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Surface modification of nanofibrous poly(acrylonitrile-*co*-acrylic acid) membrane with biomacromolecules for lipase immobilization

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ABSTRACT

In this work, poly(acrylonitrile-*co*-acrylic acid) (PANCAA) was electrospun into nanofibers with a mean diameter of 180 nm. To create a biofriendly microenvironment for enzyme immobilization, collagen or protein hydrolysate from egg skin (ES) was respectively tethered on the prepared nanofibrous membranes in the presence of 1-ethyl-3-(dimethyl-aminopropyl) carbodiamine (EDC)/*N*-hydroxyl succinimide (NHS). Confocal laser scanning microscopy (CLSM) was used to verify the surface modification and protein density on the nanofibrous membranes. Lipase from *Candida rugosa* was then immobilized on the protein-modified nanofibrous membranes by covalent binding using glutaraldehyde (GA) as coupling agent, and on the nascent PANCAA nanofibrous membrane using EDC/NHS as coupling agent, respectively. The properties of the immobilized enzyme were assayed. It was found that different pre-tethered biomacromolecules had distinct effects on the immobilized enzyme. The activity retention of the immobilized lipase on ES hydrolysate-modified nanofibrous membrane increased from 15.0% to 20.4% compared with that on the nascent one, while it was enhanced up to more than quadrupled (activity retention of 61.7%) on the collagen-modified nanofibrous membrane. The kinetic parameter, K_m and V_{max}, were also determined for the free and immobilized lipases. Furthermore, the stabilities of the immobilized lipases were obviously improved compared with the free one.

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1. Introduction

Biotransformations catalyzed with enzymes have been pursued extensively, largely as a result of their chemio-, regio-, and stereoselectivity, mild reaction conditions and high level of catalytic efficiency [1–4]. While practical applications prefer immobilized enzymes since they offer easy catalyst recycling, feasible continuous operations and simple product purification. For enzyme immobilization, the support materials have a great impact on the performance of the immobilized enzymes. The improvement of biocatalytic efficiency can be achieved by manipulating the structure of supports for enzyme immobilization. Among various supports, nanofibrous membranes have gained widespread attentions due to their large surface area-to-volume ratio and fine porous structure. They can provide relatively high quantity of enzyme loading per unit mass and low diffusion resistance necessary for high reaction rate and conversion. What's more, in comparison with nanoparticles, nanofibrous membranes are easier to recycle from reaction media and thus benefit continuous operation [5–18].

Electrospinning has been recognized as an effective way to fabricate polymeric nanofibers with diameter ranging from several micrometers down to tens of nanometers. Among various polymers, acrylonitrile-based homo- and co-polymers were most recently fabricated into nanofibrous materials with reinforcing, superhydrophobic, and/or catalytic properties [17–25]. In our previous work [17], novel nanofibrous membranes that possess reactive carboxyl groups were fabricated from poly(acrylonitrile-*co*-maleic acid) (PANCMA) by the electrospinning process. Lipase was covalently immobilized onto these membrane surfaces via the activation of carboxyl groups. It was found that the enzyme loading and the activity retention of immobilized enzyme on the nanofibrous membrane were much higher than those on the PANCMA hollow fiber membrane. Therefore, the process described in our work presents a convenient approach to fabricate nanofibrous membranes with reactive groups for enzyme immobilization.

However, similar to many other synthetic polymer materials, the relatively poor biocompatibility of acrylonitrile-based polymers probably causes some non-biospecific interactions between enzymes and the supports surface, thus, resulting in partial denaturation of enzyme protein and loss of enzyme activity. Lipases have two forms, the closed form with its active site covered by

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a polypeptide chain called lid and the open form with the lid displaced and its active site exposed to the reaction medium. Some non-biospecific interactions between lipases and the supports surface may lead to the transition from the open form to the closed form. To suppress these unfavorable interactions, surface modification is a major approach that can enhance the surface biocompatibility of supports while keeping the bulk properties intact. It was demonstrated that surface modification with natural macromolecules could create a favorable microenvironment for the immobilized enzyme to retain its activity and enhance its stabilities. Collagen, one of the main proteins in extracellular matrix, has been successfully used to modify biomaterials such as polyurethane [26], polystyrene [27] and titanium [28]. Protein hydrolysate from egg skin (ES), which is easily available and low in cost, includes different molecular weight polypeptides and a little polysaccharide. Both biomacromolecules contain reactive groups (amino groups) and could be used to form biomimetic layers on the poly(acrylonitrile-*co*-acrylic acid) (PANCAA) membrane surface for enzyme immobilization. Furthermore, collagen is a triple helix structure called tropocollagen. Unlike the whole cylindrically shaped collagen, protein hydrolysate from egg skin is a mixture of various fragmental peptides with various lengths. Therefore, it may provide a possibility to directly compare the interaction difference of fragmental polypeptide-chain/enzyme and protein-unit/enzyme at similar condition by tethering them on the same support for enzyme immobilization. To create biofriendly surfaces for immobilized enzyme, these two biomacromolecules were tethered on the PANCAA nanofibrous membranes via covalent binding. Lipase was immobilized on the modified nanofibrous membranes and the performance of the immobilized enzymes was investigated.

