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Review

Enzyme immobilization on electrospun polymer nanofibers: An overview

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ABSTRACT

Enzyme immobilization has attracted continuous attention in the fields of fine chemistry, biomedicine, and biosensor. The performance of immobilized enzyme largely depends on the structure of supports. Nanostructured supports are believed to be able to retain the catalytic activity as well as ensure the immobilization efficiency of enzyme to a high extent. Electrospinning provides a simple and versatile method to fabricate nanofibrous supports. Compared with other nanostructured supports (e.g. mesoporous silica, nanoparticles), nanofibrous supports show many advantages for their high porosity and interconnectivity. This review mainly discusses the recent advances in using nanofibers as hosts for enzyme immobilization by two different methods, surface attachment and encapsulation. Surface attachment refers to physical adsorption or covalent attachment of enzymes on pristine or modified nanofibrous supports, and encapsulation means electrospinning a mixture of enzyme and polymer. We make a detailed comparison between these two immobilization approaches and highlight their distinct characteristics. The prospective applications of enzyme immobilized electrospun nanofibers in the development of biosensors, biofuel cells and biocatalysts are also discussed.

Contents

1. Introduction

In recent decades, one-dimensional nanostructured materials have attracted much attention because of their unique properties and interesting applications. One-dimensional nanomaterials are normally in the forms of fibers, wires, rods, belts, tubes, spirals, or rings. They can be generated by various methods. Among them, electrospinning seems to be the simplest, by which one can fabricate nanofibers that are exceptionally long in length, uniform in diameter and diversified in composition. These unique features ensure the potential applications of electrospun nanofibers in many aspects, such as templates [1,2], reinforcement [3,4], filtration [5,6], catalysis [7,8], biomedical and pharmaceutical applications [9–11], electronic and optical devices [12–14]. Especially in the area of biocatalysis, electrospun nanofibers show distinctive characteristics and superiority.

Enzymes are well-known green catalysts that possess a high degree of specificity. The specificity involves discrimina-

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tion between substrates (substrate specificity), similar parts of molecules (regiospecificity), and optical isomers (stereospecificity) [15–20]. The mildness and specificity of enzymes endow them with a high efficiency for applications in fine-chemical/pharmaceutical synthesis, food processing, biosensors fabrication, bioremediation, and protein digestion in proteomic analysis [21–25]. However, the applications of enzymes are limited by their instability and non-reusability. Enzyme immobilization is an effective way to overcome these limitations to some extent. First, the multiple-point attachment to the support can restrict the undesirable conformational change of enzyme proteins in unfriendly environments. Second, insoluble supports can be recycled much more easily than soluble enzymes.

The results of immobilization, including the performance of immobilized enzymes, strongly depend on the properties of supports, which are usually referred to as material types, compositions, and structures, etc. So far, different nanostructured materials have been used as supports, such as mesoporous silica, nanotubes, nanoparticles, and nanofibers. They stand out of other supports because of their extremely high surface area-to-volume ratios, which can provide large specific surface areas for highly efficient immobilization as well as stabilize enzymes [26]. However, some of the nanostructured materials have disadvantages that are difficult to overcome. For example, mesoporous silica usually confines enzyme molecules on its inner surface, which limits the diffusion of substrate to/off the enzyme and results in lower enzyme activity. Nanoparticles and nanotubes are known to remarkably decrease mass transfer limitation, while their dispersion and recycling are more difficult. On the contrary, electrospun nanofibers have a great potential to overcome these problems, and may be promising supports for enzyme immobilization. Briefly, the qualification of nanofibers as excellent supports is attributed to: (i) a variety of polymers can be electrospun and meet different requirements as supports, (ii) the high porosity and the interconnectivity of electrospun supports endows them with a low hindrance for mass transfer, and (iii) the nanofiber surfaces can be modified to benefit enzyme activity. Although each nanofiber provides the surface for hosting enzymes, the collection of randomly arrayed nanofibers usually forms a non-woven mesh (or membrane) with reusability. From this point of view, the as-spun membranes have also been explored as filters [5,27,28]. The enzyme-immobilized nanofibrous membranes have functions of biocatalysis and separation simultaneously which is generally accepted as the fundamental requirement for enzymatic membrane-bioreactor [29–31]. Applications in biosensors [32,33] and biofuel cells [34] are also allowed for these nanofibrous membranes because of their porosity.

