Evaluation of dynamic mechanical and cytotoxic properties of electrospun poly (lactic acid)/cellulose nanocrystalline composite membranes

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Abstract. In this paper, polylactic acid (PLA) /cellulose nanocrystalline (CNC) composite fiber membranes were prepared by electrospinning technique. The microstructure of the fiber membranes produced was all transformed from a smooth surface to a nanoporous surface by varying the CNC loading level. The influence of CNC content on the morphology, thermal dynamic properties, and cytotoxic properties of PLA/CNC composite nanofiber membranes was characterized. The results show that water molecules can enhance the hydrogen bonding networks between PLA and CNC. when the cellulose nanocrystal content is less than 10%, the composite membranes have good dynamic mechanical properties and no significant cytotoxic effect.

1. Introduction

Polylactic acid (PLA), a biodegradable thermoplastic polyester derived from sustainable resources [1], is considered one of the most attractive biopolymers due to its renewable, biodegradable, biocompatible, and superior physical properties [2]. It can be used as a medical suture, an absorbable anti-adhesion material [3,4], and a biological scaffold in the biomedical field [5]. However, polylactic acid-based biomedical anti-adhesion and medical scaffold materials have problems such as low mechanical strength, high manufacturing cost, and a high price, which affect their promotion and application value. Moreover, the polylactic acid has poor water absorption performance, which is disadvantageous for the cells of the living body to enter the gap of the membrane for adhesion and growth.

The water content in the human body accounts for about 70%. By improving the water absorption of polylactic acid and strengthening the mechanical properties of composite materials, the application scope of polylactic acid as a medical antimicrobial dressing can be broadened [6]. However, electrospun pure PLA fibers suffer from weak mechanical properties and low thermal stability [7], which limit their industrial applications. Therefore, improving the mechanical properties of electrospun PLA nanofibers is highly desired for consumer applications, especially since PLA-based fibers must meet controllable mechanical requirements during transportation, reprocessing, and recycling. Cellulose nanocrystalline (CNC) is a kind of renewable material which is directly extracted from natural cellulose. Different kinds of CNC were blended with PLA solution, and nanofiber membranes were obtained by electrospinning. It is found that CNC can form a hydrogen bond with the PLA matrix, which can improve its thermal and mechanical properties and even memory function [8-10].
In this study, PLA/CNC composite nanofiber membranes were prepared by electrospinning method and the effects of CNC incorporation on the morphology, the dynamic mechanical properties, and cytocompatibility of PLA/CNC composite nanofiber membranes were systematically evaluated.

2. EXPERIMENTAL

2.1. Materials
PLA was bought from Zhejiang Haizheng Company, China; Cellulose nanocrystals (CNCs) with a length of 167-400 nm and a diameter of 17-42 nm was provided by Beijing Canglang Shanshui Environmental Protection Technology Co., Ltd, China; N, N-dimethylformamide (DMF) (analytical grade, ≥99.5%), was purchased from Beijing Tongguang Fine Chemical Co., Ltd., China; dichloromethane (DCM) (analytical grade, ≥99.5%) was offered by Sinopharm Chemical Reagent Company, China.

2.2. Electrospinning
A certain amount of CNC was put in 20 mL CHCl2/DMF 75/25 (v/v) to obtain a well-dispersed suspension by agitating magnetically for 3 h and then by ultrasonic shaking for 30 min. PLA was dissolved overnight in the CNC suspension by shaking to form a PLA/CNC homogeneous solution. To study the effect of CNC content on the properties of PLA/CNC composite nanofiber, the CNC was loaded in PLA solution with a concentration of 1%, 3%, 5%, 6%, 8%, 10%, 20% (based on the PLA weight). The prepared electrospinning solutions were designated as PLA, PLA/CNC3, PLA/CNC5, PLA/CNC7, PLA/CNC10, and PLA/CNC20. The electrospinning process was utilized by applying a voltage of 18 kV to the polymer solution at a feed rate of 0.8 mL/h using a 22 G blunt-tipped needle. All electrospinning experiments were carried out at constant temperature and humidity (25°C, 30%).

2.3. Characterization
The surface as well as the cross-section of PLA/CNC fibers were observed by scanning electron microscope (SEM, JSM-6366LV, JEOL Ltd., Japan) at an accelerating voltage of 15 kV. Before observation by SEM, all samples were mounted on aluminum stubs and sputter-coated with gold for 40s. The average diameter of PLA/CNC composite nanofiber was obtained by analyzing SEM images using the Nano Measurer software.

The dynamic mechanical analysis was carried out using a DMA Q800 (USA). Rectangular specimens with dimensions of 10 mm×5 mm×0.1 mm were cut from the nanofiber membranes and tested in tensile mode. The measurement was carried out in the temperature range of 37 to 180°C at a heating rate of 5°C /min with a 1 Hz frequency and a strain amplitude of 10 μm. Three specimens were tested for each nanofiber membranes.

The cytotoxicity assay was based on ISO 10993.5-2009, all test samples were tested in three parallel replicates.

