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Improved Mechanical Properties of Poly(butylene succinate) Membrane by Co-electrospinning with Gelatin

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Abstract Gelatin, a natural proteinous polymer, was used to co-electrospin with poly(butylene succinate) (PBS) in order to improve the mechanical properties of PBS membrane and facilitate its applications in biomedical field. The PBS/gelatin blend membranes have narrower distribution of fiber diameter and smoother surface than neat PBS membrane. The contact angles, water absorption rates and water uptakes of the PBS/gelatin blend membranes were measured, showing increased hydrophilicity. The interaction between PBS and gelatin was investigated by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and differential scanning calorimetry (DSC). The mechanical properties of PBS/gelatin blend membranes in both dry and wet states were evaluated by uniaxial tensile tests. In the dry state, the PBS/gelatin blend membrane containing 10% gelatin has a 3-times increase in tensile strength without any adverse effect on ductility because of the existence of interaction between the two blend components, little change in crystallinity of PBS, and possible interaction between any adjacent fibers; the tensile strength and elongation at break are even better in the wet state attributed to some gelatin on fiber surfaces, which act as a binder in the presence of water. The potential applications of PBS/gelatin blend membranes were demonstrated by successful immobilization of thrombin, a clinically-used hemostatic drug. The thrombin-loaded membrane could be used for rapid hemostasis.

Keywords PBS; Gelatin; Electrospinning; Mechanical properties; Blending

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INTRODUCTION

Poly(butylene succinate) (PBS) is one of the most promising biodegradable and biocompatible aliphatic polyesters[1–8]. It is synthesized from 1,4-butandiol and succinic acid by polycondensation. As a thermoplastic polymer having commercial availability and good processability, PBS is a good alternative to non-degradable polyolefins in packaging applications. Research has also been devoted to its applications as biomaterials such as stent material[6]. Since polymer electrospun membranes have shown various applications in biomedical fields such as tissue engineering, wound dressing, drug or protein delivery, and enzyme immobilization[9–23], we[20] recently investigated the potential biomedical application of PBS electrospun membranes and found that they are highly effective in rapid hemostasis after immobilization of thrombin, an effective hemostat for topical injury. The advantages of this type of rapid hemostatic dressing over clinically-used thrombin solution include easy handling, long shelf life, simplicity of application, and no requirement for pre-application preparation. However, poor mechanical properties are their major drawbacks, due to presumably weak interaction among fibers, which results in loose fiber packing.

Gelatin is derived from collagen, a natural polymer. It is biodegradable, biocompatible and commercially available at relatively low cost, therefore widely used in biomedical field[24–33]. For example, gelatin sponge is a clinically-used hemostatic dressing[26]. Using its proteinous and hydrophilic nature to advantage, gelatin is often co-electrospun with other polymers such as polycaprolactone (PCL), poly(lactic-*co*-glycolic acid) (PLGA), poly(L-lactide-*co*-ε-caprolactone) (PLCL), chitosan, polyurethane (PU) and polyester urethane urea (PEUU) to obtain membranes with improved hydrophilicity and biocompatibility, and the blend membranes have shown potential applications in wound dressing, skin and myocardial regeneration, *etc*[27–33]. Incorporating a natural polymer such as collagen, gelatin and soluble eggshell membrane protein (SEP) to a synthetic polymer such as poly(lactic acid) often decreases the mechanical properties[33–35]. However, a literature survey has found that the tensile strength of chitosan/gelatin (1/1, *W*/*W*) blend membrane is more than doubled, and that of PLCL/gelatin (9/1, *W*/*W*) electrospun membrane is even tripled, compared to those of neat chitosan and PLCL electropsun

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membranes[29, 32]. The reason behind these improvements could be certain miscibility or compatibility between the two components arisen from intermolecular hydrogen bonding or rigid-filler-like behavior of hard gelatin[29, 32].

The goal of this work is to improve the mechanical properties of PBS electrospun membrane by co-electospinning with gelatin. The tensile strength of PBS/gelatin (9/1, *W*/*W*) blend membrane is four times of that of neat PBS membrane. The interaction between PBS and gelatin was investigated by ATR-FTIR and DSC. The effects of gelatin on wettability, water absorption rate, and water uptake of the membrane were evaluated. To demonstrate potential application of PBS/gelatin blend membrane, thrombin was successfully immobilized onto the membrane, showing its effectiveness in rapid hemostasis.