Several reviews have summarized the applications of supports with different nanostructures for enzyme immobilization [26,34,35]. The present paper is focused on electrospun nanofibrous supports, describing the role of the nanofiber surface (pristine and modified) and enzyme immobilization approaches (surface attachment and encapsulation). Prospective applications for these enzyme-immobilized nanofibrous membranes are also discussed.

2. Surface attachment of enzymes on nanofibers

2.1. Enzyme immobilization on pristine nanofibers

The first example dealing with nanofibers as supports for enzyme immobilization has been described by Jia et al. [36]. In their study, polystyrene synthesized with a hydroxyl-containing initiator was electrospun into nanofibers having reactive surfaces. Then, α-chymotrypsin was covalently attached onto these surfaces. The amount of loaded enzyme was up to 1.4 wt.% of the nanofibers. It showed that over 27.4% of the external nanofiber surface was covered with a monolayer of enzyme. Specific activity of the immobilized α-chymotrypsin was over 65% of that of the native enzyme in aqueous solution. Low diffusional limitation for the immobilized enzyme was reasonably deduced from this result. The stabilities of immobilized α-chymotrypsin to storage and organic solvents (e.g. isooctane and hexane) were much higher than those of the free ones. Pristine silk fibroin (SF) nanofibers were also used for α-chymotrypsin immobilization [37]. Interesting results were found with regard to the stabilities of immobilized enzymes on SF nanofibers with different diameters. α-Chymotrypsin on SF nanofibers with 205 nm diameter retained more than 90% of the initial activity after 24-h storage in aqueous solution and showed the highest storage stability. However, the highest stability in ethanol was obtained from α-chymotrypsin on SF nanofibers with 320 nm diameter which retained more than 45% of the initial activity.

In our group, nanofibers electrospun from poly(acrylonitrile-*co*-maleic acid) were used for lipase immobilization [38]. The amount and activity retention of lipase immobilized on the nanofibers were 21.2 mg/g fibers and 37.6%, respectively, while those on the corresponding hollow fiber membrane were only 2.36 mg/g membrane and 33.9%. Combined with the kinetic parameters, it can be concluded that nanofibers presents a low diffusion restriction on substrate. However, low activity retention for the immobilized lipase suggests that loss of enzymatic activity still exists. It can be partly attributed to the immobilization reaction and enzyme-support interactions. Despite that, this study provides a simple route to fabricate reactive-groups-containing nanofibers for the covalent immobilization of enzymes. To improve the performances of the immobilized enzymes, the reactive groups on the nanofibers are potential for further modification of the nanofiber surface. This will be discussed later.

Different from the above results, Li et al. [39] immobilized lipase by an amidination reaction using pristine polyacrylonitrile

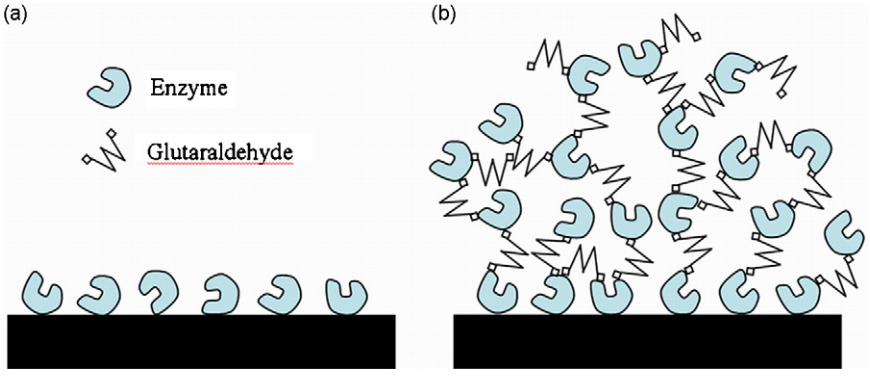

Fig. 1. Enzyme dispersion on the nanofiber in monolayer (a) and aggregate (b).

nanofibers as supports. Their results of activity retention showed that lipase immobilized by the conjugation method gave higher activity than those immobilized by other methods. Further explanation is needed for these results.

