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Review

Enzymatic biofuel cells fabricated by nanomaterials and their uses as implantable, wearable, and biosensing devices

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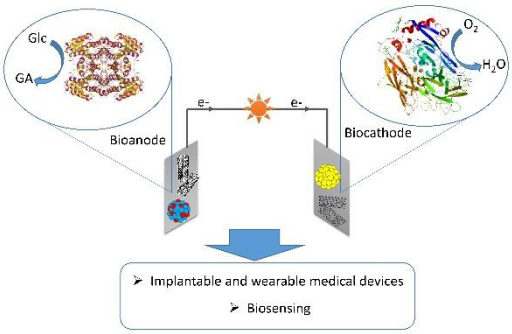
Abstract

Enzymatic biofuel cell (EBFC) is a particular sort of fuel cell (FC) that oxidizes its fuel using enzymes as catalysts instead of valuable metals. The enzymes such as laccase (Lac) or bilirubin oxidase (BOD) in cathode compartment and glucose dehydrogenase (GDH) or glucose oxidase (GOD) in anode compartment can be applied as biocatalysts in EBFCs. Power is produced by reducing oxidant (O_2) and oxidizing natural fuels including, glucose (Glc), fructose, and alcohols. Within the different conductive nanomaterials, excellent electrical conductivity, different shapes of carbon and metallic materials with particular morphology steady mechanical and thermal characteristics have significant roles in EBFC. Furthermore, EBFC has gained special consideration as a domain of nanotechnology applications. It is predicted to develop the applications of EBFCs as an implantable power source in the production of pacemakers, transmitters, miniaturized sensors, artificial organs, etc. Herein, we review recently published articles to sumerise the function of EBFCs, the enzymes used for EBFCs construction, and EBFCs applications in biosensing and as implantable and wearable medical devices.



Keywords: *Enzymatic biofuel cell, Carbon material, Metallic nanoparticles, Medical devices.*

Introduction

Fuel cells (FCs) are electrochemical energy-exchange devices; they transform the substrate chemical energy to electrical energy using metal catalysts.[1,2] Enzymatic biofuel cell (EBFC) is a particular sort of FC which oxidizes its fuel using enzymes as a catalyst rather than valuable metals. EBFCs, though currently restricted to available equipment, are promising energy-exchange devices regarding their relatively low-priced constituents and fuels and serve as possible power supplies or bionic implants.[3-5] EBFC is performed on a similar inclusive basis to all FCs: (i) electrons from a parent molecule are divided using a catalyst, (ii) the catalyst is forced to go around an electrolyte curtain by a wire to produce an electric current. EBFCs are different from stereotype FCs in two ways: (i) the fuels they accept and (ii) the catalysts they apply.[6-7] Regular FCs use metal catalysts such as platinum and nickel. In contrast, EBFCs use enzymes extracted from living cells (FCs that exploit entire cells to catalyze the fuel are called microbial FCs).[3,7] It is facile for enzymes to generate energy in high quantities, and so they have the advantage of economies of scale and are perfect for application in biofuel cells (BFCs).[8] During the FC's running, carbon monoxide is formed by the interplay of the carbon molecules with oxygen, which makes most organic compounds inappropriate fuels through FCs with metal catalysts. Carbon monoxide will rapidly "poison" the precious metals which the cell action depends on and render them ineffective.[9] The EBFCs fuel is exceptionally inexpensive; fuels such as sugars and other biofuels that can be cultivated and yielded to large extents. Biofuels are available in almost any part of the world, which makes them an exceptional engaging choice from a logistics stance, and still further, for environmentalists concerned with embracing sustainable energy sources.

The driving force or motive power required for rewarding reaction catalysis is a valuable enzymatic characteristic that makes EBFC a good choice in various applications and operates at potentials close to the substrate of enzymes. Moreover, the active sites surrounded by the protein matrix present many essential functions, internal electron coupling, selectivity for the substrate, the capacity to attach to other proteins (or the electrode), and acidic/basic properties.[10] Enzymes from thermophilic organisms can tolerate a more extensive range of temperatures. As, the normal state of enzymes operation is typically between 20 to 50 °C

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and at pH 4.0 to 8.0.[11-12] A drawback of applying enzymes is their sizes; for large enzymes, a low current density per unit electrode area is generated owing to the confined space. To solve this problem, immobilization on conducting carbon supports or 3-dimensional (3D) electrodes are applied, which offer a higher surface area. These electrodes are expanded into 3D space, which dramatically expands the surface area for enzymes to attach and intensifying the current.[11-13]

In this study, we review the function of EBFCs, the enzymes used for their construction, and EBFCs applications such as implantable and wearable medical devices and biosensing in recently published articles.

EBFCs operation and development

As a whole, Glc-driven EBFCs transform the chemical energy latent in Glc to electrical power with the aim of Glc oxidation at an enzyme-modified anode and a decrease of an ultimate electron acceptor (mainly molecular oxygen) at an enzyme-modified cathode.[14-15] The used conventional oxygen-reducing cathodic enzymes are including the multicopper oxidases like bilirubin oxidase (BOD) and laccase (Lac).[16-17] In general, EBFCs produce lower power densities compare to their traditional counterparts (i.e., direct methanol/ethanol FCs, solid oxide FCs, lithium-ion batteries, etc.). However, EBFCs guarantee the ease of miniaturization, applications as wearable and implantable devices, inherent biocompatibility, moderate optimal operating conditions, and high peculiarity.[18-19]