EXPERIMENTAL

Materials

Poly(butylene succinate) (PBS, M_w = 6.3 × 10^4) was provided by the Technical Institute of Physics and Chemistry, Chinese Academy of Sciences (TIPCCAS). Gelatin (AR) was purchased from Shandong Xiya Chemical Industry Co., Ltd. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, 99.5%) was purchased from Aladdin Industrial Corporation. Trisodium citrate ($Na_3C_6H_5O_7·2H_2O$) and hydrogen tetrachloroaurate ($HAuCl_4·4H_2O$) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Lyophilized thrombin (Thr) powder (500 U/batch) is a prescribed reagent, obtained from Changchun Yuanda Guoao Pharmaceutical Co., Ltd. and stored at 4 °C. Fibrinogen (Fib, type 1-s, 65%–85% proteins) from Sigma-Aldrich is also a prescribed reagent, obtained from Beijing Biodee Biotechnology Co., Ltd. and stored at −18 °C. All the materials and reagents were used without further purification. Deionized water was used to prepare the aqueous solutions.

Electrospinning

PBS and gelatin were dissolved in HFIP by stirring at 40 °C for 24 h. The total concentration was fixed at 20 wt%. The mass proportions of PBS/gelatin were 9/1, 7/3 and 5/5. A solution was put into a 10 mL syringe with a 12# blunt end needle. Electrospinning was conducted at ambient conditions using an electrospinning machine (HD-2335) fabricated by Beijing Ucalery Technology Development Co., Ltd. The applied voltage was 5.5 kV. The collector was a grounded metal drum (diameter of 8 cm) with an aluminum foil attached to it, put away from the tip of needle with a receiving distance of 15 cm and a rotation speed of 60 r/min. The feeding rate was 0.9 mL/h. The fibrous membrane was dried in a vacuum oven at 40 °C for 24 h. The PBS/gelatin blend membranes containing PBS/gelatin ratios of 9/1, 7/3 and 5/5 are denoted as PG91, PG73 and PG55 in figures and tables, and their thicknesses are around 0.1 mm.

Adsorption of Gold Nanoparticles

In order to confirm the presence of some gelatin on the fiber surfaces, a gold sol containing monodisperse spherical gold nanoparticles of 16 nm was prepared according to a published procedure[36–39] and filtered through PBS/gelatin (9/1) blend membrane. After thorough rinsing with water, the membrane is in wine red color, indicating successful adsorption of gold nanoparticles.

Morphology Characterization

A JSM7401 FESEM was used to observe the morphology of the electrospun fibers at an accelerating voltage of 3 kV. Before observation, the samples were sputter-coated with gold for 40 s. The diameters of 50 fibers were measured using JEOL software (SMILEVIEW) and averaged.

Measurements of Porosity

The porosity (*B*) of the blend membranes can be calculated by the following formula:

$$B = \left(1 - \frac{m}{S\,d\,(\rho_1 x + \rho_2 y)}\right) \times 100\%$$

where *m*, *S* and *d* are mass, area and thickness of the membranes, respectively; ρ_1, ρ_2 are the density of PBS and gelatin, respectively; *x*, *y* are the mass ratio of PBS/gelatin in the membrane, respectively. Thickness (*d*) of a membrane was measured by a screw micrometer.

Measurements of Contact Angles, Water Penatration Rates and Water Uptakes

The water contact angles were measured using a POWEREACH type contact angle meter (Shanghai Zhongchen Digital Equipment Corporation). During the measurements, the time taken for a droplet (about 15 μL) from contacting the membrane to completely penetrate into the membrane was recorded as penetration rate of a water droplet.

The water uptakes of the membranes were measured by immersing membranes in deionized water for 2 h. After taken out and gently wiped by the surfaces with filter paper to remove excess water, the membranes were accurately weighed. The water uptake (*P*) was calculated as P (%) = $[(W_t - W_0)/W_0] \times 100$, in which W_t and W_0 are the weights of wet and dry membranes, respectively. All results were an average of two measurements.

Attenuated Total Reflectance FTIR Spectroscopy (ATR-FTIR)

The ATR-FTIR spectra of PBS/gelatin blend membranes were recorded on a Thermo-Nicolet 6700 machine.

