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Wearable biofuel cells based on the classification of enzyme for high power outputs and lifetimes

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ABSTRACT

Wearable enzymatic biofuel cells would be the most prospective fuel cells for wearable devices because of their low cost, compactness and flexibility. As the high specificity and catalytic properties of enzymes, enzymatic biofuel cells (EBFCs) catalyze the fuel associated with the redox reaction and get electrical energy. Available biofuels such as glucose, lactate and pyruvate can be harvested from biofluids of sweat, tears and blood, which afford cells a favorable use in implantable and wearable devices. However, the development of wearable enzymatic biofuel cells requires significant improvements on the power density and enzymes lifetime. In this paper, some new advances in improving the performance of wearable enzymatic biofuel cells are reviewed based on the bioanode and biocathode by classifying single-enzyme and multi-enzyme catalysis system. Thereinto, the bioanode usually contains oxidases and dehydrogenases as catalyst, and the biocathode utilizes the catalysis of multi-copper oxidases (MCOs) in the single system. For further enhancing the power density, efforts to develop multi-enzyme catalysis strategies are discussed in bioanode and biocathode respectively. Moreover, some potential technologies in recent years, such as carbon nanodots, CNT sponges and mixed operational/storage electrode are summarized owing to notable efficiency and the capability of enhancing electron transfer on the electrode. Finally, major challenges and future prospects are discussed for the high power output, stable and practical wearable enzymatic biofuel cells.

1. Introduction

Recently, various wearable electronic devices, such as smart watches, fitness bands and wearable ECG detectors, have brought great convenience to people. Nevertheless, the choice of suitable power source for wearable devices is a major challenge that cuts across almost wearable electronic applications because of their miniaturization and biocompatibility. Researchers are working on developing intelligent adaptive algorithms, low-power consuming and body-compliant power sources for reducing energy shortage of wearable electronic devices (Bandodkar and Wang, 2016). Thereinto, biofuel cells are considered as a potential power source. Biofuel cells provide a mode of electrical energy generation, which is based on the transformation of chemical energy of biofuels from living organism directly into electricity via redox reaction (Holzinger et al., 2012). Their possible types include that using enzymes rather than precious metals as the catalyst to oxidize biofuels. Glucose, lactate and pyruvate are the common biofuels for wearable devices, as they can be harvested from biofluids of sweat, tears or blood. (Bandodkar et al., 2016, 2017). Microbial biofuel cells and enzymatic biofuel cells (EBFCs) have been widely explored in recent years. However, some limiting factors of microbial fuel cells render them unattractive for wearable application. For example, the cytotoxicity of microbes poses health concerns during potential wearable applications (Bandodkar, 2017). By contrast, EBFCs, the bioelectronic devices which can convert the chemical energy of biofuels into electrical energy with single-enzyme or multi-enzyme catalysis, are capable to overcome these issues. Hence, EBFCs would be the most popular biofuel cells with continuous and uninterrupted electrical energy (Kim et al., 2017).

When enzymes are immobilized onto the surface of electrodes, the common approaches for the electric wiring of active site to the electrode can be categorized as mediated electron transfer (MET) and direct electron transfer (DET). As the active site of enzyme contains coenzyme molecule which is tightly bound and buried inside the center of enzyme, the distance between the active site of enzyme and the surface of electrode is too far for an efficient electron transfer (Cosnier et al.,

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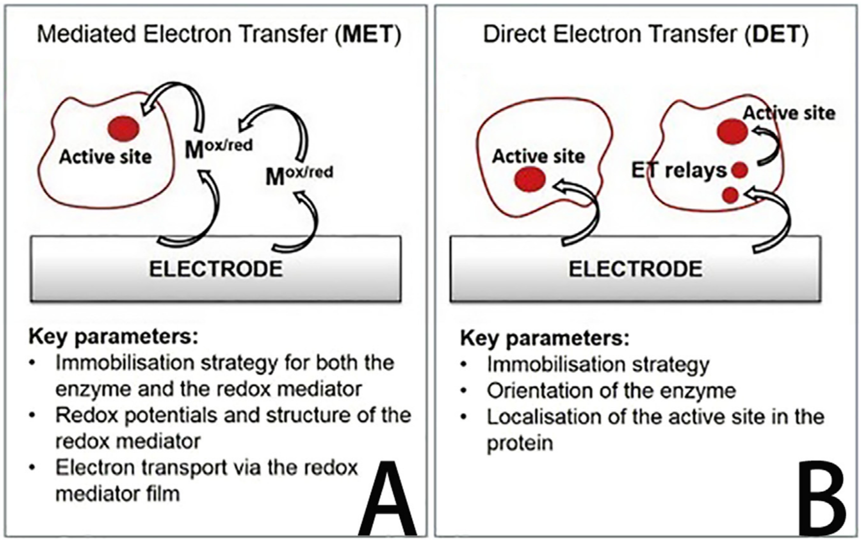

Fig. 1. Principle and key parameters of electron transfer pathways for an immobilized enzyme (A) indirect or mediated electron transfer (MET) via a small redox mediator (Mox/red). (B) Direct electron transfer (DET). (Cosnier et al., 2016).

2016; Suroviec, 2017). The unfavorable condition requires the use of mediators (some small molecules having the appropriate redox potential and activity) that act as electron shuttles to facilitate electron transfer. So this approach is named as MET (Fig. 1A). Although mediators can help in increasing the current density and the kinetics of electron transfer, resorting to them would also increase the overpotential at the electrodes and thus decrease the voltage of the EBFCs (Zebda et al., 2011). What's more, it is complicated to synthesize and immobilize the mediators. To solve these shortcomings, researchers could look into completely obviating the need for mediators by developing biofuel cells that rely on DET (Fig. 1B) (Bandodkar, 2017; Ivnitski et al., 2006; Zeng et al., 2015). This has been possible thanks to the advances in nanomaterial, especially the wide application of carbon nanotubes (CNTs). CNTs become prominent material for the application of bioelectronic devices owing to their biocompatibility, particular structure, and their exceptional electronic and mechanical properties (Gross et al., 2016; Holzinger et al., 2012). In addition, CNTs have shown to be appropriate to establish electronic communication with redox enzymes since the thin diameter allows them to approach the redox active sites and provide efficient electrical nano-pathway for DET.

Although skin-worn biofuel cells (Bandodkar et al., 2017; Jia et al., 2013a, 2013b), ocular biofuel cells (Falk et al., 2013; Reid et al., 2016, 2015) and some other wearable biofuel cells have been reported, there are still many challenges before they meet the practical demands. For example, the conformability and mechanical resiliency are indispensable for wearable devices, and the stable immobilization of enzymes and mediators is also an important cause limiting the development of wearable enzymatic biofuel cells. Aiming at these challenges, this review will detail some recent progresses for wearable enzymatic biofuel cells based on the classification of enzyme for high power outputs and long lifetimes, including the reaction mechanisms, sensitive materials, and fabrication methods of biofuel cells.

2. The development status and trend of bioanode

Redox processes involved in enzymatic biofuel cells are generally catalyzed by enzymes in their purified form or within an organism. In the bioanode, available enzymes often have high specificity towards fuels (glucose, lactate etc.) for achieving oxidation reaction and release electrons. Recently, oxidases and dehydrogenases are widely used in the single-enzyme catalysis system. And the combination of glucose oxidase (GOx) with catalase or horseradish peroxidase (HRP) is always applied for the multi-enzyme system to remove H_2O_2 and speed catalysis.

2.1. Single-enzyme catalysis for bioanode

2.1.1. Oxidase catalysis

It is commonly recognized that glucose and lactate are the substrates widespread in biofluids, and GOx and lactate oxidase (LOx) have the properties of high specificity, activity and stability towards glucose and lactate. Therefore, GOx and LOx are commonly used for wearable enzymatic biofuel cells. For glucose oxidation reaction in the presence of GOx, glucose is oxidized to gluconolactone. At the same time, two electrons are transferred from glucose to the redox center of GOx, with the cofactor-flavin adenine dinucleotide (FAD), is reduced to $FADH_2$ (Eq. (1)). Subsequently, the generated $FADH_2$ can return back FAD through an oxidation reaction. Re-oxidation reactions of $FADH_2$ can result from electron transfer to the O_2 dissolved in the solution (via Eq. (2)) or to the electrode if the electrons are able to jump from the GOx active site to the electrode surface (via Eq. (3)) (Gao et al., 2010; Heller, 1990). Thereinto, Eq. (2) shows the oxidation of $FADH_2$ to FAD in the presence of O_2, and Eq. (3) shows the electrons transfer between the active site of GOx and the electrode. In addition, H_2O_2 can also be electrochemically oxidized to O_2 in the system through a two-electron process presented by Eq. (4) (Garcia-Perez et al., 2016; Wang and Li, 2011):

$$FAD + Glucose \rightarrow FADH_2 + Gluconolactone \tag{1}$$

$$FADH_2 + O_2 \rightarrow FAD + H_2O_2 \tag{2}$$

$$FADH_2 \leftrightarrow FAD + 2H^+ + 2e^- \tag{3}$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \tag{4}$$

Furthermore, the electron transfer would be achieved with the help of mediators if the distance between the active site of enzyme and the surface of electrode is longer than the tunneling distance ($\approx$1.5 nm)(De Poulpiquet et al., 2014). Similarly, the oxidation of lactate catalyzed by LOx could be presented in the same way.

2.1.1.1. The development of DET for oxidase catalysis.

Obviously, DET is becoming a trend for wearable enzymatic biofuel cells owing to its easy fabrication and optimal cell voltage, and the development of nanomaterials has made it more convenient to achieve DET.

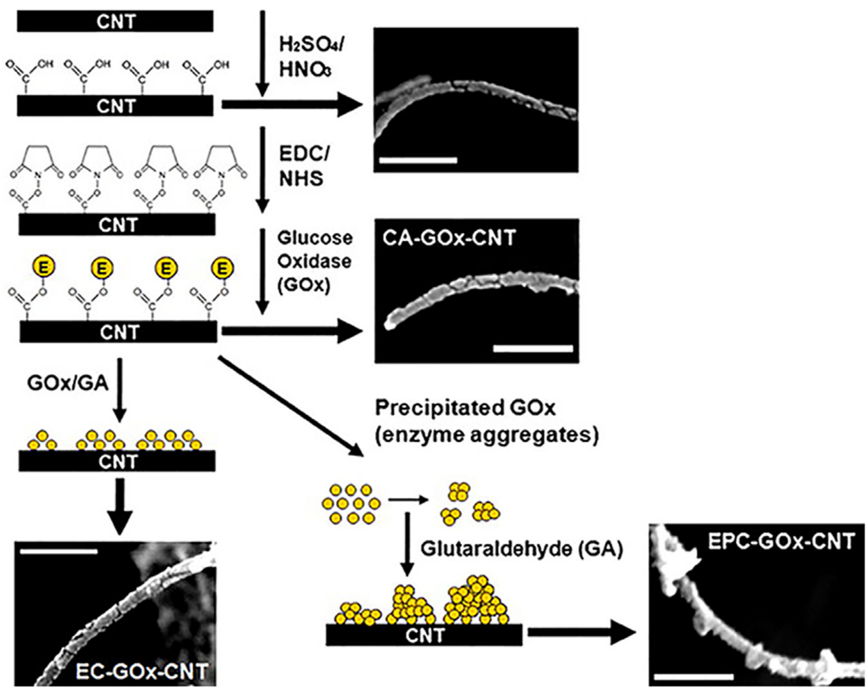

Fig. 2. Schematic illustration of three different approaches to GOx immobilization on CNTs. GOx molecules were covalently attached to carboxylated CNTs via a linker of EDC/NHS (CA-GOx). The EPC-GOx sample was prepared using the GA treatment after GOx molecules were precipitated in the vicinity of CA-GOx-CNTs. The SEM images of CA-GOx-CNTs, EC-GOx-CNTs, and EPC-GOx-CNTs are shown at each step, and the scale bar in each SEM image denotes 200 nm (Kim et al., 2011).