2. Experimental

2.1. Materials

PANCAA with a viscosity-averaged molecular weight (M_v) of 2.4×10^5 g/mol was synthesized by a water phase precipitation co-polymerization process. The molar content of acrylic acid in this co-polymer is about 15.6% by elemental analysis method. Type | collagen (from bovine Achilles tendon), lipase (from *Candida rugosa*), 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *p*-nitrophenyl palmitate (*p*-NPP) were purchased from Sigma and used as received. Fluorescein isothiocyanate (FITC) (HPLC grade) was commercially obtained from Fluka. Coumassie brilliant blue G250 (CBBG) for the Bradford protein assay was purchased from Sinopharm Chemical Reagent Co. Ltd. and bovine serum albumin (BSA, BP0081) was obtained from Sino-American Biotechnology Co. *N*-Hydroxyl succinimide (NHS) and glutaraldehyde (GA) are biological grade. Other reagents are of analytical grade without further purification.

2.2. Preparation of PANCAA nanofibrous membranes by electrospinning

PANCAA was dissolved in dimethylformamide (DMF) at 60 °C with gentle stirring for 12 h to form a homogeneous solution of 4 wt.%. Electrospinning was carried out using a syringe with a 1.2 mm diameter stainless steel spinneret at an applied electrical potential difference of 13 kV over 15 cm gap between the spinneret and the collector. The feed rate of solution from the needle outlet was kept constant at 1.0 mL/h by a microinfusion pump (WZ-50C2, Zhejiang University Medical Instrument Co., Ltd., China). The collector is a flat plate wrapped with conductive aluminium film. It usually took 3 h to obtain nanofibrous membrane with sufficient thickness and uniform fiber diameter of about 180 nm. All the nanofibrous membranes were dried under vacuum at 80 °C for 24 h to remove residual solvent before use.

2.3. Preparation of ES hydrolysate

Hydrolysis of egg shells was followed Li's method with some modification [29]. Egg shells were collected and immersed in water for 2 h. ES was peeled from egg shell, thoroughly washed with de-ionized water, and dried in vacuum oven at room temperature. A sample of 2 g ES and 4 g $Ba(OH)_2 \cdot 8H_2O$ was dissolved in 40 mL de-ionized water and stirred for 5 h at 60 °C. After this, 10% H_2SO_4 solution was added into the mixture to terminate the hydrolysis and modulate the pH value of the solution to 4.0. The mixture was filtrated and the collected solution was concentrated by distillation and then dried under vacuum at 60 °C for 24 h to remove residual water before use. The molecular weight of ES hydrolysate was characterized by GPC.

2.4. Preparation of nanofibrous membranes modified with ES hydrolysate or collagen

An appropriate amount of nascent PANCAA nanofibrous membrane was thoroughly washed with de-ionized water, and then rinsed with phosphate buffer solution (PBS, 50 mM, pH 7.0). After this, the membrane was submerged into an EDC/NHS solution (10 mg/mL in PBS (50 mM, pH 7.0), the molar ratio of EDC to NHS = 1:1) and shaken gently for 2 h at room temperature. The activated membrane was taken out, washed several times with PBS (50 mM, pH 7.0) and then submerged into the ES hydrolysate solution (0.5 or 2 mg/mL in PBS (50 mM, pH 7.0)) or collagen solution (0.5 mg/mL or 2 mg/mL in 10 wt.% acetic acid water solution) and shaken gently for 3 h at 4 °C. The ES hydrolysate-modified membrane was washed with PBS for 10–12 times. The collagen-modified membrane was washed with acetic acid solution (10 wt.%) for 10–12 times and the residual acids were removed by rinsing with abundant PBS.

