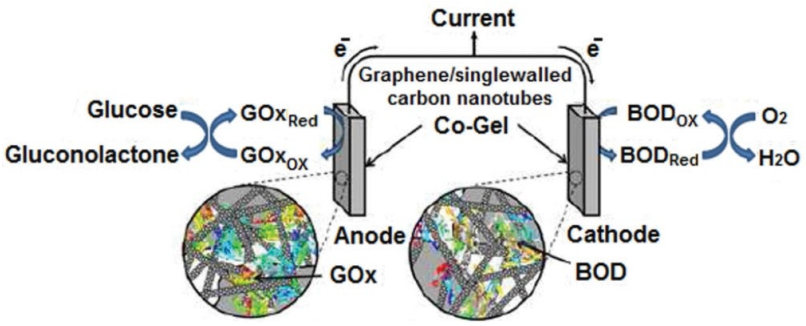

Fig. 10. Schematic representation of the membrane/mediator-free enzymatic glucose biofuel cell utilizing free standing co-gels of graphene/single-walled carbon nanotube as electrodes [96].

precipitation and crosslinking approaches (Fig. 11). They indicated that with using enzyme adsorption, precipitation and crosslinking approach they could improve the loading and stability of enzymes on PANFs. Beneficial this immobilization approach and mediators, these enzymatic biofuel cells showed power density output of 37.4 μW cm^{-2} that is significantly higher than that without mediators (3.1 μW cm^{-2}).

In the last decades, the PtCu bi-metal nano-materials were applied as electrodes for the fuel cells [102,110,61]. Also, according to the recent studies PtCu bimetal was employed as nano-batteries autonomous nanomotors [111].

Ammam and Fransaer introduced a new type of electrode material based on bi-metal PtCu connected wires immersed in a solution of benzene sulfonic acid-phenol or 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)-phenol, then subjected to simultaneous alternating current and direct current electric fields [112]. These electrodes can boost the power outputs of biofuel cells as they can generate sufficient power in the physiological electrolyte (10.8 μW mm^{-2} in phosphate buffer solution, pH 7.4). In order to improve the power outputs of their designed glucose biofuel cells authors added enzymes GOx at the anode and LAC at the cathode which yielded 13.3 μW mm^{-2}.

Microfluidic enzymatic biofuel cells are an attractive alternative to conventional fuel cells that can develop portable electronics [113] or medical implants [63]. In such fuel cells, fuel and oxidant streams flow in parallel and the electrochemical reactions are localized at the anode and the cathode within the respective streams. Therefore the need for a separation membrane was eliminated [114]. But incompatibility of enzymes with lithography processes and also the lack of suitable enzyme immobilization techniques [115], have limited the researche in this field.

Beneyton *et al.* developed a microfluidic glucose biofuel cell using LAC and GOx covalently bound onto single-walled carbon nanotube electrodes in a Y-junction microchannel on a glass substrate (Fig. 12) [116]. The single-walled carbon nanotube electrodes were patterned onto a glass slide using lift-off lithography and a new covalent enzyme immobilization technique was developed that is compatible with lithography processes. The direct covalent bonding of the enzyme to carboxylated single-walled carbon nanotube electrodes which was used in this biofuel cell was offered as a robust immobilization technique for microfluidic application. This microfluidic glucose biofuel cell with laminar flow of different streams of fuels and oxidants within microchannel delivered up to 1.65 μW cm^{-2} at 235 mV.

Implanted Enzymatic Glucose Biofuel Cells

The power supply for artificial organs is the noticeable application of glucose-based biofuel cells through the implantation in the human body. The electrochemical reactions in glucose fuel cell electrodes can perform indefinitely due to the ubiquity of glucose and oxygen in the extra cellular