Meanwhile, the amount of immobilized enzymes is still the key advance for DET to improve the performance of EBFCs. The conventional avenues to enzyme immobilization on electrodes are physical adsorption and covalent attachment (CA). However, these strategies unfortunately pose some disadvantages, such as the instability of physical adsorption and denature or inactivity of enzymes during covalent attachment (Lai et al., 2016). For these problems, one of the brilliant successes in enzyme immobilization is the enzyme coating (EC) approach, which is based on the chemical cross-linking of highly concentrated enzyme molecules on nanostructured materials. Based on enzyme coating approach, Kim et al. (2011) reported a highly stable GOx coating on CNTs by enzymes precipitate coatings (EPCs). In their experiment, enzymes were precipitated in the presence of salting-out ammonium sulfate, and the GA solution was added after 30 min of enzyme precipitation (Fig. 2). As the comparison, the covalent attachment was achieved by the condensation reaction between the amino group of GOx and the carboxyl group of acid-treated CNTs. Thereinto, the mixture of N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS) were used as the linker (Jiang et al., 2003). Combining with air-breathing Pt cathodes, three EBFCs with different bioanodes (fabricated by CA, EC and EPCs respectively) were compared. The result (maximum power density about 900 μW/cm^2, electron transfer rate constant of 0.2/s) demonstrated the approach of EPCs was effective in improving both the sensitivity and the thermal stability of enzyme electrodes. Furthermore, the power density and lifetime of biofuel cells were also improved as GOx precipitate coatings were highly stable and improved the enzyme loading (Kim et al., 2011, 2017). Additionally, Garcia-Perez et al. (2016) encapsulated the cross-linked GOx aggregates with the highly conductive mesoporous carbons to form the GOx-nanocomposite. Considering that graphitized mesoporous carbons (GMCs) have strong aggregation tendency, they trapped the cross-linked GOx aggregates into the three-dimensional mesoporous carbon network. GA was used to act as the chemical cross-linker, and larger GOx aggregates were formed with the increasing of GA concentration. The highest power density (22.4 μW/cm^2 at 0.24 V) was obtained with the 0.13% GA treated sample at the enzyme anode, which was 4.5 times higher than the power density obtained from GOx aggregates alone. Thus, the conclusion could be gotten that the GMCs offer a high carbon surface area in a close proximity of the GOx and have the ability to enhance electron transfer rate.

2.1.1.2. The application of oxidase catalysis based on MET. Although DET is becoming a trend for most scientists lately, there are still a number of researchers who develop EBFCs with MET. This is primarily because the electrical wiring of enzymes via MET is quantitative, and MET frequently leads to higher current density than can be achieved by DET (Cosnier et al., 2016; Rasmussen et al., 2016). Jia et al. (2013b) reported a kind of epidermal biofuel cells in the form of tattoo. The biofuel cells were structured on an easy-touse temporary tattoo platform and generated electrical energy by the lactate in human perspiration. Tetrathiafulvalene (TTF) was used as the mediator between CNTs and LOx, as its relatively low toxicity and low oxidation potential comparing with ferrocene derivatives and meldola blue (MDB). Thereinto, the property of low oxidation potential is essential for achieving high open circuit potential (OCP) of biofuel cells. Besides, a biocompatible chitosan (Chit) overlayer was used to protect the biocatalytic structure (Fig. 3A). The redox reactions that occur within the tBFCs was shown in Fig. 3B. Combining with the platinum black cathode, the real power from the tattoo cells varied between 5 and 70 μW/cm^2. The concrete results depend on the fitness level of individuals and the concentration of lactate in there perspiration. Furthermore, the same group developed textile biofuel cells using Tetrathiafulvalene 7,7,8,8-tetracyanoquinodimethane (TTF-TCNQ) as the mediator to improve the output levels and comfort level of wearable enzymatic biofuel cells (Jia et al., 2014). Similarly, TTF-TCNQ has been widely applied in the field of biosensor owing to its low toxicity and low redox potential (Kulys et al., 1992; Pauliukaite et al., 2007). The textile BFCs with LOx achieved larger power density mediated by TTF-TCNQ. And the maximum power density was up to 100 μW/cm^2 along with an OCP of 0.36 V.

Actually, TTF-TCNQ are not only used as the mediators, but also as catalysts for some specific biofuels. For example, Falk et al. (2013) chose TTF-TCNQ as the catalyst for ascorbate to build stable miniature membrane-less EBFCs. Specially, the EBFCs were based on contact lenses for harvesting energy from human lachrymal liquid. Ascorbate is the new fuel in human lachrymal liquid as it has not been exploited previously in EBFCs, and it can be explored as the power source for the

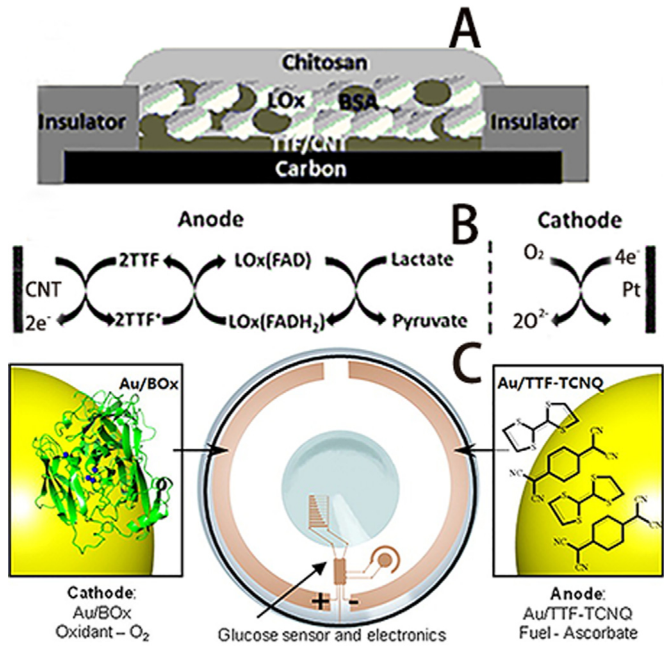

Fig. 3. (A)Illustration of the epidermal tBFC and the constituents of the anode (Jia et al., 2013b); (B) Redox reactions that occur within the tBFC (Jia et al., 2013b). (C)Conceptual scheme of a bionic contact lenses (Falk et al., 2013).

glucose sensing devices on contact lenses. In addition, three-dimensional nanostructured gold electrode was selected as the electrode substrate (Fig. 3C). When operated in human tears, the biofuel cells with bilirubin oxidase (BOx) as cathodic catalyst exhibited the following characteristics: an OCP of 0.54 V, a maximum power density of 3.1 μW/cm^2 at 0.25 V and 0.72 μW/cm^2 at 0.4 V, and a stable current density output of over 0.55 μA/cm^2 at 0.4 V for 6 h of continuous operation. Following this, another research about contact lenses biofuel cells was shown by Reid et al. (2016). Different with design of Falk, Reid used LOx to catalyze lactate in human tears and immobilized LOx into a dimethylferrocene-modified linear polyethyleneimine ($FcMe_2$-LPEI) redox polymer. Meanwhile, BOx and anthracene-pyrene modified CNTs were used in the biocathode. The EBFCs had a maximum current density of 22 ± 4 μA/cm^2 and a maximum power density of 2.4 ± 0.9 μW/cm^2 at 0.163 V with an OCP of 0.44 ± 0.08 V.

Moreover, Ramanavicius focused on the application of phenanthroline derivatives (PnDs) as the mediator for EBFCs (Ramanavicius et al., 2015). PnDs are rigid planar, hydrophobic, electron-poor heteroatomic systems, in which nitrogen atoms are well placed to act cooperatively in the binding of metal ions (Bencini and Lippolis, 2010). In addition to the unique mediator, the group selected GOx from Penicillium funiculosum 46.1 as catalyst for the anode, which has not been investigated in other researches related to the development of EBFCs. The whole EBFCs, with HRP as cathodic biocatalyst, showed an OCP about 640 mV, and calculated maximum power density was 4.2 μW/cm^2 at the voltage of 530 mV.

For mediators, a significant number of compounds based on organic metal complexes, quinonic compounds or redox polymers have been explored with proper redox mediating properties (Agnès et al., 2013; Christwardana et al., 2017; Fan et al., 2015; Lang et al., 2014; Miyake et al., 2011a; Xia et al., 2013; Yu et al., 2010; Zebda et al., 2013). For example, osmium complex (Gao et al., 2010; Liu et al., 2011, 2015), ferrocyanide or ferrocene (Palmore and Kim, 1999), ferritina (Inamuddin and Naushad, 2016) and 2.2′-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) (Zebda et al., 2009) have been commonly used as mediators in the application of biosensor, biofuel cells and so on. Besides, with the advantages of low solubility in water and low redox potential, naphthoquinone(NQ) was also integrated in the multi-walled carbon nanotubes (MWCNTs) matrix to increase the density of wired enzymes per volume unit by Reuillard (Reuillard et al., 2013). Actually, the power density of EBFCs with the help of mediators sometimes can be higher than that achieved by DET. This phenomenon is mainly caused by the possibility to obtain DET is strongly related to the location of the active site inside the protein, the conformational morphology of the protein, and the ability of the electrode to access the redox center. These mediators, however, are not all meet the requirements of wearable enzymatic biofuel cells, which strongly depend on their solubility, redox potential and some other specific indicators. Some mediators are toxic, hazardous and polluting to their immediate environment, as a result they are unsuitable for implanted and wearable devices. In addition, some mediators are environment sensitive gradually decompose thus leading to poor biofuel performance (Bandodkar, 2017). Hence, the type of DET will be still the trend and population with the development of new advances in the next decades.

2.1.2. Dehydrogenase catalysis

Although the application of oxidases has attracted lots of attention from scientists and researchers, there are some obvious drawbacks for wearable enzymatic biofuel cells. The main disadvantage of oxidases is their sensitivity to O_2 that would decrease the lifetime and efficiency of EBFCs (Bao et al., 2003; Karaśkiewicz et al., 2012; Scodeller et al., 2010). Moreover, it can be obtained that H_2O_2 would be produced in the reaction process from the Eq. (2) during the oxidase catalysis. The presence of H_2O_2 would limit catalytic activity of oxidases under physiological conditions (Bon et al., 2011; Fujita et al., 2011; Miyake et al., 2009, 2011b; Saleh et al., 2011; Tanne et al., 2010; Wen et al., 2010; Yehezkeli et al., 2011). It is not only because of the high toxicity of H_2O_2, but also its excessive coverage of the oxidase active sites (Christwardana et al., 2017).

According to these limitations, some scientists have made their effort on the study about the dehydrogenases. Glucose dehydrogenase (GDH) catalyzes glucose to D-glucono-δ-lactone selectively and is insensitive to dioxygen. At the same time, the application of GDH can prevent the production of H_2O_2. Pyrroloquinoline quinine (PQQ)-dependent glucose dehydrogenase (PQQ-GDH) and β-nicotinamide adenine dinucleotide (NAD)-dependent GDH are the two types of GDH that have been commonly applied (Chen et al., 2013). Yu et al. (2016) reported a kind of oxygen-independence EBFCs using GDH as the anodic catalyst. In their study, MDB was chosen as redox mediator and combined with the MWCNTs via π–π interaction in order to acquire the lower catalytic potential. In addition, they introduced a solid-state silver oxide/silver (Ag_2O/Ag) cathode to completely achieve the goal of molecular oxygen independence. The hybrid EBFCs showed a maximum current density of 4.1 mA/cm^2 at 0.01 V and a maximum power output of 0.281 mW/cm^2. Specially, as the presence of Ag_2O/Ag, the EBFCs could operate under anaerobic condition, while the maximum power output could reach to 0.275 mW/cm^2 at 0.34 V. Another notable example about the application of GDH was reported by Giroud et al. (2015). They used the NQ-modified pyrene (pyr-$(NQ)_2$) to mediate electrons with PQQ-GDH catalyzing glucose. Similarly, the pyr-$(NQ)_2$ was absorbed on the MWCNTs via π-π interactions like the experiment of Yu. In the presence of 50 μM free PQQ, anode catalytic current densities were as high as −124.5 ± 0.4 and −166.2 ± 2.7 μA/cm^2 at 0 and 0.3 V, respectively. Under the same conditions, electrodes without pyrene functionalizations generated only the current densities of −15.1 ± 6.2 and −34.6 ± 10.3 μA/cm^2 at 0 and 0.3 V, respectively. It is obvious that there is a large increase in glucose oxidative current densities after modifying by pyr-$(NQ)_2$. Following this, the same group introduced another application of NAD-dependent GDH with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Giroud et al., 2017). They functionalized the MWCNTs electrode by bifunctional itroaromatic molecule, and the functional nitroaromatic molecule could be linked by π-π interactions on MWCNTs. DTNB has the particularity of possessing both electroactive nitro groups and negatively charged carboxylic groups, and can be used as the biocatalyst for nicotinamide adenine dinucleotide hydrate (NAD^+) regeneration. The activated GDH/DTNB-Pyr/MWCNTs bioanode showed great activity towards glucose oxidation with high electrocatalytic current density of 2.03 mA/cm^2 at 0.1 V in quiescent solution.