2.5. FITC labeling and CLSM examination

A common protocol described previously [30] was used for FITC labeling of amino group carrying nanofibrous membrane. Confocal laser scanning microscopy (CLSM, Leica, Germany) was then applied to verify the biomacromolecules (such as ES hydrolysate and collagen) located on the surface of PANCAA nanofibrous membrane. The modified membrane was immersed in 5.0 mL PBS (50 mM, pH 7.0), containing freshly dissolved FITC (0.1 mg/mL). The labeling was performed in a dark room and shaken gently in a water bath at 4 °C for 24 h. The same labeling protocol was also applied to the original PANCAA nanofibrous membrane for comparison. To remove physically adsorbed FITC, both nanofibrous membranes were extensively washed with PBS (50 mM, pH 7.0). The nanofibrous membranes labeled with FITC were observed by CLSM equipped with He-Ne laser. High-resolution images of the labeled membrane were taken with a 63× NA 1.4 lambda blue oil objective. These images were obtained by fixing the excitation wavelength at 488 nm. Relative fluorescent intensity was obtained with a 20× NA 0.7 dry objective at the xyz-scan mode.

2.6. Immobilization of lipase onto the modified nanofibrous membranes

Lipase solution (8 mg/mL) was prepared by adding appropriate amount of lipase powder to PBS (50 mM, pH 7.0). Lipase was immobilized onto the ES hydrolysate or collagen-modified membrane by

a GA activation procedure. A sample of 5 mg membrane was submerged into 10 mL GA solution which was composed of 25% GA water solution and de-ionized water (5%, v/v) and shaken gently in a water bath for 2 h at 4 °C. The activated membrane was taken out, washed with PBS (50 mM, pH 7.0) for 6 times to remove excess GA, and then submerged in 10 mL of lipase solution. Immobilization was carried out at 4 °C in a shaking water bath for 2 h. After this, the resultant lipase-immobilized membrane was taken out and washed with 3.0 mL PBS (50 mM, pH 7.0) for 3 times (each in a shaking water bath for 20 min, and the washings together with the reacting solution were collected for the determination of enzyme concentration). As a control, lipase was also immobilized onto the original PANCAA nanofibrous membrane, which was described in references [17]. A sample of 5 mg membrane was submerged into 10 mL EDC/NHS solution (10 mg/mL in PBS, the molar ratio of EDC to NHS = 1:1) and shaken gently in a water bath for 2 h at 4 °C. The activated membrane was taken out, washed with PBS (50 mM, pH 7.0) for 6 times, and then submerged in 10 mL of lipase solution. Immobilization was carried out at 4 °C in a shaking water bath for 2 h. The washing procedure was similar to that mentioned above.

Protein concentrations in solutions were determined with Coomassie brilliant blue reagent following Bradford's method [31]. The amount of immobilized protein on the membrane was determined by measuring the initial and final concentrations of protein within the lipase solutions and washings. BSA was used as standard to construct the calibration curve. The immobilization capacity of the protein on the membrane was defined as the amount of protein (mg) per gram of the membrane. Each reported value was the mean of at least three experiments, and the standard deviation was within ca. ± 5%.

2.7. Activity assay of free and immobilized lipase

Activity for the free and immobilized lipases in aqueous medium was determined according to the method reported previously [5]. In the standard condition, a reaction mixture was composed of 1.0 mL ethanol containing 14.4 mM *p*-NPP and 1.0 mL PBS in an Erlenmeyer flask. The reaction was started by addition of 0.10 mL of free lipase preparation or the immobilized lipase preparation. The mixture was incubated at a certain temperature under reciprocal agitation at 120 strokes per minute. After 5 min of reaction, the reaction was terminated by adding 2.0 mL of 0.5 M Na_2CO_3 followed by centrifuging for 10 min (10,000 rpm). The supernatant of 0.50 mL was diluted 10-fold with de-ionized water and measured at 410 nm in an UV–vis spectrophotometer (UV-1601, Shimadzu, Japan) against a blank without enzyme and treated in parallel. The reaction rate was calculated from the slope of the absorbance vs time curve. A molar extinction coefficient of $14.5 \times 10^3\ M^{-1}\ cm^{-1}$ for *p*-nitrophenol (*p*-NP), which was determined from the absorbance of standard solution of *p*-NP in the reaction medium, was used.