EBFCs operate based on terms of (i) open-circuit voltage (OCV) (i.e., monitored discrepancy in redox between anodes and cathodes without current) and (ii) highest power density (i.e., the amounts of energy generated per unit area; mW/cm^2). These two methods are the most useful ways for reporting EBFCs performance.[2] Power densities in the range of multiple mW/cm^2 have lately been recorded with OCVs near 1 V, which is approximately the maximum conceptual voltage obtainable in a Glucose/O_2 FC. The thermodynamic redox potentials of the reactions occurring on the cathode and anode surface control the Glucose/O_2 FC.[19-20] Such amounts have stimulated the *in vivo* study of various such systems.[21-22] The performance of these systems is determined by the active enzyme density monitored at the electrode surface area.[17] In comparison with established energy systems, EBFCs are unique due to being cost-effective, enabling enzyme selectivity towards the fuel, generating electricity from renewable sources, and being usable in physiological pH and temperature. Also, they have lower levels of energy density and power density and serve as an appropriate candidate for powering up implantable medical devices.[3,13,23]

The EBFCs functionality depends on the loaded enzyme (i.e., the quantity of employed enzyme immobilized at the electrode surface per unit surface) that is clarified as the rate of substrate turnover detected as electric current per unit area. Stability is another important problem in the possible application of EBFCs, both throughout the process and supply.[24] EBFCs not only take the requisites but also meet the need for consistent power generation.[25] Currently, for the broad applicability of these enzyme-based devices, stability is one of the significant obstacles to be tackled.

Enzymes used in EBFCs

Numerous mixtures of bioanode structures containing various enzymes like glucose oxidase (GOD), glucose dehydrogenase (GDH), alcohol dehydrogenase (ADH), and cellobiose dehydrogenase (CDH) have been applied. The multicopper enzymes are the most suitable options for biocathodes to achieve efficient electrocatalytic power generation from BFCs.[26-27]

Bioanode enzymes

GOD is one of the enzymes usually utilized in EBFC that oxidizes Glc into gluconic acid.[28] By its immobilization as a catalyst at the anode surface, it releases protons and electrons, which stimulates the oxygen reduction and water generation at the cathode surface.[29] It is the most favorable enzyme in EBFCs owing to the accessibility and remarkable selectivity towards Glc.[23] GOD is a globular protein with a dimeric structure, which is comprised of a redox-active core and a protein shell. Flavin adenine dinucleotide (FAD) is buried deep on its core within the protein shell. The distance between the FAD and the GOD surface is ca 1.7 nm, and the total enzyme molecule size is ca 8 nm × 5.5 nm × 6 nm.[30] It is unconvincing that direct electron transferring happens owing to the location of FAD and the magnitude of the 160 kDa protein. As yet, many researchers have discovered a peak around −0.5 to −0.4 V that is quasi-reversible (relating to the reference electrodes and pH) at electrodes modified with GOD, and they asserted to achieve direct electron transferring.[31] It is require to work under anaerobic states for bioanodes based on GOD, because molecular oxygen is a natural electron-transfer mediator for GOD and causes to current loss; molecular oxygen competes with electrode for attaining electrons from the enzyme.[32]

GDH and CDH are other prevalent enzymes with Glc as the substrate. Dehydrogenases can operate in aerobic conditions because GDH and CDH do not react with O_2. GDH has three redox cofactors; (i) pyrroloquinoline quinone (PQQ) and FAD are as two prosthetic classes, and (ii) nicotinamide adenine dinucleotide (NAD^+) is as cosubstrate. The immobilization of NAD^+ on the surface of electrodes makes the bioanodes synthesizing procedure to be difficult because NAD^+-dependent GDHs need the free NAD^+ in the electrolyte when they oxidize Glc. Conversely, PQQ- and FAD-dependent GDHs can tackle this issue because of conforming chemical bonds with PQQ and FAD.[32] Furthermore, ADH and formate dehydrogenase (FDH) can also be used as Glc-based biocatalysts in the EBFC anode.

Most EBFCs use fuels such as biological and small organic molecules. Also, inorganic molecules can be employed as fuels at bioanode fabrication for obtaining electronic communication between electrode surfaces and suited enzymes. Human sulfite oxidase (hSO) acts as a catalyzer and can convert sulfite into sulfate ions (equation 5). Sulfite oxidation occurs at the molybdenum (Mo)-containing cofactor, whereas the produced electrons can be transmitted from the active site (Mo) to the artificial electron acceptors or other natural redox partners by a heme domain.[33] Zeng et al. (2015) applied hSO for the first time, and Tang et al.(2019) used hSO to fabricate of EBFCs.[33]

The fundamental reactions of bioanode enzymes and their fuels were presented in the following sections:[32,33]

$$GOD(FAD) + Glc \rightarrow GOD(FADH_2) + Gluconic\ acid \quad (1)$$

$$GDH(PQQ) + Glc \rightarrow GDH(FQQH_2) + Gluconic\ acid \quad (2)$$

$$FDH(NAD^+) + Formic\ acid \rightarrow FDH(NADH) + Carbon\ dioxide \quad (3)$$

$$ADH(NAD^+) + Ethanol \rightarrow ADH(NADH) + Acetaldehyde \quad (4)$$

$$hSO + SO_3^{2-} + H_2O \rightarrow hSO + SO_4^{2-} + 2H^+ + 2e^- \quad (5)$$

Biocathode enzymes

Multi-copper oxidases, including BOD, Lac, and ascorbate oxidase (AO) are well known and massively explored. These enzymes are immobilized on the surface of solid supports and are widely applied as cathodes in EBFC, and they have been indicated to carry out oxygen reduction reaction (ORR). The critical electrons for ORR are transferred directly from the electrode to the enzyme without mediators. As for the cathode, it has been proven that BOD reveals a higher ORR durability and kinetic in compare to AO and Lac.[34]