We mentioned that Falk et al. (2013) designed the EBFCs based on the contact lenses to generate electrical energy by catalyzing the ascorbate in human lachrymal liquid. Before that, the same group ever designed another membrane-less and cofactor-free EBFCs based on contact lenses (Falk et al., 2012). Interestingly, they used corynascus thermophilus cellobiose dehydrogenase (*Ct*CDH) in the bioanode to catalyze glucose. Based on three-dimensional nanostructured gold electrodes, the performance of 0.57 V OCP, about 1 $\mu W/cm^2$ maximum power density at a cell voltage of 0.5 V, and more than 20 h operational half-life were observed.

2.2. Multi-enzyme catalysis for improving bioanode efficiency

2.2.1. Enzyme cascade

The current and power output levels of EBFCs have been greatly improved by loading more enzymes, enhancing electron transfer rate etc. However, only a small part energy of the biofuel can be released by the oxidation reaction, and most of the energy is left in the oxidized product. For example, glucose can be only oxidized at C1 position to its corresponding lactone with only two electrons lost by the glucose. Additionally, the oxidation of lactate just allows for 2 of the total 12 electrons to be harnessed. Thereby, Shao and coworkers tried to enhance the current density and coulombic efficiency by enzyme cascade reaction (Shao et al., 2013). They developed a bioanode by the co-immobilization of pyranose dehydrogenase from Agaricus meleagris (*Am*PDH) and the dehydrogenase domain of cellobiose dehydrogenase from Corynascus thermophiles (*rec*DH*Ct*CDH). The two enzymes were entrapped into Os-complex modified electrodeposition polymers (Os-EDPs). Since *Am*PDH oxidizes glucose at both the C2 and C3 positions whereas *rec*DH*Ct*CDH oxidizes glucose at the C1 position, electrochemical measurements reveal that maximally 6 electrons can be harvested from one glucose molecule at the bi-enzyme anode via a cascade reaction. And the bioanode showed an increasing maximum current density about 100 $\mu A/cm^2$. Furthermore, Sokic-Lazic et al. (2011) described an enzyme cascade for complete oxidation of lactate. More importantly, they employed the Kreb's cycle biomimic in the lactate/air biofuel cells. Kreb's cycle is the main metabolic process responsible for metabolism of complex fuels in living cell. And Kreb's cycle enzymes exist in the mitochondria of a living cell and they are responsible for the complete oxidation of pyruvate (Sokic-Lazic and Minteer, 2009). Enzymes employed among them included: pyruvate dehydrogenase (PDH), citrate synthase (CS), aconitase (Aconitase), isocitric dehydrogenase (IDH), α-ketoglutarate dehydrogenase (KDH), succinyl CoA synthetase (Biocatalytics), fumarase, and malate dehydrogenase (MDH) (Fig. 4). Combining with the gas diffusion biocathode that contains 20 wt% Pt on carbon, the completed lactate/air biofuel cells gave a maximum power density of 800 ± 110 $\mu W/cm^2$ and a maximum current density 2700 ± 1200 $\mu A/cm^2$.

2.2.2. Bi-enzyme catalysis exploited in EBFC anodes

Although researchers have noticed the fact that GDH can prevent producing H_2O_2, the GDH is not extensively applied in catalyzing redox reaction. The PQQ-GDH need complex condition of solubilization and purification, and water-soluble PQQ-GDH has low selectivity and poor thermal stability (Chen et al., 2013; Zafar et al., 2012). In addition, for NAD-dependent GDH, the need for the addition of the soluble NAD cofactor complicates the catalysis to some extent. What's more, the electrochemistry of both the oxidized form (NAD^+) and the reduced form (NADH) of NAD cofactor suffer from their irreversible characteristic. And the direct oxidation of NADH at unmodified electrodes requires a high overpotential owing to its sluggish electron transfer rates (Chen et al., 2013; Saleh et al., 2010; Wilson and Turner, 1992; Yamazaki et al., 2000). Thus, some researchers began to explore bi-enzyme catalysis system to overcome the hazards of H_2O_2 and further enhance the performance of EBFCs. Reuillard designed a bi-enzyme system combining GOx and catalase as biocatalysts, for decomposing H_2O_2 and removing the O_2 at the bioanode (Reuillard et al., 2013). They selected NQ as the mediator and integrated NQ in 3D MWCNTs matrix to increase the density of wired enzymes per volume unit. The EBFCs with laccase as the cathodic catalyst, exhibited high current density of 4.47 mA/cm^2 and maximum power output of 1.54 mW/cm^2, 1.92 mW/mL and 2.67 mW/g. Using the same GOx and catalase, Christwardana et al. (2017) formed another bi-enzyme bioanode using polyethylenimine (PEI) as a bridge linking the co-immobilization enzymes and CNTs. Under the neutral condition, the PEI is positive polarity, and the acid-treated CNTs, GOx and catalase are all negative polarity. So they can be tightly connected by electrostatic attraction (Fig. 5). The combination was just like a layer-by-layer structure to promote chemical bonding and increase the amount of the immobilized enzymes (Liu et al., 2016). Adopting (GOx-Cat)/PEI/CNTs and Lac/PEI/CNTs as bioanode and biocathode respectively, maximum power density of the membraneless EBFCs was 180.8 ± 22.3 $\mu W/cm^2$.

Apart from catalase, HRP is also a good choice to decompose H_2O_2. And the bi-enzyme bioanode modified by GOx and HRP also showed great performance in the application of EBFCs. Chung et al. (2018) ever designed bioanodes mixing GOx and HRP with different mass ratio. Specially, they chose terephthalaldehyde (TPA) as cross-linker to build the [(TPA/HRP/GOx)]/PEI/CNTs structure. Here, the composite was cross-linked by C=N bonds through the aldol condensation reaction of free amine groups on the enzyme surface and aldehyde groups of TPA. And the 2:5 ratio of HRP and GOx was determined as the best catalyst after comparing the performances of EBFCs adopting four various catalysts. In the study, Pt-C sprayed on proton exchange membrane was used as biocathode. Eventually, the results showed high sensitivity, high electron transfer rates and a maximum power density of 2.0 ± 0.1 mW/cm^2.

3. Recent progress of biocathode configuration

Biofuel cells usually operate with two catalytic electrodes where the bioanode oxidizes the fuel and the biocathode catalyzes in most cases the reduction of O_2. Accordingly, paying attention to the modification of biocathode is also a crucial way to improve the performance of EBFCs. In the biocathode, the formal potential of the H_2O/O_2 redox couple is relatively high and O_2 is abundant in the air, so the O_2 is the most desirable oxidant (So et al., 2017). The cathode reaction often can be achieved by either relying on conventional oxygen-reducing noble metal, e.g., Pt, Pd or Ru, or utilizing the multi-copper oxidases (MCOs) like laccase and bilirubin oxidase (BOx). Although noble metal-based cathodes provide suitable current density, they are costly, susceptible to poisoning and offer low OCP, which are unsuitable for wearable enzymatic biofuel cells. On the contrast, enzyme-based biocathodes are much cheaper than noble metal, offer higher OCP and produce fewer byproducts as enzyme specificity (Bandodkar and Wang, 2016). At the present time, the selection of suitable enzymes and the arrangement of the electrode structures are the main considerations to be valued for improving the performance of biocathodes (So et al., 2017).

3.1. Catalysis based on multi-copper oxidases

Most oxygen-reducing biocathodes utilize enzymes from MCOs family, which are well known to perform efficient reduction of O_2 to H_2O. These enzymes contain four copper centers. A mononuclear copper center (T1) is on the enzyme surface and responsible for the electron transfer between the substrates. Also, there is a type2 (T2)/type3 (T3) trinuclear copper cluster at depth of enzyme where O_2 is reduced to

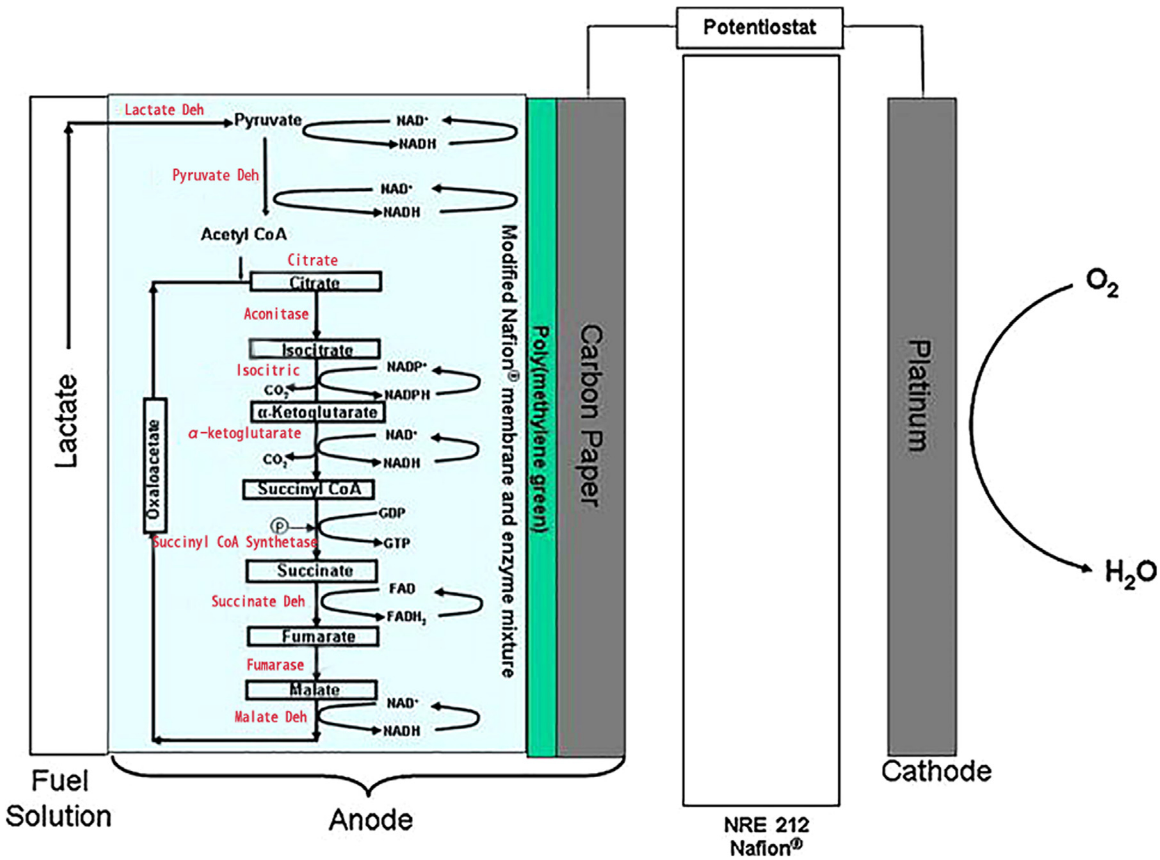

Fig. 4. Schematic of the chemistry occurring in the lactate/air biofuel cell with enzyme cascade (Sokic-Lazic et al., 2011).

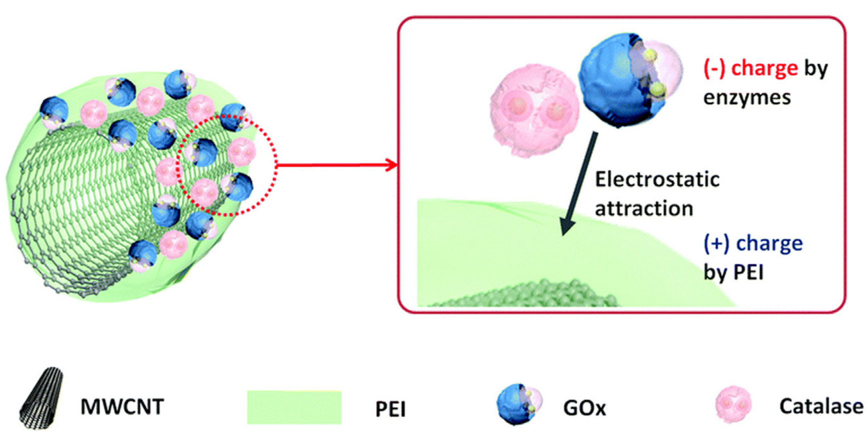

Fig. 5. Schematic showing the synthetic procedure for the CNTs/PEI/(GOx-Cat) catalyst (Christwardana et al., 2017).

water (Cosnier et al., 2016; Jamshidinia et al., 2017). In the family of multi-copper oxidases, laccase and BOx are most commonly used for EBFCs.

