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Research review paper

Challenges in biocatalysis for enzyme-based biofuel cells

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Abstract

Enzyme-based biofuel cells are attracting attention rapidly partially due to the promising advances reported recently. However, there are issues to be addressed before biofuel cells become competitive in practical applications. Two critical issues are short lifetime and poor power density, both of which are related to enzyme stability, electron transfer rate, and enzyme loading. Recent progress in nanobiocatalysis opens the possibility to improve in these aspects. Many nano-structured materials, such as mesoporous media, nanoparticles, nanofibers, and nanotubes, have been demonstrated as efficient hosts of enzyme immobilization. It is evident that, when nanostructure of conductive materials are used, the large surface area of these nanomaterials can increase the enzyme loading and facilitate reaction kinetics, and thus improving the power density of biofuel cells. In addition, research efforts have also been made to improve the activity and stability of immobilized enzymes by using nanostructures. It appears to be reasonable to us to expect that progress in nanostuctured biocatalysts will play a critical role in overcoming the major obstacles in the development of powerful biofuel cells.

Keywords: Biofuel cell; Nanoparticle; Nanofiber; Nanotube; Nanobiocatalysis; Nanomaterials; Enzyme immobilization; Enzyme stabilization; Electrospinning

Contents

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1. Introduction

The concept of biofuel cells has been known for almost one century since the first microbial biofuel cell was demonstrated in 1912 (Potter, 1912). In the 1960s, NASA showed a keen interest in power generation from human wastes on the space shuttles. That inspired a wide range of R&D efforts for biofuel cells. Biofuel cells generating power from various substances, such as urea and methane, were built and tested during that period. The first enzyme-based biofuel cell was reported in 1964 using glucose oxidase (GOx) as the anodic catalyst and glucose as the "fuel" (Yahiro et al., 1964). Exciting advances have been made since that time (Bockris and Srinivasan, 1969; Govil and Saran, 1982; Aston and Turner, 1984; Palmore and Whitesides, 1994); still, the performance of biofuel cells, in terms of power density, lifetime, and operational stability, falls far below that of chemical fuel cells. Nevertheless, recent research showed a renewed interest in biofuel cells. Instead of considering biofuel cells as a general device for power generation, most of the recent studies have been directed toward special applications, such as implantable devices, sensors, drug delivery, micro-chips, and portable power supplies (Katz and Willner, 2003b; Barton et al., 2004; Heller, 2004). To satisfy the needs for these special applications, the biocatalysts are being challenged for their extreme performance.

Concurrently, recent advances in nanoscale science and technology are fueling a new wave of revitalization in the field of biocatalysis. Synergizing with materials chemistry, various nanostructures have manifested their great potential in stabilizing and activating enzymes with performances well beyond the scope of traditional immobilization technologies. Especially, the large surface area, which these nanostructures provide for the attachment of enzymes, will increase the enzyme loading and possibly improve the power density of biofuel cells. In that sense, nanoscale engineering of the biocatalysts appears to be critical in the next stage advancement of biofuel cells. In this review, the potentials of nano-structured biocatalysts are examined to explore the opportunities for developing the next generation of biofuel cells.

2. Enzyme-based biofuel cells

Biofuel cells belong to a special class of fuel cells where biocatalysts such as microorganisms or enzymes are employed instead of metallic inorganic catalysts. The biocatalyst in a biofuel cell may simply promote the production of simple fuels, such as hydrogen or methane, from more complicated biochemical substrates, such as sugars. These simple fuels are then oxidized by inorganic catalysts at the surface of the electrodes to produce electricity. This type of biofuel cell is classified as "secondary" or "indirect." A challenging issue in developing indirect biofuel cells is the choice of operation conditions. Biocatalysts mostly prefer ambient temperature whereas metal-catalyzed fuel cell reactions usually require elevated temperature. So far only H_2–O_2 fuel cells were tested for indirect biofuel cells, possibly due to this discrepancy in the operating condition (Palmore and Whitesides, 1994; Katz et al., 2003).

The other type, "primary" or "direct" biofuel cells, is the focus of most current research. In this type of biofuel cell, biocatalysts are directly involved in the redox reaction or reaction chain for the generation of electricity. Fig. 1 shows a scheme of a primary enzyme-based biofuel cell. Preferably, enzymes are immobilized on electrodes to facilitate the repeated use of the catalysts. The fuel is enzymatically oxidized at the anode, producing protons and electrons. At the cathode, the

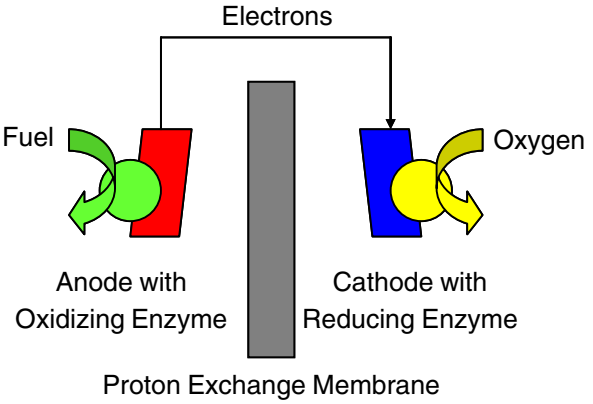

Fig. 1. Schematic of enzyme-based biofuel cells.

oxidant (usually oxygen or peroxides) reacts with electrons and protons, generating water. One of the critical challenges in developing direct biofuel cells is inefficient electron conduction between biocatalysts and electrodes.

Direct electron transfer (DET) between an enzyme and electrode has only been observed with several enzymes, such as cytochrome *c*, laccase, hydrogenase, and several peroxidases, including microperoxidases (Varfolomeev et al., 1996; Ghindilis et al., 1997; Schuhmann, 2002; Freire et al., 2003). The close contact of the enzyme active sites to the surface of the electrode is critical for DET. For laccase-catalyzed direct electro-reduction of oxygen, a critical distance between the enzyme active site and the electrode surface was proposed to be 20 Å (Yaropolov et al., 1981). A distance greater than this critical distance slowed down the overall reaction, which is rate-determined by electron conduction; whereas a shorter distance made the electron conduction so efficient that the enzymatic reaction kinetics became a rate-determining step. A similar phenomenon was also reported for horseradish peroxidase, where the critical distance was 18 Å (Kulys and Samalius, 1984).

In many of other cases, however, DET is limited by the thick and nonconductive protein shell that hosts the active site of an enzyme. To overcome this barrier, enzymes can be transformed to be conductive via chemical modification (Willner et al., 1996; Guiseppi-Elie et al., 2002; Zhao et al., 2002; Cai and Chen, 2004). Another popular strategy is the use of redox mediators that facilitate the transportation of electrons by shuttling between the enzyme active sites and the surface of electrodes. This approach has been reported for both microbial and enzymatic biofuel cells (Lewis, 1966; Govil and Saran, 1982; Palmore and Whitesides, 1994; Katz et al., 2003). Even though the mediators introduce an additional step in the redox reaction chains from fuel to electron generation, much higher efficiency of biofuel cells was usually observed. One of the challenges in using mediators, which are usually small and easily diffuse away, is how to retain them in the biofuel cells where a continuous feeding of fuels is required.

Poor power density and short lifetime are two bottleneck problems in the real application of biofuel cells. To address these issues, much efforts and significant improvements have been made during the last decade. For example, GOx and microperoxidase-11 have been monolayer-assembled on gold electrodes (0.4-cm-diameter disks) and applied in a glucose/cumene peroxide biofuel cell (Katz et al., 1999a). A power output of 520 μW was observed, corresponding to 4.1 mW/cm^2 based on the projective electrode area. Much improved power density per volume or weight was achieved with miniaturized glucose/O_2 biofuel cells (Heller, 2004). In that work, enzymes such as GOx and bilirubin oxidase (BOD) were entrapped in Os-containing redox polymers on the surface of two 7-μm carbon fibers. A power output of 4.3 μW was achieved with a total fiber volume of 0.0026 mm^3, representing 1.65 mW/mm^3. In another study, a cell lifetime of up to 45 days was reported with enzymes entrapped in a modified Nafion membrane (Minteer et al., 2004; Moore et al., 2004).