One enzyme unit was the amount of lipase liberating 1.0 μmol of *p*-NP per min in these conditions. Specific activity was defined as the enzyme units per milligram of protein. Activity retention was defined as the ratio of the activity of the amount of the enzyme coupled on the nanofibrous membrane to the activity of the same amount of free enzyme.

2.8. Stability measurements

In the thermal stability study, the free and immobilized lipases were stored in PBS at 50 °C for 2 h. Samples were periodically withdrawn for activity assay. The residual activities were determined as above.

For the reusability measurement, 5 mg immobilized lipase and 2.0 mL of substrate were added in an Erlenmeyer flask. After 5 min reaction, the released *p*-NP was measured as in activity assay. The immobilized lipase was taken out the flask and washed with PBS to remove any residual substrate on the nanofibrous membrane. It was then reintroduced into fresh substrate and the same measurement was repeated 10 times. In order to prevent the influence of storage time on the enzyme activity, there was just 30 s between two following cycles and all measurements were carried out in one day.

The storage stability was determined as follows. Free and immobilized lipases were respectively stored in PBS at 4 °C for a month. Parts of them were periodically withdrawn for activity assay. The residual activities were then determined as described above.

3. Results and discussion

3.1. Attaching ES hydrolysate and/or collagen on the PANCAA nanofibrous membranes

ES hydrolysate with a wide molecular weight from 200 to 50,000 g/mol was obtained by hydrolyzing ES (Table 1). There were more than half of the ES hydrolysate with molecular weight below 3000 g/mol, which indicated that the ES hydrolysis was complete and the ES hydrolysate we used was a mixture of fragmental peptides with various lengths. This ES hydrolysate and collagen have abundant amino acid residues on their polypeptide chains. The amine groups present in ES hydrolysate and collagen can be used to bind them onto the surface of PANCAA nanofibrous membrane via an EDC/NHS activation process. The residual amino groups on the modified nanofibrous membrane were then labeled with FITC and imaged by CLSM. Typical results are given in Fig. 1(a). Comparing with the original nanofibrous membrane, the modified ones were obviously observed in the fluorescent form, which meant there were many residual amino groups on the modified membrane surface. It indicates that ES hydrolysate or collagen was tethered on the nanofibrous membranes. Relative fluorescent intensity can be used to qualitatively verify the tethered biomacromolecules on the membrane surfaces. Data from the measurements of fluorescence emission (Fig. 1(b)) show that higher biomacromolecules concentration led to higher fluorescent intensity. To obtain a higher enzyme loading on the nanofibrous membranes, the concentration of ES hydrolysate and collagen was 2.0 mg/mL in the following experiments.

3.2. Immobilization of lipase

Lipase from *C. rugosa* was immobilized on the nascent and modified nanofibrous membranes using EDC/NHS and GA as coupling agent, respectively. As shown in Table 2, bound protein on the nascent, ES hydrolysate-modified and collagen-modified PANCAA nanofibrous membranes is 11.69, 9.47 and 9.15 mg/g, respectively. Comparing with that on the PANCMA hollow fiber membrane in the previous work [17], there is a sharp increase of the amount of bound protein on the nanofibrous membrane (in Table 2). This result can be explained by the fact that the remarkable large surface area-to-volume ratio of the nanofibrous membrane can provide more potential reaction sites for the covalent coupling of enzyme. On the other hand, the enzyme loading on the PANCAA nanofibrous membrane is almost half of that on the PANCMA nanofibrous membrane. It is reasonable that the fiber diameter of the PANCMA nanofibrous membrane used in the previous work is about 100 nm, while that of PANCAA nanofibrous membrane is about 180 nm. The effect of the fiber diameter on the amount of immobilized protein is also given and the enzyme loading on the PANCMA nanofibrous membrane

Table 1
GPC results of ES hydrolysate from egg shell membrane.

Name	M_n	M_w	M_P	Polydispersity	Area (%)
Peak1	152	376	277	2.469252	33.50
Peak 2	2,822	2,948	2,705	1.044698	36.33
Peak 3	16,027	22,518	33,684	1.404985	23.28
Peak 4	54,386	56,600	76,668	1.040707	6.89