3.1.1. Multi-copper oxidases orientation

Considering the T1 center located near the surface of MCOs and the relationship between four copper centers of MCOs and catalytic reaction, recent developments in the fabrication of MCO-catalysis biocathodes mainly focus on the controlled orientation of these enzymes on electrodes. Strategies have been employed to favor the orientation of enzymes and shorten the distance between the electrode and T1 center (Cosnier et al., 2016), as orientating the T1 copper centers to face the CNTs sidewalls can accelerate electron transfer. The common implementation methods encompass the use of aromatic, hydrophobic or hydrophilic molecules (Giroud et al., 2017). Taking laccase for example, laccase exhibits affinity towards appropriately sized aromatic moieties by virtue of a hydrophobic pocket around the T1 center (Sosna et al., 2010). These aromatic moieties would orient laccase on the electrode when attached to the electrode surface, and lead to a stable and catalytically efficient biocathode (Blanford et al., 2007; Cracknell et al., 2011; Karaśkiewicz et al., 2012; Stolarczyk et al., 2012).

Excepting the role of mediator (Giroud et al., 2015; Reuillard et al., 2013), naphthalene and its derivatives have the ability to orient MCOs toward the conductive surface of the biocathodes. A notable example is that the NQ-modified pyrene (pyr-$(NQ)_2$) can not only be a mediator in the bioanode, but can be responsible for orienting laccase and BOx in the biocathode (Giroud et al., 2015). The maximum current density of laccase/pyr-$(NQ)_2$/MWCNTs and BOx/pyr-$(NQ)_2$/MWCNTs biocathodes can reach 189.5 ± 22.4 μA/cm^2 and 277.4 ± 52.6 μA/cm^2 respectively. Besides, Karaśkiewicz et al. (2012) applied laccase bioconjugates and naphthylated CNTs to achieve the proper orientation of laccase and extend electron conducting channel between enzymes and electrodes. And the SWCNTs were connected with naphthalene by covalently bound. Combining naphthalene functionalized biocathode with GDH modified bioanode, the EBFCs demonstrated an OCP of 0.52 ± 0.02 V and a maximum power density of 131 ± 4 μW/cm^2.

Likewise, DTNB was also used in both bioanode and biocathode. Because DTNB has the particularity of possessing both electroactive nitro groups and negatively charged carboxylic groups, it can be favor of enzyme immobilization, enzyme orientation and electrocatalytic activity. Integrating with the GDH/DTNB-Pyr/MWCNTs bioanodes, Giroud used the DTNB to orient BOx in the biocathode and constructed glucose/O_2 biofuel cells (Giroud et al., 2017). Thereinto, the BOx/DTNB-Pyr/MWCNTs modified biocathode was accomplished via π-π interactions of a pyrene derivative. The EBFCs eventually exhibited an OCP of 0.64 V with an associated maximum current density of 2.1 mA/cm^2, and a maximum power density of 0.5 mW/cm^2 at 0.36 V.

In brief, the orientation of MCOs can shorten the distance between the electrode and T1 center and accelerate electron transfer to some extent. However, it is difficult to apply for the wearable biofuel cells as the toxicity of aromatic moieties. The thought of orienting MCOs towards the surface of biocathodes is awaiting some new technologies to solve.

3.1.2. Multi-copper oxidases immobilization

Various immobilization techniques including entrapment, adsorption, covalent attachment, and self-immobilization have been reported for biocathode. However, the most appropriate MCOs immobilization method has not been determined yet and few researchers are focusing on developing reliable immobilization techniques on the biocathode.

The layer-by-layer structure in the bioanode to immobilize enzymes has been previously discussed (Christwardana et al., 2017; Liu et al., 2016). The stack layers were attached together by electrostatic attraction of oppositely charged species. Likewise, Christwardana (2017) also designed the layer-by-layer structure to immobilize laccase in the biocathode. In this work, the CNTs modified by carboxylic groups (CNT-COOH) were employed as supporting material to increase laccase attachment via physical and chemical bonding between the carboxylate group of CNT-COOH and the amine group of laccases. PEI was used to enhance the connection between laccases and CNTs via electrostatic attraction. GA was also selected as cross-linker in this design. And strong covalent bonds (C=N bonds) were formed between GA and layer-by-layer structure consisting of laccase and PEI. A maximum power density of 0.2 mW/cm^2 was achieved in the glucose/O_2 biofuel cells, with GA/[Lac/PEI/Lac/CNTs] as cathodic catalyst and Pt/C as anodic catalyst.

Furthermore, Nazaruk designed another powerful connection between laccase and CNTs for electron transport by implementing bioconjugates with polymerized laccase (Nazaruk et al., 2012). They prepared the laccase/single-walled carbon nanotubes (SWCNTs) bioconjugates to modify the biocathode for the direct electron transfer in the laccase-CNTs conjugate network. The SWCNTs with amino groups were treated with GA and then reacted with polymerized or non-polymerized laccase. Additionally, they chose three different SWCNTs derivatives to compare the performance of modified electrodes (Fig. 6). The best electrode was obtained with conjugates based on SWCNTs–$(CH_2)_2$–NH_2 as the linker, and resulted in a high current density of 0.6 mA/cm^2 and a maximum power density of 1 mW/cm^2 in hybrid biofuel cells with Zn anode.

3.1.3. Thermophilic laccase

For the wearable enzymatic biofuel cells harvesting energy from human sweat, the practical application temperature would sometimes be high, especially for the wearers working in high-temperature environment. Thus, some thermostable enzymes need to be found and applied in wearable enzymatic biofuel cells to prevent enzymes inactivation in special conditions. The group of Beneyton ever studied the immobilization of thermophilic laccase (Beneyton et al., 2011). They selected thermophilic laccase named CotA, which could be gotten from Bacillus subtilis endospore coat. The bacterial laccase CotA is a good alternative catalyst for the cathode compartment because of its high stability and activity across a broad range of temperatures (Martins et al., 2002). They functionalized the enzyme via electrochemical reduction of in situ generated aminophenyl monodiazonium salts, which was a pioneer work for extremophilic CotA immobilization on GCE. The obtained results of CotA-modified electrodes showed an optimal operation temperature of 45–50 °C and stable catalytic activity for at least 7 weeks.

3.2. Bi-enzyme catalysis enhancing biocathode activity

MCOs have been commonly applied for biocathodes of EBFCs, as their ability to achieve electrocatalytic reduction of O_2 at low overpotentials. Yet, there are a lot of drawbacks limiting the application of MCOs. For example, the deactivation of MCOs may be induced by some inhibitors, like chloride ions or urates of these copper oxidases (Agnès et al., 2013). Hence, the bi-enzyme system of combining HRP and GOx was applied in the biocathode for most researches. GOx catalyzes the reduction of O_2 to H_2O_2 in the presence of glucose while HRP reduces H_2O_2 into H_2O. That is to say, the HRP-based cathode is fuelled by H_2O_2. To design a bi-enzyme system at biocathode, Reuillard et al. (2014) took advantages of the glycosylated domain of both GOx and HRP for binding these enzymes on boronic acid functionalized MWCNTs. Especially, this work described the non-covalent functionalization of MWCNTs by pyrene derivatives bearing boronic acid. Two types of pyrene groups, with or without a spacer, were used: pyrene-1-boronic acid (pyrene 1) and 1-(4-boronobenzyl)-3-(4-(pyren-1-yl) butanamido) pyridin-1-ium bromide (pyrene 2) (Fig. 7A). When ABTS was as the mediator for MET, the comparison of the catalytic current values for H_2O_2 (504 and 252 mA/cm^2 for pyrene 1 and pyrene 2 MWCNTs electrodes, respectively) corroborated the anchoring of a two time higher amount of enzyme via pyrene 1. Afterwards, the pyrene 1 functionalized MWCNT biocathode with the co-immobilization of GOx and HRP, showed a maximum current density about 30 μA/cm^2 for glucose.

It is clear that the biocathode for low-overpotential reduction of O_2 can be designed by combining HRP with GOx under physiological condition. A high ratio is needed to wire a maximum amount of HRP, while a lower amount of GOx is necessary to limit the overproduction of H_2O_2. In 2013, Agnès and coworkers ever designed a bi-enzyme catalyst system to construct biofuel cells on double-walled carbon nanotubes (DWCNTs) (Agnès et al., 2013). The GOx and HRP were co-absorbed on the biocathode, while GOx and catalase were used to modify the anode with NQ as the mediator (Fig. 7B). It is worth noting that the HRP was responsible for the H_2O_2 reduction to get H_2O, and catalase was used to oxidize H_2O_2 to generate O_2. Besides, the dispersions of bio-functionalized DWCNTs were deposited on flexible carbon cloth by controlled drop casting. This novel type of EBFCs in combination with the efficient wiring of HRP and catalase on DWCNTs films, achieved an excellent OCP value of 0.45 V and a maximum power output of 30 μW/cm^2 in 5 mM glucose solutions. What's more, Jia et al. (2012) constructed graphite rod (GR), carbon microfibers (CMF) and CNTs to be the electrode substrate. And a biocathode was modified with pyrenehexanoic acid (PHA) and HRP by co-immobilization of GOx. Firstly, they used the chemical deposition process to generate CMF onto graphite rod GR (Chen et al., 2007), and then CNTs were generated as branches of the CMF. In addition, PHA was used to break the hydrophobicity of the pristine CMF and CNTs through π-π stacking, and thus the non-covalent binding of HRP and GOx could be achieved. Benefiting from local and efficient generation of H_2O_2 and the unrestricted diffusion of glucose

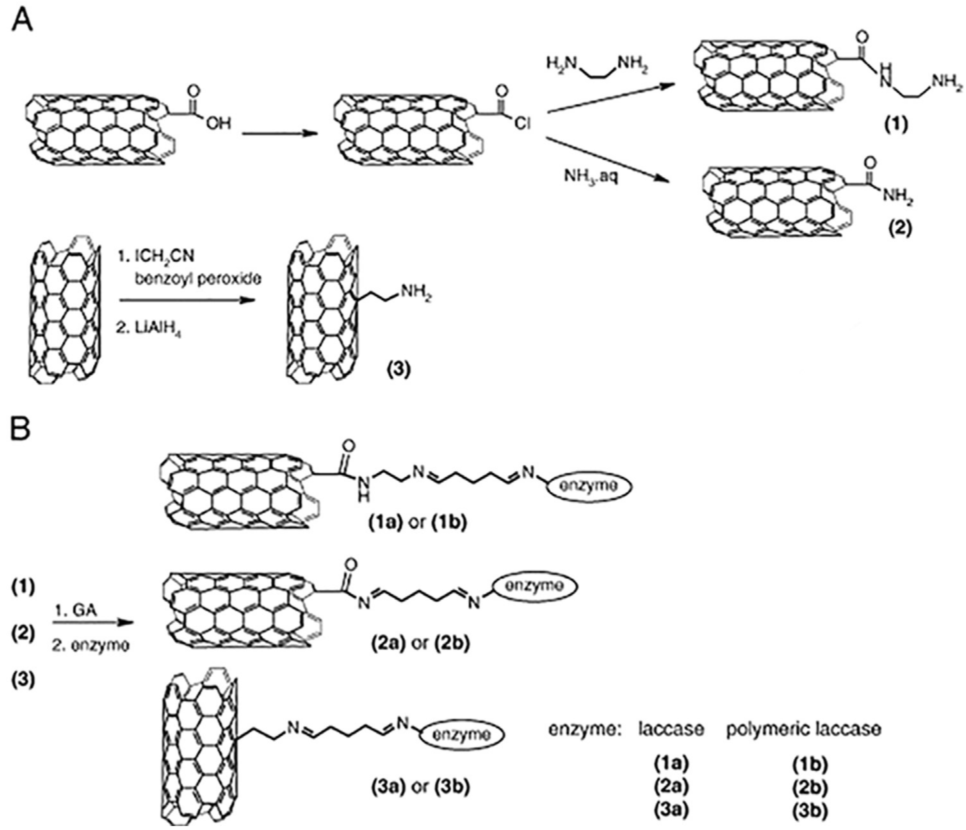

Fig. 6. (A) Schematic representation of functionalized carbon nanotubes: (1) SWCNT–CO–NH–$(CH_2)_2$–(NH_2), (2) SWCNT–CO–NH_2 and (3) SWCNT–$(CH_2)_2$–(NH_2). (B) Scheme of laccase conjugation with carbon nanotubes (Nazaruk et al., 2012).

and O_2 through the composite material, the biocathode resulted a maximum current density of about 300 μA/cm^2 at 0.7 V. In conclude, these results demonstrate that the biocathode based on the bi-enzyme catalysis maybe a desirable replacement of conventional biocathodes based on MCOs for wearable biofuel cells.

