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Gelatin and Gelatin−Hyaluronic Acid Nanofibrous Membranes Produced by Electrospinning of Their Aqueous Solutions

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Introduction

Electrospinning, as a simple and effective method for producing ultrathin fibers with diameters in the submicron range, has drawn a great deal of attention in recent years. The electrospun fibrous products exhibit some special characteristics, such as high specific surface area, high porosity, and very small pore structures on the fibers. The topological structure of electrospun products can mimic the extracellular matrix (ECM) and enhance the cell migration and proliferation. Therefore, electrospun fibrous materials are especially suitable for biomedical applications, including tissue engineering scaffolds, wound dressings, drug delivery devices, medical implants, and others.[1−6] Recently, electrospinning of natural biopolymers has become a hot issue because natural biopolymers have better biocompatibility and biodegradability and are more suitable for the human body than synthetic alternatives.[7,8] However, the electrospinning processability of these natural polymers is normally very poor. Generally speaking, electrospinning of these natural biopolymers is more difficult than that of synthetic polymers.[6,9−17]

Gelatin (GE) is a natural biopolymer derived from collagens by controlled denaturation that contains many functional groups (glycine, proline, glutamic acid, hydroxyproline, arginine, alanine, aspartic acid, and other amino acids).[18−21] Gelatin exhibits excellent biocompatibility and biodegradability properties and has been widely used and studied in regard to many biomedical applications, including wound or burn dressings; surgical treatments; tissue engineering of bone, skin, and cartilage, and others, in chemically modulated form or blended with other biopolymers.[22−26] A number of studies on the preparation of gelatin in various forms for biomedical applications have been reported. Only a few studies focus on fabricating gelatin fibers through electrospinning.[8,16,17] However, some organic solvents such as 2,2,2-trifluoroethanol (TFE)[8,16] and formic acid[17] must be employed in the fabrication of GE nanofibers. The trace toxic organic solvent in electrospun products is still a problem for biomedical applications. Water is an ideal solvent for both fabrication processes and biomedical applications of gelatin, and electrospinning carried out in neutral pH could avoid the degradation of gelatin caused by acidic conditions. Achievement of the electrospinning of aqueous gelatin solution could not only avoid the trace presence of the toxic solvent in the produced fibers but also result in the fabrication of functional fibrous biomedical products containing water-soluble drugs and other biopolymers. However, it was believed that the electrospinning of aqueous gelatin solutions was impossible, even above the gelation temperature.[8,16,17]

The main aims of this work were to explore the possibility of electrospinning aqueous gelatin solutions and attempt to determine the key issues that hinder this electrospinning process. In this study, another natural polymer, hyaluronic acid (HA), which has a high molecular weight, was used to help the study of the effect of viscosity on the electrospinning of aqueous GE solution. As a result, GE/HA blend nanofibrous membranes as well as pure GE nanofibrous membranes were produced as novel biomaterials with potential biomedical applications. This study also provides a novel and simple way to fabricate gelatin-based composite fibers with other water-soluble biopolymers.

Experimental Section

Materials. Polymers of gelatin type A (approximately 220 Bloom, viscosity 4.8 mPa·s), extracted from porcine skin by an acidic process, were purchased from Sanhesheng Gelatin Co. (Wenzhou, China). Hyaluronic acid (HA) (sodium salt, MW = 2 000 000) was purchased from Dali Co. (Nanning, China). Doubly distilled water and ethanol were obtained from Beijing Chemical Co. (Beijing, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) powder was purchased from Fluka. All materials were used without further purification.

Preparation of Spinning Solution. To achieve faster evaporation of the solvent system, all solvent used in this study was a mixture of doubly distilled water (d) and ethanol (e) (volume ratio of water to ethanol = 9/1). Pure aqueous gelatin solutions with concentrations of 4.5%, 10%, and 15% (w/v, with weight in grams and volume in milliliters) were prepared using the above mixed solvent under gentle stirring for 40 min at 40 °C. A 9% (w/v) aqueous GE solution prepared under gentle stirring for 40 min at 40 °C was mixed with HA solutions of different concentrations [0.09%, 0.45%, 0.9%, 1.35%, 1.8%, 2.25%, and 2.7% (w/v)] at 40 °C for 40 min. Finally, mixed solutions with different GE/HA weight ratios (100/1, 100/5, 100/10, 100/15, 100/20, 100/25, and 100/30) and constant gelatin concentration [4.5% (w/v)] were prepared.

