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Review

Wired enzymes in mesoporous materials: A benchmark for fabricating biofuel cells

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ABSTRACT

Evolution of fuel cells using metallic inorganic catalysts has led to the development of biofuel cells with potential applications in implantable devices. However, the main disadvantages in real world applications of enzymatic biofuel cells are short lifetime and low power density. Many efforts have been devoted to immobilize redox enzymes on surfaces to allow efficient electrical communication with electrodes and to provide an adequate habitat for biochemical activity. In this context, nanocavities of mesoporous materials offer a tailored environment for protein immobilization. Mesostructured platforms with high surface area and stability have been developed to enhance mass transport, charge transfer from biocatalysts to electrodes and enzyme stability, leading to biofuel cells with improved power density (up to 602 μW cm^{-2} at physiological conditions) and overall performance (high stability after 30 h of continuous operation and after 10 days of storage). This review discusses recent developments using mesoporous materials as novel platforms for effective electronic charge transfer in the context of current and emerging technologies in enzymatic fuel cell research, emphasizing their practical implications and potential improvements leading to a major impact on medical science and portable electronics.

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Over the last decade, interest in enzyme-based bioelectronics has flourished because of its potential applications in implantable and non-invasive medical devices. Redox enzymes have enabled integration with diverse electrode designs to develop several commercial biosensors [1]. More recently, hybrid systems in which enzymes are wired on electrodes are filling other useful niches including industrial biocatalyst and biofuel cell (BFC) development [2].

BFCs constitute a promising alternative to classical fuel cells that use metallic inorganic catalysts usually affected by poisoning or metal leaching. In BFC systems, specific enzymatic reactions are used to generate direct chemical to electrical energy conversion. BFCs offer ease of design, lower operational temperature, higher environmental compatibility and broader range of available fuels compared to traditional fuel cells. Additionally, enzymes are easy to mass-produce and genetically engineer through biotechnology techniques, while common biofuels (e.g. sugars) can be obtained on a massive scale. On the other hand, BFCs can make use of glucose present in biological fluids, which is interesting for applications in implantable devices. These characteristics render BFCs as an extremely cheap and portable alternative for energy production.

However, the main current bottle-neck problems in real applications of enzymatic BFCs are their short lifetime, low power density and electrode surface fouling by biological components [3–5]. To address these issues, much effort has been devoted to protein engineering approaches for obtaining more stable enzyme forms and for optimizing the accessibility of their prosthetic active sites in order to enhance the electronic charge transfer with electrode surfaces [6]. In addition, significant improvements have been made by using improved electrode materials and enzyme immobilization technologies [7]. Versatile devices normally require enzyme immobilization on electrode surfaces to establish more efficient electrical communication with the biocatalysts. In principle, enzymes are typically immobilized using different routes as illustrated in Scheme 1A: a) physical adsorption [8], b) covalent binding [9], c) cross-linking [10] and d) encapsulation [11–16]. Each immobilization method presents several advantages and limitations. Enzyme physical adsorption on electrode surfaces stands out as the simplest approach for protein immobilization. However, this type of interaction is frequently too weak to prevent the enzyme leaching into the reaction media, and the active site can be altered during the adsorption process. A common method to diminish enzyme leaching is the covalent binding of enzymes on surfaces, at the cost of additional synthetic steps. Again, the most important penalty paid by covalent post-immobilization is the alteration of the structural conformation of the anchored enzymes.

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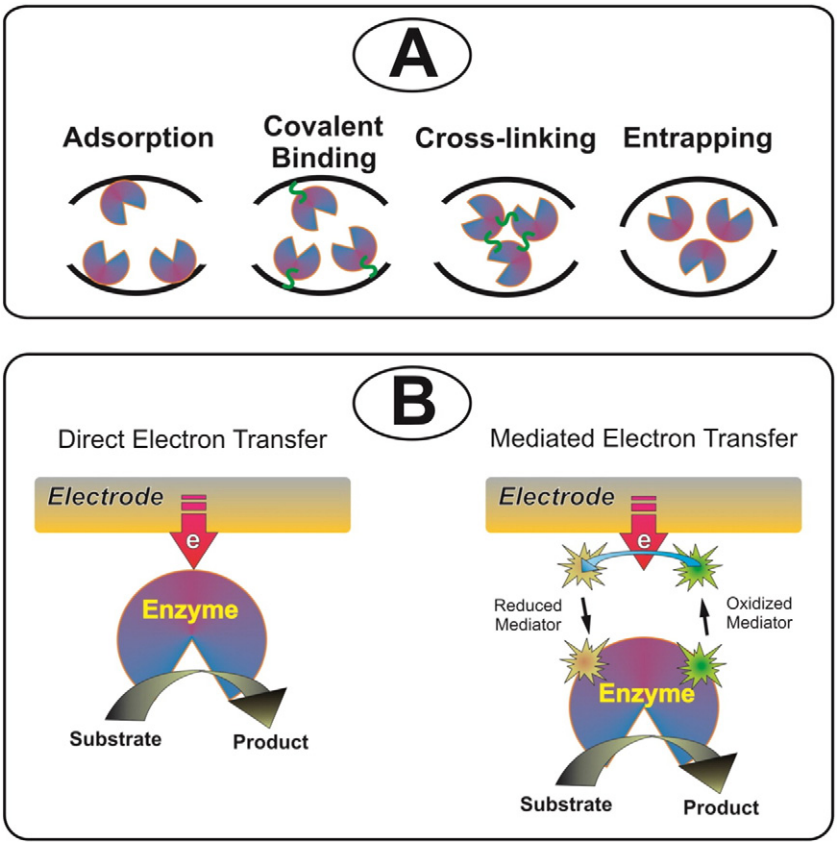