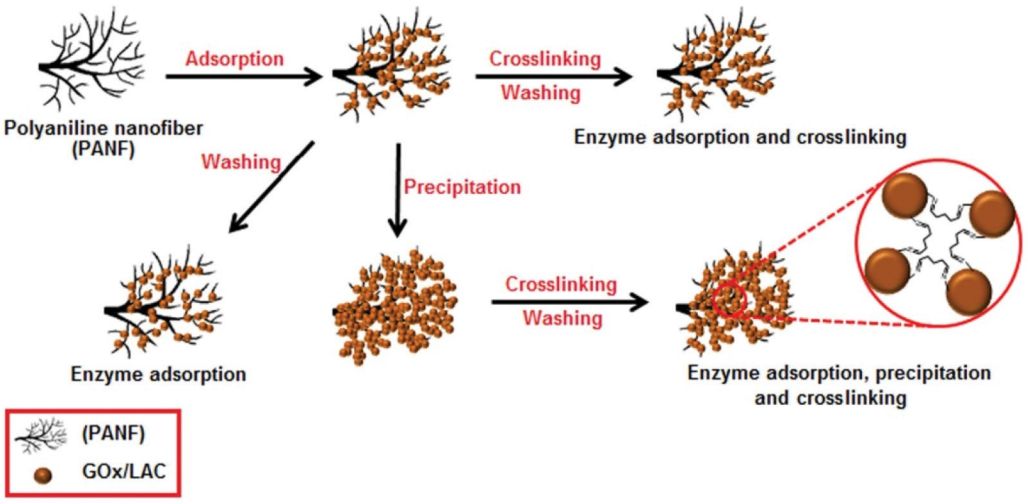

Fig. 11. Schematic illustrations of three different enzyme immobilization methods using PANFs [99]. Magnified figure of enzyme adsorption, precipitation and crosslinking represents crosslinking between enzymes molecules.

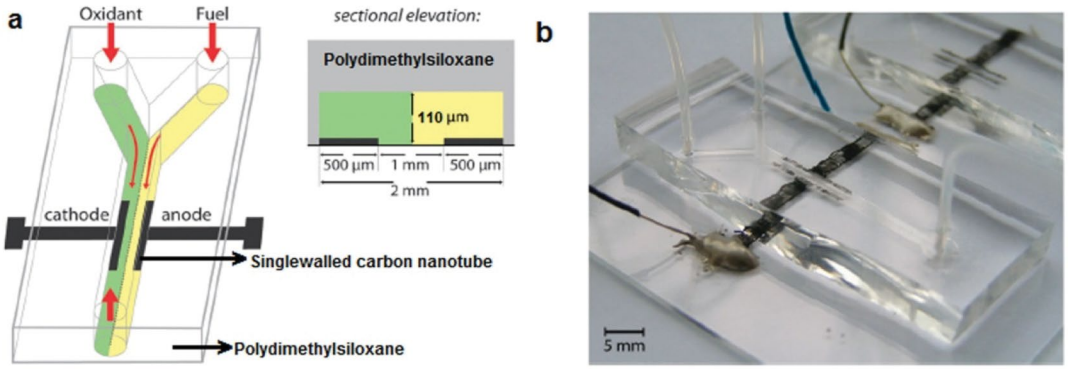

Fig. 12. (a) Scheme of the microfluidic Glucose biofuel cells. Dimensions are provided in the sectional elevation. (b) Photograph of the microfluidic Glucose biofuel cells [116].

fluid (ECF) at constant levels of 5×10^{-3} M and 45×10^{-6} M, respectively [32,67,117,118].

Since this glucose fuel cells are localized inside the body, the efficiency of the electrocatalyst under physiological conditions (around pH 7 and at 37 °C) is particularly important. Biocompatibility and in certain cases hemocompatibility of medical implanted glucose fuel cells are very challenging to prevent from any rejection or immune response from the body. Blood, interstitial fluids [119] and cerebrospinal fluid [32] are physiological fluids for the positioning of biofuel cells. In order to supply the glucose and oxygen sources for glucose fuel cells, they have to be partially in contact with the body fluids with using the selective membranes [35].

In vitro study on implantable glucose fuel cells has been performed in model solutions [32,117,120] or in human serum or blood. Several papers have been reported on *in vivo* implantation of glucose fuel cells in living creatures such as mollusks (snail and clams) [121,122], lobsters (animals that glucose is obtained from their hemolymph) [4], rats [119,123-126] and rabbits (animals that glucose is obtained from their blood vessels) [127]. It should be noted that in the case of insects, the trehalose is the anodic fuel source instead of glucose [128].