Noticeably, these improvements have been mostly achieved by choosing proper electrode materials and an improved means of enzyme immobilization to promote electron communications between enzymes and electrodes. As efforts continue to make the design of biofuel cells more efficient, we anticipate that the catalytic performance of biocatalysts themselves will eventually become a more important issue for the successful application of biofuel cells.

2.1. Immobilization of biocatalysts

Enzyme immobilization can be achieved either chemically or physically. Most of the enzyme-based biofuel cells reported so far have been constructed with physically immobilized enzymes. One common approach is to adsorb the enzymes onto conductive particles such as carbon black or graphite powder. Hydrogenase and laccase have been physically adsorbed on carbon black particles to construct composite electrodes (Tarasevich et al., 2002). Pizzariello et al. (2002) reported a glucose/H_2O_2 biofuel cell using ferrocene-modified composite electrodes. GOx or HRP was first adsorbed on synthetic graphite particles, and then the enzyme-adsorbed particles were suspended with 2-hexadecanone and ferrocene in a solvent of chloroform. The composite electrodes were prepared by spray-printing the suspension on a polyester substrate. The biofuel cell had been continuously worked for 30 days with negligible voltage drop, albeit the power density was low.

Another approach for physical immobilization is to entrap the enzymes in polymeric matrices, which usually retain the enzyme better than surface adsorption. Minteer et al. (2004) reported a method to entrap enzymes in Nafion membrane. According to this method, NAD^+-dependent dehydrogenases (such as alcohol dehydrogenase, aldehyde dehydrogenase, formaldehyde dehydrogenase, glucose dehydrogenase, and lactic dehydrogenase) were physically mixed with tetralky-

lammonium bromide-modified Nafion solution, which was then cast on methylene green-modified glassy carbon electrodes. The immobilized enzymes were treated at 140 °C for 25 min. Cofactor NAD^+ was co-immobilized via ion exchange in the Nafion membrane. Interestingly, no statistical difference in enzyme activity was observed before and after the heat treatment. Ethanol/O_2 biofuel cells constructed using this method generated a power density as high as 2.04 mW/cm^2 (Minteer et al., 2004). Cyclic voltammetry measurements indicated that mass transfer, not the reaction kinetics, is the limiting factor in such a biofuel cell.

Heller and co-workers explored the use of redox polymers to construct miniature biofuel cells (Chen et al., 2001; Mano et al., 2002, 2003a,b; Heller, 2004; Soukharev et al., 2004). Two types of redox polymers were developed, both containing Os redox centers but with different redox potentials. The polymer of higher redox potential, 0.58~0.79 V vs. standard hydrogen electrode (SHE), was used for the cathode whereas the one with a lower redox potential, 0.02~0.32 V vs. SHE, was used for the anode. Enzymes were mixed with the redox polymers along with a crosslinker, poly(ethylene glycol) (400) diglycidyl ether. The electrodes were built by casting the enzyme-polymer solution onto 7-μm carbon fibers. A recent report showed that a glucose–oxygen biofuel cell was capable of delivering a power density up to 0.35 mW/cm^2 at 0.88 V (Soukharev et al., 2004).

The efficient covalent binding of enzymes and mediators has also been demonstrated. Katz et al. reported results of biofuel cells using co-immobilized enzyme-cofactor-mediator complexes on metal electrodes (Willner et al., 1998a,b; Katz et al., 1999a,b, 2001, 2003; Katz and Willner, 2003a,b). The strategy was to modify the electrode surface with a monolayer of redox mediator–cofactor arrays and then integrate the immobilized cofactor with enzymes via bioaffinity. For example, a redox monolayer was formed by covalently grafting pyrroloquinoline quinone (PQQ) to a cystamine modified Au-electrode, followed by attaching of N6-(2-aminoethyl)-NAD^+ to the PQQ monolayer. Lactate dehydrogenase (LDH) was then adsorbed to the PQQ-NAD^+ monolayer via bioaffinity and was further stabilized by cross-linking using glutaraldehyde (Bardea et al., 1997; Katz et al., 1998). Similarly, this method was also used to construct biocathodes, such as an H_2O_2 electrode using microperoxidase-11 and an O_2 electrode with cytochrome *c*/cytochrome oxidase (Katz and Willner, 2003b). In other studies, GOx-FAD was assembled on an Au electrode with mediators such as PQQ (Willner et al., 1996), nitrospiropyran (Blonder et al., 1998), rotaxane (Katz et al., 2004), C-60 (Patolsky et al., 1998), and Au nanoparticles (Xiao et al., 2003). Because the affinity between FAD and GOx was strong, no crosslinking was required for this type of electrode.

In particular, reconstituted GOx with Au nanoparticle showed higher activities than native enzymes with the natural electron acceptor, oxygen. The unusually higher enzyme activity was attributed to the enhanced efficiency of electron conduction via the Au nanoparticle (Xiao et al., 2003). However, a recent surface plasmon resonance and electrochemical study on this system revealed that the overpotential was caused by the non-conductive dithiol-linkers between Au nanoparticles and the bulk Au electrode (Lioubashevski et al., 2004). When glucose is oxidized, electrons were first transferred from the enzyme to the Au nanoparticle. Since the dithiol-linkers are not conductive, electrons are accumulated on the Au nanoparticles, leading to an overpotential problem. This overpotential problem with any non-conductive linkers should be addressed for the facile electron transfer from the enzyme reaction to the electrodes, which can improve the power density of biofuel cells.

2.2. Enzyme stability

Several factors regulate the lifetime of biofuel cells, which has always been a concern for their practical application. For the mediated biofuel cells, the lifetime of redox mediators represents another concern (Allen and Bennetto, 1993; Barton et al., 2004). In most cases, the stability of biocatalysts themselves determines the lifetime of biofuel cells. Most enzymatic fuel cells usually last only a few days (Willner et al., 1998b; Katz et al., 1999a; Kang et al., 2004). Immobilization can help to extend the lifetime of enzymes. A miniature biofuel cell with GOx and BOD immobilized in Os-containing redox polymer lasted 20 days at 37 °C (estimated by extrapolating the power decay curve reported in the reference) (Mano et al., 2002). Moore et al. (2004) reported more promising results using tetrabutylammonium bromide modified Nafion membranes to entrap dehydrogenases. The half life of the native parent enzyme is only 7~8 h in solution whereas an active lifetime of more than 45 days was achieved after immobilization. Furthermore, biofuel cells based on this immobilization technique showed no significant power decay during several weeks of continuous operation (Winder, 2003).

2.3. Mass transfer

Three mass processes subject to transfer limitations are involved in biofuel cell reactions: 1) diffusion of the

fuel or oxidant to the active sites of the biocatalysts; 2) proton transfer through the membrane; and 3) diffusion of redox mediators between electrodes and biocatalysts, or alternatively, the electron transfer between the active sites of catalysts to the electrodes. Similar to chemical fuel cells, high resistance for the mass transfer process of fuels tends to build a concentration difference between the bulk phase and the sites of reaction, thus slowing down the reactions and leading to polarization of the electrodes. Often the performance of porous composite electrodes is limited by the mass transfer of fuels. Typical engineering methodologies, such as patterned electrode design and the introduction of convective transport by forced flow or mechanical stirring, may help to alleviate the problem.