Scheme 1. A) Possible enzyme immobilization approaches for BFCs design. B) Alternative electron transfer mechanisms in BFCs.

Several organic synthesis strategies are available for protein immobilization on functionalized surfaces [17]. Therefore, cross-linking is a simple and effective method to immobilize enzymes on various surfaces with high stability because it prevents enzymes from leaching and consequently improves the enzymatic stability. This approach can be particularly benefited by the use of porous materials for enzyme immobilization [18,19]. A major disadvantage of this method is the formation of poorly controlled aggregates; in addition, the substrates for enzyme catalysis may not easily diffuse thru the cross-linked enzyme structure. Finally, another strategy for enzyme immobilization in porous materials, which suppresses leaching, is to directly form polymeric or inorganic frameworks in the presence of enzymes. This leads to crosslinked networks that have good mechanical strength and stability. However, soft synthesis procedures and biocompatible precursors are mandatory in order to maintain the bioactivity and function of immobilized enzymes [20].

Another extremely important issue in BFC design, is to achieve an efficient electronic charge transfer between the enzyme and the electrode surface. In this framework, enzyme immobilization strategies have to fulfill multiple requirements: a) "protein friendly" environments in order to minimize enzyme denaturing processes, b) open structures for substrate and by-product diffusion that avoid enzyme leaching, and c) enhanced electrical communication between the redox enzymes and electrode surfaces for effective electron shuttling [3,21]. In this context, the development of robust and highly accessible nanomaterials applied to BFC research could provide a series of advantages, namely improve charge transfer and mass transport, greater enzyme stability and immobilization, and increased surface area of electrodes with high conductivity. As a matter of fact, energy extraction/conversion from a BFC device highly depends on the wiring efficiency of the enzyme with the electrode, and is one of the critical aspects in BFC design. Namely, electron transfer in a BFC can occur through two mechanisms: mediated electron transfer (MET) and direct electron transfer (DET) (Scheme 1B). MET requires redox molecules capable of shuttling electrons between the enzyme and the electrode [22,23]. MET poses some drawbacks, such as higher costs, thermodynamic loss between mediator and enzyme, leading to voltage losses and mediator liability imposing limited lifespan of BFCs [24]; in addition, in some cases, specialized organic chemistry synthesis techniques are required. Conversely, DET mechanisms can eliminate the inconveniences associated with MET processes, as electrons tunnel directly between the electrode and the enzyme. The direct electrochemical process can significantly improve the energy conversion efficiency: BFCs can operate close to the thermodynamic redox potential of enzymes maximizing the open-circuit voltage (OCV). However, the vast majority of DET-based BFCs yield low power outputs and the design of effective DET systems is still a challenging and controversial task [24,25]. In this sense, functional nanostructured electrodes with co-immobilized enzymes, can act as electrical wires facilitating the occurrence of the electronic charge transfer process with the following remarks: a) reduction of the electron tunneling distance between the active enzyme site and the electrode surfaces b) higher enzyme loading due to higher specific surface areas with improved stability and enhanced kinetics of the immobilized enzyme [3] and c) possibility to process the nanomaterials in diverse forms [26,27] such as nanoparticles, thin films, nanofibers, nanocomposites and mesoporous materials. These characteristics open the path of high-performance enzymatic BFCs.

The requirement of electrodes with increased surface areas and open frameworks for diffusion of reactants and by-products in the enzymatic energy generation can be fulfilled by using mesoporous materials for bioelectrochemical applications. These mesoporous frameworks can be tailored in order to control the pore geometry (shape, interconnectivity), pore size in the range of 2–50 nm, and surface chemistry. Within the mesoporous structure, catalysis, sensing, sorption and enzyme wiring can take place. Accordingly, a diversity of carbon [28], metal-oxide [29] and composite [30] mesoporous materials has attracted much

attention owing to their high surface area, short charge diffusion lengths and fast diffusion rates. These attractive properties were used for the development of BFCs with improved power density and overall performance. This review addresses recent advances in mesoporous materials for BFC design, critically analyzing the advantages and disadvantages of each design in terms of performance and lifetime, and emphasizing their practical implications and potential improvements leading to a major impact on clinical research and portable electronics.

1. Mesoporous carbon materials for BFC design

Mesoporous carbon materials are a new class of carbon nano-materials with high specific surface area, uniform pore distribution, high thermal/mechanical stability and flexible surface functionalization [31].