3.3. The gas diffusion structure of biocathode

We have known that biocathode catalyzes in most cases the reduction of O_2, therefore the unrestricted diffusion of O_2 is a critical point for preventing an unbalanced reaction rate between biocathode and bioanode. As the low solubility of O_2, oxygen-reducing electrocatalysis is governed by mass-transfer of O_2 to the electrode surface; this may limit the power output of biofuel cells. However, a gas diffusion electrode has the ability to supply gaseous substrates from gas phase, which is necessary to increase the concentration of substrates. For those low solubility gaseous substrates in the dissolved system, the gas diffusion electrode can also solve the mass-transfer limitation. Several hydrophobized carbon materials like ketjen black (KB), carbon black and CNTs are often selected as the electrode matrix for gas diffusion electrodes (Iijima, 1991). In 2016, So and coworkers designed a gas diffusion biocathode with water-dispersed MWCNTs (So et al., 2016). The relatively obvious features of this electrode were no any binders or surfactants, and water-dispersed MWCNTs compatible with BOx were deposited on a water-proof carbon cloth (WPCC). Faster interfacial electron transfer and larger steady-state catalytic reduction current density were shown in their biocathodes. The results demonstrated the possibility of constructing high-performance and efficient gas diffusion biocathodes. Subsequently, the group further improved the permeability of gas diffusion electrode (So et al., 2017). In the following work, novel CNTs with hollow structures (H-CNTs) were used to replace MWCNTs for increasing the gas permeability. Similarly, this gas diffusion system was also deposited on WPCC. They found that the catalytic current density of the BOD/H-CNTs/WPCC electrode

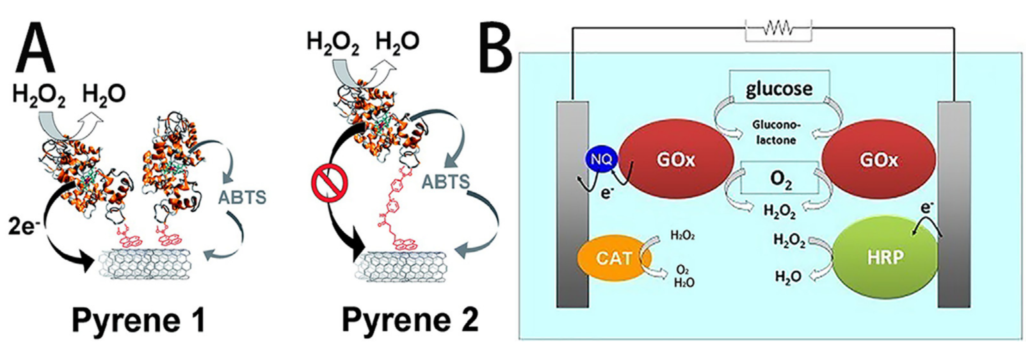

Fig. 7. (A) Schematic representation of the electrocatalytic mechanism for pyrene 1/HRP and pyrene 2/HRP MWCNTs electrodes (Reuillard et al., 2014). (B) Schematic representation of the EBFCs with bi-enzyme system (Agnès et al., 2013).

continued to increase as the amount of H-CNTs increased. In addition, they also investigated that the BOD/H-CNTs/WPCC electrode with ABTS as mediator could proceed effectively and cover a weak point of BOx as low DET activity at neutral pH.

4. Potential technologies for wearable biofuel cells

4.1. Carbon-based new material

In the last decades, rapid advancements in carbon-based nanomaterials have provided exciting prospects in biosensors and biofuel cells, such as CNTs, buckypaper, carbon nanodots (CNDs), graphene oxide (GO) and CNT sponges. These newly emerging carbon-based nanomaterials own the properties of high conductivity and good biocompatibility. Besides, these materials have displayed great capacities for enhancing electron transfer and shown to be ideal materials for bioelectrochemical applications (Zhao et al., 2015).

4.1.1. Buckypaper

Buckypaper is known as the thin sheet made from an aggregate of CNTs or CNTs grid paper. This material is typically fabricated by vacuum filtration of dispersion of CNTs, for obtaining a thin film with typical thickness in the range of 20–300 µm. Buckypaper is light, flexible, more compact and easily processed into different shapes and sizes (Cosnier et al., 2016; Gross et al., 2016). As the nanotubes in buckypaper are insoluble and can be modified with a variety of functional groups, it can be applied for sensors or electrodes. It can be drawn that the properties of buckypaper are suitable for wearable enzymatic biofule cells. Despite free-standing buckypaper tends to be brittle and difficult to manipulate, it is still desired for device integration and to decrease contact resistance and mass transport limitations (Gross et al., 2016).

The group of Reid ever designed a kind of contact lenses biofuel cells with LDH as the anodic enzyme, and they fabricated the biofuel cells by curing buckypaper electrodes on contact lenses (Reid et al., 2015). The buckypaper anode consisted of poly(methylene green), a hydrogel matrix containing LDH and NAD^+. The buckypaper cathode was modified by 1-pyrenemethyl anthracene-2-carboxylate, and then BOx was immobilized within a polymer. Based on the buckypaper electrodes, the contact lenses biofuel cells delivered a maximum current density of $61.3 \pm 2.9\,\mu A/cm^2$ and a maximum power density of $8.01 \pm 1.4\,\mu W/cm^2$ at an OCP of 0.413 ± 0.06 V. In addition, Gross et al. (2016) prepared and optimized a new class of reinforced free-standing buckypapers for biocathode. They combined original bifunctional polynorbornene copolymers with CNTs and investigated buckypaper to couple anthraquinone via amide bond formation for anthraquinone modified electrodes. They further improved electrical wiring of laccase via the hydrophobic pocket near the laccase T1 site. Besides, polynorbornene copolymer-carbon nanotube buckypaper can be used to control the immobilization and orient moieties of MCOs via amide bond formation, just like the function of NQ. Ultimately, the maximum current density observed here was $170 \pm 8\,\mu A/cm^2$. Furthermore, the bi-enzyme modified biocathode could be developed on the buckypaper in the similar way (Elouarzaki et al., 2015). Elouarzaki et al. immobilized HRP on the redox buckypaper followed by electropolymerization of pyrrole on concanavalin A. Subsequently, the GOx were additionally immobilized. In their work, pyrene-modified 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) or bis-Pyr-ABTS were included as the cross-linker (Fig. 8). The resulting biocathode was not only unrestrained under physiological conditions, but was markedly more stable and efficient for oxygen reduction. Besides, it showed a high onset potential at 0.6 V and a stable current density of 1.1 mA/cm^2 at 0.1 V.

4.1.2. Carbon nanodots

Carbon nanodots (CNDs) are the carbon-based nanomaterials that possess discrete and quasi-spherical structure. Therefore, CNDs have

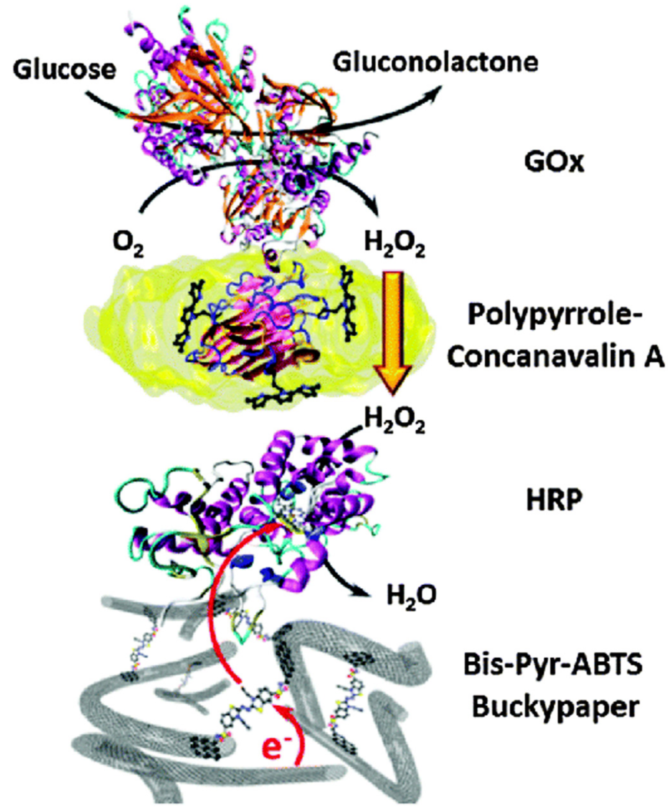

Fig. 8. Sketch of the biocathode functioning based on bis-pyrene-ABTS BP after adsorption of HRP and subsequent electropolymerization of pyrrole–concanavalin A for the immobilization of GOx (Elouarzaki et al., 2015).

the properties of large specific surface area and strong adsorption ability, which are propitious to increase the number of immobilized enzymes and strengthen energy conversion efficiency. In the work of Zhao, CNDs were used as immobilization supports and electron carriers to promote electrons transfer between GOx and BOx (Zhao et al., 2015). In the preparing process, Nafion solution was used as binder to spread on the electrode surface, and GOx/CNDs/GC and BOx/CNDs/GC electrodes were both fabricated by the drop-cast method. The constructed EBFCs displayed an OCP as high as 0.93 V and a maximum power density of 40.8 $\mu W/cm^2$ at 0.41 V. Obviously, it is reasonable that CNDs are used to be biocatalysts supports and electrochemical signal carriers.

4.1.3. Graphene oxide

Grapheme oxide (GO) represents a new class of two-dimensional nanostructure, and the emergency of GO has attracted considerable attention from researchers. Such attention is owing to its excellent electric properties and huge potential in capacitor, batteries, biofuel cells etc. Furthermore, the reduced form of GO (rGO), is getting more and more popular in the application of enzymatic biosensors and biofuel cells. RGO partially restores the remarkable physical and electrical properties of graphenes, while still benefiting from the presence of oxygen functional groups. Ravenna et al. (2015) introduced a typical application of rGO for bioanode. In this work, they added phenothiazine (PTZ) into GO matrix as mediator for its low formal redox potentials. And then the redox mediator could reduce GO to rGO in the presence of enzymes. Interestingly, the results of the experiment demonstrated that an irreversibly oxidized form of PTZ, named 3H-phenothiazine-3-one (phenothiazone, PTZ-O), was synthesized in rGO matrix. They chose NAD-dependent GDH as anodic catalyst to construct GDH/PTZ-O/rGO bioanode, and a maximum power density of 345 $\mu W/cm^2$ was generated by the hybrid biofuel cells. In this system, the cathode was controlled by a potentiostat and was biased continuously to 400 mV. Besides, Palanisamy and coworkers presented the single step fabrication of electrochemically reduced graphene oxide (ERGO)/GOx biocomposite without any binders and cross-linkers (Palanisamy et al., 2012). And the fabricated (ERGO)/GOx biocomposite was used to be the bioanode with ferrocene monocarboxylic acid (FMCA) mediator. In the biocathode, laccase was chosen to catalyze the reduction of O_2 based on the MWCNTs/zinc oxide (ZnO) electrode. Finally, the fabricated biofuel cell delivers a power density up to 54 nW cm/^{2} and an OCP of 0.055 V.

4.1.4. 3D CNT sponges electrode

CNT sponges are the sponge-like bulk material consisting of self-assembled, interconnected CNT skeletons, which were firstly proposed by Gui et al. (2010). The characteristics of CNT sponges include: a density close to the lightest aerogels, a porosity of > 99%, high structural flexibility and robustness, and wettability to organics in pristine form. CNT sponges can be easily processed into various shapes and compressed to large-strains repeatedly in air or liquids without collapse. Moreover, CNT sponges own great conductivity, thermal stability and mechanical flexibility, which exceedingly meet requirements of wearable enzymatic biofuel cell. He et al. (2018) introduced CNT sponges into the field of electrodes, and designed poly(3,4-ethylenedioxythiophene) (PEDOT)/3D CNT sponges electrodes to fabricate the all-solid state symmetric supercapacitor. The CNTs framework can be synthesized by chemical vapor deposition (CVD) method, and then CNTs composite with PEDOT by the electrodeposition to form the PEDOT/CNTs sponges electrode (Fig. 9). As a result of high conductivity and good mechanical strength of free-standing electrodes, this sponge-like CNT skeleton and equably coated PEDOT corporately contributed to the high mass energy density and power density. This PEDOT/CNTs sponge electrode showed highest mass-specific capacitance of 147 F/g at 0.5 A/g, tuned by the PEDOT mass loading, and exhibited good cyclic stability with the evidence that more than 95% of capacitance remained after 3000 cycles. Besides, the symmetric supercapacitor showed the maximum energy density of 12.6 Wh/kg and maximum power density of 10 kW/kg. Although there has not been a specific application of CNT sponges on EBFCs or wearable devices, the application prospects would be extremely extensive.