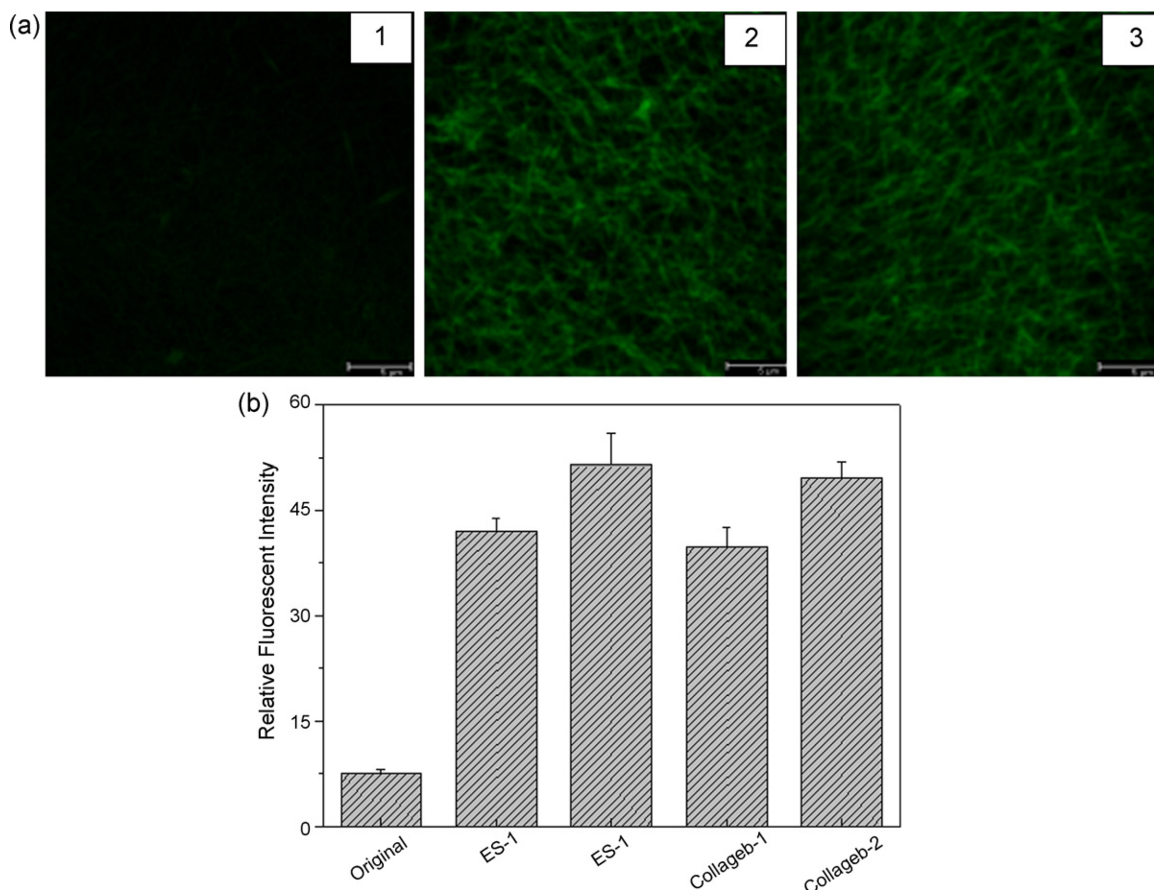

Fig. 1. (a) Images of the nanofibrous membrane: (1) original PANCAA nanofibrous membrane; (2) ES hydrolysate-modified nanofibrous membrane; (3) collagen-modified nanofibrous membrane. (b) Fluorescent intensities of samples determined by CLSM (Original: PANCAA nanofibrous membrane; ES-1: 0.5 mg/mL ES hydrolysate; ES-2: 2 mg/mL ES hydrolysate; Collagen-1: 0.5 mg/mL collagen; Collagen-2: 2 mg/mL collagen).

Table 2
Activity and kinetic parameters (V_{max}, K_m) for the free and immobilized lipases under optimum conditions.

Sample	Bound protein (mg/g)	Specific activity (U/mg)	Activity retention (%)	V_{max} (U/mg)	K_m (mM)
Free lipase[a]	–	42.1	100	46.4	0.45
Lipase immobilized on nascent PANCMA hollow fiber membrane [17]	2.36 ± 0.06	14.3	33.9 ± 1.6	16.1	1.36
Lipase immobilized on nascent PANCMA nanofibrous membrane [17]	21.2 ± 0.71	15.8	37.6 ± 1.8	16.5	0.98
Free lipase[b]	–	23.0	100	25.2	0.48
Lipase immobilized on nascent PANCAA nanofibrous membrane	11.69 ± 0.45	3.4	15.0 ± 0.8	3.61	0.95
Lipase immobilized on ES hydrolysate-modified PANCAA nanofibrous membrane	9.47 ± 0.36	4.7	20.4 ± 1.1	5.40	1.01
Lipase immobilized on collagen-modified PANCAA nanofibrous membrane	9.15 ± 0.28	14.2	61.7 ± 1.6	16.5	0.98