The mass transfer resistance of mediators may also become significant when polymeric mediators are used. The apparent diffusion coefficients of redox polymers are usually 10^{-9}~10^{-8} cm^2/s, much less than the typical value of small diffusional mediators (10^{-6}~10^{-5} cm^2/s). The careful design of materials may help to improve the diffusion coefficient of redox polymers. For example, redox centers were grafted as side groups to polymeric backbones. Since the grafted side groups interact with each other via molecular oscillation, electrons and/or proton pass along the polymeric backbones, mimicking a macroscopic mass transfer process of the mediator (the redox center). A 1000-fold increase of the apparent diffusion coefficient was reported recently by increasing the length of the spacers between the polymer backbone and the redox center (Mano et al., 2002, 2003a; Kim et al., 2003).

2.4. Power density and enzyme loading

One of the major issues in developing biofuel cells is the power density, which is usually measured by power generation per surface area of electrode, or per weight or volume of the cell. High enzyme loading is critical for high output current density. For example, when GOx was randomly packed as a monolayer on a flat surface, an enzyme loading was only 1.7×10^{-12} mol/cm^2 (0.27 μg/cm^2), which was determined by the physical size of the enzyme. Assuming all the enzyme molecules are as active as in aqueous solutions with a typical turnover number of 600 s^{-1}, the upper limit of the current density was calculated to be only about 0.2 mA/cm^2 (Willner et al., 1996). GOx is known as one of the most efficient redox enzymes. For other enzymes with lower specific activity, the theoretical current density should be even lower. Since enzymatic biofuel cells typically work at a voltage lower than 1 V, theoretically this current density can only afford a power density of less than 0.2 mW/cm^2.

Numerous efforts were dedicated to the improvement of power density by increasing the enzyme loading in various ways. For example, multiple-layer enzyme assemblies were tested in improving the enzyme loading for biofuel cells. When compared to the performance of biofuel cells reported about two decades ago, the power density of recently developed biofuel cells was about 1~2 orders of magnitude higher (Schroder et al., 2003; Winder, 2003; Niessen et al., 2004a,b). These advances have pushed the technology of biofuel cells one step further toward commercial applications. In fact, biofuel cells with a power density of more than 1 mW/cm^2 may have been already powerful enough for the construction of cellular phone chargers (Narayanan and Valdez, 2003).

3. Nano-structured biocatalysts

Many attempts have been made to immobilize enzymes using various nanostructures such as mesoporous media, nanoparticles, nanofibers, and nanocomposites. Especially, the large surface area afforded by these nanostructures usually leads to high enzyme loading, which can be used in improving the power density of enzyme-based biofuel cells. Enzyme stabilization in nanostructures has also been reported in many papers, which can be used in extending the lifetime of enzyme-based biofuel cells. Sometimes, the apparent enzyme activity could be improved because of the relieved mass transfer limitation of substrates in nanostructures when compared to macro-scale matrices in conventional enzyme immobilization. Both enzyme stabilization and activation together with high enzyme loadings in various nanostructures will significantly improve enzyme-based biofuel cells.

3.1. Mesoporous media

Mesoporous materials have attracted much attention for many applications because of their controlled porosity and high surface areas (Schmidt-Winkel et al., 1999; Ying et al., 1999; Lee et al., 2001; Davis, 2002; Kim et al., 2002; Schuth and Schmidt, 2002; Lee et al., 2004). Especially, enzyme immobilization has been extensively studied recently using mesoporous materials as the hosts (Diaz and Balkus, 1996; Takahashi et al., 2000; Wang et al., 2001; Han et al., 2002; Lei et al., 2002; Fan et al., 2003a,b). After the first report of enzyme immobilization into MCM-41 (pore size: 4 nm) by Diaz and Balkus (1996), a variety of mesoporous

media have been tested as the hosts of enzyme immobilization, including SBA-15 (pre size: 5–13 nm), mesocellular foam (MCF, pore size 15–40 nm), and mesoporous carbons. Recently, several modifications of mesoporous silica were successfully done for the quick adsorption of enzymes, such as the enlargement of the inlet pore size and the modified morphologies of materials (Fan et al., 2003a; Lei et al., 2004). Immobilized enzymes in mesoporous materials have found their applications in biosensors (Liu et al., 1997b,a; Heilmann et al., 2003; Liu et al., 2003), peptide synthesis (Xing et al., 2000), and pulp bio-bleaching (Sasaki et al., 2001). We anticipate that more and more diversified applications will be reported in the near future, including biofuel cells.

One of the most frequently used approaches in immobilizing enzymes into mesoporous materials is a simple adsorption (Diaz and Balkus, 1996; Takahashi et al., 2000; Han et al., 2002; Lei et al., 2002; Fan et al., 2003a,b). The stability of adsorbed enzymes in mesoporous materials is dependent on many factors, including the pore size of mesoporous materials and charge interaction. The pore size of mesoporous materials affects the adsorption and leaching of enzymes in a more direct way (Diaz and Balkus, 1996; Takahashi et al., 2000; Takahashi et al., 2001; Yiu et al., 2001; Fadnavis et al., 2003; Fan et al., 2003a; Lei et al., 2004; Vinu et al., 2004b). The pore size of mesoporous materials should be similar to or larger than that of enzymes for successful enzyme adsorption. The size-matching between pore size and the molecular diameter of enzymes is important in achieving high stability of adsorbed enzymes (Takahashi et al., 2000; Takahashi et al., 2001). In other words, mesoporous materials with large pore size usually end up with poor enzyme stability by allowing the adsorbed enzymes to leach-out very quickly from mesopores. The charge interaction plays a key role in determining the enzyme stability in mesoporous materials (Han et al., 2002; Lei et al., 2002; Vinu et al., 2004a,b). If the charge of mesopores is opposite to the net surface charge of enzymes, it will make a stable enzyme system because of the attractive interaction between two opposite charges, which acts against the leaching of enzymes from the mesopores. On the other hand, when enzymes and mesopores have the same charge, enzyme stability becomes poor due to the repulsion between enzymes and the internal surface of mesopores, leading to a serious leaching of enzymes out of mesoporous materials. The charge status of enzymes and mesopores can be controlled by changing the pH of buffer solution (Vinu et al., 2004a,b) and functionalizing mesoporous materials with various functional groups such as amino or carboxyl groups (Lei et al., 2002).

Due to the lack of strong binding force between enzyme molecules and the supports, one serious problem with the adsorption approach is enzyme leaching, resulting in poor enzyme loading and stability. To prevent this problem, Wang et al. (2001) covalently attached an enzyme (α-chymotrypsin) into mesoporous silica and investigated the stability of both native and immobilized enzymes in anhydrous methanol. The measured half-life of the covalently attached enzyme was over 1000-fold higher than that of the native enzyme. The enhanced stability in methanol, excluding the possibility of enzyme autolysis, demonstrated that the covalent binding provided effective protection against enzyme inactivation caused by structural denaturation. Wang et al. hypothesized that since the concave curvature of mesopores is comparable to the convex curvature of the surface of enzymes, thus mesopores provide an ideal configuration for multipoint covalent attachment of enzyme molecules, resulting in better stabilization compared to a flat surface (Fig. 2). It

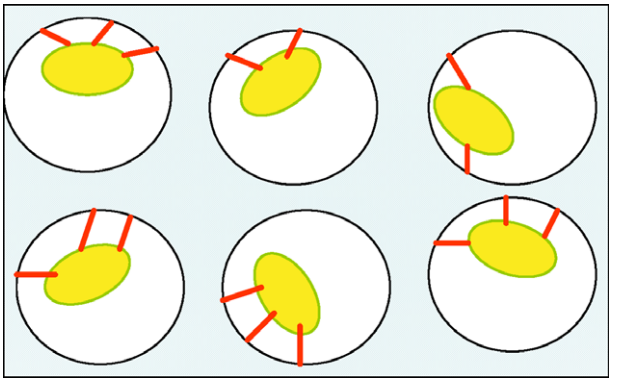

Fig. 2. Covalent binding of enzyme molecules onto mesoporous glass. The curvature of the pores provides the potential for multiple-point attachment whereas flat surface mostly achieves single-pint binding (Wang et al., 2001).

is generally accepted that more covalent attachments per enzyme molecule result in a more stable form of enzymes (Mozhaev et al., 1990).