Electrospinning. The environmental temperature of the electrospinning process was controlled at 40 ± 3 °C, which is above the initial gelation temperature (37 °C) of aqueous gelatin solution. The electrospinning solutions were placed into a 5-mL syringe with a capillary tip having an inner diameter of 0.3 mm. A syringe pump was used to feed polymer solution into the needle tip at a rate of 60 μL/min. A high-voltage power supply (The Beijing Machinery & Electricity Institute, Beijing, China) was employed to generate the electric field (0−50 kV). The applied voltage was fixed at 22 kV, and the tip-to-collector distance was fixed at 15 cm. Three types of collectors were used for obtaining different morphologies of fibrous membranes: (a) aluminum foil for nonwoven fibers, (b) two parallel copper wires (1.5 mm in diameter) separated by 1.5 cm for aligned fibers, and (c) a square (1.5 cm × 1.5 cm) copper frame (composed of copper wire with a diameter of 1.5 mm) for crossed fibers. All collectors were connected

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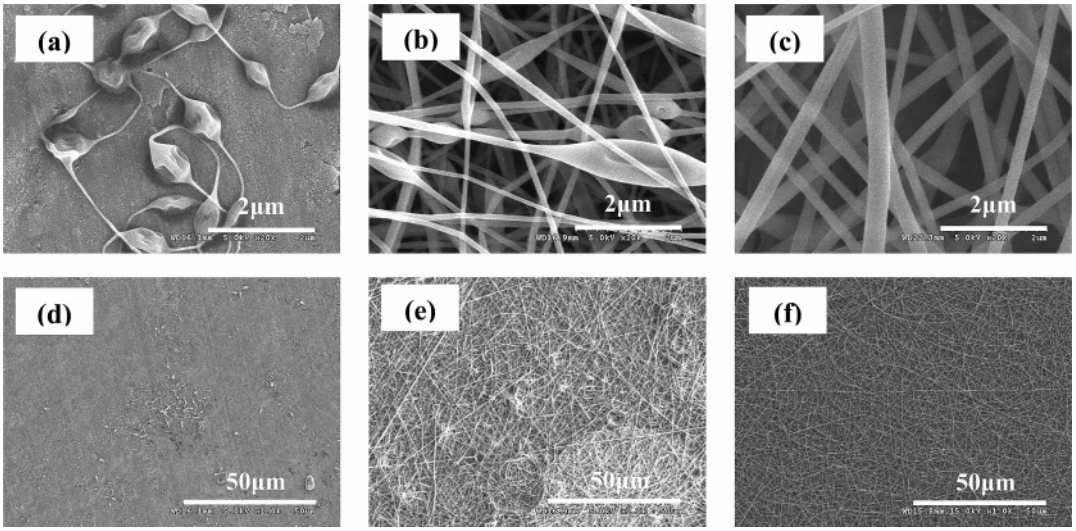

Figure 1. SEM images of GE fibers electrospun from gelatin solutions with different concentrations (solvent = mixture of water and ethanol with a water-to-ethanol volume ratio of 9/1): (a,d) 4.5, (b,e) 10, and (c,f) 15% (w/v). Magnification: (a−c) 20 000×, (d−f) 1000×.

Table 1. Surface Tensions and Conductivities of the Spinning Solutions

GE (%, w/v) or GE/HA composition (w/w)	surface tension (mN/m)	conductivity (μS/cm)
GE 4.5	42.528	785
GE 10	41.648	1282
GE 15	42.387	1537
GE/HA 100/1	43.569	841
GE/HA 100/5	43.332	1053
GE/HA 100/10	45.461	1291
GE/HA 100/15	44.852	1668
GE/HA 100/20	44.819	1959
GE/HA 100/30	44.653	2410

to ground. The fibers produced were placed into a vacuum oven at 50 °C for 30 min to evaporate residue solvents.