[a] 1150 U/mg solid of the free lipase was used in the previous studies [17].
[b] 810 U/mg solid of the free lipase was used in this study.

decreased from 21.2 to 12.5 mg/g as the fiber diameter increase from 100 to 220 nm [17]. Similar results have been reported by Jia et al. [6]. In this regard, it demonstrates clearly that increasing the outer surface area of the supports is the efficient way to enhance the enzyme loading.

The activities of the immobilized enzymes on the PANCMA and PANCAA membranes under optimum reaction conditions are listed in Table 2. It was found that the activity of the immobilized enzyme on the nascent PANCAA nanofibrous membrane is obviously lower than that on the PANCMA one [17]. The main reason is that the activity of the crude enzyme used in the previous studies is 1150 U/mg solid, while that of crude enzyme used in this work is 810 U/mg solid. In this work, compared with that on the nascent nanofibrous membrane, the activity retention of lipase increases from 14.9% on the original PANCAA one to 20.4% and 61.7%, respectively on the ES hydrolysate-modified and collagen-modified nanofibrous membranes. Although ES hydrolysate loses the natural conformation of protein, it introduces spacer arms with different lengths, which reduce the interaction between enzyme and support, and thus benefit the activity of the immobilized enzyme to some extent. In the case of the collagen-modified nanofibrous membrane, since collagen as one natural macromolecule possesses excellent biocompatibility and hydrophilicity, the collagen-layer on the support surface creates a biocompatible microenvironment and offers a more effective interfacial activation for the immobilized lipase. Therefore, the activity of the immobilized enzyme on the collagen-modified nanofibrous membrane is much higher than that on the nascent and ES hydrolysate-modified ones.

The kinetics parameters, maximum reaction rates (V_{max}) and Michaelis–Menten constants (K_m) from the double reciprocal plot are shown in Table 2. Compared with the free lipase, immobilized lipases obviously reveal lower V_{max} and higher K_m values. The increase in the K_m values is either due to the conformational changes of the enzyme resulting in a lower possibility to form substrate–enzyme complex or due to the lower accessibility of the substrate to the active site of the immobilized enzyme caused by the increased diffusion limitation. We can also see that the K_m values of the immobilized lipase on the nanofibrous membranes are lower than that on the hollow fiber membrane. This result is attributed to the fact that the nanofibrous membrane, possessing the advantages of a large surface area-to-volume ratio and high porosity, could create a more favorable interface for the mass transfer of substrate or product to or from the active site of enzyme.

3.3. Effect of pH and temperature on the activity

The pH dependence of the immobilized lipase activities was compared with that of the free enzyme for *p*-NPP hydrolysis in the pH range of 4.4–9.2 at 37 °C. It can be seen from Fig. 2 that the optimum pH value for the free lipase is about 7.0, while those for the immobilized lipases shift to the alkaline region at about 7.5 respectively for ES hydrolysate-modified and collagen-modified nanofibrous membranes. It can be explained as that the isoelectric point (pI) value of protein is about 4.0–6.5, when pH value is higher than 6.5, the peptide chains has negative charge and attracts H^+ in the solution making the surrounding of peptide much more acid than bulk solution. Furthermore, the relative activities of the immobilized lipases were improved in a broad pH range compared with the free one.

Effect of temperature on the activity of the free and immobilized lipase for *p*-NPP hydrolysis at pH 7.0 in a temperature range of 20–55 °C is shown in Fig. 3. It was found that the optimum temperature for lipase activity shifts from about 37 °C of the free enzyme to 45, 48 and 48 °C of the enzymes immobilized on the nascent, ES hydrolysate-modified and collagen-modified nanofibrous membranes, respectively. The extended maximum temperature range for the immobilized lipases reveals its higher stability to temperature than the free one. This can be attributed to the conformational limitation on the enzyme as a result of covalent bond formation between the enzyme and the support at high temperature. In addition, the improved resistance of protein to thermal denaturation is also an important factor.