The first example of implanted glucose biofuel cell which could generate the sufficient power from a mammal's body fluids was reported by Zebda *et al.* [125]. This biofuel cell that designed based on carbon nanotube/enzyme electrodes (GOx in anode and LAC in cathode) was sealed in a biocompatible Dacron® bag and was implanted in the abdominal cavity of a rat with the wiring to the external devices organized on the head of the animal. An ultra-low power boost converter that is a kind of charge pumps was interfaced between the electrical output of biofuel cell and the electronic devices for their short-time activation (Fig. 13A). An average V_{oc} of 0.57 V and a power output of 38.7 mW were achieved from this implanted cell that represents a promising solution to several issues for electronic medical devices.

In other work, MacVittie *et al.* implanted two pairs of the biocatalytic cathodes-anodes into two different lobsters and connected in series [4]. They could generate V_{oc} up to 1.2 V from their designed biofuel cell system which was able to activate a digital watch as a simple example-model device (Fig. 13B). This glucose fuel cell included a (PQQ) GDH modified anode and LAC modified cathode immobilized on a buckypaper conductive support. The hemolanph glucose and hemocyanin (oxygen-carrying pigment in hemolymph) are the glucose and oxygen sources in this fuel cell respectively with 2:1 stoichiometric ratio at 4-10 °C.

Cinquin *et al.* have reported the implantation of glucose fuel cells in the retroperitoneal space of freely moving rats as a high-vascularized area which is used from interstitial fluids instead of blood [119]. Their designed bioelectrodes was based on composite graphite discs containing GOx and ubiquinone at the anode and polyphenol oxidase (PPO) and quinone at the cathode that could produce a stable power of more than 7.52 mW ml^{-1} and a peak power of 24.4 mW ml^{-1} (Fig. 13C) which is better than pacemaker requirements. Cinquin and *et al.* indicated that the use of PPO in cathode in contrast to LAC and BOD could efficiently operate at pH 7 and was not inhibited by the products present in physiological fluids.

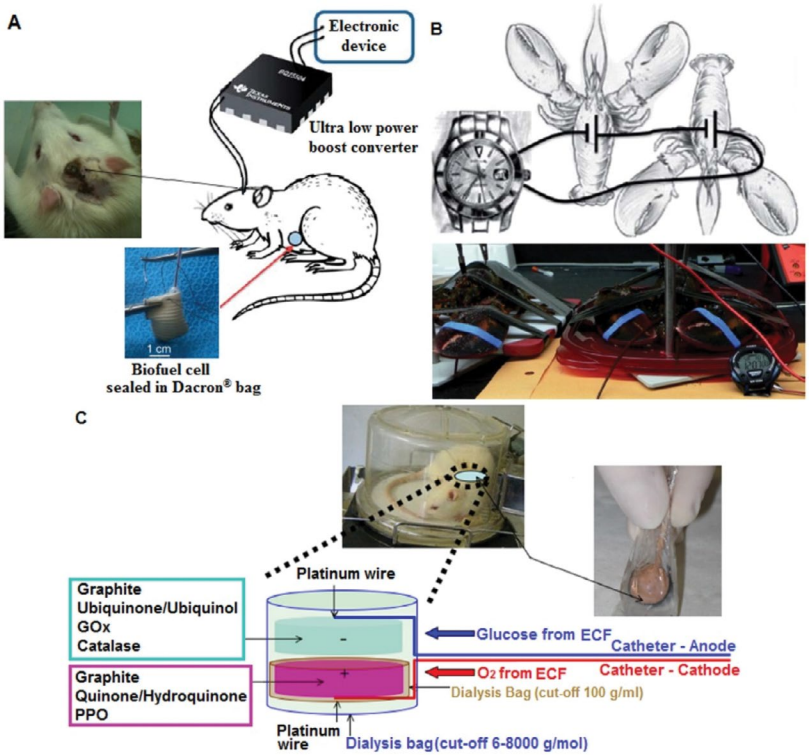

Fig. 13. (A) A biocompatible biofuel cell, implanted in the abdominal cavity of a rat [125]. (B) The biofuel cell composed of two pairs of the biocatalytic cathodes-anodes implanted in two lobsters wired in series and used for powering an electronic watch [4]. (C) The principle of an implantable quinone-ubiquinone glucose fuel cell which is inserted into the retroperitoneal space in left lateral position of a rat. The current generated from the oxidation of ubiquinol combined with the reduction of quinone. Ubiquinol and quinone are enzymatically generated by GOx and PPO, respectively [119].