4.2. Mixed operational/storage electrode

Constant and steady electrical output is the basic and indispensable condition for wearable biofuel cells. And it is noteworthy that future power management of enzymatic biofuel cells in electronic devices will rely on discontinuous discharge cycles (Reuillard et al., 2015). To the best of our knowledge, apart from focusing either on the electrocatalytic or the charge-storage properties of electrodes separately, some works about dual-feature devices have been in operation. For instance, Reuillard et al. (2015) designed the discharge/reactivation cycles to solve the problem of deactivation of laccase, and studied the possibility to discontinuously produce significant amounts of electricity via EBFCs over a long period. They designed MWCNTs-based glucose EBFCs with the mixture of GOx and catalase as bioanode catalysts and laccase as biocathode catalyst, respectively. Between the constant load discharges, the anode was stored at room temperature in a phosphate buffer solution (pH 7) containing glucose, and the biocathode was stored at room temperature in a phosphate buffer solution (pH 5) for the reversible reactivation of laccase. During one year, the phosphate buffer solution containing glucose was replaced every three days, and the phosphate buffer solution of pH 5 was replaced every few days. One year later, the EBFCs were still able to deliver 22% of its initial maximum power density. Although the reactivation of laccase biocathode was investigated in acidic conditions and this approach is hard to be applied in the actual wearable biofuel cells, the idea about constructing a hybrid discharge/reactivation cycle device is worthy to be considered.

Besides, another emerging technology is introducing electrochemical capacitors into EBFCs circuit to build supercapacitor/biofuel hybrid devices, which enable the accumulation of charge in the EBFCs, resulting in output pulses of higher power. Hybrid electric power devices for simultaneous generation and storage of electrical energy have been reported recently (Agnes et al., 2014; Xiao et al., 2017). Almost supercapacitor/BFC hybrids are based on the fabrication of hybrid composite modified electrodes with integration of enzymes and capacitive materials. And the main feature is their ability to generate cyclic, high power pulses from the discharge of the supercapacitor, which can be recharged towards to OCP via the internal EBFCs in the following open-circuit mode. Agnes et al. (2014) constructed the supercapacitors/biofuel cells hybrid device based on CNTs matrix. In the device, redox enzymes served for continuous charging of the capacitors. They used the CNTs matrix to be an electrode material and build double layer supercapacitor. Specially, the CNTs/enzyme matrix was constructed by compression of the CNTs under an applied force of 1×10^4 N in a hydraulic press. The bioanode disks were compressed by the mixture solution of MWCNTs, GOx and catalase, and the mixture solution of laccase and MWCNTs were compressed to biocathode. When a 30 mA current pulse was applied, a maximum power of 16 mW at 0.5 V was obtained for the setup with a 1 cm electrode distance between the negative and the positive electrodes. Meanwhile, this supercapacitor/biofuel cell hybrid system remained stable for at least 40,000 pulses of 2 mW. Furthermore, Xiao et al. (2017) designed another symmetric supercapacitors/biofuel hybrid devices based on the nanoporous gold electrode (NPG). In their work, PEDOT and the redox polymer [Os(2,2′-bipyridine)$_2$(polyvinylimidazole)$_{10}$Cl]$^{+/2+}$(Os(bpy)$_2$PVI) were electrodeposited onto the porous gold electrodes with the co-immobilization of enzymes. GDH and BOx were selected as anodic and cathodic catalysts respectively. GDH/Os(bpy)$_2$PVI/PEDOT/NPG and BOx/Os(bpy)$_2$PVI/PEDOT/NPG electrodes were subsequently assembled into a dual-functioning device comprising a EBFC and a capacitor. Once charged by the internal EBFC, the device could discharge as a supercapacitor at a current density of 2 mA/cm^2 providing maximum power density of 608.8 μW/cm^2. Besides, the hybrid device exhibited good operational stability for 50 charge/discharge cycles and about 7 h at a

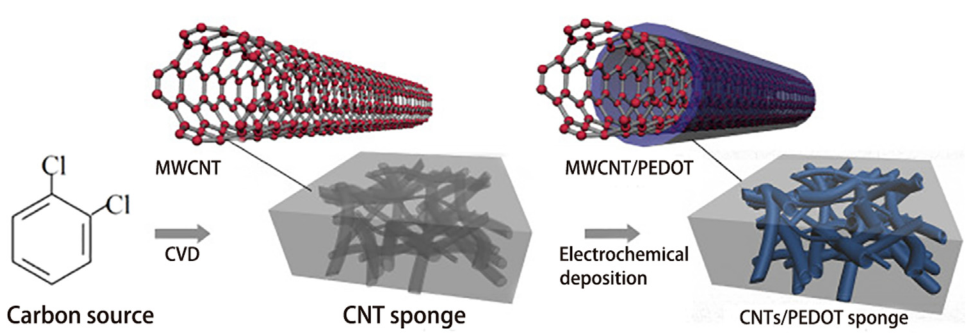

Fig. 9. A schematic illustration of the fabrication process of the CNTs/PEDOT sponge composite (He et al., 2018).

Table 1
Detailed structures and parameters of some typical EBFCs.

Fuel	Anode	Anode mediator	Cathode	OCP	Maximum power density	Maximum current density	Reference
Lactate	Au/CNTs/NQ/LOx/Chit	NQ	Au/CNTs/Ag_2O	0.5 V	1.2 mW/cm^2 at 0.2 V	__	(Bandodkar, 2017)
Lactate	CNTs/TTF/LOX/Chit	TTF	Pt black	__	5–70 μW/cm^2	14 mA/cm^2	(Jia et al., 2013b)
Lactate	Buckypaper/Poly-MG/LDH	Poly-MG	Buckypaper/ BOx	0.413 ± 0.06 V	8.01 ± 1.4μW/cm^2	61.3 ± 2.9μA/cm^2	(Reid et al., 2015)
Ascorbate	Au/TTF-TCNQ	TTF-TCNQ	Au/BOx	0.54 V	3.1μW/cm^2 at 0.25 V	0.55μA/cm^2 at 0.4 V	(Falk et al., 2013)
Lactate	CNTs/$FcMe_2$-LPEI/LOx	$FcMe_2$-LPEI	CNTs/Ar-pyr/ BOx	0.44 ± 0.08 V	2.4 ± 0.9μW/cm^2 at 0.163 V	22 ± 4μA/cm^2	(Reid et al., 2016)
Lactate	GMC/GOx/GA		Pt	0.48 V	22.4μW/cm^2 at 0.24 V	150μA/cm^2	(Garcia-Perez et al., 2016)
Glucose	CNTs/TTF-TCNQ/LOx	TTF-TCNQ	Pt	0.67 V	100μW/cm^2 at 0.36 V	__	(Jia et al., 2014)
Glucose	GRE/PD/GOx	PD	GRE/HRP	640 mV	4.2μW/cm^2 at 0.53 V	__	(Ramanavicius et al., 2015)
Glucose	CNTs/NQ/GOx-Cat	NQ	CNTs/GOx-HRP	450 mV	30μW/cm^2 at 0. 3 V	127μA/cm^2	(Agnès et al., 2013)
Glucose	CNTs/PEI/ GOx-Cat		CNTs/PEI/Lac	__	180 ± 22.3μW/cm^2	1.4 mA/cm^2	(Christwardana et al., 2017)
Glucose	CNTs/Cat/NQ/GOx	NQ	CNTs/Lac	0.76 V	1.54 mW/cm^2	4.47 mA/cm^2	(Reuillard et al., 2013)
Glucose	Au/*Ct*CDH		Au/BOx	0.57 V	1μW/cm^2 at 0. 5 V	__	(Falk et al., 2012)
Glucose	MDB-MWCNTs/GDH		Ag_2O/Ag	0.59 V	0.275 mW/cm^2 at 0. 34 V	__	(Yu et al., 2016)
Glucose	MWCNTs/pry-$(NQ)_2$/PQQ-GDH	pry-$(NQ)_2$	MWCNTs/ pry-$(NQ)_2$/Lac MWCNTs/ pry-$(NQ)_2$/BOx	__	12–20μW/cm^2	50–80μA/cm^2	(Giroud et al., 2015)
Lactate	MWCNTs/DTNB-pyr/GDH		MWCNTs/Lac MWCNTs/BOx	0.64 v	0.5 mW/cm^2 at 0. 36 V	2.1 mA/cm^2	(Giroud et al., 2017)
Lactate	Dehydrogenases (LDH, PDH, CS, Aconitase, IDH, KDH, succinyl CoA synthetase, SDH, fumarase, MDH)		Pt	__	827 ± 21μW/cm^2	3320 ± 110 μA/cm^2	(Sokic-Lazic et al., 2011)
Glucose	CNTs/PEI/[GOx/HRP/TPA]		Pt-C	__	2.0 ± 0.1 mW/cm^2	__	(Chung et al., 2018)
Glucose	Pt		[CNTs/Lac/PEI/ Lac]/GA	__	0.2 mW/cm^2	__	(Christwardana, 2017)
Glucose	CND/GOx		CND/BOx	0.93 V	40.8μW/cm^2 at 0. 41 V	100μA/cm^2	(Zhao et al., 2015)
Glucose	rGO/PTZ-O/GDH	PTZ-O	Potentiostat at 400 mV	__	345μW/cm^2	__	(Ravenna et al., 2015)
Glucose	CNTs Matrix/GOx		CNTs Matrix/Lac	1 ± 0.1 V	2 mW/cm^2	__	(Agnes et al., 2014)

discharge current density of 0.2 mA/cm^2. It is clear that hybrid devices could be continuously recharged via biocatalytic energy conversion to generate high power discharge cycles. There is no doubt that dual-feature system is a possible approach to settle down the trouble of power supply, but there are lots of difficulties before commercialization.

5. Conclusions

In the present review article, we have detailed the recent scientific advances and bottlenecks of wearable enzymatic biofuel cells. These strategies improved the performance of EBFCs from the standpoints of maximum current density, maximum power density or lifetime. The significant parameters about part of EBFCs were listed in Table 1. Electron transfer would occur during the redox reaction of some biofluids, and several enzymes have been explored to catalyze redox reaction and accelerate electron transfer. Electron transfer methods are always divided into DET and MET two types. Thereinto, MET needs the help of mediators to transfer electrons which can lead to higher catalytic current, and DET is becoming a trend for its convenient and optimal cell voltage with the development of carbon-based nanomaterials. From the aspect of catalytic electrodes, the bioanode usually contains oxidases and dehydrogenases as catalyst, and the biocathode use the catalysis of multi-copper oxidases (MCOs) in the single system. Besides, the combination of GOx and HRP or catalase has been explored in bioanodes and biocathodes for further enhancing the performance of EBFCs. In addition, several nanomaterials newly emerging have displayed great abilities to enhance electron transfer of redox reaction, such as buckypaper, CNDs, GO and CNT sponges. And the idea of mixed operational/storage system has infinite prospects for achieving discontinuous discharge cycles. In conclusion, the observed OCP can be increased by using direct electron transfer or redox mediators with redox potentials closer to those of the enzyme/cofactor. As for the low current and power density, these limits can be improved through efficient substrate diffusion, enhancing electron transfer rates between enzymes and electrodes, improving catalytic activity, loading more enzymes and utilizing enzyme cascades for deep and complete oxidation pathways, as well as introducing supercapacitors/biofuel hybrid devices.

Since the invention of EBFCs in 1960s, researchers have spent significant time and effort to overcome their bottlenecks. Although several available wearable biofuel cells have been provided and reported can charge for a watch and light a LED, the field of wearable biofuel cells is still in its infancy. A lot of parameters are still hard to meet the requirements of commercialization, such as long duration, enough and stable power output, conformability and mechanical resiliency. In brief, the reasonable allocation of bioanode and biocathode is the critical factor for improving the performance of wearable enzymatic biofuel cells. With the fast development of modern material, biological and electronic science, it is hopeful that wearable biofuel cells would be commonly applied for wearable and implantable devices one day. Great potential of wearable biofuel cells will nevertheless continue to attract enormous interest in these exciting and potentially revolutionary technologies.