As biocompatible and biodegradable materials, nanofibers of poly(ε-caprolactone) and poly(D,L-lactic-*co*-glycolic acid)-*b*-poly(ethylene glycol)-NH_2 (PLGA-*b*-PEG-NH_2) block copolymer were used as supports to covalently immobilize lysozyme [40]. In addition to exhibiting considerable enzyme loading and enzymatic activity, these nanofibers are biodegradable. It is helpful to the post-treatment on supports.

Although nanofibers have large surface area-to-volume ratio, the immobilized enzymes only form monolayer on each fiber (see schematically in Fig. 1(a)), which largely limits the enzyme loading. Therefore, Kim et al. [41] introduced enzyme-aggregate coatings on the electrospun polymer nanofibers (Fig. 1(b)). In their work, seed enzyme molecules were covalently attached on the nanofibers electrospun from a mixture of polystyrene and poly(styrene-*co*-maleic anhydride). Then, additional enzyme molecules and aggregates from solution were cross-linked to the seed enzyme molecules. The results showed that the initial apparent activity of the α-chymotrypsin-aggregate-coated nanofibers was nine times higher than that of nanofibers with just one layer of covalently attached α-chymotrypsin molecules. The α-chymotrypsin-aggregate-coated nanofibers also showed considerable stability even after being shaken more than 1 month under vigorous conditions. It was due to the high stability of the pre-organized superstructure of cross-linked enzyme aggregates that were covalently attached to the nanofibers [42]. Cross-linking reaction could damage the activity of enzymes, however, the extremely high enzyme loading overcame it and still led to high overall enzymatic activity. This approach is especially potential for the manufacturing of enzyme-based biosensors and biofuels, which normally require high enzyme loading to ensure wide linear range in analytic detection.

Enzyme loading on a hydrophobic support is often limited because of the worse accessibility of enzymes to the support surface. Nair et al. developed a simple method to resolve this problem [43]. PS (polystyrene)/PSMA (containing maleic anhydride) were electrospun into hydrophobic composite nanofibers. These hydrophobic nanofibers were treated with aqueous alcohol solution. Thereafter, the tightly aggregated nanofibers could be dispersed in water to form a loosely entangled structure. This structure was stable enough for enzyme immobilization. The results indicated that this method increased the amount of enzyme loading up to eight times. It also augmented the steady-state conversion for a continuous flow reactor filled with the enzyme-loaded nanofibers.

2.2. Enzyme immobilization on modified nanofibers

From the above description, it is reasonable to choose nanofibers as the supports for enzyme immobilization with regard to both enzyme loading and enzymatic activity. Depending on the kinds of nanofiber materials, immobilization approaches as well as the treatment methods to the supports, the behavior and loading of enzyme can be tailored flexibly. However, the loss of enzymatic activity cannot be avoided. As can be seen from the studies mentioned above [37,38], the activity retention of enzyme is lower than 100%. Generally speaking, this can be ascribed to the following factors. First, multipoint attachment to support restricts the freedom of the immobilized enzyme so that it is difficult for the enzyme protein to adapt suitable conformation for catalysis. Second, non-biospecific interactions tend to occur between enzyme and support, which results in undesired change on the conformation of enzyme (Fig. 2(a and b)) and the variation of microenvironment [44]. Third, especially for redox enzyme, electron transfer involved in the catalysis process may be retarded by the support. These factors are not independent, and all of them can be related to the surface chemistry of support. Therefore, the specific activity and stabilities of immobilized enzymes can be improved through tailoring the surface chemistry of nanofibers. Several modes of surface modifications are indicated in Fig. 2(c–e).

2.2.1. Modification towards biocompatible surface

Tailoring the surface chemistry towards biocompatibility is commonly used for promoting the activity of immobilized enzymes, which is stimulated by biomimetics methodology. By mimicking the natural mode in living cells where enzymes present