Cross-linking. EDC was used as a cross-linker to achieve the cross-linking reactions of GE and GE/HA fibrous membranes in this study. Pure GE fibrous membranes and GE/HA (100/25) fibrous membranes were cut into 1.5 cm × 1.5 cm pieces and immersed in EDC solution [50 mM, 8/2 (v/v) mixture of ethanol and water used as the solvent] for 24 h at 4 °C. After cross-linking, the samples were washed with warm water (30 °C) three times and immersed in pure water at 37 °C for 24 h to investigate the water resistance of the fibrous membranes. After that, the samples were placed in a vacuum oven at 50 °C for 24 h to observe the morphologies by SEM.

Characterization. The morphologies of the electrospun fibers were observed by means of scanning electron microscopy (SEM, Hitachi S-4300). The surface tensions of the GE and GE-based solutions were measured with a surface tension meter (Dataphysics) at 40 °C. The conductivities of the GE and GE-based solutions were measured with a conductivity meter (DDS-307A, Rex Shanghai) at 40 °C. The shear viscosities of the electrospinning solutions were measured at 40 °C with an Advanced Rheometric Expansion System (Ares, TA Instruments), using a 17 × 16 mm couette geometry for the measurements. The shear rate was controlled from 1 to 1000 s^{-1}.

Results and Discussion

Electrospinning of Aqueous Gelatin Solutions. GE can be dissolved in warm water; however, it begins to form a gel when the solution temperature is below 37 °C. To date, the electrospinning of aqueous gelatin solution had not been reported.

To the best of our knowledge, the formation of a gel by aqueous GE solutions at room temperature can be attributed to strong H-bonding, which is sensitive to temperature. Therefore, when the aqueous GE solution is below 37 °C, strong H-bonding induces gel formation and leads to the depressed mobility of the solution. However, when the GE solution is raised above 37 °C, the H-bonding can be destroyed, and a stable polymer solution can be obtained. On the other hand, GE is an ampholyte polymer, and aqueous GE solution at an optimum temperature that is near the phase-separation or micelle-formation temperature should have relatively low surface tension, unlike HA aqueous solution, which has a high surface tension.[27]

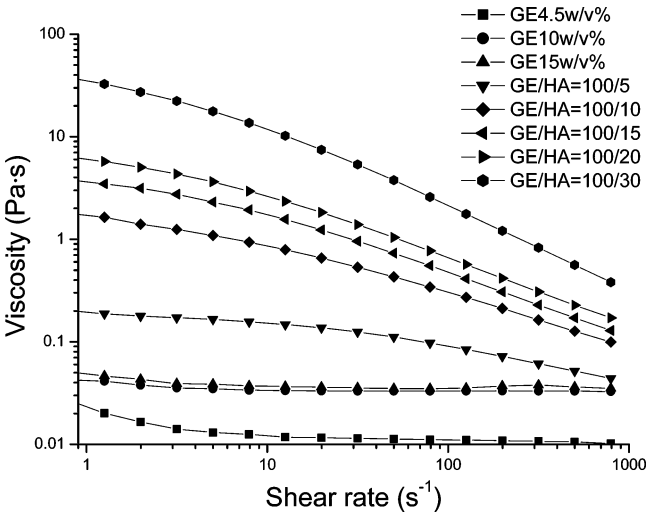

Figure 2. Viscosities of pure gelatin solutions [GE concentration = 4.5%, 10%, and 15% (w/v)] and GE/HA blended solutions (GE/HA weight ratio = 100/5, 100/10, 100/15, 100/20, and 100/3). The solvent in all cases was a mixture of water and ethanol (volume ratio of water to ethanol = 9/1).

In this study, the environmental temperature and the aqueous GE solution temperature were controlled at 40 °C to overcome the problems of gel formation and water evaporation. Initially, 4.5% (w/v) aqueous GE solution was selected for electrospinning. We found that, in this case, the jet was not stable, and a bead-on-string morphology with several big beads was obtained, as shown in Figure 1a,d. The results indicate that whipping instability with small jet loops occurred and that it is possible to electrospin aqueous GE solutions. Therefore, the optimum electrospinning parameters were explored further. Then, aqueous GE solutions with increased concentrations were used for