The potential usefulness of carbon mesoporous materials for BFC design was demonstrated by adsorbed laccase (LAC) on a carbon aerogel catalyzing the oxygen reduction without the addition of any redox mediator [32]. Carbon aerogel is composed of nanometer-sized covalently linked particles with very high porosity, large surface area and electrical conductivity. In this preliminary study, the material on which the enzymes were adsorbed had an average pore size of 22 nm, which was big enough to allow permeation of the enzymes. The achieved current density reached 10 mA cm^{-2} and the results demonstrated that enzyme catalytic turnover rate constant was sufficiently large to allow diffusion-controlled reduction of O_2 under large mass transfer conditions. Moreover, the biocathode showed a stable current intensity for 10 days.

Later, highly ordered mesoporous carbons (OMCs) attracted much attention owing to their extremely high surface area, monodisperse pore sizes, and high thermal stability and conductivity. The first report using a mesoporous material to develop a BFC describes a compartment-less glucose/O_2 BFC using OMCs as substrates for bioelectrodes [33]. Improved electrochemistry on OMCs compared to carbon nanotubes (CNTs) was suggested because of the presence of more edge-plane-like defective sites in the former ones. In this work, in which Meldola's blue was use as a mediator for the bioxidation of glucose by glucose dehydrogenase (GDH) and LAC for the electroreduction of O_2, the OCV and the maximum power output (P max) of OMC-based BFC were 0.82 V and 38.7 μW cm^{-2} (at 0.54 V) respectively (Table 1). The performance of this BFC proved to be better than CNT-based BFC prepared in similar conditions. However, we must note that the glucose concentration used was 60 mM, which is one order of magnitude higher than typical physiological concentrations of this biofuel (ca. 5 mM). Also, although OMCs have good conductivity, in this study reasonable current output required the use of mediators. On the other hand, mediator-less electrocatalysis of glucose oxidase (GOX) immobilized on OMCs of the FDU-15 type resulted in a maximum current of 590 μA cm^{-2} and the potential application in a BFC was also demonstrated [34], although the electron exchange rate constant (*Ks*) was much lower than MET based BFC using OMCs, as expected (4.1 s^{-1} vs. 39.8 s^{-1}). In this case, *Ks* could be also be disfavored as enzyme aggregates were prepared in situ. The BFC lifetime was not analyzed in this study.

A highly hydrophilic OMC material synthesized by incorporating a polymer (polyvinyl alcohol) showed an excellent performance when incorporated as a bioanode in a mediator-less glucose/O_2 BFC [35]. In this work, transmission electron microscopy (TEM) images revealed the presence of mesopores and micropores (pore size < 2 nm) (Fig. 1A). The outside surface of OMCs and part of pores may allow GOX immobilization. In this case, surface hydrophilicity favored enzyme immobilization and the diffusion of reactants to the enzyme active sites. Moreover, the authors hypothesized that micropores could be a stoichiometric electron acceptor and host for a variety of electron-donating guest species [36,37]. Although the estimated *Ks* was 3.98 s^{-1}, at physiological concentration of glucose, the catalytic electrooxidation of glucose reached 150 μA cm^{-2} and the OCV and the P max of hydrophilic OMC-based BFC (1.2 V and 110 μW cm^{-2} (at 0.72 V) respectively)

Table 1
Summary of mesoporous-based BFCs and comparison with other BFCs of high performance.