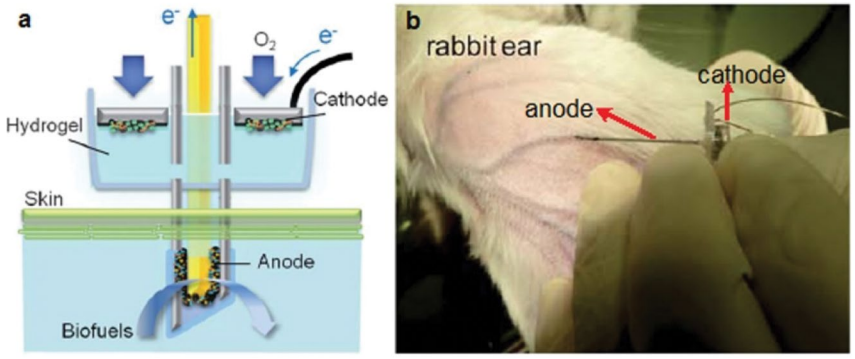

Fig. 14. (a) Schematic structure of a biofuel cell designed to utilize biochemical energy in living organisms [127]. (b) Photographs of the assembled biofuel cell for power generation from a rabbit vein [127].

Since the oxygen concentration in the natural organisms (that generally have a skin), is lower than that of biofuels like sugars, a partially implanted miniature biofuel cell was introduced by Miyake *et al.* which was consist of a gas-diffusion biocathode for utilizing the abundant oxygen in air, and a needle bioanode inserted in a vain of a rabbit ear to access its blood glucose (Fig. 14b) [127]. BOD-modified face of the carbon paper-based biocathode coated with a hydrophobic ketjenblack to control excess penetration of liquid which leads to a fourfold improvement in its performance. The anode was prepared using a SUS needle and glucose dehydrogenase. Drilling the side pores in the wall of the needle could enhance the supply of biofluids to the inner anodes. Then, the anode was coated with 2-methacryloyloxyethyl phosphorylcholine as an anti- biofouling agent in order to make it

biocompatible. An ionconducting agarose hydrogel as the inner matrix surrounds the electrodes (Fig. 14a). Miyake *et al.* claimed that such needle-based biofuel cells can be expected to serve in the future as the power unit of biodevices for environmental or healthcare monitoring.

For the first time the operation of an intravenously implanted miniaturized glucose/O_2 hybrid enzyme-Pt micro-biofuel cell with a high output power density in a living rat was introduced by Sales *et al.* [123]. An efficient enzymatic bioanode was developed based on a flexible carbon fiber microelectrode modified with neutral red redox mediator and GOx. Also, a flexible carbon fiber microelectrode modified with platinum nanoparticles was stabilized on polyamidoamine dendrimer and used as a cathode. The mechanical flexibility of carbon fiber microelectrodes facilitates the insertion of this fuel cell in a catheter (Fig. 15). Furthermore, these microelectrodes have the excellent chemical properties for efficient electron transfer and enzyme immobilization. Sales *et al.* believe that the glucose/O_2 biofuel cells based on flexible carbon fiber microelectrodes opens new avenues for the improvement of integrated electrochemical systems and presents the possibility for exploring new electrode components to maximize the efficiency of implantable devices.

Halámková *et al.* presented a very first example of an implanted membraneless glucose fuel cell composed of two enzyme modified multi-walled carbon nanotube buckypaper-based electrodes which provided direct non-mediated electron transport [121]. In this implanted biofuel cell (PQQ)GDH on the anode and LAC on the cathode were linked to the carbon nanotubes using a heterobifunctional cross-linker, 1-pyrenebutanoic acid succinimidyl ester (PBSE), which provides covalent binding with amino groups of protein lysine residues through the formation of amide bonds and interacts with carbon nanotubes *via* π-π stacking of the polyaromatic pyrenyl moieties (Fig. 16) [130]. This biofuel cell was applied as the first implanted biofuel cell which continuously operated in a well living and free-moving snail and produces electrical power over a long period of time (several months) using physiologically produced glucose as a fuel. The authors demonstrated that metabolically regenerated glucose can recharge the living battery for continuous production of electricity. So that the partial restoration of the electrical output in real time was observed upon feeding the snail, but full restoration required more time because of slow metabolic processes and slow glucose diffusion. The electrodes were inserted into the snail through two holes cut in the shell and placed into the hemolymph (a good source of glucose and oxygen) between the body wall and internal organs (Fig. 17).