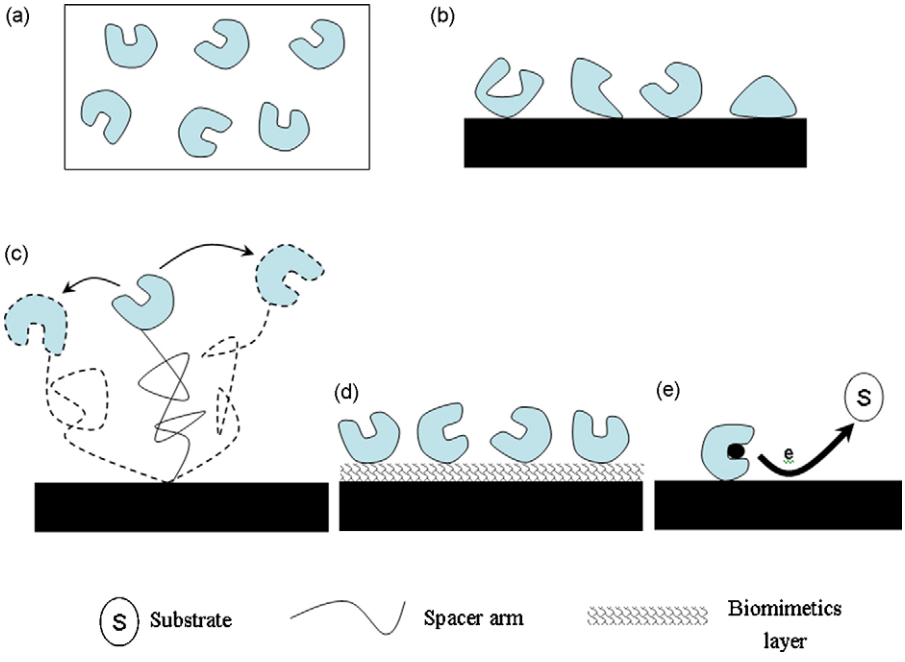

Fig. 2. Effect of surroundings on the enzymatic activity: (a) native state; (b) surface induced undesired conformational change; (c) increased mobility by the flexible spacer; (d) reduced nonspecific interactions by the biomimetic layer; (e) fastened electron transfer by the electrical conductivity of the support.

mostly, the biomimetic systems are supposed to be able to stabilize the structure of enzymes and retain their activities.

It has been mentioned earlier that the nanofibers electrospun from poly(acrylonitrile-*co*-acrylic acid) was used for lipase immobilization [38]. A main reason to choose this polymer is its availability of functional groups, which can react not only with lipase, but also with other molecules (e.g. biomacromolecules). The latter provides a simple route for surface modification that increases the surface biocompatibility of nanofibers. Therefore, in the follow-up study, the carboxyl-containing nanofibers were modified with chitosan or gelatin to build dual-layer biomimetic surface [45]. Chitosan is the principal derivative of chitin and can be acquired at low cost. As a natural polyaminosaccharide, chitosan offers a set of characteristics unique from common biomaterials, including physiological inertness, antibacterial properties, remarkable affinity toward proteins, good gel forming properties as well as good chelation of heavy metal ions (the application of chitosan-based materials for enzyme immobilization has been reviewed in detail by Krajewska [46,47]). Gelatin is derived from collagen and shows biological properties almost identical with those of collagen. It is promising also because of its commercial availability at low cost. Tethering these two biomacromolecules on the nanofiber surface can combine the biocompatibility of them with the mechanical strength of nanofiber. Moreover, the abundant reactive groups on the backbone of chitosan or gelatin can provide sufficient bonding sites for enzyme immobilization. Our results demonstrate the tethering of chitosan or gelatin both increase the activity retention of immobilized lipase, with little sacrifice of enzyme loading [45].

In another study also stimulated by biomimetics, phospholipid analogues were anchored onto the nanofibers to enhance the activity of immobilized lipase [48]. Phospholipids, the principal components of natural biomembranes, have been proved to be inherently biocompatible with various proteins [49]. Polymer surfaces modified with phospholipid analogues have been shown to interact with proteins without three-dimensionally conformational changes and hence to reduce protein adsorption significantly [50]. Therefore, phospholipid moieties are often incorporated into the backbones or side chains of polymers to fabricate biomimetic surfaces. Researches have shown that membranes modified with phospholipid moieties exhibited excellent biocompatibility [51,52]. Because of their alkoxyl groups, the studied phospholipid moieties can also render the surface moderate hydrophobicity to some extent [52]. It is essential for lipase adsorption, because lipase can be activated in the presence of hydrophobic interface [53]. In the natural state, some elements of secondary structure (termed the 'lid') cover the active sites of lipase and make them inaccessible to substrates, referred to as the so-called 'closed state' of lipase. On the other hand, significant conformational rearrangements take place at a hydrophobic interface, yielding the 'open state' of lipase [54]. Generally, phospholipids anchored on the support surface have two effects on the immobilized lipase, namely stabilization and activation.