Material of interest	Anode	Cathode	Fuel concentration	P max	OCV	References
Ordered mesoporous carbons (OMCs)	GDH/OMCs–MDB/GC	LAC/OMCs/GC	60 mM glucose	38.7 μW cm^{-2} (at 0.54 V)	0.82 V	Zhou et al. [33]
Hydrophilic OMCs	GOX/OMCs/GC	Pt wire	10 mM glucose	110 μW cm^{-2} (at 0.72 V)	1.2 V	Guo et al. [35]
Mesoporous carbon MSU-F-C	GOX/MSU-F-C/GC (NER)	Pt wire	200 mM glucose	66.4 μW cm^{-2} (V not informed)	Not informed	Kwon et al. [39]
Hollow core mesoporous shell carbon (HCMSC) nanospheres	GOX/HCMSC-PAH/Fc/flexible carbon cloth	Pt foil	5 mM glucose	Not informed	0.38 V	Olyveira et al. [40]
Mesoporous carbon nanoparticles	GOX/Fc-MeOH/MCNP/GC	BOD/ABTS/MCNP/GC	80 mM glucose[a]	95 μW cm^{-2} (V not informed)	0.5 V[a]	Trifonov et al. [41]
CNT/porous silicon (pSi) composite	GOX/CNT/Au-coated pSi	LAC/CNT/Au-coated pSi	4 mM glucose	1.38 μW cm^{-2} (at 0.99 V)	~0.15 V	Wang et al. [44]
Nanostructured silica sol–gel/CNT composites	GOX/Fc-MeOH/mesoporous SiO_2/CNT/Gold	BOD/ABTS/mesoporous SiO_2/CNT/Gold	100 mM glucose	120 μW cm^{-2} (at 0.24 V)	0.48 V	Lim et al. [45]
Mesoporous titania/silver nanoparticles (AgNP) composite	GOX/mesoporous titania/AgNP/meso porous silica/glass	LAC/mesoporous titania/AgNP/meso porous silica/Glass	5 mM glucose	602 μW cm^{-2} (at 0.68 V)	0.91 V	Bellino et al. [47]
CNT fibers	GOX/PVPO/CNT fibers	BOD/PPO/CNT fibers	15 mM glucose	740 μW cm^{-2} (at 0.57 V)	0.83 V	Gao et al. [49]
Woven biscrolled CNT yarns	GOX/Os-mediator/PEDOT-multiwall CNT	BOD/Os-mediator/PEDOT-multiwall CNT	7 mM glucose	1180 μW cm^{-2} (at 0.40 V)	0.7 V (at 60 mM glucose)	Kwon et al. [54]
Carbon fiber (CF) sheets	GDH/NADH/Vitamin K_3/CF electrode	BOD/$K_3[Fe(CN)_6]$/CF electrode	400 mM glucose	1450 μW cm^{-2} (at 0.3 V)	0.8 V	Sakai et al. [50]
CNT 3D matrix	GOX/naphthoquinone/catalase/CNT-based 3D electrode	LAC/CNT-based 3D electrode	50 mM glucose	1540 μW cm^{-2} (at 0.4 V)	0.76 V	Reuillard et al. [51]
3D graphene–SWCNT hybrid	GOD/3D graphene–SWCNT hybrid electrode	LAC/ABTS/3D graphene–SWCNT hybrid electrode	30 mM glucose	2270 μW cm^{-2} (V not informed)	1.2 V	Prasad et al. [52]

[a] Values not explicitly reported, but estimated from graphical results.

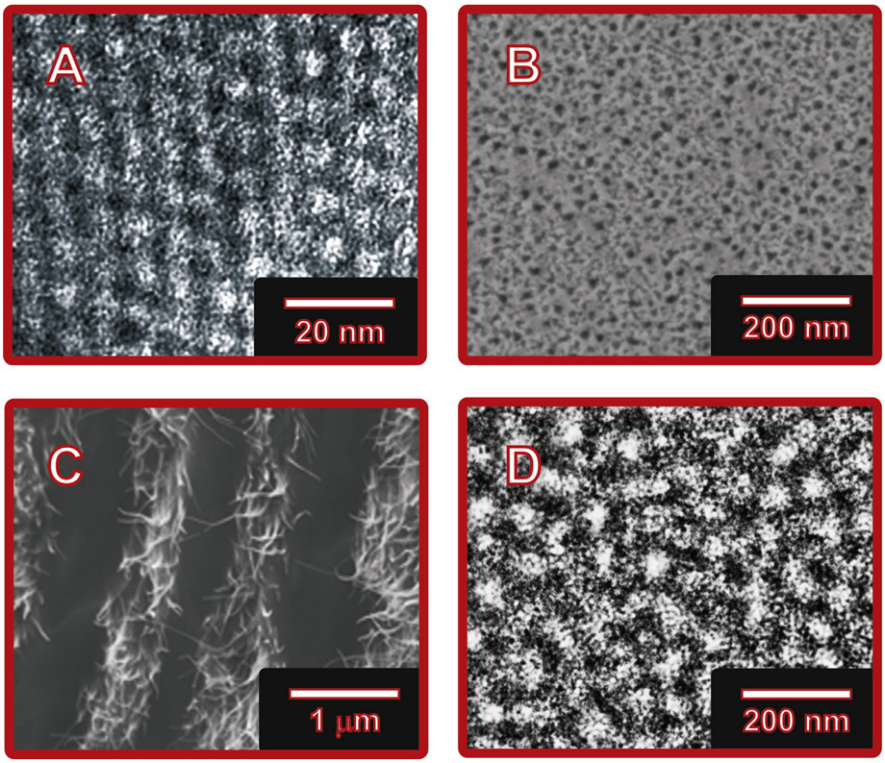

Fig. 1. Representative images of some typical materials involved in BFC designs discussed in the review. A) TEM image of hydrophilic ordered mesoporous carbon (adapted from Journal of Power Sources, 195, High-performance biofuel cell made with hydrophilic ordered mesoporous carbon as electrode material, 4090–4097, Copyright 2010, with permission from Elsevier [35]). B) SEM image of porous silicon (adapted from Electrochemistry Communications, 11, Membrane-less and mediator-free enzymatic biofuel cell using carbon nanotube/porous silicon electrodes, 34–37, Copyright 2009, with permission from Elsevier [44]). C) SEM cross-section images of the fracture surface of a biscrolled CNT yarn cathode (adapted by permission from Macmillan Publishers Ltd: Nature Communications [54], copyright 2014). D) TEM imagine of hierarchical mesoporous film showing bimodal porosity (adapted from Nano-Designed Enzyme-Functionalized Hierarchical Metal-Oxide Mesoporous Thin Films: En Route to Versatile Biofuel Cells; M.G. Bellino, G.J.A.A. Soler-Illia; Small 10. Copyright (c), 2014 [47]).