The mesoporous environment can also be fabricated as nanometer-scale reactors for multi-enzyme catalysis with co-immobilized enzymes and cofactors (El-Zahab et al., 2004). LDH, glucose dehydrogenase (GDH), and cofactor (NADH) were covalently co-immobilized in porous silica particles with pore sizes of 30 or 100 nm in diameter. NADH is converted to NAD^+ during the LDH-catalyzed reduction of pyruvate to lactate whereas NADH is regenerated from NAD^+ via GDH-catalyzed oxidation of glucose. This approach can be directly applied in the construction of electrodes for biofuel cells. Co-immobilization of enzyme and redox mediators via covalent binding will prevent the leaching problem during the continuous feeding of fuels.

Recently, several rigorous approaches have been proposed for developing a stable enzyme system in mesoporous media. Ma et al. (2004) partly closed the inlet of mesopores by using a silane monomer such as vinyltrimethoxysilane (VTMS) after the enzyme was adsorbed into the mesoporous silica. It was demonstrated that the treatment resulted in a good entrapment of enzymes by preventing enzyme leaching, but did not inhibit the transfer of a smaller substrate and product than the enzyme molecules (Fig. 3C). Wang and Caruso (2004) made a coating on the surface of enzyme-adsorbed mesoporous silica with an organic/inorganic composite shell. This approach resulted in high enzyme loading and stability, and the entrapped enzymes were protected from proteolysis since proteases cannot penetrate through the coating layers (Fig. 3D).

The above two approaches demonstrated the prevention of enzyme leaching, but this requires a rigorous optimization to prevent the complete closure of mesopores that can lead to a serious mass-transfer limitation of the substrate. Recently, Lee et al. (2005) used a bimodal mesoporous silica for enzyme immobilization via a ship-in-a-bottle approach, which employs adsorption of enzymes followed by crosslinking using glutaraldehyde (GA) treatment. The crosslinked enzyme aggregates (CLEA) in the main mesocellular pores (37 nm in size) would not leach out through narrower window pores (13 nm in size), resulting in an impressive stability and activity with an extremely high loading of enzymes (Fig. 3E). For example, CLEA of α-chymotrypsin (CLEA-CT) in a mesoporous silica could hold 0.5 g CT in 1 g of silica, which is comparable to the maximal loading of CT in mesoporous silica. CLEA-CT showed no decrease in activity in a rigorously shaking condition

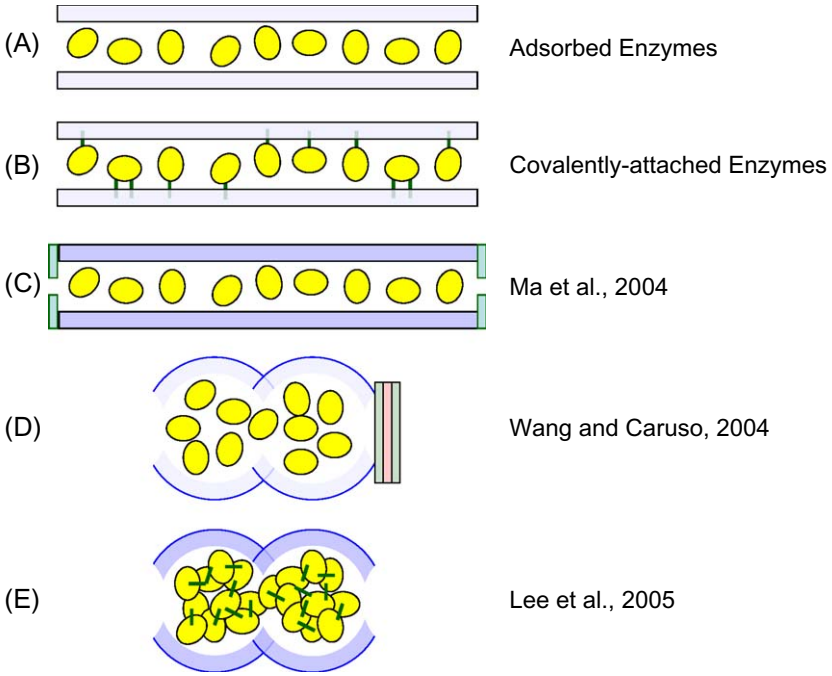

Fig. 3. Several recent advances in the development of stable enzyme systems in mesoporous silica: (A) adsorption; (B) covalent attachment (Wang et al., 2001); (C) partial closure of mesopore inlets (Ma et al., 2004); (D) nanocomposite shell on the particle surface (Wang and Caruso, 2004); and (E) crosslinked enzyme aggregates via a ship-in-a-bottle approach (Lee et al., 2005).

for more than a month whereas the adsorption method resulted in a half life of 3.6 days in the same condition. Since this ship-in-a-bottle approach can be easily expanded to many other enzymes, the stabilized enzyme activity of CLEAs in mesoporous media will make a major impact in many applications, including biofuel cells.

3.2. Nanoparticles

Micrometer-sized materials have been extensively examined as a carrier for enzyme immobilization (Xu et al., 1996; Govardhan, 1999; Haring and Schreier, 1999). Recently, there has been growing interest in the use of nanoparticles as a host for enzymes (Daubresse et al., 1996; Martins et al., 1996; Caruso and Schuler, 2000; Liao and Chen, 2001; Jia et al., 2003). The effective enzyme loading on nanoparticles could be achieved up to 6.4 or 10 wt.% due to a large surface area per unit mass of nanoparticles (Jia et al., 2003). Further theoretical and experimental studies revealed that particle mobility, related to particle size and solution viscosity, could affect the intrinsic activity of the particle-attached enzymes (Jia et al., 2003).

Despite these promising features provided by nanoparticle-attached enzymes, their dispersion in reaction solutions and the subsequent recovery for reuse are often a daunting task. A simple solution to this problem is to use magnetic nanoparticles, which can be separated from the reaction medium simply by using a magnet. This strategy was demonstrated by a study in which a lipase was attached to γ-Fe_2O_3 nanoparticles via covalent bonds (Dyal et al., 2003). For the covalent attachment of enzyme molecules, the nanoparticle surface was activated with either acetyl or amine groups that can directly react with or be connected by glutaraldehyde to the amine groups on the surface of enzyme molecules. An enzyme loading up to 5.6 wt.% was achieved on γ-Fe_2O_3 nanoparticles with an average size of 20 ± 10 nm in diameter. Although the immobilized enzyme displayed much lower activity than native enzymes (less than 1% compared to native enzymes), their operational stability was greatly enhanced. During a period of one month, there was only ~15% loss of activity observed.

3.3. Nanofibers and nanotubes

Nanoparticles provide the upper limits in terms of balancing the contradictory issues including surface area, mass-transfer resistance, and effective enzyme loading. However, their dispersion in reaction solutions and the subsequent recovery for reuse are difficult as mentioned above. It appears that the use of nanofibers would overcome this problem while still keeping the advantageous features of nanometer-sized materials. Electrospinning has proven to be a simple and versatile method to prepare nanofibers from a variety of materials (Reneker and Chun, 1996; Fang and Reneker, 1997; Norris et al., 2000; MacDiarmid et al., 2001; Megelski et al., 2002; Frenot and Chronakis, 2003; Li et al., 2003; Wnek et al., 2003; Li et al., 2004; Li and Xia, 2004).

Electrospun nanofibers provide a large surface area for the attachment or entrapment of enzymes. In the case of porous nanofibers, they can reduce the diffusional path of the substrate from the reaction medium to the enzyme active sites because of the reduced dimension in size, leading to better enzyme activity. Electrospinning can generate non-woven mats or well-aligned arrays of nanofibers with controllable compositions and sizes in a matter of minutes (Reneker and Chun, 1996; Fang and Reneker, 1997; Norris et al., 2000; MacDiarmid et al., 2001; Megelski et al., 2002; Frenot and Chronakis, 2003; Li et al., 2003; Wnek et al., 2003; Li et al., 2004; Li and Xia, 2004). Electrospun nanofiber mats are durable and easily separable and can also be processed in a highly porous form to relieve the mass-transfer limitation of the substrate through the mats.