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References

Agnes, C., Holzinger, M., Le Goff, A., Reuillard, B., Elouarzaki, K., Tingry, S., Cosnier, S., 2014. Supercapacitor/biofuel cell hybrids based on wired enzymes on carbon nanotube matrices: autonomous reloading after high power pulses in neutral buffered glucose solutions. Energy Environ. Sci. 7 (6), 1884–1888.

Agnès, C., Reuillard, B., Le Goff, A., Holzinger, M., Cosnier, S., 2013. A double-walled carbon nanotube-based glucose/H_2O_2 biofuel cell operating under physiological conditions. Electrochem. Commun. 34, 105–108.

Bandodkar, A.J., 2017. Review-wearable biofuel cells: past, present and future. J. Electrochem. Soc. 164 (3), H3007–H3014.

Bandodkar, A.J., Jeerapan, I., Wang, J., 2016. Wearable chemical sensors: present challenges and future prospects. ACS Sens. 1 (5), 464–482.

Bandodkar, A.J., Wang, J., 2016. Wearable biofuel cells: a review. Electroanalysis 28 (6), 1188–1200.

Bandodkar, A.J., You, J.-M., Kim, N.-H., Gu, Y., Kumar, R., Mohan, A.M.V., Kurniawan, J., Imani, S., Nakagawa, T., Parish, B., Parthasarathy, M., Mercier, P.P., Xu, S., Wang, J., 2017. Soft, stretchable, high power density electronic skin-based biofuel cells for scavenging energy from human sweat. Energy Environ. Sci. 10 (7), 1581–1589.

Bao, J., Furumoto, K., Yoshimoto, M., Fukunaga, K., Nakao, K., 2003. Competitive inhibition by hydrogen peroxide produced in glucose oxidation catalyzed by glucose oxidase. Biochem. Eng. J. 13 (1), 69–72.

Bencini, A., Lippolis, V., 2010. 1,10-Phenanthroline: a versatile building block for the construction of ligands for various purposes. Coord. Chem. Rev. 254 (17), 2096–2180.

Beneyton, T., El Harrak, A., Griffiths, A.D., Hellwig, P., Taly, V., 2011. Immobilization of CotA, an extremophilic laccase from Bacillus subtilis, on glassy carbon electrodes for biofuel cell applications. Electrochem. Commun. 13 (1), 24–27.

Blanford, C.F., Heath, R.S., Armstrong, F.A., 2007. A stable electrode for high-potential, electrocatalytic O_2 reduction based on rational attachment of a blue copper oxidase to a graphite surface. Chem. Commun. 17, 1710–1712.

Bon, S.C.Y., Lalo, H., Wang, Z., Etienne, M., Gajdzik, J., Kohring, G.W., Walcarius, A., Hempelmann, R., Kuhn, A., 2011. Multiscale-tailored bioelectrode surfaces for optimized catalytic conversion efficiency. Langmuir 27 (20), 12737.

Chen, C., Xie, Q., Yang, D., Xiao, H., Fu, Y., Tan, Y., Yao, S., 2013. Recent advances in electrochemical glucose biosensors: a review. RSC Adv. 3 (14), 4473–4491.

Chen, X., Li, N., Eckhard, K., Stoica, L., Xia, W., Assmann, J., Muhler, M., Schuhmann, W., 2007. Pulsed electrodeposition of Pt nanoclusters on carbon nanotubes modified carbon materials using diffusion restricting viscous electrolytes. Electrochem. Commun. 9 (6), 1348–1354.

Christwardana, M., 2017. Combination of physico-chemical entrapment and crosslinking of low activity laccase-based biocathode on carboxylated carbon nanotube for increasing biofuel cell performance. Enzym. Microb. Technol. 106, 1–10.

Christwardana, M., Chung, Y., Kwon, Y., 2017. Co-immobilization of glucose oxidase and catalase for enhancing the performance of a membraneless glucose biofuel cell operated under physiological conditions. Nanoscale 9 (5), 1993–2002.

Chung, Y., Tannia, D.C., Kwon, Y., 2018. Glucose biofuel cells using bi-enzyme catalysts including glucose oxidase, horseradish peroxidase and terephthalaldehyde crosslinker. Chem. Eng. J. 334, 1085–1092.

Cosnier, S., Gross, J., A., Le Goff, A., Holzinger, M., 2016. Recent advances on enzymatic glucose/oxygen and hydrogen/oxygen biofuel cells: achievements and limitations. J. Power Sources 325, 252–263.

Cracknell, J.A., Mcnamara, T.P., Lowe, E.D., Blanford, C.F., 2011. Bilirubin oxidase from Myrothecium verrucaria: x-ray determination of the complete crystal structure and a rational surface modification for enhanced electrocatalytic O_2 reduction. Dalton Trans. 40 (25), 6668–6675.

De Poulpiquet, A., Ciaccafava, A., Lojou, E., 2014. New trends in enzyme immobilization at nanostructured interfaces for efficient electrocatalysis in biofuel cells. Electrochim. Acta 126, 104–114.

Elouarzaki, K., Bourourou, M., Holzinger, M., Le Goff, A., Marks, R.S., Cosnier, S., 2015. Freestanding HRP–GOx redox buckypaper as an oxygen-reducing biocathode for biofuel cell applications. Energy Environ. Sci. 8 (7), 2069–2074.

Falk, M., Andoralov, V., Blum, Z., Sotres, J., Suyatin, D.B., Ruzgas, T., Arnebrant, T., Shleev, S., 2012. Biofuel cell as a power source for electronic contact lenses. Biosens. Bioelectron. 37 (1), 38–45.

Falk, M., Andoralov, V., Silow, M., Toscano, M.D., Shleev, S., 2013. Miniature biofuel cell as a potential power source for glucose-sensing contact lenses. Anal. Chem. 85 (13), 6342–6348.

Fan, S., Hou, C., Liang, B., Feng, R., Liu, A., 2015. Microbial surface displayed enzymes based biofuel cell utilizing degradation products of lignocellulosic biomass for direct electrical energy. Bioresour. Technol. 192, 821–825.

Fujita, K., Nakamura, N., Murata, K., Igarashi, K., Samejima, M., Ohno, H., 2011. Electrochemical analysis of electrode-immobilized dehydrogenases in hydrated choline dihydrogen phosphate-type ionic liquid. Electrochim. Acta 56 (20), 7224–7227.

Gao, F., Viry, L., Maugey, M., Poulin, P., Mano, N., 2010. Engineering hybrid nanotube wires for high-power biofuel cells. Nat. Commun. 1, 2.

Garcia-Perez, T., Hong, S.-G., Kim, J., Ha, S., 2016. Entrapping cross-linked glucose oxidase aggregates within a graphitized mesoporous carbon network for enzymatic biofuel cells. Enzym. Microb. Technol. 90, 26–34.

Giroud, F., Milton, R.D., Tan, B.-X., Minteer, S.D., 2015. Simplifying enzymatic biofuel cells: immobilized naphthoquinone as a biocathodic orientational moiety and bioanodic electron mediator. ACS Catalysis 5 (2), 1240–1244.

Giroud, F., Sawada, K., Taya, M., Cosnier, S., 2017. 5,5-Dithiobis(2-nitrobenzoic acid) pyrene derivative-carbon nanotube electrodes for NADH electrooxidation and oriented immobilization of multicopper oxidases for the development of glucose/O_2 biofuel cells. Biosens. Bioelectron. 87, 957–963.

Gross, A.J., Robin, M.P., Nedellec, Y., O'Reilly, R.K., Shan, D., Cosnier, S., 2016. Robust bifunctional buckypapers from carbon nanotubes and polynorbornene copolymers for flexible engineering of enzymatic bioelectrodes. Carbon 107, 542–547.

Gui, X., Wei, J., Wang, K., Cao, A., Zhu, H., Jia, Y., Shu, Q., Wu, D., 2010. Carbon nanotube sponges. Adv. Mater. 22 (5), 617–621.

He, X., Yang, W., Mao, X., Xu, L., Zhou, Y., Chen, Y., Zhao, Y., Yang, Y., Xu, J., 2018. All-solid state symmetric supercapacitors based on compressible and flexible free-standing 3D carbon nanotubes (CNTs)/poly(3,4-ethylenedioxythiophene) (PEDOT) sponge electrodes. J. Power Sources 376, 138–146.

Heller, A., 1990. Electrical wiring of redox enzymes. Acc. Chem. Res. 23 (5), 128–134.

Holzinger, M., Le Goff, A., Cosnier, S., 2012. Carbon nanotube/enzyme biofuel cells. Electrochim. Acta 82, 179–190.

Iijima, S., 1991. Helical microtubules of graphitic carbon. Nature 354 (6348), 56–58.

Inamuddin, Beenish, Naushad, M., 2016. Fabrication of bioanode by using electrically conducting polythiophene via entrapment technique. Korean J. Chem. Eng. 33 (1), 120–125.

Ivnitski, D., Branch, B., Atanassov, P., Apblett, C., 2006. Glucose oxidase anode for biofuel cell based on direct electron transfer. Electrochem. Commun. 8 (8), 1204–1210.

Jamshidinia, Z., Mashayekhimazar, F., Ahmadi, M., Molaeirad, A., Alijanianzadeh, M., Janfaza, S., 2017. Investigation of direct and mediated electron transfer of laccase-based biocathode. J. Electrochem. Sci. Technol. 8 (2), 87–95.

Jia, W., Bandodkar, A.J., Valdes-Ramirez, G., Windmiller, J.R., Yang, Z., Ramirez, J., Chan, G., Wang, J., 2013a. Electrochemical tattoo biosensors for real-time non-invasive lactate monitoring in human perspiration. Anal. Chem. 85 (14), 6553–6560.

Jia, W., Jin, C., Xia, W., Muhler, M., Schuhmann, W., Stoica, L., 2012. Glucose oxidase/horseradish peroxidase co-immobilized at a CNT-modified graphite electrode: towards potentially implantable biocathodes. Chem. Eur. J. 18 (10), 2783–2786.

Jia, W., Valdes-Ramirez, G., Bandodkar, A.J., Windmiller, J.R., Wang, J., 2013b. Epidermal biofuel cells: energy harvesting from human perspiration. Angew. Chem. Int. Ed. 52 (28), 7233–7236.

Jia, W., Wang, X., Imani, S., Bandodkar, A.J., Ramírez, J., Mercier, P.P., Wang, J., 2014. Wearable textile biofuel cells for powering electronics. J. Mater. Chem. A 2 (43), 18184–18189.

Jiang, K., Schadler, L.S., Siegel, R.W., Zhang, X., Zhang, H., Terrones, M., 2003. Protein immobilization on carbon nanotubes via a two-step process of diimide-activated amidation. J. Mater. Chem. 14 (1), 37–39.

Karaśkiewicz, M., Nazaruk, E., Żelechowska, K., Biernat, J.F., Rogalski, J., Bilewicz, R., 2012. Fully enzymatic mediatorless fuel cell with efficient naphthylated carbon nanotube–laccase composite cathodes. Electrochem. Commun. 20, 124–127.

Kim, B.C., Zhao, X., Ahn, H.K., Kim, J.H., Lee, H.J., Kim, K.W., Nair, S., Hsiao, E., Jia, H., Oh, M.K., Sang, B.I., Kim, B.S., Kim, S.H., Kwon, Y., Ha, S., Gu, M.B., Wang, P., Kim, J., 2011. Highly stable enzyme precipitate coatings and their electrochemical applications. Biosens. Bioelectron. 26 (5), 1980–1986.

Kim, J.H., Hong, S.G., Wee, Y., Hu, S., Kwon, Y., Ha, S., Kim, J., 2017. Enzyme precipitate coating of pyranose oxidase on carbon nanotubes and their electrochemical applications. Biosens. Bioelectron. 87, 365–372.

Kulys, J., Simkeviciene, V., Higgins, I., 1992. Concerning the toxicity of two compounds used as mediators in biosensor devices: 7, 7, 8, 8-tetracyanoquinodimethane (TCNQ) and tetrathiafulvalene (TTF). Biosens. Bioelectron. 7 (7), 495–501.

Lai, J., Yi, Y., Zhu, P., Shen, J., Wu, K., Zhang, L., Liu, J., 2016. Polyaniline-based glucose biosensor: a review. J. Electroanal. Chem. 782, 138–153.