(Fig. 2A), values superior to previous OMC-based BFC using mediators even at higher concentration of biofuel. These results show a promising enhanced electrocatalytic activity of GOX immobilized on as-prepared OMC material, but the BFC lifetime was not evaluated in this study.

A mesocellular carbon foam, MSU-F-C [38], with a large pore size by means of the controlled incorporation of a carbon precursor into the pores of a mesocellular silica foam template, has been used as a GOX hosting nanoscale enzyme reactor (NER) to improve the performance of a BFC [39]. This mesoporous carbon has a bottleneck pore structure with mesocellular pores of 26 nm connected with window mesopores of 17 nm and surrounded by small pores 4.7 nm in diameter. In addition, it also has micropores of around 0.6 nm, which occupy about 15% of the total pore volume. The NER assembly is based on enzyme adsorption within the mesoporous matrix followed by enzyme chemical crosslinking. Enzyme stabilization in these reactors is based on a ship-in-a-bottle mechanism, which prevents leaching of crosslinked enzymes in mesocellular pores through smaller connecting mesopores. Moreover, the authors suggest that multipoint covalent linkages on the surfaces of biomolecules may inhibit enzyme denaturation and improve the electron transfer, by shortening the distance between the enzyme and the surfaces of the conductive mesoporous carbons. In this particular case, a high mass transfer rate could be achieved because of the large window mesopores that behave as effective substrate transport channels. The glucose/O_2 BFC yields a P max of 1.69 μW cm^{-2} without the addition of a free redox mediator but, using a concentration of 200 mM glucose, exceedingly higher than physiological values. This power density was improved adjusting the following variables: a) by adding the freely diffusing mediator benzoquinone (up to 14.23 μW cm^{-2}), b) by increasing the enzyme load using Nafion® conductive polymer in the space between the MSU-F-C and the carbon paper used as support (up to 27.06 μW cm^{-2}), c) by inserting a gold mesh as an electrode collector (up to 36.57 μW cm^{-2}) or d) by co-immobilizing GOX and ferrocenecarboxylic acid to enhance electron transfer from GOX to the electrode (up to 66.35 μW cm^{-2}). In addition, the bioanode remained stable for 32 h under stirring, keeping 73% of initial activity. In all cases and under similar conditions, NER based BFC had a better performance than MSU-F-C based BFC without crosslinking of GOX, demonstrating that the approach of NER is useful for stabilizing enzyme activity and expediting the overall electron transfer. However, NER based BFC performance, even at higher glucose concentrations, was not as good as hydrophilic OMC based BFC [35].

An approach to facilitate mass transport of fuel was designed using a flexible carbon cloth electrode and hollow core–mesoporous shell carbon (HCMSC) nanospheres of ca. 305 nm in diameter as bioanode materials for adsorbing GOX [40]. These spheres are uniform particles with large surface area and 3D interconnected multimodal porosity. Hollow macropores and interstitial spaces between the packed nanospheres were connected to the mesoporous channels in the shell, and can facilitate mass transport by being efficient reservoirs of fuel. In this study, using ferrocene as mediator molecule and GOX adsorbed on the surface of the nanospheres in the bioanode, the OCV reached scarcely 0.38 V and the maximum current was 100 μA cm^{-2} at 5 mM glucose (similar to previous approaches considering the low glucose concentration), while the P max was not reported.

Carbon can be processed in the form of nanoparticles, and assemblies of these building blocks on electrode surfaces enable the design of mesoporous structures. Recently, mesoporous carbon nanoparticles (CNP) less than 50 nm in diameter and with a pore size of ~6.3 nm were implemented to design electrically contacted enzyme electrodes [41]. In this study, mediator molecules were encapsulated within the CNP pores which were capped by cross-linked enzymes. Nafion® was also used as a co-additive to help in adhering and stabilizing the enzyme on the electrodes. It was postulated that this configuration protects the relay in the CNP matrix and provides the conformation of an integrated