Nanofibers with phospholipid moieties were also used for lipase immobilization [48]. The nanofibers were electrospun from the copolymer of acrylonitrile and 2-methacryloyloxyethyl phosphorylcholine (MPC). Phospholipid moieties usually present in zwitterionic state in an aqueous medium, which was used to adsorb enzyme through electrostatic interaction. We found that the introduction of phospholipid moieties obviously enhanced the activity of lipase, while retained the enzyme loading. These results suggested that a biomimetic layer from the phospholipid moieties provided a stable, highly hydrophilic and biocompatible external environment, leading to an effective interfacial activation for the immobilized lipase. This piece of work provides a simple route to fabricate biocompatible nanomaterials based on biomimetics methodology. It also offers an insight into the correlation between biomimetic surroundings and the immobilized enzymes. Although the effects of phospholipids on enzymes have been studied extensively [55,56], the interactions between these two molecules, especially when they are located on the surface of nanomaterials, have not been clarified yet. Considering the interactions among phospholipids, enzymes and nanomaterial surfaces, it can be predicted that a further study about enzyme immobilization on these biomimetic nanofibers will be of great interest.

Blending is another effective way to enhance the surface biocompatibility of polymer materials. Polysulfone is an engineering plastic with high mechanical strength and formability. Materials (i.e. membrane) made from polysulfone alone tend to exhibit hydrophobicity and limited biocompatibility, while those from its blends with hydrophilic and biocompatible components (e.g. poly(*N*-vinyl-2-pyrrolidone)—PVP) have significantly improved biocompatibility [57]. Therefore, lipase was immobilized onto the polysulfone-based nanofibers through physical adsorption [58]. PVP or PEG was used as additive, aiming to tailor the surface properties of polysulfone nanofibers. The results showed that the activity of immobilized lipase increased with the content of PVP or PEG in the nanofibers, though the adsorption amount of lipase was not influenced. It was attributed to the enrichment of PVP or PEG towards the nanofiber surface. Compared with other nanofibers for lipase immobilization, the composite nanofibers showed much lower adsorption capacity for enzyme protein and activity retention. The reason is still unknown. Nevertheless, upon proper modification, the electrospun polysulfone nanofibers are still potential supports for lipase immobilization.

2.2.2. Modification towards enzyme mobility

The flexibility of immobilized enzyme can be improved by keeping the enzyme apart the support surface. A common method is to introduce spacer arms onto the surface [59–61]. The flexible spacers can offer the enzyme more freedom of movement and minimize the steric hindrance caused by the solid support, so that the microenvironment for the immobilized enzyme is closed to that for the free one. Wang et al. introduced PEG as the spacer arm onto the alkali-hydrolyzed cellulose nanofibers for the covalent immobilization of lipase [62]. The fibrous structure was retained throughout each process such as alkaline hydrolysis, activation, coupling, and activity assays. The immobilized lipase was revealed to present high stability to non-polar solvents and high temperature. Meanwhile, the results suggested that the molecular structure of PEG made the major difference in the catalysis of lipase rather than its chain length. The highly hydrophilic PEG layer could offer essential water, which ensured the conformational flexibility of immobilized enzyme in organic media [63].

2.2.3. Modification towards electrical conductivity

Electron transfer tends to be involved in the reaction catalyzed by redox enzyme. It is apparent that if electron transfer is guaranteed, the catalytic reaction of enzyme will be sustainable. This is why electrically conductive polymers were applied to enzyme immobilization, especially when used in electrochemical biosensors [64–68]. Blending insulating polymers with electrically conductive nanomaterials is also an effective method to fabricate suitable supports for enzyme immobilization [69,70].