BOD is obtained from plants, fungi, and bacteria.[35,36] The BOD has been extensively used for the diagnostic analysis of bilirubin in serum. Lately, BOD has drawn interest in applying the cathode of BFC as an enzymatic catalyst due to its better activity at neutral pH. It has high thermal stability and low susceptibility to the presence of chloride ions. BOD demonstrated lower redox potentials (near +650 mV versus NHE at pH 7.0). It is a monomeric protein with a molecular mass of 60 kDa and is composed of three domains. T1Cu acts as an important element in electron transfer from the substrate to a trinuclear Cu center. A pair of T3Cu atoms (T3aCu and T3bCu) and one T2Cu comprise the trinuclear Cu center. This center is responsible for the reduction of oxygen. T3aCu and T3bCu ions in the trinuclear Cu center have a connection to T1Cu via Cys457-His459 and His458-Cys457 amino acids. They form a Y shape and it is nearly 13 angstroms between Cu centers.[37]

Also, there are four Cu atoms in the active site of Lac. They are classified into three groups, and act similar to BOD coppers. They are responsible for the reduction of oxygen to water.[38] Lac can catalyze the oxidation of many phenolic substrates such as monophenols, diphenols, polyphenols, methoxy phenols, and aminophenols.[32] Lac is applicable in numerous industries, including chemical synthesis,[39] biobleaching dyes decolorization,[40] bioremediation for the oxidation and elimination of organic pollution in water and wastewater,[41,42] biosensing for the advancement of the phenolic compound biosensor,[43--47] and EBFCs fabrication. Lac can be applied as a biorecognition element in enzymatic biosensors to measure phenol and phenolic compounds, such as catechol in water and wastewater samples.[48] At pH values between 2.5 and 4.5, Lac is excessively active, and it is so sensitive to the physiological environment, being paralyzed in solutions containing chloride ions. However, it is an interesting choice for the cathode of EBFC because its redox potential can be as high as +780 mV against the normal hydrogen electrode [NHE; trametes hirsuta Lac measured at pH 6.0].[37]

AO is a dimeric enzyme of 140 kDa with three specific domains in each monomer. This enzyme can be separated into monomers at neutral to alkaline pHs. These two subunits consist of four copper ions with structures and responsibilities similar to BOD and Lac copper ions. Accordingly, AO is a glycoprotein, and the catalytic activity of this enzyme may be regulated by glycan moieties. Its catalytic performance seems to be enhanced when it losses sugars-deglycation (or nonenzymatic deglycosylation) because exoglycosidase treatment probably cause to better catalytic sites exposure.[49]

Mediated electron transferring

The complicated 3D structure of enzymes causes weak electrical communication between the redox active sites of the enzyme and the electrode. It is related to the oxidase/hydrogenase enzymes immobilized onto the surface of enzymatic electrodes. Redox mediators or chemical modification can be applied to be electrically conductive and overcome this obstacle.[26] To prevent energy waste, the redox potential of utilized mediators should be as near as practicable to enzyme redox potential. This causes to regulate the bioelectrodes voltage output. The general mediators for the anode are including quinone, ferrocene, and their derivatives like methylene green, azure dyes, ferri-/ferrocyanide, phenazines, small redox proteins, and redox polymers.[32] These redox active materials make more accessible the relay of electrons between the surface of electrodes and the enzyme's active sites. Nevertheless, their disadvantages are non-biodegradability and non-biocompatibility. Ferritin (Frt), a redox and an iron storage protein, has been employed as an eco-friendly, biodegradable, and biocompatible redox mediator. It appears to be a promising choice to overcome these flaws owing to its similar redox potential to that of GOD enzyme. It is a widespread protein that is available in all cells and can hold iron up to 4500 molecules in its core. As a redox mediator, it is a capable option for better electron transfer in EBFC anodes because its working potential is close to the enzymes' oxidation potential. The number of iron atoms in the Frt shell affects the number of transmitted electrons and the rate of electron transmission, unveiling the evident potential of Frt to hit the target of a biomaterial as a redox mediator for electron transfer in the bioanodes.[26,50,51]

Fabrication and modification of EBFCs using nanomaterials

Enzyme immobilization

It has been established that a pivotal factor in the process of EEBC is enzyme immobilization. The development of enzyme immobilization methods and electrode materials that permit close interaction between the electrode and the enzyme active site to decrease the necessary electron transfer distance is a major subject in the EBFC field[52] and a crucial stage in the fabrication of enzyme-modified electrodes.[53] However, due to insufficient steadiness and little reusability, enzymes should be immobilized by a practical technique as they are roughly unstable and cannot be reused.[54] There have been used several methods to immobilize them, such as entrapment by inorganic or organic polymer,[55] applying the 3D matrix,[56] adsorption by solid supports,[57] and forming a covalent bond using functional groups.[58] It can be argued that the biocatalytic strength greatly relies on the base platform material through immobilization and enzymatic interaction. Though, owing to the platform material effect on the biocatalytic system features, choosing the support material is the toughest decision.[29] Using covalent immobilization of enzyme in stabilized matrixes, including nanostructured and mesostructured metal oxides, conducting polymers, metal nanoparticles, sol-gel matrixes, mesostructured silica, graphene, and carbon nanotubes (CNTs) makes electron transfer faster and results in enhanced enzyme stability. They are essential materials that immobilize the enzyme and improve the electrical conductivity between the electrode surface and its active site.[23]

Carbon materials

Throughout the past years, large numbers of carbon materials, namely carbonized polymers, CNTs, 3D carbon composites, and graphene, have been one of the interesting topics extensively implemented in the realm of EBFCs that is owing to their numerous superiorities, i.e., excellent electrical conductivity, large specific

surface area, unique porous network structures, and good biocompatibility. Commonly, combining different materials including conducting polymers and metal nanomaterials, with conventional carbon nanomaterials through physical adsorption or chemical bonding has been recommended as a practical approach to create novel carbon composites for EBFCs. Hybrid materials are one of the consequential studies focuses owing to their synergistic behavior reached from the assets of both dual-layer capacitive substances, including CNTs, graphene, and their other derivatives, and pseudo-capacitive materials like conducting transition metal oxides and polymers. It is a commonplace conception that the efficient relation between carbon nanomaterials and the conjugated backbone of conducting polymers, for example polyaniline (PANI), facilitates the diffusion of the charge through the components of the hybrid assembly, which is followed by the advancement in the conductivity of the hybrid systems.