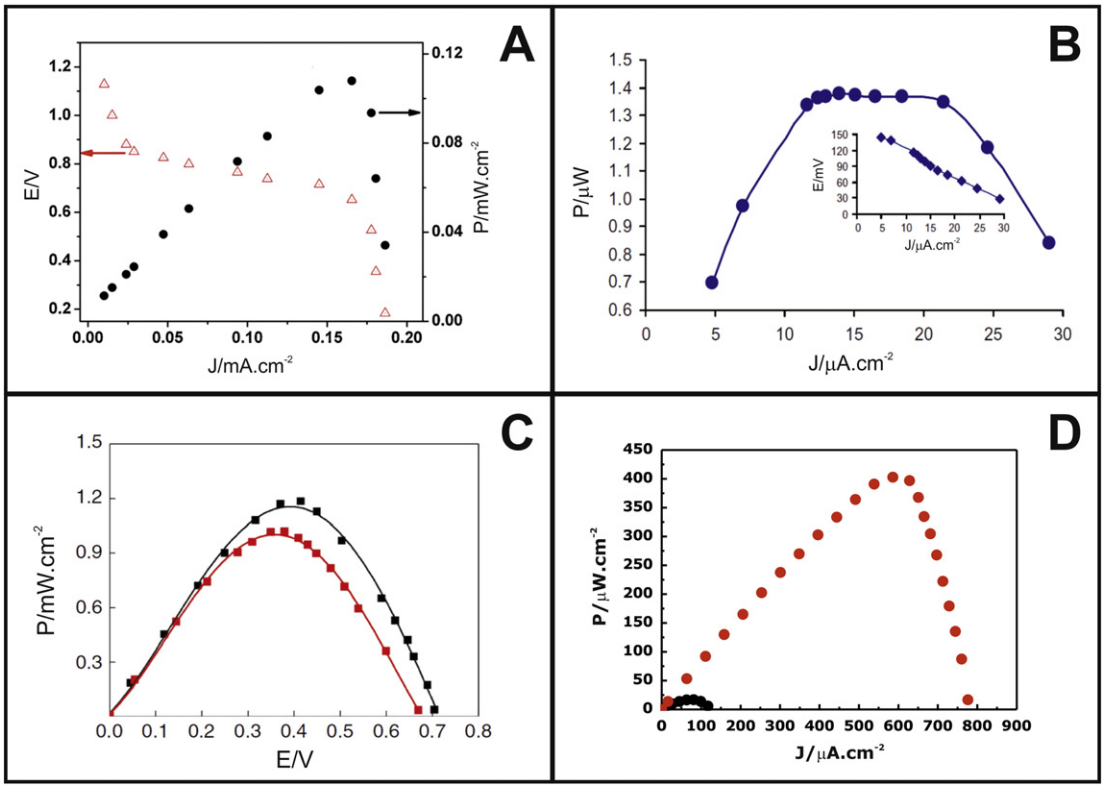

Fig. 2. Representative performances of some typical BFCs discussed in the review. A) Hydrophilic ordered mesoporous carbon based BFCs: polarization curve (opened red triangles) and dependence of the power output on the current density (filled black circles) at 10 mM glucose (adapted from Journal of Power Sources, 195, High-performance biofuel cell made with hydrophilic ordered mesoporous carbon as electrode material, 4090–4097, Copyright 2010, with permission from Elsevier [35]). B) BFCs using carbon nanotube/porous silicon electrodes: Power vs. current density at different loads in 4 mM glucose solution (inset: polarization curve) (adapted from Electrochemistry Communications, 11, Membrane-less and mediator-free enzymatic biofuel cell using carbon nanotube/porous silicon electrodes, 34–37, Copyright 2009, with permission from Elsevier [44]). C) Areal power density as a function of cell voltage for a biscrolled CNT yarn BFC at 7 mM glucose (filled black squares and curve) and in human serum (filled red squares and curve) (adapted by permission from Macmillan Publishers Ltd: Nature Communications [54], copyright 2014). D) Dependence of power density on current density for a BFC made with uniform (black circle) and hierarchical (red circle) mesoporous titania/silver nanoparticle nanocomposite scaffolds in 50 mM glucose solution under air at 20 °C (adapted from Nano-Designed Enzyme–Functionalized Hierarchical Metal–Oxide Mesoporous Thin Films: En Route to Versatile Biofuel Cells; M.G. Bellino, G.J.A.A. Soler-Illia; Small 10. Copyright (c), 2014 [47]).

structure. Again, the use of soluble mediators allowed reaching a higher electron transfer rate (*Ks*: 995 s^{-1}) for the catalyzed oxidation of glucose by GOX. In this system, the BFC consisting of a GOX/mesoporous CNP anode and bilirubin oxidase/mesoporous CNP cathode including mediators for both catalytic reactions, yielded a P max of 95 μW cm^{-2} at 80 mM glucose. These values are lower than power densities obtained with other mediator-less designs at even lower glucose concentrations [35]. In addition, there was also no indication of the device lifetime.

A different mesoporous carbon structure was recently analyzed as a platform for bio-oxidation of hydrogen [42]. This consisted of a herringbone carbon nanofiber (CNF) mesoporous film which allowed a mediator-less hydrogen oxidation by adsorbed hydrogenases with high catalytic efficiency. Nanofibers exhibit a nanostructure based on graphene layers obliquely stacking, with lots of reactive edge sites, high surface area and hierarchical pore volumes (micropores below 2 nm in size and mesopores of 2–25 nm). It was postulated that while microporosity could improve gas transport inside the carbon film, mesoporosity would be suitable for enzyme entrapment. This, together with the hydrophobicity of the chemically treated carbon material may result in a stable and very high efficient bioelectrode. In fact, it was demonstrated that the catalytic current was limited by mass transport inside the CNF film and reached 4.5 mA cm^{-2} with sufficient hydrogen supply. The electron transfer rate was 48 s^{-1}, which is a high turnover frequency considering a DET based bioelectrode. Even though this study did not develop nor evaluate a BFC bioelectrode performance, results are very promising and the use of hydrogen produced from biomass can be envisioned as a fuel for a future H_2/O_2 BFC.