In our work, multiwalled carbon nanotubes (MWCNTs) were filled into poly(acrylonitrile-*co*-acrylic acid) nanofibers for covalent immobilization of catalase [71]. When the mass ratio of MWCNTs to the polymer was 30%, the activity of the immobilized catalase was increased by 47% without reduction in the enzyme loading. It is partly attributed to the superb electrical conductivity of MWCNTs, which can also form charge-transfer complex with

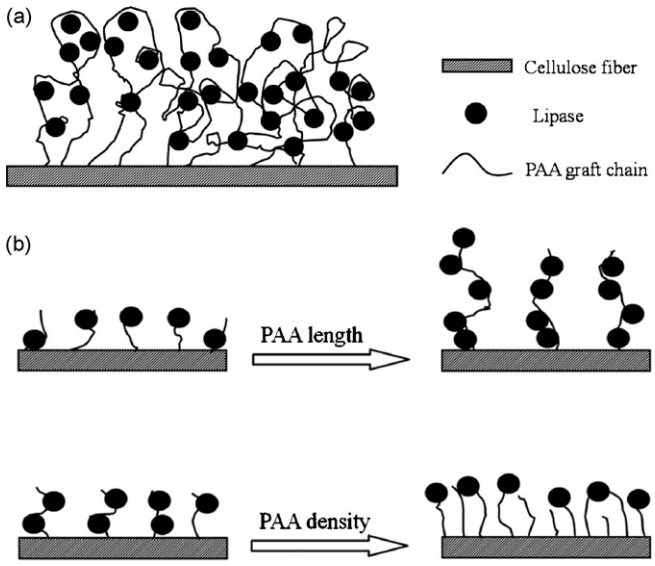

Fig. 3. Structural modes of PAA chains on the cellulose fibers: (a) gel-like graft chains; (b) brush-like graft chains. In brush-like model, PAA length and density shows great effects on the adsorptions of lipase [81]. Copyright© (2005 and Wiley). Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

polyacrylonitrile. This specificity is thought to assist the electron transfer between the hydrogen peroxide molecules and the intermediate catalase, thus enhancing the activity of the immobilized catalase. Catalase immobilized on the composite nanofibers also showed higher storage stability than that on the pristine nanofibers [72], which is associated with the hydrophilization and biocompatibility from MWCNTs. This composite nanofibrous support was also used to immobilize another redox enzyme, horseradish peroxidase, whose activity was also obviously enhanced by the filled MWCNTs.

There is a similar case where catalase was covalently immobilized onto the nanofibers from MWCNTs co-electrospun with poly(acrylonitrile-*co*-acrylic acid) bearing metalloporphyrin pendents [73]. Porphyrin was recognized as an electron donor. The results showed that the activity of the immobilized catalase was enhanced most when both MWCNTs and metalloporphyrin were incorporated into the nanofibers, indicating their cooperating effect.

The outstanding electrical conductivity of MWCNTs endows them potential in redox enzyme immobilization. Despite the fact of activity enhancement for redox enzymes, there is no evidence to directly relate this effect with the electrical conductivity. Therefore, for the above work, it is necessary to verify how the composite nanofibers interacted with the immobilized enzymes. On the other hand, this method (i.e. blending for enhancing activity) also shows several attractive features. First, this modification of nanofibers is accomplished simply by co-electrospinning. By adjusting the electrospinning parameters, the nanofibers can be deposited onto various collectors, which provides a feasibility to modify the enzyme electrode (or sensor) with these composite nanofibers. Second, MWCNTs increase the mechanical stability of nanofibers, and make them more durable under operating conditions. As the cost of MWCNTs declines, the composite nanofibers can be applied in the large-scale catalysis.

2.2.4. Others

Surface modifications can also bring along other benefits for enzyme immobilization. For example, the microstructure of surface modification layer can be modulated to tailor the behavior of immobilized enzymes [74] (e.g. the nanofibrous membrane system studied by Chen and Hsieh [75]). In their study, poly(acrylic acid) (PAA) was grafted onto the cellulose nanofibers for physical adsorption of lipase. Gel-like (Fig. 3a) or brush-like PAA layer (Fig. 3b) was formed on the nanofibers depending on the modes of