Because of these attractive features, electrospun nanofibers have generated much attention as supports for enzyme immobilization (Jia et al., 2002; Smith et al., 2002; Al-Sheheri, 2003; Wang and Hsieh, 2003; Xie and Hsieh, 2003; Zeng et al., 2003; Bruno et al., 2004; Gouma et al., 2004; Hsieh et al., 2004; Wang and Hsieh, 2004; Zeng et al., 2004; Chua et al., 2005; Kim, 2005; Wu et al., 2005). As a first report, α-chymotrypsin was covalently attached to the polystyrene nanofibers of 120 nm diameter (Jia et al., 2002). The observed enzyme loading was achieved up to 1.4% (wt/wt), corresponding to over 27.4% monolayer coverage of the external surface of nanofibers. The specific activity of the nanofibrous enzyme was over 65% of that of the native enzyme in aqueous solution, indicating a relatively low diffusional limitation. When the nanofibrous α-chymotrypsin was used in organic solvents, such as hexane and isooctane, it exhibited over three orders of magnitude higher activity than that of its native counterpart. The half-life of the nanofibrous enzyme in anhydrous methanol was 18-fold higher than that of the native enzyme, suggesting that the covalent bonding improved the enzyme stability against structural denaturation.

Recently, Kim et al. (2005) successfully developed an active and stable enzyme system using electrospun nanofibers. They fabricated the enzyme aggregate coatings on the surface of electrospun polymer nanofibers. This approach employs the covalent attachment of seed enzyme molecules onto nanofibers, followed by the glutaraldehyde (GA) treatment crosslinking additional enzyme molecules or aggregates onto the covalently attached seed enzyme molecules (Fig. 4C). The apparent activity of α-chymotrypsin coatings based on per unit mass of fibers was nine times higher than that of covalently attached enzymes on nanofibers. The operational stability of enzyme coatings was greatly improved with no measurable loss of enzyme activity over a month of observation under rigorous shaking. This new approach of enzyme coatings on nanofibers, yielding high activity and stability, creates an economically viable enzyme system for using expensive enzymes with potential applications in various fields, such as biofuel cells, bioconversion, bioremediation, and biosensors.

Carbon nanotubes (CNTs), single-walled (SW) or multi-walled (MW), typically have a diameter ranging from a few to several tens of nanometers and a length of up to hundreds of micrometers. Their unique physicochemical properties have attracted extensive research in a wide spectrum of scientific areas such as scanning probe microscopy (Poggi et al., 2002), electrochemical actuators (Baughman et al., 1999), and biosensors (Sotiropoulou and Chaniotakis, 2003; Sotiropoulou et al., 2003). Enzyme-polymer-single-walled carbon nanotube (SWNT) composites were prepared and examined for biocatalytic performance (Rege et al., 2003). Improved enzyme activity was observed in comparison to similar enzyme-containing composites without using SWNTs. It was discussed that the use of SWNTs, which possesses a high specific surface area, may effectively adsorb enzyme molecules and retain the enzyme within the polymer matrix, whereas other forms of enzyme-composites may suffer from enzyme loss via leaching when they were placed in contact with aqueous solutions. The stable and active enzyme system on conductive CNTs will make a great impact in the field of biofuel cells.

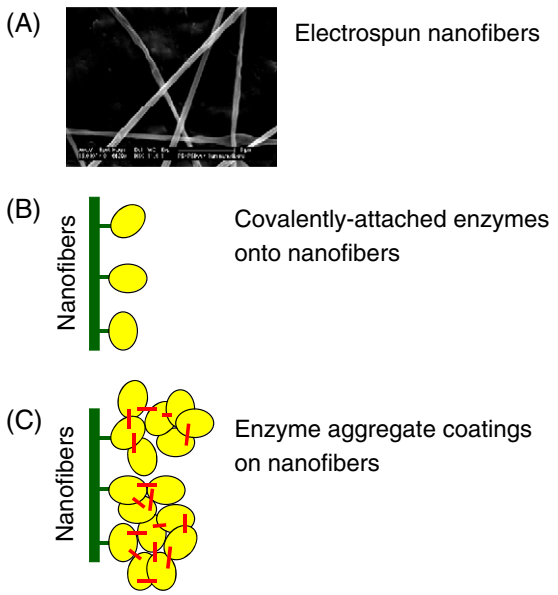

Fig. 4. Enzyme immobilization on electrospun nanofibers: (A) electrospun nanofibers; (B) covalent attachment (Jia et al., 2002); (C) enzyme coatings on nanofibers (Kim, 2005).

3.4. Single enzyme nanoparticles

As an innovative means of enzyme stabilization, Kim and Grate (2003) have developed an approach to develop single-enzyme nanoparticles (SENs) that dramatically stabilize the enzyme by surrounding each enzyme molecule with a porous composite organic/inorganic network of less than a few nanometers thick. The synthetic procedure, consisting of enzyme modification and two orthogonal polymerization steps, yields nanoparticles containing a single enzyme molecule. In experiments with α-chymotrypsin (CT), the incorporation into the nanostructure dramatically increased the enzymatic stability. For example, the half-lives of free CT and SEN-CT were 12 h and 143 days, respectively. When stored in buffer solution at 4 °C, SEN-CT showed a negligible decrease in CT activity over 5 months. As an extension, it was also demonstrated that the trypsin can be stabilized in a form of SENs. Furthermore, the nanoscale structure around the enzyme is sufficiently thin that it does not impose a significant mass transfer limitation on the substrate. This unique synthetic approach, leading to a stable and active form of enzymes, is different from conventional enzyme modification and enzyme immobilization.

Since SENs are still nanometer scale (less than 10 nm in size for the case of SEN-CT), they could be further immobilized in nano-structured matrices (Fig. 5). Nano-structured matrices, providing a large surface area for the attachment of SENs, can improve the loading of SENs, leading to increased power density in biofuel cells. In that sense, using nano-structured matrices will be a powerful approach in developing miniaturized biofuel cells that are limited by the surface

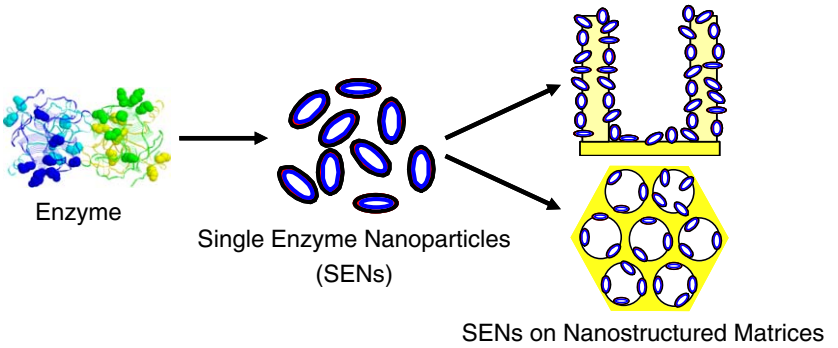

Fig. 5. Immobilization of SENs on nano-structured matrices, such as well-aligned carbon nanotubes and nanoporous media (Kim and Grate, 2003).

area for the attachment of enzymes. As an exemplary demonstration, SEN-CTs were adsorbed into mesoporous silica with a high surface area, and this approach resulted in a good volumetric enzyme activity and secondary stabilization because of the protection of SENs in nanoporous silica. The combination of SENs (active and stable form of enzyme) and mesoporous materials (immobilization supports with a large surface area and tunable pore size) will result in an ideal enzyme system for various applications, including enzyme-based biofuel cells.