Lang, Q., Yin, L., Shi, J., Li, L., Xia, L., Liu, A., 2014. Co-immobilization of glucoamylase and glucose oxidase for electrochemical sequential enzyme electrode for starch biosensor and biofuel cell. Biosens. Bioelectron. 51 (2014), 158–163.

Liu, J., Sun, S., Shang, H., Lai, J., Zhang, L., 2016. Electrochemical biosensor based on bienzyme and carbon nanotubes incorporated into an Os-complex thin film for continuous glucose detection in human saliva. Electroanalysis 28 (9), 2016–2021.

Liu, J., Sun, S.H., Liu, C.C., Wei, S.S., 2011. An amperometric glucose biosensor based on a screen-printed electrode and Os-complex mediator for flow injection analysis. Measurement 44 (10), 1878–1883.

Liu, J., Zhang, L.L., Fu, C.Y., 2015. Os-complex-based amperometric bienzyme biosensor for continuous determination of lactate in saliva. Anal. Methods 7 (15), 6158–6164.

Martins, L.O., Soares, C.M., Pereira, M.M., Teixeira, M., Costa, T., Jones, G.H., Henriques, A.O., 2002. Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of theBacillus subtilis endospore coat. J. Biol. Chem. 277 (21), 18849–18859.

Miyake, T., Haneda, K., Nagai, N., Yatagawa, Y., Onami, H., Yoshino, S., Abe, T., Nishizawa, M., 2011a. Enzymatic biofuel cells designed for direct power generation from biofluids in living organisms. Energy Environ. Sci. 4 (12), 5008–5012.

Miyake, T., Oike, M., Yoshino, S., Yatagawa, Y., Haneda, K., Kaji, H., Nishizawa, M., 2009. Biofuel cell anode: nad+/glucose dehydrogenase-coimmobilized ketjenblack electrode. Chem. Phys. Lett. 480 (1–3), 123–126.

Miyake, T., Yoshino, S., Yamada, T., Hata, K., Nishizawa, M., 2011b. Self-regulating enzyme− nanotube ensemble films and their application as flexible electrodes for biofuel cells. J. Am. Chem. Soc. 133 (13), 5129–5134.

Nazaruk, E., Karaskiewicz, M., Żelechowska, K., Biernat, J.F., Rogalski, J., Bilewicz, R., 2012. Powerful connection of laccase and carbon nanotubes. Electrochem. Commun. 14 (1), 67–70.

Palanisamy, S., Cheemalapati, S., Chen, S.M., 2012. An enzymatic biofuel cell based on electrochemically reduced graphene oxide and multiwalled carbon nanotubes/zinc

oxide modified electrode. Int. J. Electrochem. Sci. 7 (11), 11477–11487.
Palmore, G.T.R., Kim, H.-H., 1999. Electro-enzymatic reduction of dioxygen to water in the cathode compartment of a biofuel cell. J. Electroanal. Chem. 464 (1), 110–117.
Pauliukaite, R., Malinauskas, A., Zhylyak, G., Spichiger-Keller, U.E., 2007. Conductive organic complex salt TTF-TCNQ as a mediator for biosensors. An overview. Electroanal.: Int. J. Devoted Fundam. Pract. Asp. Electroanal. 19 (24), 2491–2498.
Ramanavicius, A., Kausaite-Minkstimiene, A., Morkvenaite-Vilkonciene, I., Genys, P., Mikhailova, R., Semashko, T., Voronovic, J., Ramanaviciene, A., 2015. Biofuel cell based on glucose oxidase from Penicillium funiculosum 46.1 and horseradish peroxidase. Chem. Eng. J. 264, 165–173.
Rasmussen, M., Abdellaoui, S., Minteer, S.D., 2016. Enzymatic biofuel cells: 30 years of critical advancements. Biosens. Bioelectron. 76, 91–102.
Ravenna, Y., Xia, L., Gun, J., Mikhaylov, A.A., Medvedev, A.G., Lev, O., Alfonta, L., 2015. Biocomposite based on reduced graphene oxide film modified with phenothiazone and flavin adenine dinucleotide-dependent glucose dehydrogenase for glucose sensing and biofuel cell applications. Anal. Chem. 87 (19), 9567–9571.
Reid, R.C., Jones, S.R., Hickey, D.P., Minteer, S.D., Gale, B.K., 2016. Modeling carbon nanotube connectivity and surface activity in a contact lens biofuel cell. Electrochim. Acta 203, 30–40.
Reid, R.C., Minteer, S.D., Gale, B.K., 2015. Contact lens biofuel cell tested in a synthetic tear solution. Biosens. Bioelectron. 68, 142–148.
Reuillard, B., Abreu, C., Lalaoui, N., Le Goff, A., Holzinger, M., Ondel, O., Buret, F., Cosnier, S., 2015. One-year stability for a glucose/oxygen biofuel cell combined with pH reactivation of the laccase/carbon nanotube biocathode. Bioelectrochemistry 106, 73–76.
Reuillard, B., Le Goff, A., Agnes, C., Holzinger, M., Zebda, A., Gondran, C., Elouarzaki, K., Cosnier, S., 2013. High power enzymatic biofuel cell based on naphthoquinone-mediated oxidation of glucose by glucose oxidase in a carbon nanotube 3D matrix. Phys. Chem. Chem. Phys. 15 (14), 4892–4896.
Reuillard, B., Le Goff, A., Holzinger, M., Cosnier, S., 2014. Non-covalent functionalization of carbon nanotubes with boronic acids for the wiring of glycosylated redox enzymes in oxygen-reducing biocathodes. J. Mater. Chem. B 2 (16), 2228–2232.
Saleh, F.S., Mao, L., Ohsaka, T., 2010. Development of a dehydrogenase-based glucose anode using a molecular assembly composed of nile blue and functionalized SWCNTs and its applications to a glucose sensor and glucose/O 2 biofuel cell. Sens. Actuators B 152 (1), 130–135.
Saleh, F.S., Mao, L., Ohsaka, T., 2011. Development of a dehydrogenase-based glucose anode using a molecular assembly composed of nile blue and functionalized SWCNTs and its applications to a glucose sensor and glucose/O_2 biofuel cell. Sens. Actuators B 152 (1), 130–135.
Scodeller, P., Carballo, R., Szamocki, R., Levin, L., Forchiassin, F., Calvo, E.J., 2010. Layer-by-layer self-assembled osmium polymer-mediated laccase oxygen cathodes for biofuel cells: the role of hydrogen peroxide. J. Am. Chem. Soc. 132 (32), 11132–11140.
Shao, M., Zafar, M.N., Sygmund, C., Guschin, D.A., Ludwig, R., Peterbauer, C.K., Schuhmann, W., Gorton, L., 2013. Mutual enhancement of the current density and the coulombic efficiency for a bioanode by entrapping bi-enzymes with Os-complex modified electrodeposition paints. Biosens. Bioelectron. 40 (1), 308–314.
So, K., Onizuka, M., Komukai, T., Kitazumi, Y., Shirai, O., Kano, K., 2016. Binder/surfactant-free biocathode with bilirubin oxidase for gas-diffusion-type system. Electrochem. Commun. 66, 58–61.
So, K., Ozawa, H., Onizuka, M., Komukai, T., Kitazumi, Y., Shirai, O., Kano, K., 2017. Highly permeable gas diffusion electrodes with hollow carbon nanotubes for bilirubin oxidase-catalyzed dioxygen reduction. Electrochim. Acta 246, 794–799.
Sokic-Lazic, D., de Andrade, A.R., Minteer, S.D., 2011. Utilization of enzyme cascades for complete oxidation of lactate in an enzymatic biofuel cell. Electrochim. Acta 56 (28), 10772–10775.
Sokic-Lazic, D., Minteer, S.D., 2009. Pyruvate/air enzymatic biofuel cell capable of complete oxidation. Electrochem. Solid-State Lett. 12 (9), F26–F28.
Sosna, M., Chrétien, J.-M., Kilburn, J.D., Bartlett, P.N., 2010. Monolayer anthracene and anthraquinone modified electrodes as platforms for Trametes hirsuta laccase immobilisation. Phys. Chem. Chem. Phys. 12 (34), 10018–10026.
Stolarczyk, K., Sepelowska, M., Lyp, D., Żelechowska, K., Biernat, J.F., Rogalski, J., Farmer, K.D., Roberts, K.N., Bilewicz, R., 2012. Hybrid biobattery based on arylated carbon nanotubes and laccase. Bioelectrochemistry 87, 154–163.
Suroviec, A.H., 2017. Layer-by-layer assembly of glucose oxidase on carbon nanotube modified electrodes. Methods Mol. Biol. 1504, 203–213.
Tanne, C., Gobel, G., Lisdat, F., 2010. Development of a (PQQ)-GDH-anode based on MWCNT-modified gold and its application in a glucose/O_2-biofuel cell. Biosens. Bioelectron. 26 (2), 530–535.
Wang, Y., Li, M.Q., 2011. Effects of superfine grinding on the properties of wheat bran. Mod. Food Sci. Technol. 27 (3), 271–274.
Wen, D., Deng, L., Zhou, M., Guo, S.J., Shang, L., Xu, G.B., Dong, S.J., 2010. A biofuel cell with a single-walled carbon nanohorn-based bioanode operating at physiological condition. Biosens. Bioelectron. 25 (6), 1544–1547.
Wilson, R., Turner, A.P.F., 1992. Glucose-oxidase - an ideal enzyme. Biosens. Bioelectron. 7 (3), 165–185.
Xia, L., Liang, B., Li, L., Tang, X.J., Palchetti, I., Mascini, M., Liu, A.H., 2013. Direct energy conversion from xylose using xylose dehydrogenase surface displayed bacteria based enzymatic biofuel cell. Biosens. Bioelectron. 44, 160–163.
Xiao, X., Conghaile, P.O., Leech, D., Ludwig, R., Magner, E., 2017. A symmetric supercapacitor/biofuel cell hybrid device based on enzyme-modified nanoporous gold: an autonomous pulse generator. Biosens. Bioelectron. 90, 96–102.
Yamazaki, T., Kojima, K., Sode, K., 2000. Extended-range glucose sensor employing engineered glucose dehydrogenases. Anal. Chem. 72 (19), 4689–4693.
Yehezkeli, O., Tel-Vered, R., Raichlin, S., Willner, I., 2011. Nano-engineered flavin-dependent glucose dehydrogenase/gold nanoparticle-modified electrodes for glucose sensing and biofuel cell applications. ACS Nano 5 (3), 2385–2391.
Yu, E.H., Krewer, U., Scott, K., 2010. Principles and materials aspects of direct alkaline alcohol fuel cells. Energies 3 (8), 1499–1528.
Yu, Y., Xu, M., Bai, L., Han, L., Dong, S., 2016. Recoverable hybrid enzymatic biofuel cell with molecular oxygen-independence. Biosens. Bioelectron. 75, 23–27.
Zafar, M.N., Wang, X., Sygmund, C., Ludwig, R., Leech, D., Gorton, L., 2012. Electron-transfer studies with a new flavin adenine dinucleotide dependent glucose dehydrogenase and osmium polymers of different redox potentials. Anal. Chem. 84 (1), 334–341.
Zebda, A., Cosnier, S., Alcaraz, J.P., Holzinger, M., Le Goff, A., Gondran, C., Boucher, F., Giroud, F., Gorgy, K., Lamraoui, H., Cinquin, P., 2013. Single glucose biofuel cells implanted in rats power electronic devices. Sci. Rep. 3, 1516.
Zebda, A., Gondran, C., Le Goff, A., Holzinger, M., Cinquin, P., Cosnier, S., 2011. Mediatorless high-power glucose biofuel cells based on compressed carbon nanotube-enzyme electrodes. Nat. Commun. 2, 370.
Zebda, A., Renaud, L., Cretin, M., Innocent, C., Pichot, F., Ferrigno, R., Tingry, S., 2009. Electrochemical performance of a glucose/oxygen microfluidic biofuel cell. J. Power Sources 193 (2), 602–606.
Zeng, T., Pankratov, D., Falk, M., Leimkuhler, S., Shleev, S., Wollenberger, U., 2015. Miniature direct electron transfer based sulphite/oxygen enzymatic fuel cells. Biosens. Bioelectron. 66, 39–42.
Zhao, M., Gao, Y., Sun, J., Gao, F., 2015. Mediatorless glucose biosensor and direct electron transfer type glucose/air biofuel cell enabled with carbon nanodots. Anal. Chem. 87 (5), 2615–2622.