Table 1
Some typical cases of enzyme immobilization on electrospun nanofibers

Enzyme species	Support	Immobilization method	Protein loading (mg/g fibers)	Activity retention (%)	Ref.
α-Chymotrypsin	PS NF[a]	Chemical	14.0	65	[36]
α-Chymotrypsin	SF NF[b]	Chemical	56.6	66.78	[37]
Lipase	PANCMA NF[c]	Chemical	21.2 ± 0.71	37.6 ± 1.8	[38]
Lipase	PAN NF	Chemical[d]	21.2 ± 1.3	81.3 ± 1.1	[39]
Lipase	As-spun PS/PSMA NF[e]	Chemical	5.6 ± 2.2	16.5	[43]
Lipase	Dispersed PS/PSMA NF[f]	Chemical	42.4 ± 18.5	16.5	[43]
Lipase	Chitosan-tethered PANCMA NF	Chemical	22.5 ± 0.75	45.6 ± 1.8	[45]
Lipase	Gelatin-tethered PANCMA NF	Chemical	20.7 ± 0.75	49.7 ± 1.8	[45]
Lipase	PAN NF	Physical	23.2 ± 1.6	56.4 ± 0.7	[48]
Lipase	PANCMPC NF[g]	Physical	22.9 ± 1.5	76.8 ± 0.6	[48]
Lipase	PSF NF	Physical	0.8 ± 0.12	6.2 ± 0.32	[58]
Lipase	PSF/PVP NF	Physical	0.59 ± 0.09	26.7 ± 0.42	[58]
Lipase	PSF/PEG-200 NF[h]	Physical	1.24 ± 0.15	18.7 ± 0.23	[58]
Catalase	PANCAA NF[i]	Chemical	23.9 ± 0.62[j]	33.11	[77]
Catalase	PANCAA/MWCNT NF	Chemical	31.1 ± 4.54	47.90	[77]
Catalase	PANAACoPP NF[k]	Chemical	18.9 ± 4.03	39.3	[79]
Catalase	PANCAACoPP/MWCNT NF	Chemical	22.81 ± 4.82	48.5	[79]
Horseradish peroxidase	PANCAA NF	Chemical	21.8 ± 1.22	14.02	[78]
Horseradish peroxidase	PANCAA/MWCNT NF	Chemical	25.1 ± 1.69	23.56	[78]

[a] Functional polystyrene nanofibers.
[b] Regenerated silk fibroin nanofibers.
[c] PANCMA NF: poly(acrylonitrile-*co*-maleic acid) nanofibers.
[d] Amidination reaction.
[e] Pristine polystyrene/poly(styrene-*co*-maleic anhydride) composite nanofibers.
[f] Aqueous alcohol solution treated polystyrene/poly(styrene-*co*-maleic anhydride) composite nanofibers.
[g] PANCMPC: poly(acrylonitrile-*co*-(2-methacryloyloxyethyl phosphorylcholine)).
[h] PEG-200: PEG with average molecular weight of 200 g/mol.
[i] PANCAA: poly(acrylonitrile-*co*-acrylic acid).
[j] Hereafter, enzyme loading takes place of protein loading.
[k] PANAACoPP: terpolymer from acrylonitrile, acrylic acid and metalloporphyrin with Co^{2+}.

surface-initiation. The results indicated that these two structures had distinct effects on the adsorption behavior and the activity of lipase. Gel-like structure showed a stronger ability to seize enzyme molecules (Fig. 3a), while the adsorption efficiency decreased with the extent of entanglement and the thickness of grafting layer. This structure also hindered the diffusion of substrate and restricted the conformational freedom of immobilized enzyme, leading to a lower activity of lipase. Brush-like structure exhibited a lower capacity to entrap enzymes. The adsorption of lipase was able to achieve higher efficiency as the grafted PAA chains got fewer and longer (Fig. 3b, up), the activity of the immobilized lipase was higher as PAA grafts got fewer and shorter (Fig. 3b, down).

As a summary (Table 1), surface modifications have been well combined with the nanotechnology strategy to enhance the activities and the stabilities of immobilized enzymes. However, only preliminary study has been explored on the effects of nanofiber surfaces on the immobilized enzymes. Many problems still remain to be resolved. For example, up to now, only apparent data on the behaviors of the immobilized enzymes have been given to illustrate the role of nanofiber surfaces. Nearly all the surface modification methodologies of nanofibers were copied from those of other structured supports, with few considerations of the characteristics of nanofibers themselves. Therefore, more in-depth studies are essential in the near future.