A common approach is carbonizing of nanostructured polymers, like polypyrrole (PPy) and PANI, to obtain various kinds of microporous carbon nanomaterials. Kang et al. affirmed that the nanofiber such as PANI would be pyrolyzed to a spherical or granular configuration at carbonization temperatures higher than 1000°C. To preserve its primary structure, they generated a 3D PANI@Gr composite based on the powerful covalent bonds and carbonized it at 1600°C.[31] Subsequently, for achieving more power density in another Glc/O_2 BFC, a 3D PANI1600@CNT composite was provided. Kang et al. fabricated a unique 3D PANI1600@CNTs composite that had a structure like a rhizobium. For synthesize of this composite, aniline monomers were in-situ polymerized around and along the functionalized CNTs and later carbonized at 1600 °C. The carbonized PANI could gather the CNTs into a 3D network due to the performing uniformly conductive “glue” and joining the obtained tubes together.[31] In another study, Kang et al. (2019) carbonized a tube of rectangular polypyrrole (RPPy) at a high temperature to produce an innovative carbon tube and used it for the formation of EBFCs with elevated function. When carbonized RPPy is used, the Lac or GOD modified electrodes showed an outstanding bioelectrochemical performance.[59]

A beneficial technique for preparing thin films via multiwalled carbon nanotubes (MWCNTs) films is electrophoretic deposition (EPD) that it obtains surfaces with appropriate thickness as EPD-MWCNT. Generally, utilizing an applied electric field, MWCNT powders can be driven to reach an electrode surface after uniformly suspended in a desirable liquid. Afterward, the MWCNTs assemble on the surface electrode via an opposite charge and generate a cohesive film. Zhong et al. utilized EPD-MWCNT films as electrode supports to construct nanostructured MWCNT films for high-yield EBFCs. They modified EPD-MWCNT films with Lac and FAD-GDH to prepare the biocathode and bioanode, respectively. These EBFCs exhibit greater power density than that of EBFCs relying on MWCNT films using drop-casting or buckypapers.[60]

Chung and Kwon fabricated [(TPA/HRP/GOD)]/PEI/CNT catalysts containing polyethylenimine (PEI) polymer beside terephthalaldehyde (TPA) cross-linker and two enzymes of horse radish peroxidase (HRP) and GOD. It was proved that the [(TPA/HRP/GOD)]/PEI/CNT catalyst induced improvements of EBFC performance and catalytic activity as a result of proper elimination of toxic H_2O_2 molecules by HRP, outstanding Glc reactivity by GOD, and strong bonding of the structure by TPA.[61]

When enzymes like GOD are directly deposited onto CNT fibers they act as conductive supports for EBFCs and can further solutions to overcome the low power efficiency of direct electron transferring -EBFCs.[62]

Graphene and its derivatives have presented novel possibilities for the planning and creation of next-generation EBFCs. With considering these outstanding features, graphene has a high capacity to provide solutions for the previously noted problems in EBFCs area. For instance, biological compatibility is beneficial to preserve the activity of enzymes; more sites to immobilize enzymes can be obtained because of its large surface area; the better electron mobility and conductivity assist the electron transmission between electrodes and redox enzymes.[32] Moreover, the fabricated electrode based on 3D graphene may consequently be attractive support for the enzyme immobilization with effective heterogeneous electron transfer in electrocatalytic systems. Up to now, a great number of studies addressing graphene-based materials and their functions have been reviewed. Babadi et al. (2019)[23] fabricated a 3D bio-nanocomposite using graphene/GOD for raising enzyme lifetime and enzyme immobilization with an improved electron transfer rate. The better electrical conductivity of the 3D graphene promotes the direct electron transferring between the modified GCE and the active site of the GOD and reduces the resistance to electron flow.

Tang et al. (2019) reported a modified electrode based on 3D graphene suited to hSO immobilization. For electrode fabrication, carbon papers were coated with graphene oxide, and then graphene-polyethylenimine composites were drop-casted. The negatively charged hSO can be assimilated electrostatically on the positively charged matrix on carbon papers' electrodes coated with graphene oxide.[33] Using the electroreduced 3D graphene support leads to rise of local microenvironment electronic conductivity, decline in the electron transfer resistance of the interface between the electrolyte and the electrode surface, and obtain a greater immobilization of hSO through electrostatic interaction. Tang et al. reported that the proposed hSO bioelectrode displayed a substantial catalytic rate and efficiency compare to the other hSO bioelectrodes.[33]

The aggregation of many horn-shaped sheaths of single-walled graphene sheets results in individual particles called carbon nano-horn. Kuroishi et al. fabricated a film-like EBFC using the carbon nano-horn and drawing on micro-electro-mechanical systems technology.[63]

Metallic nanoparticles

Ji et al. (2020)[64] showed a heme mimicking nanostructure, which enabled the efficiency of EBFC to increase by employing the double function of iron- and nitrogen-co-doped CNT (Fe–N/CNT) catalysts. The Fe–N/CNT is straightly utilized for ORR as a cathode catalyst when mingled with GOD and PEI to build GOD/PEI/[Fe–N/CNT]. For the fabrication of the GOD/PEI/[Fe–N/CNT], PEI was employed to strengthen the physical interplay through electrostatic affinity and entanglement while the GOD was doped onto the Fe–N/CNT surface. It was an original study that exhibits the potential of the heme mimicking nanocatalyst as both cathodic and anodic catalysts for EBFCs.