4. Conclusions

There is growing interest in enzyme-based biofuel cells as a source of renewable and sustainable power. They are attractive for special applications, such as implantable devices, sensors, drug delivery, microchips, and portable power supplies. Several drawbacks, such as short lifetime and low power density, have limited enzyme-based biofuel cells from being used for practical applications. Recent developments in the newly emerging nanobiocatalysis appear to be promising because they provide some solutions in overcoming the present bottle-neck problems. Better understanding and further developments of nanobiocatalysis will expedite the improvement of biofuel cells, and high performance biofuel cells may soon take a role in the dynamic energy market.

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References

Allen RM, Bennetto HP. Microbial fuel-cells: electricity production from carbohydrates. Appl Biochem Biotechnol 1993;39–40:27–40.

Al-Sheheri HA. The use of electrospinning technology in enzymes preservation and chemical warfare protective clothing applications. The University of Akron, Dissertation, 2003.

Aston WJ, Turner APF. Biosensors and biofuel cells. Biotechnol Genet Eng Rev 1984;1:89–120.

Bardea A, Katz E, Bueckmann AF, Willner I. NAD^+-dependent enzyme electrodes: electrical contact of cofactor-dependent enzymes and electrodes. J Am Chem Soc 1997;119:9114–9.

Barton SC, Gallaway J, Atanassov P. Enzymatic biofuel cells for implantable and microscale devices. Chem Rev 2004;104:4867–86.

Baughman RH, Cui C, Zakhidov AA, Iqbal Z, Barisci JN, Spinks GM, et al. Carbon nanotube actuators. Science 1999;284:1340–4.

Blonder R, Willner I, Bueckmann AF. Reconstitution of apo-glucose oxidase on nitrospiropyran and FAD mixed monolayers on gold electrodes: photostimulation of bioelectrocatalytic features of the biocatalyst. J Am Chem Soc 1998;120:9335–41.

Bockris JOM, Srinivasan S. Fuel cells: their electrochemistry. New York: McGraw-Hill; 1969.

Bruno FF, Drew C, Nagarajan R, Wang X, Kumar J, Samuelson LA. Conductive polymer complexes from macromolecule inspired biocatalysis. Polym Mater Sci Eng 2004;90:234–5.

Cai C, Chen J. Direct electron transfer of glucose oxidase promoted by carbon nanotubes. Anal Biochem 2004;332:75–83.

Caruso F, Schuler C. Enzyme multilayers on colloid particles: assembly, stability, and enzymatic activity. Langmuir 2000;16:9595–603.

Chen T, Barton SC, Binyamin G, Gao Z, Zhang Y, Kim H-H, et al. A miniature biofuel cell. J Am Chem Soc 2001;123:8630–1.

Chua K-N, Lim W-S, Zhang P, Lu H, Wen J, Ramakrishna S, et al. Stable immobilization of rat hepatocyte spheroids on galactosylated nanofiber scaffold. Biomaterials 2005;26:2537–47.

Daubresse C, Grandfils C, Jerome R, Teyssie P. Enzyme immobilization in reactive nanoparticles produced by inverse microemulsion polymerization. Colloid Polym Sci 1996;274:482–9.

Davis Mark E.. Ordered porous materials for emerging applications. Nature 2002;417:813–21.

Diaz JF, Balkus Jr KJ. Enzyme immobilization in MCM-41 molecular sieve. J Mol Catal, B Enzym 1996;2:115–26.

Dyal A, Loos K, Noto M, Chang SW, Spagnoli C, Shafi KVPM, et al. Activity of candida rugosa lipase immobilized on g-Fe_2O_3 magnetic nanoparticles. J Am Chem Soc 2003;125:1684–5.

El-Zahab B, Jia H, Wang P. Enabling multienzyme biocatalysis using nanoporous materials. Biotechnol Bioeng 2004;87:178–83.

Fadnavis NW, Bhaskar V, Kantam ML, Choudary BM. Highly efficient "tight fit" immobilization of α-chymotrypsin in mesoporous MCM-41: a novel approach using precursor immobilization and activation. Biotechnol Prog 2003;19:346–51.

Fan J, Lei J, Wang L, Yu C, Tu B, Zhao D. Rapid and high-capacity immobilization of enzymes based on mesoporous silicas with controlled morphologies. Chem Commun 2003a;2140–1.

Fan J, Yu C, Gao F, Lei J, Tian B, Wang L, et al. Cubic mesoporous silica with large controllable entrance sizes and advanced adsorption properties. Angew Chem Int Ed 2003b; 42:3146–50.

Fang X, Reneker DH. DNA fibers by electrospinning. J Macromol Sci, Phys 1997;B36:169–73.

Freire RS, Pessoa CA, Mello LD, Kubota LT. Direct electron transfer: an approach for electrochemical biosensors with higher selectivity and sensitivity. J Braz Chem Soc 2003;14:230–43.

Frenot A, Chronakis IS. Polymer nanofibers assembled by electrospinning. Curr Opin Colloid Interface Sci 2003;8:64–75.

Ghindilis AL, Atanasov P, Wilkins E. Enzyme-catalyzed direct electron transfer: fundamentals and analytical applications. Electroanalysis 1997;9:661–74.

Gouma P, Simon S, Jha PK, Sawicka K. Bio-composite oxides for resistive detection of pathogens. Chem Senses 2004;20:72–3.

Govardhan CP. Crosslinking of enzymes for improved stability and performance. Cur Opin Biotechnol 1999;10:331–5.

Govil G, Saran A. Biochemical fuel cells. J Indian Chem Soc 1982;59:1226–8.

Guiseppi-Elie A, Lei C, Baughman RH. Direct electron transfer of glucose oxidase on carbon nanotubes. Nanotechnology 2002;13: 559–64.

Han Y-J, Watson JT, Stucky GD, Butler A. Catalytic activity of mesoporous silicate-immobilized chloroperoxidase. J Mol Catal, B Enzym 2002;17:1–8.

Haring D, Schreier P. Cross-linked enzyme crystals. Curr Opin Chem Biol 1999;3:35–8.

Heilmann A, Teuscher N, Kiesow A, Janasek D, Spohn U. Nanoporous aluminum oxide as a novel support material for enzyme biosensors. J Nanosci Nanotechnol 2003;3:375–9.

Heller A. Miniature biofuel cells. Phys Chem Chem Phys 2004; 6:209–16.

Hsieh Y-L, Xie J, Wang Y, Chen H, Li L, Zhang L, et al. Generation of polymer-based nano-porous fibers and protein or enzyme membrane compositions. PCT Int Appl WO 2004044281.

Jia H, Zhu G, Vugrinovich B, Kataphinan W, Reneker DH, Wang P. Enzyme-carrying polymeric nanofibers prepared via electrospinning for use as unique biocatalysts. Biotechnol Prog 2002;18: 1027–32.

Jia H, Zhu G, Wang P. Catalytic behaviors associated with enzymes attached to nanoparticles: the effect of particle mobility. Biotechnol Bioeng 2003;84:406–14.

Kang C, Shin H, Zhang Y, Heller A. Deactivation of bilirubin oxidase by a product of the reaction of urate and O_2. Bioelectrochemistry 2004;65:83–8.

Katz E, Willner I. A biofuel cell with electrochemically switchable and tunable power output. J Am Chem Soc 2003a;125:6803–13.

Katz E, Willner I. Biofuel cells based on monolayer-functionalized biocatalytic electrodes. In: Geckeler KE, editor. Advanced macromolecular and supramolecular materials and processes. New York: Kluwer Academic/Plenum Publishers; 2003b. p. 175–96.

Katz E, Heleg-Shabtai V, Bardea A, Willner I, Rau HK, Haehnel W. Fully integrated biocatalytic electrodes based on bioaffinity interactions. Biosens Bioelectron 1998;13:741–56.