Some authors extended this research to demonstrate *in vivo* operating batteries based on glucose fuel cells implanted in clams and integrated in electrical circuitries [122]. In order to increase the power produced by their implanted glucose fuel cell, they connected single biofuel cells implanted in clams in parallel and serial circuitries, using three cells in each circuitry (Fig. 18). They indicated that the living batteries generated V_{oc}, short circuitry current (I_{sc}) and P_{max} of *ca.* 800 mV, 25 mA, 5.2

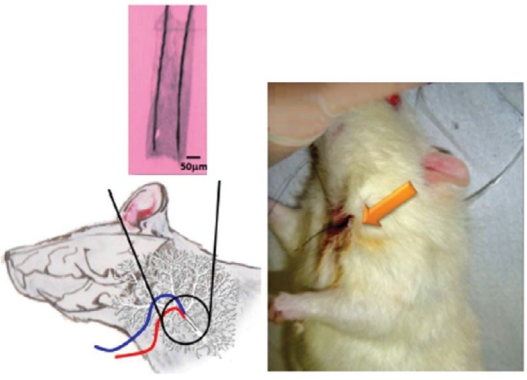

Fig. 15. An optical microscope image of the flexible carbon fiber-based biofuel cell inside the catheter which was surgically implanted in the jugular vein of a rat [123,129].

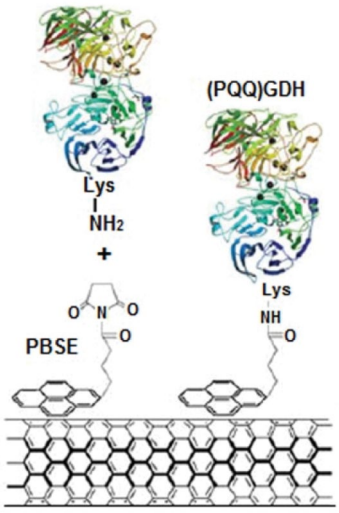

Fig. 16. Immobilization of the PQQ-GDH on carbon nanotubes with the help of the heterobifunctional linker PBSE [131].

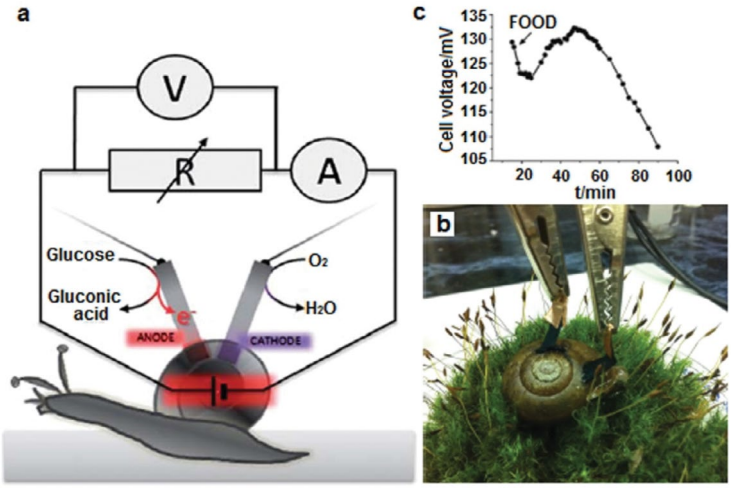

Fig. 17. (a) Circuit for the implanted biofuel cell. (b) Photograph of a snail with implanted biocatalytic electrodes connected with crocodile clips to the external circuitry. (c) Restoring the cell voltage in real time upon feeding the snail [121].