Gholami et al.,[65] developed a mediatorless/membraneless EBFC using bipolar electrochemistry (BPE). For the fabrication of bioanode, they electroplated an Au-bipolar electrode through BPE

to derive Au nanostructures (AuNSs) for the immobilization of the FAD-GDH enzyme. Also, BOD immobilization was prepared based on the electropolymerization of thiophene-3-carboxcylic acid (TCA) on an Au microfilm as a bipolar electrode. This biocathode demonstrated a high electrocatalytic activity toward direct ORR.

Kwon et al. (2019)[62] fabricated layer by layer (LbL)-assembled hybrid gold nanoparticles (AuNPs)-coated carbon nanotube fibers (Au-CFs) electrodes with good operational stability, high ORR activity, and remarkable electrical conductivity. They demonstrated that Au-CF electrodes could be applied as conductive supports for anodes as well as electrocatalytic cathodes. The LbL-assembled GOD multilayers (m-GOD/Au-CF) bioanode provided a favorable immobilized enzyme conformation as well as an effective electron communication between the highly conducive Au-CF electrode and the immobilized GOD. Moreover, the internal resistance was decreased and enzymatic reaction and charge transfer were facilitated. The stable formation of electrochemically GOD layers and active AuNPs arrays utilizing small NH_2-functionalized organic linkers (tris-(2-aminoethyl)amine (TREN)) improved performances. Therefore, under a fixed external resistance, the hybrid EBFCs generated a high-power output, superior to conventional CNT-based EBFCs measured under the same conditions.

Kang et al.,[61] studied a catalyst for promoting ORR EBFC performance. It consisted of Lac and AuNPs- naphthalenethiol (NPT), which were linked to PEI and CNT. They reported that CNT/PEI more immobilization of Lac and AuNPs-NPT was occurred and CNT/PEI acts as electron relay between Lac and AuNPs/PEI by electron collection effect and between NPT and AuNPs by thiol-gold bond.

Developing hybrid devices can be done by integration of supercapacitors with EBFCs to harvest higher power output. Xiao et al. prepared a supercapacitor/BFC hybrid device by immobilizing redox enzymes using electrodeposited poly(3,4-ethylenedioxythiophene) (PEDOT) and redox polymer of $[Os(2,2'\text{-bipyridine})_2(\text{polyvinylimidazole})_{10}Cl]^{+/2+}$ ($Os(bpy)_2PVI$) on dealloyed nanoporous gold. Tuning the deposition circumstances can easily control the deposited layer thickness. The hybrid device displayed good operational stability for 50 charge/discharge cycles and ca. 7 hours at a discharge current density of 0.2 mA cm^{-2}.[66]

The incorporation of AuNPs into an electroactive polymeric framework improved the redox activity of the bio-electrodes. Mishra et al. developed the GOD bioanode fabrication using an interfacial polymerization-based strategy named the Au@PANI nanofiber network. Its combination with a similarly constructed Lac biocathode for developing of EBFC demonstrated increasing of electrocatalytic activity of the metal-polymer nano-framework-based EBFC.[67]

Enzyme-copper hybrid nanoflowers can be prepared to yield a flower-like morphology with high stability and activity. Chung et al. used GOD and Lac nanoflowers as anode and cathode electrodes, respectively, to fabricate an EBFC. Both greatly enhanced stability performance and high power density output were obtained. It is anticipated that enzyme nanoflowers can be utilized for various enzymatic catalysis-based applications such as biosensors and biocatalysis.[61] Based on enzyme nanoflowers, we constructed Lac nanoflower biocathode and GDH bioande to develop a novel Glc/O_2 EBFC. To prepare the bioanode, GCE was modified by the LbL structure of GOD nano-sheets, GDH, and NAD^+ via electrostatic affinities, and the capping layer consisted of glutaraldehyde, bovine serum albumin (BSA), and GDH. Lac nanoflowers were immobilized onto AuNPs that were electrodeposited on a gold electrode. Furthermore, a polydopamine biofilm was employed to keep the immobilized Lac nanoflowers in place. The biocathode and bioanode were integrated into a membrane free Glc/O_2 EBFC system. The corresponding stability and performance of developed EBFC were assessed *in vitro*. The results demonstrated its high-performance and acceptable stability to employ it in implantable medical devices.[68]

Different applications of EBFCs

EBFCs can be applied as an energy source to power-miniaturized and implantable devices.[27] EBFCs are utilized as high-quality sources for supply power to artificial human organs, namely insulin pumps, brain simulators, pacemakers, point of care (POC) diagnostic of Glc concentration in the bodily fluid, e.g. blood, urine, saliva, and a plethora of security implementations.[27]

With increasing the power demands of wearable electronic devices, the on-body energy-extracting methods has been developed. One of the proposed productive methodologies is implementing of nanomaterials in EBFCs assembly to achieve high power generation. Kumar et al. (2018) have recorded the relevant and exciting approaches applied in enhancing EBFCs, with a particular emphasis on Glc as biofuel, employing various nanomaterials for EBFCs, and emphasizing the revolutions in this field.[69] The broad applications of implantable EBFCs have become very attractive in biomedical sciences. Zebda et al. focused on medical and physiological features and reviewed the improvement of electrochemistry of EBFC technologies that affect the biocompatibility of EBFCs operating inside a living body. They questioned the power source for implanting of medical instruments, facing challenges of lithium battery strategy and the ability of implantable EBFCs to be credible alternatives to grant the amount of power needed for medical devices. They concluded that the physiological limitations and related ethical considerations are necessary when using EBFCs planned to be implanted for long-term use inside a living animal and finally applicable to human clinical functions.[70]