Katz E, Filanovsky B, Willner I. A biofuel cell based on two immiscible solvents and glucose oxidase and microperoxidase-11 monolayer-functionalized electrodes. New J Chem 1999a;23: 481–7.

Katz E, Willner I, Kotlyar AB. A non-compartmentalized glucose O_2 biofuel cell by bioengineered electrode surfaces. J Electroanal Chem 1999b;479:64–8.

Katz E, Bueckmann AF, Willner I. Self-powered enzyme-based biosensors. J Am Chem Soc 2001;123:10752–3.

Katz E, Shipway NA, Willner I. Biochemical fuel cells. In: Vielstich A, Lamm A, Gasteiger HA, editors. Handbook of fuel cells — fundamentals technology and applications. Chichester: John Wiley and Sons Ltd.; 2003. p. 355–81.

Katz E, Sheeney-Haj-Ichia L, Willner I. Electrical contacting of glucose oxidase in a redox-active rotaxane configuration. Angew Chem Int Ed 2004;43:3292–300.

Kim J. Enzyme-polymer composites with high biocatalytic activity and stability. Polym Mater Sci Eng 2005;92:552–3.

Kim J, Grate JW. Single-enzyme nanoparticles armored by a nanometer-scale organic/inorganic network. Nano Let 2003;3: 1219–22.

Kim S-W, Kim M, Lee WY, Hyeon T. Fabrication of hollow palladium spheres and their successful application as the recyclable heterogeneous catalyst for suzuki coupling reactions. J Am Chem Soc 2002;124:7642–3.

Kim HH, Mano N, Zhang XC, Heller A. A miniature membraneless biofuel cell operating under physiological conditions at 05 V. J Electrochem Soc 2003;150:A209–13.

Kim BC, Nair S, Kim J, Kwak JH, Grate JW, Kim SH, et al. Preparation of biocatalytic nanofibres with high activity and stability via enzyme aggregate coating on polymer nanofibres. Nanotechnology 2005;16:S382–8.

Kulys JJ, Samalius AS. Dependence of the efficiency of bioelectrocatalytic processes on the electrode surface-state. Bioelectrochem Bioenerg 1984;13:163–9.

Lee J, Sohn K, Hyeon T. Fabrication of novel mesocellular carbon foams with uniform ultralarge mesopores. J Am Chem Soc 2001;123:5146–7.

Lee J, Han S, Hyeon T. Synthesis of new nanoporous carbon materials using nanostructured silica materials as templates. J Mater Chem 2004;14:478–86.

Lee J, Kim J, Kim J, Jia H, Kim M-I, Kwak JH, et al. Simple synthesis of hierarchically ordered mesocellular mesoporous silica materials and their successful application as a host of enzyme immobilization. Small 2005;1:744–53.

Lei C, Shin Y, Liu J, Ackerman EJ. Entrapping enzyme in a functionalized nanoporous support. J Am Chem Soc 2002;124: 11242–3.

Lei J, Fan J, Yu C, Zhang L, Jiang S, Tu B, et al. Immobilization of enzymes in mesoporous materials: controlling the entrance to nanospace. Microporous Mesoporous Mater 2004; 73:121–8.

Lewis K. Biochemical fuel cells. Bacteriol Rev 1966;30:101–13.

Li D, Xia Y. Direct fabrication of composite and ceramic hollow nanofibers by electrospinning. Nano Let 2004;4:933–8.

Li D, Wang Y, Xia Y. Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays. Nano Let 2003;3: 1167–71.

Li D, Wang Y, Xia Y. Electrospinning nanofibers as uniaxially aligned arrays and layer-by-layer stacked films. Adv Mater 2004;16:361–6.

Liao M-H, Chen D-H. Immobilization of yeast alcohol dehydrogenase on magnetic nanoparticles for improving its stability. Biotechnol Lett 2001;23:1723–7.

Lioubashevski O, Chegel VI, Patolsky F, Katz E, Willner I. Enzyme-catalyzed bio-pumping of electrons into Au-nanoparticles: a surface plasmon resonance and electrochemical study. J Am Chem Soc 2004;126:7133–43.

Liu B, Hu R, Deng J. Characterization of immobilization of an enzyme in a modified Y zeolite matrix and its application to an amperometric glucose biosensor. Anal Chem 1997;69:2343–8.

Liu B, Hu R, Deng J. Fabrication of an amperometric biosensor based on the immobilization of glucose oxidase in a modified molecular sieve matrix. Analyst 1997;122:821–6.

Liu B, Cao Y, Chen D, Kong J, Deng J. Amperometric biosensor based on a nanoporous ZrO_2 matrix. Anal Chim Acta 2003; 478:59–66.

Ma H, He J, Evans DG, Duan X. Immobilization of lipase in a mesoporous reactor based on MCM-41. J Mol Catal, B Enzym 2004;30:209–17.

MacDiarmid AG, Jones WE, Norris ID, Gao J, Johnson AT, Pinto NJ, et al. Electrostatically-generated nanofibers of electronic polymers. Synth Met 2001;119:27–30.

Mano N, Mao F, Heller A. A miniature biofuel cell operating in a physiological buffer. J Am Chem Soc 2002;124:12962–3.

Mano N, Mao F, Heller A. Characteristics of a miniature compartment-less glucose-O_2 biofuel cell and its operation in a living plant. J Am Chem Soc 2003a;125:6588–94.

Mano N, Mao F, Shin W, Chen T, Heller A. A miniature biofuel cell operating at 078 V. Chem Commun 2003b;518–819.

Martins MBF, Simoes SID, Cruz MEM, Gaspar R. Development of enzyme-loaded nanoparticles: effect of pH. J Mater Sci, Mater Med 1996;7:413–4.

Megelski S, Stephens JS, Chase DB, Rabolt JF. Micro- and nanostructure surface morphology on electrospun polymer fibers. Macromolecules 2002;35:8456–66.

Minteer SD, Akers NL, Moore CM. Enzyme immobilization for use in biofuel cells and sensors. U.S. Pat. Application Publication (2004) US 2004101741.

Moore CM, Akers NL, Hill AD, Johnson ZC, Minteer SD. Improving the environment for immobilized dehydrogenase enzymes by modifying nafion with tetraalkylammonium bromides. Biomacromolecules 2004;5:1241–7.

Mozhaev VV, Melik-Nubarov NS, Sergeeva MV, Siksnis V, Martinek K. Strategy for stabilizing enzymes Part One: Increasing stability of enzymes via their multi-point interaction with a support. Biocatalysis 1990;3:179–87.

Narayanan SR, Valdez TI. Portable direct methanol fuel cell system. In: Vielstich W, Lamm A, Gasteiger HA, editors. Handbook of fuel cells- fundamentals technology and applications. Chichester: John Wiley and Sons Ltd; 2003. p. 1133–41.

Niessen J, Schroder U, Rosenbaum M, Scholz F. Fluorinated polyanilines as superior materials for electrocatalytic anodes in bacterial fuel cells. Electrochem Commun 2004a;6:571–5.

Niessen J, Schroder U, Scholz F. Exploiting complex carbohydrates for microbial electricity generation — a bacterial fuel cell operating on starch. Electrochem Commun 2004b;6:955–8.

Norris ID, Shaker MM, Ko FK, MacDiarmid AG. Electrostatic fabrication of ultrafine conducting fibers: polyaniline/polyethylene oxide blends. Synth Met 2000;114:109–14.

Palmore GTR, Whitesides GM. Microbial and enzymic biofuel cells. ACS Symp Ser 1994;566:271–90.

Patolsky F, Tao G, Katz E, Willner I. C60-Mediated bioelectrocatalyzed oxidation of glucose with glucose oxidase. J Electroanal Chem 1998;454:9–13.

Pizzariello A, Stredansky M, Miertus S. A glucose/hydrogen peroxide biofuel cell that uses oxidase and peroxidase as catalysts by composite bulk-modified bioelectrodes based on a solid binding matrix. Bioelectrochemistry 2002;56:99–105.