3. Encapsulation immobilization of enzymes in nanofibers

The encapsulation of enzymes in the nanofibers can be achieved by direct co-electrospinning of enzymes and other components (organic or inorganic materials). Most proteins can only be dissolved in aqueous media. Therefore, in many cases, the polymers to be co-electrospun with enzymes are required to be water-soluble so that they can form homogeneous solution with the enzymes. This can reduce the surface tension of electrospinning solution, which is necessary for fabricating bead-free nanofibers [76]. The commonly used polymers include poly(vinyl alcohol) (PVA) [32,77–79], poly(ethylene oxide) (PEO) [77] and poly(*N*-vinyl-2-pyrrolidone) (PVP) [33]. These polymers are commercially available at a fair price and present good affinity to enzymes. Furthermore, PVA and PEO have dissimilar structures to natural biomacromolecules and the capability to form secondary bonding with proteins, which can dissociate the hydrogen-bonded molecules (protein, chitosan, etc.) and make the co-electrospinning of biomacromolecules easier [80–83].

The co-electrospinning method offers a simple route to immobilize enzymes into nanofibers and the enzyme loading can be substantially high (up to 50% of the fibers [79]). With these characteristics, the enzyme-immobilized nanofibers were directly applied in fabricating enzyme electrodes for biosensors [32,33]. These biosensors showed some common features, such as a low detection limit and fast response, despite their difference in nature (glucose oxidase and urease were respectively included in the nanofibers). The so-manufactured biosensor provides another advantage that the electrode can be facilely regenerated by peeling away the nanofibrous mesh.

Despite the outstanding characteristics mentioned above, the encapsulation approach has several disadvantages:

1. The enzyme molecules are not only embedded into the nanofibers, but also reside on the surface, which usually leads to loss of enzymes during measurement and storage.
2. Because most of the enzyme molecules are confined inside the nonporous fibers, the accessibility of the substrate to the enzyme is inhibited.
3. The materials to be electrospun with enzymes are only limited to several species. Even electrospun from homogeneous solution, nanofibers with beads were still formed [32,33,78]. When the nanofibers are immersed in aqueous medium, the water solubility leads to swell and disintegrate of nanofibers, resulting in enzyme leakage, thermal instability and poor reusability.
4. Cross-linking of the biocatalytic fibers tends to reduce the activity of immobilized enzymes, though it is normally used for increasing the stability of physically encapsulated enzymes [78,79]. On one hand, cross-linking can reduce the porosity (among fibers [78,79]), which limits the accessibility of substrates to the active sites of enzymes. On the other hand, cross-linking itself damages the active sites of enzymes.

Considering these obstacles, water-insoluble materials have been alternatively used for co-electrospinning with enzymes. For that purpose, a coaxial electrospinning setup was used to fabricate poly(ε-caprolactone) (PCL)/lysozyme fibers with core-shell structure [84], which has often been used for the purpose of controlled release [84–86]. In another example, surfactant was used to stabilize α-chymotrypsin in a PS/PSMA solution, which was then electrospun into biocatalytic nanofibers [87]. This two-phase electrospinning method has also been used for fabricating nanofibers with a core-shell structure [85,88,89]. Also, silica nanofibers were used to encapsulate horseradish peroxidase through electrospinning [90], and no deformation of the fibers and no enzyme leakage were observed in their study. Besides, the fibers were highly mesoporous, which facilitated the diffusion of the substrate to the enzyme.

However, a large number of water-insoluble materials (e.g. PS, PSF, and PAN) are of poor biocompatibility. If they are used to encapsulate enzymes, undesired conformational change of the enzymes will take place [91–93]. Two approaches can be used to improve the microenvironment of the encapsulated enzymes. First, additional biocompatible components are incorporated into the spinning solution to offer a biofriendly microenvironment for enzyme. For instance, people included PEG [84,86] and dextran [94] in the core protein solution for the coaxial electrospinning. Second, the biocompatibility of the polymers are improved by in situ polymerization [95,96] or side chain modification [97–99], which will also favor the enzyme catalysis.

4. Outlook

Electrospun nanofibers have been proven to be excellent supports for enzyme immobilization because they can provide large surface area-to-volume ratios, pore sizes tailored to protein molecule dimensions, functionalized surfaces, multiple sites for interaction or attachment, and low mass-transfer limitation. However, the studies of this issue are still limited in a small number as there are still problems in their large-scale application. First, nanofibers are still difficult to fabricate in batches, although multiple spinnerets have been developed [83,100,101]. Second, very few tools can be used to evaluate the effect of the nanofiber surface on the behaviors of the immobilized enzymes. Nevertheless, based on their unique advantages, people could still anticipate that the resultant biocatalytic materials would enable new and expanded uses of enzymes in practical applications such as biosensors, bioremediation, biofuel cells and bioconversions.

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