Wearable and implantable EBFCs

The progress of non-invasive portable electrochemical biosensors has drawn considerable attention. They can diagnose health conditions to investigate physiological fluids except blood analytes (e.g. saliva, tears, sweat, and urine). These fluids are easily achievable and do not necessitate any invasive measures. Textile sensors and epidermal contact lenses have been applied. Nonetheless, a power supply is essential for their constant operation, and subsequently, different energy sources like vibration, light, temperature differences can be exploited. On the contrary, EBFC is an assuring technology for wearable power sources. It can produce electricity under conditions of normal pressure, normal temperature, and neutral pH and show excellent biocompatibility and little environmental loading. Another application of EBFCs is that they work as sensors if their output is dependent on the concentration of the biomarker. Furthermore, an elementary wearable sensor that consumes less power can be constructed because additional energy is not required for working the potentiostat. One of the main problems related to use of other

Table 1. Table 1. Some wearable and implantable EBFCs and comparing their power density

Anode	Cathode	Power density	EBFC type/site	Ref.
GDH/A-CNT/PolyMG/A-CNT/fiber electrode	PTFE-CNT/BOD/A-CNT/PTFE-CNT/fiber	48–216 μW/cm^2	Wearable/cloth	[72]
Copper wire	Chit/MWCNT/Lac	-	Implantable/rat body	[48]
GDH, r (PAA-PVI-[Os(dmo-bpy)$_2$Cl]$^{+/2+}$), PEGDGE/SPCE	BOD/SPCE	15.26–38.33 nW/cm^2	Implantable/ human dermal fbroblasts	[73]
PQQ-GDH/Polythiophene/Buckypaper/ITO	BOD /Polythiophene /Buckypaper/ITO	2-10 μW	Implantable/slug	[74]

Methylene green (MG); Polytetrafluoroethylene (PTFE); acid-treated CNT (A-CNT); poly(ethylene glycol) diglycidyl ether (PEGDGE); screen-printed carbon electrode (SPCE); Indium tin oxide (ITO).

physiological fluids is the lower concentration of the target analyte compare to blood. For instance, the concentration of Glc in tears and saliva is up to 20-fold lower than in the blood. On the other hand, Glc levels in urine are observed to associate with blood Glc levels and are high, compare to saliva or tears. Urinary Glc is observed when the blood Glc level surpasses the kidneys' threshold (≥10 mM). Glucose concentration in the urine is higher than 2.5 mM for diabetic patients, which is satisfactory for supplying electronic circuits. Any differences in urinary Glc level are significant in diabetes monitoring, prevention, and treatment.[71] In the following, some recently fabricated wearable and implantable EBFCs were reviewed, and Table 1 shows some data of them.

Yin et al. assimilated two elements to design wearable EBFCs with flexible enzyme/MWCNT fibers on a cotton textile: (i) bioanode fibers for Glc oxidation and (ii) O_2-diffusion biocathode fibers for oxygen reduction. The anode and cathode fibers were provided by modifying GDH and BOD on MWCNT-coated carbon fibers, respectively.[72]

Enhancing the *in vivo* efficacy of an implanted EBFC can be achieved by improving the biocompatible diffusing polymers that are serve as buffering diffusion blockages. Sarra El Ichi-Ribault et al. (2018) used a bioelectronic device comprising an EBFC connected to a wireless teletransmission media embedded in a rabbit and monitored and checked its function *in vivo* for 2 months. At the end of the 18th day of implantation, the teletransmission media was used to wirelessly charge and discharge the implanted EBFC *in vivo* through a 100 kg load for 30 min each day. For an additional 16 days of operation, the EBFC delivered 16 mW/mL Glc continuously during every 30 min discharge each day. After 2 months of implantation, the power output vanished probably due to inflammatory mechanism.[48]

Jeon et al.[73] performed a fundamental cell culture study utilizing GDH and BOD as anode and cathode enzymes, respectively. The prepared EBFC demonstrated power densities of 15.26 to 38.33 nW/cm^2 based on the enzyme concentration in the system with an addition of 25 mM Glc. Despite the low power density, the GDH-based EBFC demonstrated growth in cell viability (~150%) and cell migration (~90%) with an approximately slight inflammatory response. Owing to the lethal concentration of H_2O_2 byproducts (~1500 μM) for GOD, the GDH-based EBFC was regarded as an auspicious implantable means for producing electricity in biomedical application.

Bollella et al. (2019)[74] used biocatalytic buckypaper electrodes modified by PQQ-dependent GDH and BOD for Glc oxidation and O_2 reduction, respectively. They used an EBFC with a small size (millimeter-scale; 2 × 3 × 2 mm^3). It was experimented in a model glucose-containing aqueous solution in human serum and as an embedded tool in a living gray garden slug (Deroceras reticulatum). The electrical power was generated in the range of 2-10 μW. This instrument microelectronic of temperature-sensing was prepared by a rechargeable supercapacitor, wireless data downloading capacity, and internal data memory particularly devised for activation by the EBFC. The power management circuit granted the optimized consumption of the power generated by the EBFC to be relied on the sensor operation activity. The total system, including power-generating EBFC and power-consuming sensor, is operated separately through harvesting electrical energy from an accessible environmental source, as represented by harvesting power from the glucose- incorporating hemolymph (blood substituting biofluid) in the slug. The data was read out wirelessly.