3. Results and discussion

3.1. Morphology of PLA and PLA/CNC composite nanofibers
Fig. 1 SEM images of PLA/CNC composite membranes

The morphology of electrospun PLA/CNC fibrous membranes with random or aligned fibers loading with various amounts of CNC are shown in Figure 1. From the figure, it is found that the composite membrane has good apparent morphology, showing a continuous random pore structure, no bead formation, and fiber diameter. The distribution is uniform and the pore size is large, indicating that cellulose nanocrystals with different content can be combined with PLA to prepare composite fiber membranes by electrospinning technique. When the content of cellulose nanocrystals is 0% to 10%, the surface of the composite membrane is smooth, and nanofibers with uniform distribution of fiber diameter can be obtained. When the content of cellulose nanocrystals is 20%, the composite membrane is occasionally accompanied by coarse fibers and some very fine particles. This may be because the cellulose liquid crystals are too viscous when the cellulose nanocrystal content is too high. The nanocrystals themselves are agglomerated together and occasionally a thick line spray occurs during electrospinning. The nanocellulose crystals have a negative charge, and the addition of nanocellulose crystals to the composite fiber film increases the viscosity of the spinning fluid and increases the surface tension. The effect of surface tension that needs to be overcome becomes greater when the spinning stream is spun under a certain high voltage, resulting in thicker fibers. In summary, when the content of cellulose nanocrystals is less than 10%, a well-formed composite fiber membrane can be obtained.
3.2. Dynamic mechanical properties

Before water absorption, the storage modulus of pure PLA is 20 MPa at 37°C. When adding 5%, 10%, and 20% CNC, the storage modulus will increase to 50 MPa, 110 MPa, and 40 MPa, when the temperature is lower than 60°C. The storage modulus of the composite material remains almost unchanged. This is because the amorphous region in PLA is in a glassy state. As the temperature rises, the glassy state transforms into a highly elastic state, and the storage modulus decreases significantly, but CNC is added. The storage modulus of the composite membrane is still higher than that of pure PLA. This is because of the interface force formed between the cellulose nanocrystals and the polylactic acid matrix. When the temperature exceeds Tg, the storage modulus of polylactic acid will drop sharply, and the storage modulus of the composite film added with cellulose nanocrystals will show a downward trend, but its storage modulus is still higher than that of pure Polylactic acid. After absorbing water, the storage modulus of pure PLA is 20 MPa at 37°C. When adding 5%, 10%, and 20% CNC, the storage modulus increases to 530 MPa, 400 MPa, and 40 MPa. When the cellulose nanocrystal content is 5% and 10%, the water response effect of the composite material is obvious, and the sensitivity to water is strong. The effect of temperature on the storage moduli of the specimens before water absorption is shown in Figure 2(a). The results show that water adsorption has little effect on the glass transition temperature of the composite membrane, but the storage modulus changes greatly.

The possible reason is that after absorbing water, a small number of water molecules are present in the composite film, and the water molecules can form intermolecular hydrogen bonds with the hydroxyl group on the surface of cellulose nanocrystals and the carbonyl group of PLA, thus enhancing the intermolecular interaction within the molecules. When the content of cellulose nanocrystals in composite membranes is too high, they may not be able to better interact with water molecules due to the agglomeration of their hydroxyl groups. It also hinders the interaction of the water molecules with the carbonyl group in the PLA matrix, resulting in no significant change in the storage modulus. A schematic diagram of hydrogen bonding in PLA/CNC composite film before and after water absorption is shown in Figure 3.
Fig. 3 Schematic diagram of hydrogen bonds between polylactic acid/CNC

3.3. Cytotoxicity

In this experiment, the test sample extracts did not tend to be cytotoxic to L-929 mouse fibroblasts. The test samples of fibrous membranes were tested without potential cytotoxicity. From the cytotoxicity test data of composite membranes in Table 1, it was found that the addition of cellulose nanocrystals did not affect the biocompatibility of the fiber membranes. Both nanocellulose crystals and PLA are biodegradable materials and their degradation products are safe and easily absorbed by the human body. As the content of cellulose nanocrystals in the composite fiber membrane increases, the cell viability of the test sample decreases from 100.00% to 78.29%. This is due to an increase in CNC loading increasing the number of CNC adhering to the surface of the PLA matrix, and the CNC undergo agglomeration, which affects cell adhesion and proliferation. The addition of cellulose nanocrystals improved the mechanical properties of the polylactic acid composite fiber membrane, resulting in partial cell detachment and a significant reinforcement effect on the fiber membrane. On the other hand, the addition of nano-cellulose crystals also improved the hydrophilicity and hydrophobicity of the composite fiber membrane to some extent. As a biocompatible fibrous membrane material, lower hydrophilicity affects the drug absorption of active-matrix and the degradation performance of the fibrous membrane material.

| x% CNC | Test sample cell viability percentage (%) | Potential cytotoxicity |
|--------|------------------------------------------|-----------------------|
| 0      | 100.00%                                  | no                    |
| 0.1    | 99.53%                                   | no                    |
| 0.3    | 96.98%                                   | no                    |
| 0.5    | 95.86%                                   | no                    |
| 0.6    | 95.43%                                   | no                    |
| 0.8    | 95.73%                                   | no                    |
| 1.0    | 96.41%                                   | no                    |
| 2.0    | 78.29%                                   | no                    |
4. Conclusions
Pure PLA and PLA/CNF nanofiber membranes based on biodegradable PLA and CNC were successfully prepared by electrospinning. When the composite membrane does not absorb water, the internal network structure of the composite membrane is a double-network structure of hydrogen bonds between PA-CNC and CNC-CNC, while with water-absorption the internal structure of the composite membrane is a multi-network structure of hydrogen bond interactions. The network structure makes the molecular arrangement more regular and shows good dynamic mechanical properties. The composite membranes have no potential cytotoxicity with low CNC contents. The addition of cellulose nanocrystals further broadened the application of PLA in biomedical dressings.

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