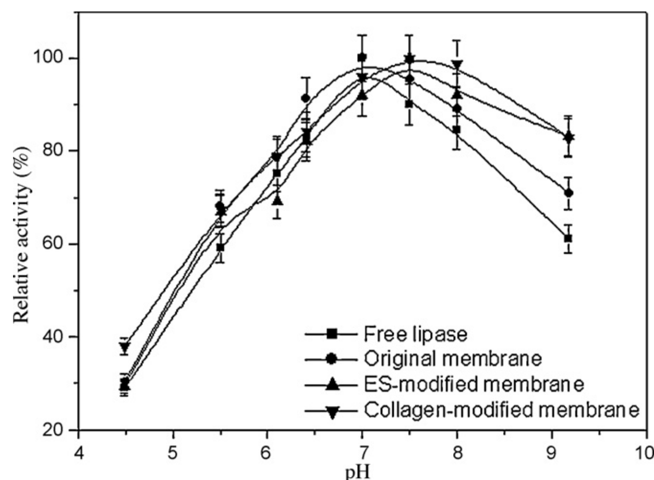

Fig. 2. Effect of pH value on the enzyme activity of the free and immobilized lipases.

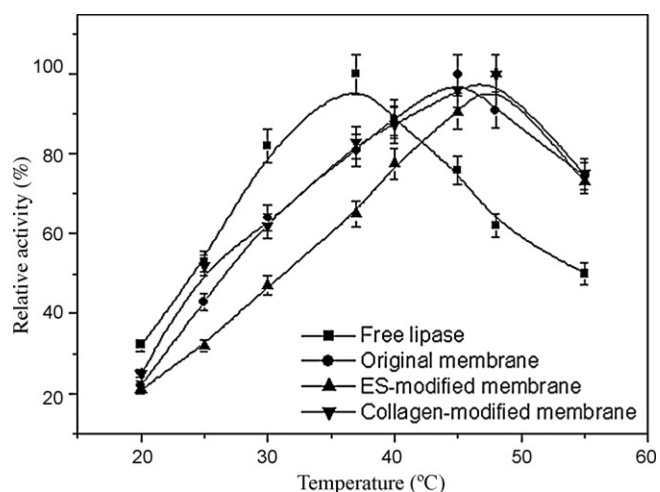

Fig. 3. Effect of temperature on the enzyme activity of the free and immobilized lipases.

3.4. Enzyme stability studies

For industry applications, enzyme stability for the immobilized enzyme is one of the significant indexes to evaluate the properties of enzyme, which can make the immobilized enzyme superior to the free one. Fig. 4 shows the thermal stability of the free and the immobilized lipases on the nanofibrous membrane. It can be seen that the free lipase lost all of its initial activity within 100 min, while the immobilized lipases can retain their initial activity of about 50% for the nascent PANCAA nanofibrous membrane, 66% for the ES hydrolysate-modified nanofibrous membrane and 70% for the collagen-modified nanofibrous membrane after a 120 min of heat treatment, respectively. It demonstrates that the thermal stability of immobilized lipase is much better than the free one. Moreover, pre-tethering biomacromolecules can improve the stability of lipase compared with the nascent one due to two possible factors.

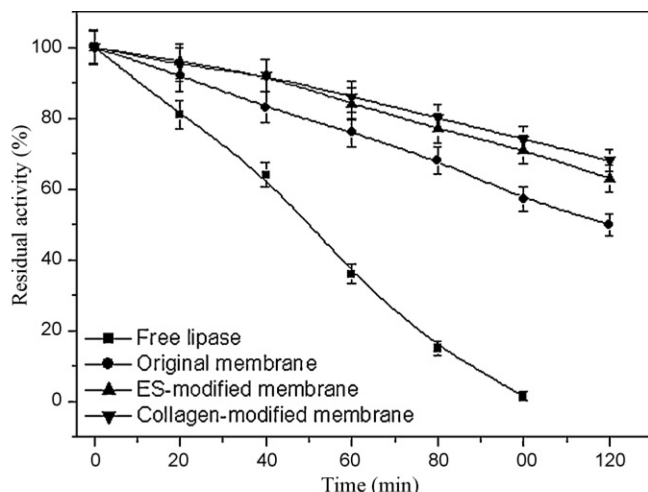

Fig. 4. Thermal stability of the free and immobilized lipases.