EBFC-based self-powered biosensors

EBFC-based self-powered biosensors can present considerable superiorities: simple miniaturization, simple instruments, and no requirement for any additional power sources. However, they also undergo restrictions like lower sensitivity or special goals.[75]

Shitanda, et al. (2019)[71] fabricated a six glucose/O_2 EBFC array arranged in series utilizing screen printing as a self-powered glucose sensor. It showed an electromotive force of 3.2 V. Porous carbon electrodes were constructed using screen printing of MgO-templated carbon on water-repellent paper to enhance the efficiency of the cathode and consequently to hinder it from being the limiting step. The bioanode included tetrathiafulvalene as a mediator and GOD as a catalyst, and the cathode contained BOD as a catalyst for O_2 reduction. A good linear relationship was achieved between the output of EBFCs and glucose concentration (1–25 mM) that comprises a range of urine glucose levels. The artificial urine components did not affect the output of the EBFC, but the output was reduced by low buffer capacity and low ion conductivity.

Also, the biosensors based on EBFCs or self-powered biosensors have been prosperously applied for discovering of toxic pollutants, immunoassays, biomolecules, tumor markers, and tumor cells.[76,77] Despite their typical counterparts, EBFCs-based self-powered biosensors had outstanding properties such as no requirement for external power supplies, excellent anti-interference function, easy miniaturization, and being inexpensive.[76] Li et al. (2020) favorably encapsulated Lac in zeolitic imidazolate framework-8 (ZIF-8) and united it using bacterial cellulose (BC)/carboxylated MWCNTs (c-MWCNTs) skeleton to fabricate BC/c-MWCNTs/ZIF-8@Lac electrode with high flexibility. BC is a green biological substance with favorable flexibility and biocompatibility. This electrode is ingeniously composed of a highly flexible self-powered sensing platform depending on BFC obtained from single-enzyme, detecting bisphenol A (BPA) as a model analyte.[77]

Table 2. Some EBFCs used as self-power biosensors

Anode	Cathode	Target	Linear range	Detection limit	Power density	Current density	Ref.
MgO-template carbon	BOD	Glucose	1–25 mM	-	0.12 $mWcm^{-2}$	0.47 $mAcm^{-2}$	[71]
BC/c-MWCNTs/ZIF-8@LAC	BC/c-MWCNTs/ZIF-8@Lac electrode	BPA	0.01–0.4 mM	1.95×10^{-3} mM	3.68 W m^{-3}	-	[77]
FAD-GDH/Th-AuNPs/ACNT/Gr	BOD/CNTs/Gr	Glucose	0.5–6.9 mM	50 µM	0.27 mW cm^{-2}	0.925 mA cm^{-2}	[78]
GDH/c-MWCNT/GCE	Aptamer/Au electrode	ATZ	10–200 nM	7.5 nM	15.3 µW cm^{-2}	-	[79]
SH-Sgc8c aptamer/AuNPs/g-C_3N_4	BOD/AuNPs	CTCs	20–2 × 105 cells mL^{-1}	10 cells mL^{-1}	-	-	[76]
FAD-GDH/P_2Mo_{18}/PMA /MWCNT	-	Glucose	1–20 mM	-	-	0.34 $mAcm^{-2}$	[80]
GDH/N-CNT/ carbon paper electrode	HCR/AuNPs	SNPs (p53 gene fragment)	0.1–500 pM	20 aM	-	-	[75]
SiO_2@AuNPs-csDNA/ GOx/AuNPs/ carbon paper electrode	Lac/PDA/AuNPs	AMP	10 pM –100 nM	3 pM	-	-	[81]
GOD/CNT/AuNPs/ITO	ITO; adding DNA functionalized PMSN to the buffer solution	miRNA-21	0.01−1000 fM	2.7 aM	-	-	[82]
GOD-AuNPs-PAE	BOD-AuNPs-PCE	miRNA-21	5 fM–100 pM	2.7 fM	132 µW·cm^{-2}	18.5 µA/cm^2	[83]

AminoCNTs (ACNTs); thionine (Th); DNA hybridization chain reaction (HCR); polydopamine (PDA); Ampicillin (AMP); positively charged mesoporous silica nanoparticles (PMSN); Paper anode electrode (PAE); Paper cathode electrode (PCE)

A 3D framework comprised of thionine and AuNPs grafted on amino-CNT/Gr support is formed as a unique platform for improving the activity of enzymes toward glucose oxidation. The well-arranged nanorods were created after the sonication of thionine with AuNPs. The assimilation of these frameworks with amino-CNT through electrochemical treatment is the main factor for useful enzyme entanglement and successive electron transmission. The performance of the bioanode combined with BOD immobilized on amino-CNT/Gr, as biocathode, in an assembly glucose/O_2 EBFC was investigated, and an OCV of 0.705 V was obtained. Under a 5-mM glucose concentration, as a normal concentration in physiological fluid, current and power densities were obtained 0.925 mA/cm^2 and 0.27 mW/cm^2, respectively. Furthermore, the proposed bioanode was capable of sensing glucose at a concentration range of 0.5 to 6.9 mM with a detection limit of 50 µM.[78]

Wang et al. (2020)[79] fabricated a self-powered aptasensor (SPA) system by immobilizing aptamers on the cathode surface to detect environmental pollutant, atrazine (ATZ), for the first time. This sensing platform was fabricated using aptamer loaded gold electrode as biocathode and GDH modified electrode as bioanode to generate electrons to recognize target. It could sense the target rapidly by a redox probe, $[Fe(CN)_6]^{3-}$, as the "key" of electron transfer switch and the difference of output power density once ATZ was trapped by the cathode.