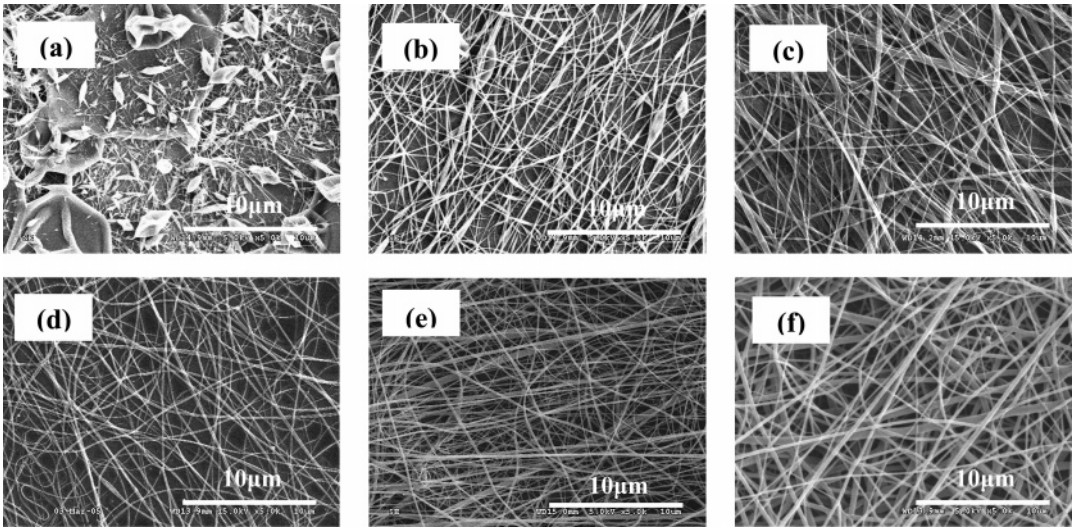

Figure 3. SEM images of gelatin and GE/HA blend fibers (5000×). The weight ratio of gelatin to HA was (a) 100/1, (b) 100/5, (c) 100/10, (d) 100/15, (e) 100/20, and (f) 100/30. In each solution the concentration of gelatin was fixed at 4.5% (w/v).

electrospinning. It can be seen from Figure 1b,e that the number of spindles among the fibers decreased and the fiber formation ability improved. As the GE concentration reached 15% (w/v), the bead-on-string morphology disappeared, and smooth, homogeneous fibers with an average diameter of 212 nm were produced (Figure 1c,f). The fiber diameter of the GE increased with increasing GE concentration. The surface tension measurements indicated that the surface tensions of GE solutions with different concentrations did not change much, as shown in Table 1. However, both the viscosity (Figure 2) and the conductivity of the aqueous GE solutions increased with increasing GE concentration. GE is a polyelectrolyte polymer, and the increase in GE concentration could lead to an increase in the conductivity of the solution. In the smooth-fiber processing window, the relatively higher conductivity and viscosity are both favorable factors for improving electrospinnability.[1,28−30] Higher-viscosity polymer solutions usually exhibit longer stress relaxation times, which could prevent the fracturing of the ejected jets during electrospinning. At the same time, the high conductivity could enhance the electric force, which helps to strengthen the whipping instability and improve the formation of smooth fibers.

Electrospinning of GE/HA Solution. Hyaluronic acid (HA), a naturally occurring linear polysaccharide and a main component of the extracellular matrix of connective tissues, has excellent properties of biocompatibility and biodegradation. HA and its derivatives have been widely used for biomedical purposes such as drug delivery, wound dressings, artificial skin, and tissue engineering scaffolds.[31−35] GE and collagen blended with HA have been studied in fabricating sponge, membrane, and fluid materials for the above biomedical applications, among others. However, the electrospinning of GE/HA blends had not previously been studied. The addition of HA was expected to improve the spinnability of aqueous gelatin solution and prepare blend fibrous products for biomedical applications.

As shown in Figure 1a, at a concentration of 4.5% (w/v), pure aqueous GE solutions cannot be electrospun into smooth fibers, and the whole process was unstable. However, when a small amount of HA was introduced into the aqueous GE solution, the electrospinning process became continuous, as shown in Figure 3b,c. When the weight ratio of GE/HA reached 100/10, the bead-on-string morphology disappeared, and smooth fibers were fabricated (Figure 3c). It was found that, when the content of HA in the GE/HA blends increased above 100/10, the electrospinning process became increasingly fluent, and the fiber diameter became larger (Figure 3d-f).

