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Nanofibrous poly(acrylonitrile-*co*-maleic acid) membranes functionalized with gelatin and chitosan for lipase immobilization

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Abstract

Nanofibrous membranes with an average diameter of 100 and 180 nm were fabricated from poly(acrylonitrile-*co*-maleic acid) (PANCMA) by the electrospinning process. These nanofibrous membranes contain reactive groups which can be used to covalently immobilize biomacromolecules. Two natural macromolecules, chitosan and gelatin, were tethered on these nanofibrous membranes to fabricate dual-layer biomimetic supports for enzyme immobilization in the presence of 1-ethyl-3-(dimethyl-aminopropyl) carbodiimide hydrochloride (EDC)/*N*-hydroxyl succinimide (NHS). Lipase from *Candida rugosa* was then immobilized on these dual-layer biomimetic supports using glutaraldehyde (GA), and on the nascent PANCMA fibrous membrane using EDC/NHS as coupling agent, respectively. The properties of the immobilized lipases were assayed. It was found that there is an increase of the activity retention of the immobilized lipase on the chitosan-modified nanofibrous membrane ($45.6 \pm 1.8\%$) and on the gelatin-modified one ($49.7 \pm 1.8\%$), compared to that on the nascent one ($37.6 \pm 1.8\%$). The kinetic parameters of the free and immobilized lipases, K_m and V_{max}, were also assayed. In comparison with the immobilized lipase on the nascent nanofibrous membrane, there is an increase of the V_{max} value for the immobilized lipases on the chitosan- and gelatin-modified nanofibrous membranes. Results also indicate that the pH and thermal stabilities of lipases increase upon immobilization. The residual activities of the immobilized lipases are 55% on the chitosan-modified nanofibrous membrane and 60% on the gelatin-modified one, after 10 uses.

Keywords: Electrospinning; Nanofibrous membranes; Biomacromolecules; Enzyme immobilization; Lipase

1. Introduction

As biocatalysts, enzymes exhibit a number of advantages such as high level of catalytic efficiency and high degree of selectivities, including chemical-, regio- and stero-selectivity [1–3]. However, there exist various practical problems in the enzyme applications, for example, high cost and instability. To overcome these problems, immobilizing enzyme onto insoluble or solid supports has been regarded as a useful strategy to improve their thermal and operational stability and recoverability.

In recent years, there is a trend to use nanostructured materials as supports for enzyme immobilization, since the large surface area to volume ratio of nanosize materials can effectively improve the enzyme loading per unit volume of support and the catalytic efficiency of the immobilized enzyme. Both nanoparticles and nanofibers were explored for this purpose [4–7]. In the cases of nanoparticles, nevertheless, their dispersion in reaction solution and the subsequent recovery for reuse are often difficult. On the contrary, nanofibers can be easily recovered from reaction media and be applied for continuous operations. Jia et al. [6] immobilized α-chymotrypsin on the surface of polystyrene nanofibers (120 nm) produced by electrospinning and showed that the enzyme loading was 1.4% (wt/wt). Jiang et al. [7] studied the feasibility of lysozyme immobilization

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by electrospinning the enzyme-containing dextran solution into nanofibrous membranes (200–400 nm).

On the other hand, for enzyme immobilization, the biocompatibility of support is also one important requirement [8–11], as the biocompatible surface can reduce some non-biospecific enzyme-support interactions, create a specific microenvironment for the enzymes and thus benefit the enzyme activity [12]. To increase the biocompatibility of the support, various surface modification protocols have often been used to introduce a biofriendly interface on the support surface, such as coating, adsorption, self-assembly and graft polymerization. Among these methods, it is relatively easy and effective to directly tether natural macromolecules on the support surface to form a biomimetic layer for enzyme immobilization. In fact, this protocol has been used in tissue engineering recently [13,14].

In our previous work [15], a dual-layer biomimetic membrane support had been prepared for enzyme immobilization, by tethering chitosan on the poly(acrylonitrile-*co*-maleic acid) (PANCMA) hollow fiber ultrafiltration membrane surface with reactive carboxyl groups. In this work, to further raise enzyme loading on the support and to reduce the diffusion resistance for the immobilized enzyme, PANCMA was fabricated into nanofibrous membranes by the electrospinning process. Two natural macromolecules, chitosan and gelatin, were then tethered on the PANCMA nanofibrous membrane surface to produce novel dual-layer biomimetic membrane supports with nanostructure, respectively. Chitosan (D-glucosamine), which is present in variety of sources and commercially obtained from wastes generated by fishing industry, is considered to be a suitable support for enzyme immobilization since it is nontoxic and user-friendly, and has protein affinity [16,17]. Gelatin (a protein) with characteristics of easily available and low in cost is derived from collagen [18]. It shows biological properties that is almost identical with those of collagen. These features make gelatin widely used in a variety of biomedical applications. Since these two biomaterials containing reactive groups (amino groups) have been successfully used as enzyme immobilization supports, it is reasonable to choose them for the formation of biomimetic layers on the PANCMA nanofibrous membrane surface for enzyme immobilization. Lipase, one kind of the most utility enzyme [19–22], was immobilized on these nanofibrous membranes with GA and 1-ethyl-3-(dimethyl-aminopropyl) carbodiimide hydrochloride (EDC)/*N*-hydroxyl succinimide (NHS) as coupling agent, respectively. The effect of immobilization process on the activity, pH and thermal stabilities, kinetic parameters and reusability of the enzyme was investigated.