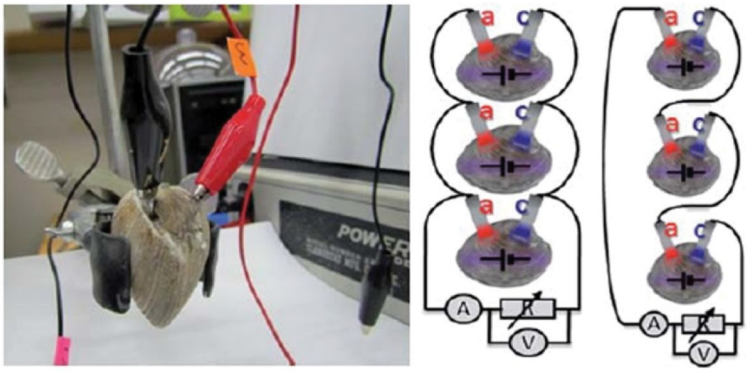

Fig. 18. Photo of a clam with implanted biocatalytic electrodes and circuitries composed of 3 clam-biofuel cells connected in parallel and in serial, respectively [122].

mW and *ca.* 360 mV, 300 mA, 37 mW for the serial and parallel connections, respectively. As expected, the voltage produced in the serial connections and current in the parallel connections show further enhancement significantly. This clam-battery was connected to a capacitor operating as a charge pump to accumulate electrical energy for activating a DC-rotary electrical motor.

In another work the same bioelectrodes were placed directly onto the surgically exposed cremaster tissue of an anesthetized rat (cremaster tissue was exposed through an incision of the scrotum) [126]. Cremaster tissue was chosen because skeletal muscle is highly irrigated and the cremaster anatomy permits its exteriorization into a flat sheet where the electrodes can be firmly placed in close contact with the muscle and its blood vessels (Fig. 19). This implantation method which is based on touching electrodes and tissue, with advantage of having larger contact surface area that generate bigger currents, might be a good alternative for the glucose fuel cells based on the electrodes implanted inside blood vessels. Castorena-Gonzalez *et al.* claimed that their implantation methodology might be complementary to the novel approach based on the epidermal tattoo-based electrodes which are located on skin [132].

CHALLENGES AHEAD OF IMPLANTED ENZYMATIC GLUCOSE BIOFUEL CELLS' DESIGNING

Despite the valuable research achievements in the implanted glucose fuel cell field, still further extensive researche to be felt to solve the remaining major problems in this field.

The Stability of Implanted Glucose-based Biofuel Cells

The stability of implanted glucose biofuel cells is a crucial parameter for their future success. In

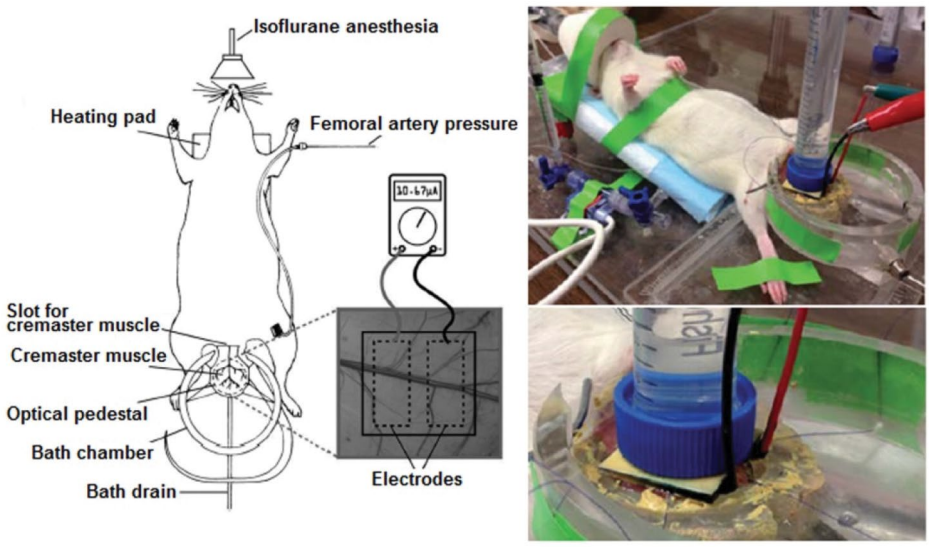

Fig. 19. Carbon nanotube-buckypaper based bioelectrodes deposited on the cremaster tissue of a rat [126].