Differential Scanning Calorimetry (DSC)

The crystallization behavior of PBS in PBS/gelatin electrospun membranes was investigated using DSC (2910M, TA Instrument) under nitrogen. Firstly, the samples were heated from room temperature to 150 °C at a rate of 10 °C/min and kept at 150 °C for 1 min, then cooled to 20 °C at a rate of 10 °C/min. The standard enthalpy of PBS is 200 J/g. The crystallinity of PBS (x_c) is calculated by the following formula:

$$x_c = \frac{\Delta H_m}{p200} \times 100\%$$

where ΔH_m is the melting enthalpy of the sample and *p* is the mass percent of PBS in the blend membrane.

Tensile Testing

Uniaxial tensile tests were carried out on a TCS-2000 tensile

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tester (Gotech) at an extension rate of 5 mm/min. The PBS/gelatin blend membranes of a size of 5 cm × 1 cm in both dry and wet states were tested. At least five measurements were conducted and averaged for each type of membrane. The samples in the dry state are those dried in vacuo and then stored under the testing conditions for at least 48 h before testing. The samples in the wet state are those immersed thoroughly in water and then taken out for testing.

Immobilization of Thrombin and Assessment of Enzyme Activity

Nine pieces of PBS/gelatin (9/1) blend membranes were immersed into 15 mL of thrombin solution (25 U/mL). The mixture was placed at 4 °C for 6 h with manual shaking once per hour, and then the membranes were taken out and dried by vacuum freeze drying.

The enzyme activities of the thrombin-loaded PBS/gelatin blend membranes were assessed according to a slightly modified method described in the Chinese Pharmacopoeia[20, 40]. Briefly, to a clean test tube containing 0.9 mL of fibrinogen solution in normal saline with a concentration of 1 mg Fib/mL were added a membrane of 1 cm^2 pre-cut into 9 identical small pieces and 0.1 mL of water at 37 °C. The mixture was gently shaken. The initial gelation time t_{gel} was recorded when Tyndall effect is observed. The enzyme activity of the immobilized thrombin was obtained using the previously reported calibration curve[20], and expressed as equivalent activity unit of free thrombin.

RESULTS AND DISCUSSION

Morphological Properties

Figs. 1(a)–1(c) show FESEM micrographs of PBS/gelatin electrospun membranes containing 10 wt%, 30 wt% and 50 wt% of gelatin. Compared to neat PBS membrane, in which some very thin fibers (about 520 nm) are present along with large fibers (about 3 μm)[20], the distribution of fiber diameters is narrow as a result of the disappearance of very thin fibers. The average fiber diameter decreases when gelatin content increases due to the decreased viscosity and increased conductivity of the electrospinning solution. The decrease in solution viscosity is visible and the increase in solution conductivity is caused by the polar groups (such as amino and carboxyl groups) of gelatin, and both factors are in favor of the decrease in fiber diameter. Macroscopically, the surface of PBS/gelatin blend membranes is much smoother than that of neat PBS membrane (Fig. 2c versus Fig. 2a) probably because of the existence of inter-fiber interaction, which is attributed to the gelatin molecules on the fiber surfaces.

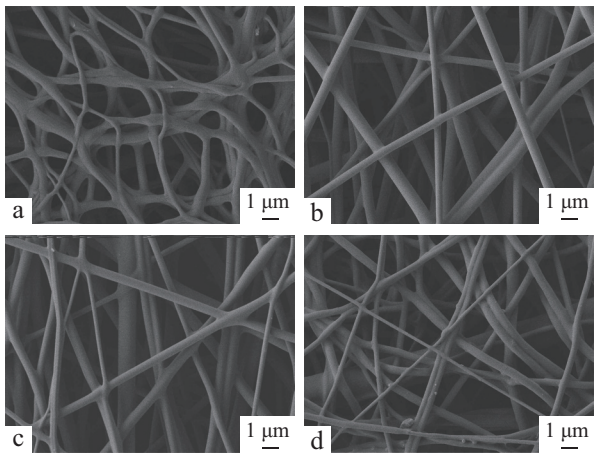

Fig. 1 FESEM images of PBS/gelatin electrospun membranes: (a) PG91, (b) PG73, (c) PG55, (d) thrombin-loaded PG91