2. Experimental

2.1. Materials

Lipase (from *Candida rugosa*), Bradford reagent, bovine serum albumin (BSA, molecular mass: 67,000 Da), EDC, NHS, *p*-nitrophenyl palmitate (*p*-NPP) and 2-morpholino- ethane sulfonic acid (MES) were purchased from Sigma and used as received. Gelatin was purchased from Gouyao Chemical Reagent Co., LTD., China. All other chemicals are of analytical grade and used without further purification.

PANCMA hollow fiber ultrafiltration membrane was fabricated in our lab according to the reported process [23–25]. The outer and the inner diameters of the hollow fiber ultrafiltration membrane are 850 and 545 μm, respectively, with water flux of 146 L/m^2 h atm, BSA rejection of 96%, and breaking strength of 135 N/cm^2. The molar fraction of maleic acid in the copolymer is 7.5%.

2.2. Preparation of the PANCMA nanofibrous membranes by electrospinning

PANCMA was dissolved in dimethylformamide (DMF) at room temperature with gentle stirring for 12 h to form a homogeneous solution (4 wt%). After air bubbles were removed completely, the solution was placed in a plastic syringe (50 mL) bearing a 1 mm inner diameter metal needle, which was connected with a high-voltage power supply (DW-P303-1AC, Tianjin Dongwen High-voltage Power Supply Plant, China). The grounded counter electrode was connected to the tinfoil collector. Typically, electrospinning was performed at 8, 10, 12 and 14 kV. The distance between the needle tip and the collector was 180 mm. The flow rate of the solution was controlled by a syringe pump (WZ-50C2, Zhejiang University Medical Instrument Co., LTD, China) to maintain at 0.3 mL/h from the needle outlet. It usually took 5 h to obtain a sufficiently thick membrane that can be detached from the tinfoil collector. The membrane on tinfoil was dried under vacuum at 60 °C before it was detached. The morphology of the electrospun nanofibrous membrane was examined under a field emission scanning electron microscope (FESEM, FEI, SIRION-100, USA).

2.3. Preparation of low molecular weight gelatin

A sample of 7.8 g gelatin with high molecular weight (HMW gelatin with molecular mass of 160,000 Da) was stirred for 1 h and dissolved absolutely in 100 mL de-ionized water to form a solution (78 mg/mL) at 80 °C. Then, 0.6 g citric acid monohydrate was added to the solution to modulate the pH value of the solution to 3.0 and stirred for 4 h at 80 °C. After this, 0.1 M NaOH solution was added to modulate the pH value of the solution to 7.0 to terminate the hydrolysis. The product was collected with rotatory evaporator at 60 °C, washed several times with de-ionized water and dried in vacuum oven at room temperature. Following this process, the low molecular weight gelatin (LMW gelatin with and molecular mass of 7000 Da) was obtained. The M_w was characterized with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The low molecular weight chitosan was prepared according to the reported process [15].

2.4. Preparation of the dual-layer biomimetic membranes

An appropriate amount of the nascent nanofibrous membrane was thoroughly washed with de-ionized water, and then rinsed with acetic acid buffer solution (50 mM, pH 5.0). After this the membrane was submerged into the low molecular weight chitosan or gelatin solution (15 mg/mL in acetic acid buffer solution, 50 mM, pH 5.0) in the presence of EDC/NHS (10 mg/mL, the molar ratio of EDC to NHS = 1:1) and shook gently for 24 h at room temperature. Finally, the modified membrane was taken out, washed several times with de-ionized water to remove free chitosan or gelatin and then dried in atmosphere. Following this process, the chitosan-modified or gelatin-modified nanofibrous membrane was obtained.

2.5. Immobilization of lipase on the dual-layer biomimetic membranes

Schematic representatives for the preparation of supports and the enzyme immobilization are shown in Fig. 1. Lipase was immobilized onto