A light-driven membrane-less and mediator-less self-powered cytosensing platform was offered through a combination of BFCs and photoelectrochemical technology for ultrasensitive detection of circulating tumor cells (CTCs). The sophisticated designed photoelectrode (SH-Sgc8c aptamer/AuNPs/g-C_3N_4) was used as an alternative anode to create the cytosensor for glucose oxidation, avoiding the introduction of the anodic enzyme. At first, the glucose could advantageously arrive at the photoanode surface and was simply oxidized by the photogenerated holes. Then the photogenerated electrons would transfer to the biocathode and the biocatalytic reduction of O_2 was occurred, which caused a high E^{OCV}. Nevertheless, CTCs could preferentially interact with Sgc8c aptamer through a particular detection. Consequently, the complex with large steric obstruction was immobilized on the photoanode surface, which could highly affect the electron transmission between glucose and photoanode surface.[76]

Two inorganic-organic hybrid materials depending on heteropolyoxometalates (POMs), including $(C_4H_{10}N)_6[P_2Mo18O_{62}]$. $4H_2O$ (P_2Mo_{18}) and $(C_6H_8NO)_4[H_2P_2W_{18}O_{62}]$. $6H_2O$ (P_2W_{18}), were used as mediators for electron transferring between MWCNT and FAD-GDH matrixes in glucose EBFC and biosensor applications. P_2W_{18} and P_2Mo_{18} were immobilized on 1-pyrenemethylamine (PMA) functionalized MWCNT deposits. A 10-fold enhancement in a catalytic current and a moderately lower OCV of -0.10 V vs SCE were detected for the electrode modified with P_2Mo_{18}. The evident excellence of P_2Mo_{18} is related, partially to its improved incorporation in the MWCNT matrix correlated to P_2W_{18}. The mediated electron transfer capacities of the POMs were also investigated in a glucose sensor setup and were satisfying for glucose detection.[80]

A prepared self-powered biosensor for recognizing of single nucleotide polymorphisms (SNPs) was fabricated by combining EBFCs with a DNA amplification technology. This self-powered biosensor demonstrated not only outstanding capacity to determine the p53 gene fragment from random sequences (e.g., single-base mutant sequences) but also showed superior sensitivity with a detection limit of 20 aM. Additionally, the results of the real cell lysate sample have laid a foundation for disease diagnostics and, potentially, act as a valuable tool for even more areas.[75]

A EBFCs-based self-powered aptasensing platform was reported for antibiotic residue detection. In this work, DNA bioconjugate, i.e., SiO_2@AuNPs-complementary strand of aptamer (SiO_2@AuNPs-csDNA) was created, which had a pivotal

role in blocking the mass transport of glucose to the bioanode. Owing to the aptamer detection of the target, SiO_2@AuNPs-csDNA bioconjugate broke away from the bioanode in the presence of the target antibiotic. Without the blockage of glucose by the DNA bioconjugate, a considerably higher OCV of the EBFCs-based aptasensor was acquired that its amplitude was relied on the antibiotic concentration.[81]

MicroRNAs (miRNAs), as a small noncoding sequence with 18 to 25 nucleotides in length, act as a pivotal factor in numerous biological processes, namely gene expression, transcription, and biological progress, involving cell proliferation, apoptosis, hematopoiesis, and differentiation. The unusual expression of miRNAs would cause the formation, invasion, and metastasis of cancer. Thus, miRNAs have been regarded as an encouraging group of biomarkers for early diagnosis. Gai et al. offered a new homogeneous self-powered biosensing technology via integration of BFC and homogeneous electrochemical procedure, which was further used for ultrasensitive miRNA observation. To fabricate such an assay protocol, the cathodic electron acceptor $[Fe(CN)_6]^{3-}$ was entrapped in the pores of positively charged mesoporous silica nanoparticles and capped by bio-gate DNAs. Once the target miRNA existed, it would stimulate the controlled release of $[Fe(CN)_6]^{3-}$, resulting in a dramatic increase of OCV. Accordingly, the "signal-on" homogeneous self-powered biosensor for the ultrasensitive miRNA assay was realized. This study shows a prosperous prototype of portable and on-site biomedical sensor.[82] In another study, the paper supported glucose/O_2 EBFC-based self-powered sensing platform was developed for visual analysis. The AuNPs paper fibers were used for modification of EBFC device. GOD and BOD were employed to prepare bioanode and biocathode, respectively. A target responsive cargo release system was designed based on mesoporous silica nanocarrier controlled by miRNA-21. Based on the H_2O_2 mediated iodide oxidation reaction to form iodine that further modulated the starch chromogenic reaction, undesired H_2O_2 could be effective eliminated, resulting in exceptionally enhanced EBFC efficiency as well providing a way for visual signal readout.[83] Summarized data of EBFCs used as self-power biosensors were shown in Table 2.

Conclusion

In comparison with the established energy systems, EBFCs are unique due to being cost-effective, enabling enzyme selectivity towards the fuel, generating electricity from renewable sources, and being useful in physiological pH and temperature. However, they have lower energy density and power density. These advantages make them an appropriate candidate for powering up implantable medical devices like micro-drug pumps and pacemakers. Also, they are even used in drug delivery, wastewater treatment, remote sensing, biosensors, and communication systems in bioelectronics.

Commonly two characteristics, including OCV and output power density, are used to describe EBFCs performance. The EBFC applications are mainly in the areas of implantation for endogenous physiological devices. For fabrication of these devices, a combination with nanomaterials renders higher power densities. EBFCs have a defect, such as comparatively low power generation due to the challenge associated with electron extraction from enzymes, compared with valuable noble metals. Concerning FCs, the use of enzymes has numerous advantages. However, the existing cutting-edge research demonstrates that the lower output, fabrication cost, shorter longevity, high maintenance, and weak viability of the enzymes are the chief obstacles in harvesting EBFC's to their entire performance. Besides, to postulate the best possible state of electrode, such as an appropriate choice of electrode materials, the variety of their mechanical and electrical characteristics and stiffness of the materials to guarantee the optimized surface-to-volume ratio is fundamental.

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