2. Mesoporous composite materials for BFC design

Ordered mesoporous silica and titania materials originally gathered attention as they can provide mechanically stable, open and uniform pore structures as well as high pore volume with large surface area, thus offering a suitable matrix for enzyme immobilization [12,13,21]. There are only a few examples in the literature in which metal-oxide mesoporous films have been used to immobilize enzymes over conductive electrodes. An approach in which a metal-oxide mesoporous material was applied is a photoelectrochemical BFC using titania films to immobilize a porphyrin sensitizer [43]. In this work, the photoanode consisted of a fluorine-doped tin oxide (FTO) conducting glass electrode modified with titania mesoporous film of 20 nm-particle size in which the sensitizer with strong light absorption was entrapped. The fuel (glucose), the enzyme (GDH) and the co-enzyme (NADH and NAD^+) were added in the photoanode solution compartment (i.e. the enzyme was not immobilized). The electron transport phenomenon here was based firstly on the electron donating process from glucose oxidation to the immobilized sensitizer, and light absorption followed by charge donation from sensitizer to the conduction band of nanocrystalline titania. Secondly, the electrons are rapidly transferred in FTO conducting glass, and the electrons are delivered to a Pt black cathode through an external circuit. In this condition, the OCV and the maximum power density were 0.74 V and 33.94 μW cm^{-2} (at 0.45 V) respectively, and it is noteworthy that this configuration showed better performance than enzymatic BFC and porphyrin-sensitized solar cells under similar conditions.

The intrinsic poor electrical properties of the metal-oxide mesoporous matrices have clearly restricted their incorporation in BFC designs. In this regard, the combination of mesoporous scaffolds with other nanostructured materials offers much more design flexibility and has emerged in order to exploit the best of both worlds: protection and immobilization properties and mass transport facilitation of metalloid/metal-oxide mesoporous materials and excellent electrical conductivity of metals and carbon materials.

One of the foregoing studies is composed of a membrane-less mediator-free enzymatic glucose/O_2 BFC using single wall CNT and gold-coated porous silicon (pSi) as electrodes [44]. In this study, CNTs were used to immobilize the enzymes and to facilitate electron transfer and pSi, with pore size of 20 nm on average, was used as a nano-fluidic platform for mass transport enhancement (Fig. 1B). However, the performance of this BFC resulted very poor with a P max of 1.38 μW cm^{-2} at 0.99 V and OCV of ~0.15 V at physiological glucose concentration (4 mM) (Fig. 2B). Although DET is claimed, no clear evidence of direct oxidation of glucose was shown.

Another glucose/O_2 MET based BFC with better performance was designed based on nanostructured silica sol–gel/CNT composite electrodes [45]. In this study, multiwall CNTs, GOX and bilirubin oxidase were encapsulated via sol–gel process in a mesoporous silica matrix and the resulting porosity was enough to allow adequate diffusion of glucose, oxygen and mediator molecules. The P max was 120 μW cm^{-2} (at 0.24 V) and the OCV was 0.48 V at 100 mM glucose, which is much higher than the physiological concentration. Considering this, its performance turned to be not superior to OMC based BFC performance [35]. DET was also demonstrated in nanostructured silica sol–gel/CNT composite electrodes containing bilirubin oxidase [46], showing that the development of mediator-less BFC using this kind of composite materials is achievable.

Recently, a modular combination of hierarchical mesoporous titania thin films, silver nanoparticles (AgNP) and enzymes (GOX and LAC) led to a highly efficient ultrathin membrane-less mediator-free glucose/O_2 BFC system, which stands at the height of the state-of-the art maximum performance [47]. In this study, 80 nm thick mesoporous titania thin films were deposited on glass substrates [48], yielding a hierarchical dual pore size distribution centered in 13 and 38 nm diameter (Fig. 1D). Bioanode and biocathode were formed by adsorption of enzymes in larger pores and photodeposition of AgNP of ca. 10 nm in smaller pores. The AgNP played a key role in this composite by improving the contact between the enzyme and the electrode. Besides, the titania matrix could also play a fundamental role in enzyme wiring and electron transfer at the oxide–metal interface. Meanwhile, the larger pores acted as cages for the redox enzymes interconnected by smaller pores that allowed the supply of reactants. Using this configuration, the OCV and P max were 0.91 V and 403 μW cm^{-2} (at 0.68 V), respectively at 20 °C and 50 mM glucose (Fig. 2D). A substantial higher incorporation of enzymes in hierarchical mesoporous thin film was demonstrated to be the reason of its outstanding better performance. The best performance, however, was achieved by the addition of an intermediate mesoporous silica layer with 3 nm diameter pores between the glass substrate and the titania film (free of AgNP and enzymes) to improve mass transport of reactants and products to and from electrodes. This configuration led to a maximum power density of 602 μW cm^{-2} (at 0.68 V) at 37 °C and 5 mM glucose, showing the potential application of this BFC in self-powered implantable devices. As of the BFC lifetime, only 10% loss in voltage output was observed after continuous operation for 30 h. Moreover, after 10 days of storage, the current density of bioelectrodes maintained more than 85% of the initial value and the maximum power output showed only an 8% decrease, all these results indicating a good lifetime of the bioelectrodes, which might be due to the protective effect of the mesoporous titania on the enzymes.