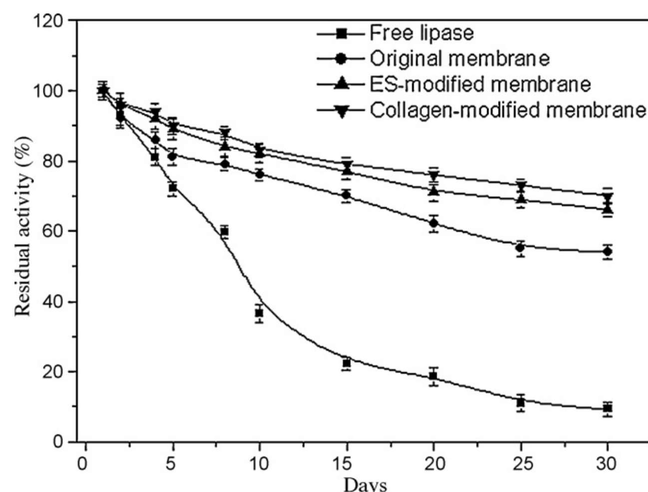

Fig. 6. Storage stability of the free and immobilized lipases at 4 °C.

On one hand, the biocompatible layer formed by the pre-tethering biomacromolecules on the nanofibrous membrane stabilizes the conformation of enzyme protein and improve the resistance of protein to thermal denaturation. Another reason should also be considered. The ES hydrolysate and collagen could be bonded with enzyme through more points because of their relative unfolded conformation. Therefore, the pre-tethering biomacromolecules could prevent the conformation transition of the enzyme and retain the enzyme activity in thermal treatment.

The reusable stability of an immobilized enzyme without appreciable loss of enzyme activity is important for the economic viability of a biocatalytic process. To evaluate the reusable stability, the lipase-immobilized nanofibrous membranes were washed with PBS after each reaction run and reintroduced into a fresh solution. This process was repeated up to 10 cycles. As shown in Fig. 5, after 10 reuses the residual activity for immobilized enzyme is about 46%, 60% and 62% for the nascent PANCAA, ES hydrolysate-modified and collagen-modified nanofibrous membranes, respectively. The activity loss could be related to the inactivation of the enzyme caused by the denaturation of the protein.

The free and immobilized lipases were stored at 4 °C and retained activities are represented in Fig. 6. Under the same conditions, activity of the immobilized lipase during storage decreases more slowly than that of free one. The free lipase loses most of its initial activity within 30 days at 4 °C. It can be seen that, the immobilized lipases on the ES hydrolysate-modified and collagen-modified nanofibrous membranes have high storage stability, and preserve about 66% and 70% of their initial activity at 4 °C, respectively. This extended stability could be attributed to the prevention of structural denaturation as a result of the covalent bonding of lipase onto the biocompatible pre-tethering proteins layer on the nanofibrous membrane surface.

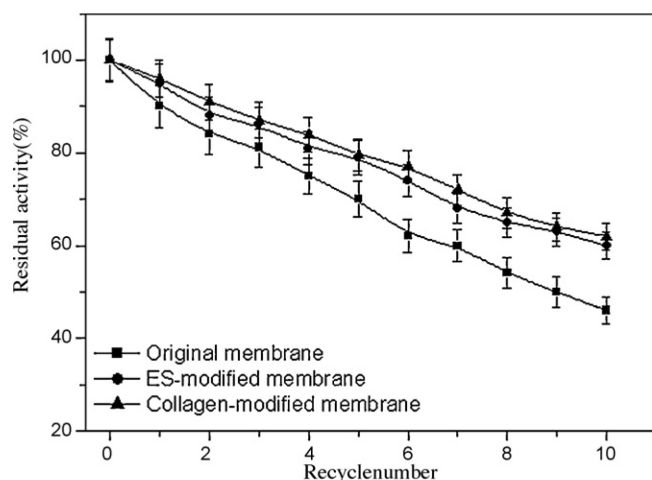

Fig. 5. Reusability of the immobilized lipases.

4. Conclusions

PANCAA was fabricated into nanofibrous membrane and two biomacromolecules, ES hydrolysate and collagen, were tethered onto the membrane surface to form a biomimetic layer for enzyme immobilization. Enhancement of both the activity retention and stabilities (such as thermal stability, reusability and storage stability) of the immobilized lipases can be found on ES hydrolysate-modified and collagen-modified nanofibrous membranes compared with that on the nascent one. The diverse effects of ES hydrolysate and collagen on the immobilized lipase are attributed to the distinctly different conformation and three-dimensional structure of ES hydrolysate and collagen. These results imply that immobilization of biomacromolecules onto synthetic nanofibrous membrane can provide biofriendly microenvironment for further tethering of enzymes. The method presented here provides an optimal support for the enzyme immobilization.

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