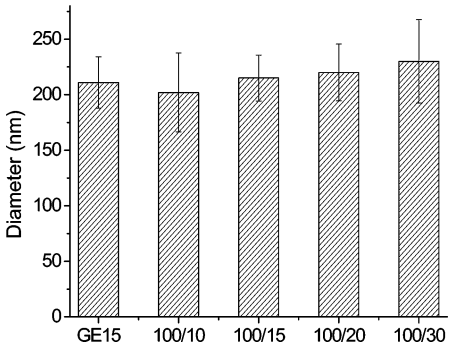

Figure 4. Average fiber diameters of pure gelatin and GE/HA blends with different compositions (weight ratio of gelatin to HA = 100/10, 100/15, 100/20, and 100/30).

The surface tensions of GE/HA mixed solutions were slightly different from those of pure aqueous GE solutions, as shown in Table 1. The conductivities of the GE/HA mixed solutions increased with increasing HA content. HA is a polyelectrolyte, and the addition of HA to GE solutions could result in an increase in ion strength, which might result in higher conductivity. It was found that the viscosities of the mixed aqueous solutions also increased greatly with increasing HA content, and the viscosity at a 1 s^{-1} shearing rate changed from 0.025 Pa·s [4.5% (w/v) GE solution] to 35 Pa·s (GE/HA = 100/30), as shown in Figure 2. The unusually high molecular weight of HA contributed to the distinctly increased viscosity of the mixed solution. It can be seen that smooth blend fibers with a narrow fiber diameter distribution were obtained, and the average diameter increase from 200 to 240 nm with increasing content of HA in the spinning solution, as shown in Figure 4. GE/HA nanofibrous membranes with different mixing ratios could have varying biophysical properties and can be expected to be used in various biomedical applications.

Control of the Topography and Fiber Size of Electrospun Products. Aligned and crossed electrospun fibers are thought to have potential applications in tissue engineering scaffolds, for the remodeling of some special engineered tissues. A 100/25 ratio of GE to HA was selected for fabricating aligned-array and crossed-array fibers using copper wire and a copper frame, respectively, as collectors. As presented in Figure 5a,b,

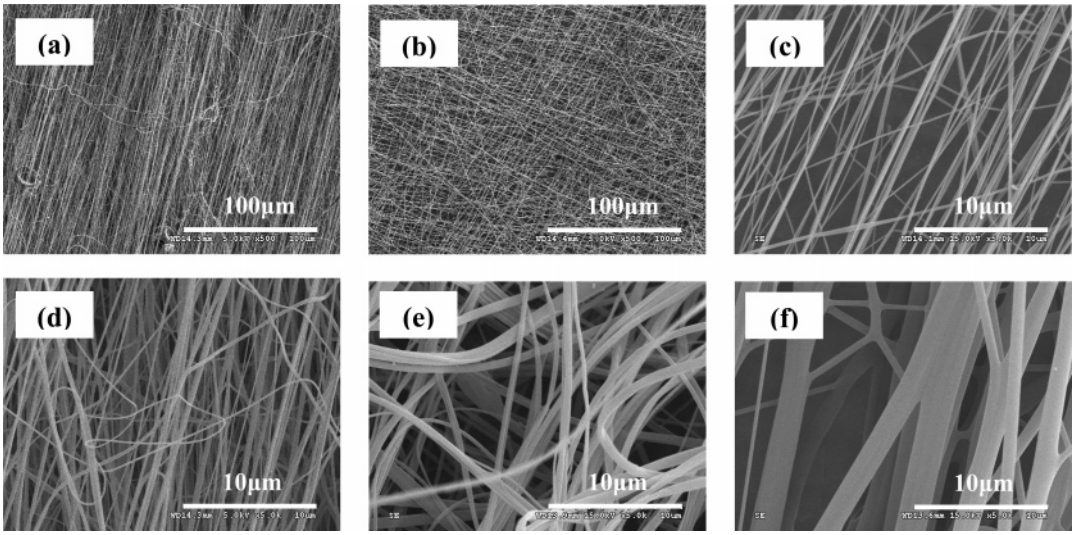

Figure 5. Control of topography and fiber size of GE/HA = 100/25 (w/w) fibrous membranes. The total concentration of the spinning solutions was (a,b,d) 5%, (c) 3%, (e) 7%, and (f) 8% (w/v). Magnification: (a,b) 500×, (c−h) 5000×.