Table 1. Comparison of the Electrical Outputs for Different Iplanted Enzymatic Biofuel Cells Operating *in vivo*[a]

Animal/location	V_{oc} (mV)	I_{sc} (μA)	P_{max} (μW)	Ref.
Rat/cremaster tissue	140	10	1.4	[126]
Rat/retroperitoneal space	270	-	2	[119]
Rat/jugular vein	220	0.5	0.0095	[123]
Rat/abdominal cavity	570	-	38.7	[125]
Rabbit/ear vein	800	1.6	0.42	[127]
Lobster/hemolymph	550	1000	160	[4]
Snail/hemolymph	530	42.5	7.45	[121]
Clam/hemolymph	350	80	10	[122]

[a]The numbers given in the table were derived from the original published values taking into account the electrode geometrical areas. For some biofuel cells the electrode areas were not reported, thus current and power cannot be derived from the published values of the current density and power density.

the case of biofuel cells used for activating the pacemaker, they could compete with the presently used batteries only if they can operate in vivo more than 10 years while at best they could be stable only for several days. It should be kept in mind that some of certain technological applications only need the short time stability of implantable biofuel cells that they are consistent with existing designed biofuel cells. Another point that should be noted is that the enzymatic glucose fuel call despite of specificity for glucose oxidation and oxygen reduction are limited by enzyme stability. When an enzymatic biofuel cell was implanted inside the body can cause an inflammatory reaction. The lifetime of the implanted enzymatic biofuel cell was reduced by the inflammatory reactions such as phagocytosis of the coated enzymes on biofuel cell electrodes. In such circumstances, after a few days of implantation fibrous tissues are formed around the electrodes and the diffusion of the biofuels was limited. The development of new biocompatible materials to protect the enzymatic biofuel cells can be effective for the advancement of this field of application [132]. Since the limited stability of biocathode over time usually affects the overall stability of the glucose biofuel cells, El Ichi *et al.* recently produced a thin film from chitosan cross-linked with genipin which was used as a biocompatible barrier on the surface of biocathodes implanted in rats [133]. They indicated that the use of a cross-linker prevents the degradation of chitosan in acidic conditions and after a long period especially *in vivo*. Their designed biocathodes (nanofibrous network of chitosan in the presence of genipin, carbon nanotubes and LAC) remained

operational after 167 days *in vivo*.

Anyway, contrary to enzymatic glucose biofuel cells, abiotic glucose fuel cells remain highly stable over long time periods. It is predictable that abiotic glucose fuel cells could one day play an important role in the field of implantable medical devises.

The Size of Implanted Glucose-based Biofuel Cells

In the implanted glucose biofuel cells, particularly the enzymatic type of them, the designed electrodes are too large to be implanted in a human body. It is because of low levels of electron transfer between the enzyme active sites and the electrode support (only about 6%) [121].

The larger electrodes, which have immobilized higher amount of enzymes on themselves, could potentially generate higher current. Heller's group has investigated different aspects of miniature biofuel cells [7]. Miniaturization of implanted glucose fuel cells could be achieved with an efficient electrical communication between the enzyme and the electrode. Among two existing ways for electron transfer between the enzyme and the electrode (DET and MET), however the DET has the great potential for miniaturization, the MET approach generally can generate higher output power than the DET. Therefore the choice of a suitable mediator can create an efficient electron transfer between the enzyme and the electrode and consequently miniaturize the glucose biofuel cells.

The Voltage Produced by Implanted Glucose-based Biofuel Cells

Three approaches have been investigated by researchers in facing with the challenge of low voltage produced by the glucose biofuel cells: (i) assembling glucose biofuel cells in series electrically, thus increasing the total output voltage [4,122,134-138], (ii) using a charge pump to storage the produced electrical energy for the burst release in short pulses or using from a DC-DC converter [62,125,139-143], and (iii) developing of nanostructured three-dimensional electrodes which improved the number of wired enzymes per surface or volume unit [134]. The voltage, current and power outputs obtained from some of *in vivo* implanted biofuel cells are summarized in Table 1 [131].

CONCLUSIONS

Glucose-based biofuel cells with employing enzymes or abiotic electrocatalysts harvest the energy from the biological sources. They can become a desirable power source for the self-powered micro-electro-mechanical systems implants. This review summarizes the recent studies on "implantable" and "implanted" types of abiotic and enzymatic glucose biofuel cells and gives comprehensive information about their structural characteristics and applications. Further improvements in terms of power output and life time of glucose-based biofuel cells can convert them into viable alternatives to lithium-ion batteries. It is expected that the nanotechnology is vital to achieve this aim.

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