Poggi MA, Bottomley LA, Lillehei PT. Scanning probe microscopy. Anal Chem 2002;74:2851–62.

Potter MC. Electrical effects accompanying the decomposition of organic compounds. Proc R Soc B, Biol Sci 1912;84:260–76.

Rege K, Raravikar NR, Kim D-Y, Schadler LS, Ajayan PM, Dordick JS. Enzyme-polymer-single walled carbon nanotube composites as biocatalytic films. Nano Let 2003;3:829–32.

Reneker DH, Chun I. Nanometer diameter fibers of polymer, produced by electrospinning. Nanotechnology 1996;7:216–23.

Sasaki T, Kajino T, Li B, Sugiyama H, Takahashi H. New pulp biobleaching system involving manganese peroxidase immobilized in a silica support with controlled pore sizes. Appl Environ Microbiol 2001;67:2208–12.

Schmidt-Winkel P, Lukens WW, Zhao DY, Yang PD, Chmelka BF, Stucky GD. Mesocellular siliceous foams with uniformly sized cells and windows. J Am Chem Soc 1999;121:254–5.

Schroder U, Niessen J, Scholz F. A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. Angew Chem Int Ed 2003;42:2880–3.

Schuhmann W. Amperometric enzyme biosensors based on optimized electron-transfer pathways and non-manual immobilization procedures. Rev Mol Biotechnol 2002;82:425–41.

Schuth F, Schmidt W. Microporous and mesoporous materials. Adv Mater 2002;14:629–38.

Smith, D., Kataphinan, W., Reneker, D., Dabney, S. Preservation of biological materials using fiber-forming techniques. WO 2002100628 (2002).

Sotiropoulou S, Chaniotakis NA. Carbon nanotube array-based biosensor. Anal Bioanal Chem 2003;375:103–5.

Sotiropoulou S, Gavalas V, Vamvakaki V, Chaniotakis NA. Novel carbon materials in biosensor systems. Biosens Bioelectron 2003; 18:211–5.

Soukharev V, Mano N, Heller A. A four-electron O_2-electroreduction biocatalyst superior to platinum and a biofuel cell operating at 088 v. J Am Chem Soc 2004;126:8368–9.

Takahashi H, Li B, Sasaki T, Miyazaki C, Kajino T, Inagaki S. Catalytic activity in organic solvents and stability of immobilized enzymes depend on the pore size and surface characteristics of mesoporous silica. Chem Mater 2000;12:3301–5.

Takahashi H, Li B, Sasaki T, Miyazaki C, Kajino T, Inagaki S. Immobilized enzymes in ordered mesoporous silica materials and improvement of their stability and catalytic activity in an organic solvent. Microporous Mesoporous Mater 2001;44–45: 755–62.

Tarasevich MR, Bogdanovskaya VA, Zagudaeva NM, Kapustin AV. Composite materials for direct bioelectrocatalysis of the hydrogen and oxygen reactions in biofuel cells. Russ J Electrochem 2002;38:335.

Varfolomeev SD, Kurochkin IN, Yaropolov AI. Direct electron transfer effect biosensors. Biosens Bioelectron 1996;11:863–71.

Vinu A, Murugesan V, Hartmann M. Adsorption of lysozyme over mesoporous molecular sieves MCM-41 and SBA-15: influence of pH and aluminum incorporation. J Phys Chem B 2004a;108: 7323–30.

Vinu A, Murugesan V, Tangermann O, Hartmann M. Adsorption of cytochrome *c* on mesoporous molecular sieves: influence of pH, pore diameter, and aluminum incorporation. Chem Mater 2004b; 16:3056–65.

Wang P, Dai S, Waezsada SD, Tsao A, Davison BH. Enzyme stabilization by covalent binding in nanoporous sol–gel glass for nonaqueous biocatalysis. Biotechnol Bioeng 2001;74:249–55.

Wang YJ, Caruso F. Enzyme encapsulation in nanoporous silica spheres. Chem Commun 2004;1528–9.

Wang Y, Hsieh Y-L. Enzyme immobilization via electrospinning of polymer/enzyme blends. Polym Prep (Am Chem Soc, Div Polym Chem) 2003;44(1);1212–3.

Wang Y, Hsieh Y-L. Enzyme immobilization to ultra-fine cellulose fibers via amphiphilic polyethylene glycol spacers. J Polym Sci, A, Polym Chem 2004;42:4289–99.

Willner I, Heleg-Shabtai V, Blonder R, Katz E, Tao G, Bueckmann AF, et al. Electrical wiring of glucose oxidase by reconstitution of FAD-modified monolayers assembled onto au-electrodes. J Am Chem Soc 1996;118:10321–2.

Willner I, Arad G, Katz E. A biofuel cell based on pyrroloquinoline quinone and microperoxidase-11 monolayer-functionalized electrodes. Bioelectrochem Bioenerg 1998a;44:209–14.

Willner I, Katz E, Patolsky F, Buckmann AF. Biofuel cell based on glucose oxidase and microperoxidase-11 monolayer-functionalized electrodes. J Chem Soc, Perkin Transact 2, Phys Org Chem 1981b;817–22.

Winder R. Alcoholic fuel. Chem Ind 2003;15–8.

Wnek GE, Carr ME, Simpson DG, Bowlin GL. Electrospinning of nanofiber fibrinogen structures. Nano Let 2003;3:213–6.

Wu L, Yuan X, Sheng J. Immobilization of cellulase in nanofibrous PVA membranes by electrospinning. J Membr Sci 2005;250: 167–73.

Xiao Y, Patolsky F, Katz E, Hainfeld JF, Willner I. "Plugging into Enzymes": nanowiring of redox enzymes by a gold nanoparticle. Science 2003;299:1877–81.

Xie J, Hsieh Y-L. Ultra-high surface fibrous membranes from electrospinning of natural proteins: casein and lipase enzyme. J Mater Sci 2003;38:2125–33.

Xing G-W, Li X-W, Tian G-L, Ye Y-H. Enzymatic peptide synthesis in organic solvent with different zeolites as immobilization matrixes. Tetrahedron 2000;56:3517–22.

Xu H-X, Li M-Q, Pan Z-Q, Ma J-B, He B-L. Immobilization of L-asparaginase on dextran magnetic nanoparticles. Shengwu Hua Za Zhi 1996;12:744–6.

Yahiro AT, Lee SM, Kimble DO. Bioelectrochemistry I Enzyme utilizing biofuel cell studies. Biochim Biophys Acta 1964;88: 375–83.

Yaropolov AI, Sukhomlin TK, Karyakin AA, Varfolomeev SD, Berezin IV. Possibility of electron tunneling transfer during enzymic catalysis of electrode processes. Dokl Akad Nauk SSSR 1981; 260:1192–5.

Ying JY, Mehnert CP, Wong MS. Synthesis and applications of supramolecular-templated mesoporous materials. Angew Chem Int Ed 1999;38:56–77.

Yiu HHP, Wright PA, Botting NP. Enzyme immobilization using siliceous mesoporous molecular sieves. Microporous Mesoporous Mater 2001;44–45:763–8.

Zeng ZSJ, Hou H, Kissel T, Wendorff JH, Greiner A. Functional polymer nanofibers and nanotubes via electrospinning: chemical modifications for selected applications. Polym Prep (Am Chem Soc, Div Polym Chem) 2003;44(2);76–7.

Zhao Y-D, Zhang W-D, Chen H, Luo Q-M. Direct electron transfer of glucose oxidase molecules adsorbed onto carbon nanotube powder microelectrode. Anal Sci Int J Jpn Soc Anal Chem 2002; 18:939–41.

Zeng J, Chen X, Liang Q, Xu X, Jing X. Enzymatic degradation of poly(L-lactide) and poly(ε-caprolactone) electrospun fibers. Macromol Biosci 2004;4:1118–25.