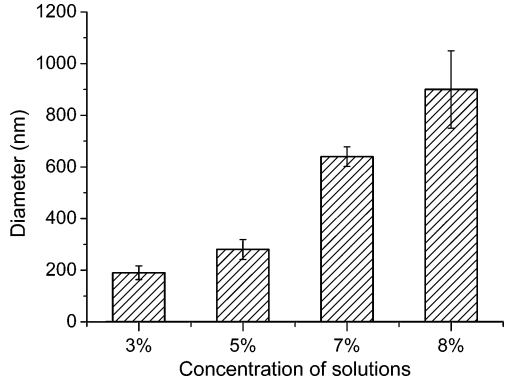

Figure 6. Average diameters of fibers produced from solutions of different total concentration at the same composition [GE/HA = 100/25 (w/w)].

aligned and crossed GE/HA blend fibers could be obtained, with average diameters changed slightly compared to those collected using aluminum foil. Fibers with orderly arrays could have biomedical applications, especially in heart and blood tissue scaffolds.[36−38] Fibers with varying average diameters were fabricated by increasing the total concentration of the spinning solutions from 3 to 8% (w/v), as shown in Figure 5c−f. The average diameters of the blend fibers could be controlled in the range between 190 nm and 1 μm (Figure 6). Fibrous membranes with different fiber sizes could have versatile properties, such as mechanical strength and cell attachment. The topographical features could also affect cell adhesion and proliferation on the electrospun fibrous membranes.[39] Therefore, the control of topography and fiber size of the electrospun products will play an important role in their potential biomedical applications.

C**ross-linking of the Electrospun Membranes.** Both GE and HA are water-soluble biopolymers, and they should be cross-linked to be water-resistant before use as biomedical materials. In this study, EDC was used as a cross-linker to carry out the cross-linking reaction. After the GE and GE/HA (100/25) cross-linked fibrous membranes had been immersed in water at 37 °C for 24 h, their morphologies changed slightly compared with those of uncross-linked fibrous membranes, and no obvious adhesion of fibers was observed, as shown in Figure 7a,b. This result indicates that the EDC cross-linking method was an effective way[24] to fabricate GE and GE/HA water-resistant

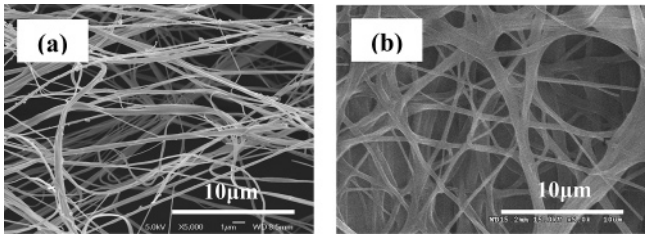

Figure 7. Morphologies of (a) GE and (b) GE/HA = 100/25 cross-linked membranes after immersion in water at 37 °C for 24 h. Magnification: 5000×.

fibrous membranes and make it possible for GE and GE/HA fibrous membranes to be used for tissue engineering scaffolds, wound dressings, and other purposes.

Conclusions

In this study, the electrospinning of aqueous GE and aqueous GE/HA solutions was investigated for the first time. The gelling behavior of aqueous GE solutions was suppressed by increasing the spinning solution temperature. For pure aqueous GE solutions, when the polymer concentration reached 15% (w/v), GE fibers could be obtained successfully by electrospinning, which indicates that aqueous GE solutions can be electrospun. GE/HA blend fibers with different weight ratios (from 100/10 to 100/30) and controlled average fiber diameters were also successfully electrospun. It was found that the addition of HA could improve the electrospinning processability of aqueous GE solutions. Measurements on the spinning solutions indicated that increased viscosity and conductivity contributed to the formation of GE and GE/HA fibers by electrospinning. Aligned- and crossed-array nanofibrous membranes were successfully prepared by changing the collection geometry. Through chemical cross-linking, water-resistant GE and GE/HA nanofibrous membranes were produced. It is expected that the GE and GE/HA blend nanofibrous membranes have potential applications as novel biomedical materials.

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