It is important to highlight that the performance of this hierarchical mesoporous nanocomposite based BFC was the highest obtained to date by any mesoporous based BFC (Table 1). However, there are several examples of other BFC designs where comparable or even better performances were obtained, though using higher concentrations of fuel. For comparison with recently developed high performance BFCs, Gao et al. described a glucose/O_2 BFC based on the immobilization of bilirubin oxidase (BOD), GOX and redox polymer mediators in CNT fibers (microwires) with a power density of 740 μW cm^{-2} in the presence of 15 mM glucose [49]. Sakai et al. reported a BFC based on BOD, glucose dehydrogenase and redox mediators entrapping on carbon fiber sheets, which delivered 1450 μW cm^{-2} using a glucose concentration of 400 mM [50]. Reuillard et al. described a BFC based on naphthoquinone-mediated oxidation of glucose by GOX and reduction of O_2 by LAC in a carbon nanotube 3D matrix. In this case, the power density achieved was 1540 μW cm^{-2} using 50 mM glucose [51]. Finally, the best power output performance was obtained by Prasad et al. [52]. They reported a MET based BFC in which GOX and LAC were immobilized in 3D graphene-single wall CNT hybrid electrodes yielding a power density of 2270 μW cm^{-2} using 30 mM glucose.

Even after considering the highest power BFCs, the specific power of the hierarchical mesoporous composite based mediator-free BFC (per unit of volume of electrode) at physiological conditions, was superior to date to any glucose/O_2 BFC: exceeded by four orders of magnitude the power density obtained by a BFC that was based on compressed CNT disks of 3 mm thick (1 mW cm^{-2}) [53] or by the BFC based on carbon fiber bioelectrodes of ca. 1.5 mm thick (1.45 mW cm^{-2}) [50], and by two orders of magnitude the power density obtained by a BFC that was based on woven biscrolled CNT yarns of 50 μm diameter (1.18 mW cm^{-2}) [54] (Table 1, Figs. 1C and 2C) or the BFC based on CNT microwires of 9.5 μm diameter (0.74 mW cm^{-2}) [49]. This suggests that the hierarchical nanocomposite matrix would allow for an improved connection of large number of enzymes and that performance of this BFC could be improved by increasing the thickness of the mesoporous ultrathin film without significantly altering its miniaturization and implantable potentiality. In addition, this is an interesting proof of principle of the assembled construction of complex nanosystems from building blocks of different nature.

3. Conclusions and perspectives

Even though BFCs are still far from classical fuel cells in terms of maximal power output, recent advances using nanostructured materials have shown to be promising in paving the way to miniaturized implantable BFC. This review has addressed recent advances in the mesoporous materials for BFC design. The immobilization of enzymes in mesoporous matrices, which present precisely defined pore sizes, large surface areas, short charge diffusion lengths and fast diffusion rates, has yielded the desired stability and, in some cases, DET from enzyme to electrodes. Within mesoporous carbon materials, hydrophilic OMC-based BFCs showed the highest power output. The best performance, however, has been achieved by the use of mesoporous/metal nanocomposite materials for BFC design, offering highest OCV and power output, and demonstrating the possibility to even improve the performance by minor changes in BFC configuration. In addition, a series of nanobuilding blocks and assembly procedures are available to be combined in order to optimize performance. Even though any of these materials are commercially available and most of them are not easy to produce (e.g. herringbone CNF mesoporous film), the modular combination of hierarchical titania thin films, AgNP and enzymes offers support versatility, low cost and ease of fabrication.

We have emphasized the basic scientific results and the practical implications for defined mesoporous structures. Better understanding and future developments of mesoporous based BFCs will definitely expedite their improvement, and high performance implantable BFCs may soon take a role in medical science and clinical research.

Abbreviations

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
BOD	bilirubin oxidase
CNT	carbon nanotubes
Fc	ferrocene
MCNP	mesoporous carbon nanoparticles
Fc-MeOH	ferrocene methanol
GC	glassy carbon
GDH	glucose dehydrogenase
GOX	glucose oxidase
LAC	laccase
MDB	Meldola's blue
MET	mediated electron transfer
NER	nanoscale enzyme reactor
OMC	ordered mesoporous carbon
OCV	open circuit voltage
Os-mediator	osmium containing mediator molecule
PAH	polyallylamine hydrochloride
PVPO	PVP-[Os (N, N′ -alkylanated-2,2′ bi-imidazole)$_3$]$^{2+/3}$
PPO	PAA-PVI-[Os (4,4′ -dichloro-2,2′-bipyridine)$_2$ Cl]$^{+/2+}$
PEDOT	poly(3,4-ethylenedioxythiophene)

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