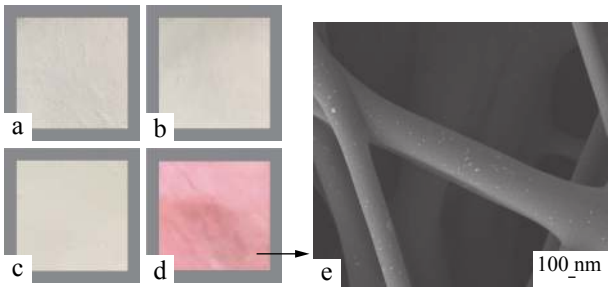

Fig. 2 Digital photos of the electrospun fiber mats (a, c) before and (b, d) after adsorption of gold nanoparticles: (a, b) neat PBS and (c, d) PG91; (e) FESEM micrograph of gold nanoparticle-decorated PG91

The presence of some gelatin on fiber surface is evidenced by adsorption of gold nanoparticles (Fig. 2). When gold sol was filtered through neat PBS membrane, no gold nanoparticles were adsorbed onto the membrane as indicated by the unchanged white color, while PBS/gelatin (9/1) membrane changed into red wine-colored, exhibiting the color of gold nanoparticles of 16 nm. As shown by FESEM image given in Fig. 2(e), there are indeed some gold nanoparticles on fiber surfaces, in either monodisperse or slightly agglomerated state. Gelatin can assist in adsorption of gold nanoparticles because there are amino, carboxyl and amide groups in gelatin molecules. Gold nanoparticles have very good affinity to amino groups because of coordination[38]. Also, the carboxyl and amide groups of gelatin can form hydrogen bonds with the carboxylate or carboxyl groups capped on the surface of gold nanoparticles. The gelatin on fiber surface is beneficial for improving hydrophilicity and mechanical properties.

Contact Angles, Water Absorption Rates and Water Uptakes

Hydrophilicity is very important for biomaterials. Therefore, the contact angles, water absorption rates and water uptakes of the PBS/gelatin blend membranes were measured and the results are listed in Table 1. All the membranes show very good wettability, rapid water penetration rate and high water uptake, favoring their applications in some fields such hemostasis. During the contact angle measurements, the time for a water droplet of about 15 μL to completely penetrate into a PBS/gelatin blend membrane (23–18 s) is shorter than that into neat PBS membrane (29 s)[20] and decreases when gelatin content increases, indicating improved hydrophilicity. This can be attributed to the presence of hydrophilic gelatin on fiber surface and is beneficial for their applications as biomaterials. The water uptakes of PBS/gelatin blend

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Table 1 Fiber diameters, contact angles, porosities, penetration rates of water droplets and water uptakes of PBS/gelatin electrospun membranes

Sample	Fiber diameter (nm)	Contact angle (°)	Porosity (%)	Penetration rate of water droplet (s)	Water uptake (%)
PG91	852 ± 301	0	75.1	23	410
PG73	817 ± 237	0	70.6	20	357
PG55	669 ± 262	0	71.1	18	332

membranes do not vary much compared to that of neat PBS (388%)[20], because the water uptake of a membrane is mainly affected by two parameters: hydrophilicity and porosity. With increasing gelatin content, porosity decreases and hydrophilicity increases. The two parameters have opposite effects on water uptake.

Evidences for the Interaction between PBS and Gelatin

The interaction between the two components is a decisive factor affecting the mechanical properties of binary polymer blends. Herein, the interaction between PBS and gelatin in PBS/gelatin electrospun membranes was investigated by ATR-FTIR and DSC. Fig. 3 shows ATR-FTIR spectra of PBS/gelatin blend membranes along with that of neat PBS membrane. Neat PBS membrane shows a characteristic peak of carbonyl group at 1712 cm^{-1}. When 50% of gelatin is blended to PBS, the carbonyl band shifts to 1716 cm^{-1}, while a movement of 7 cm^{-1} is also observed for amide I band of gelatin compared to that of PBS/gelatin (7/3) membrane. This confirms the existence of interaction between PBS and gelatin through hydrogen bonding between the carbonyl groups of PBS and the amide groups of gelatin. In the literature, the interaction between a natural protein SEP, which is mainly composed of collagen type I, and a polyester-type polymer PLA through formation of hydrogen bonding has also been reported[35].

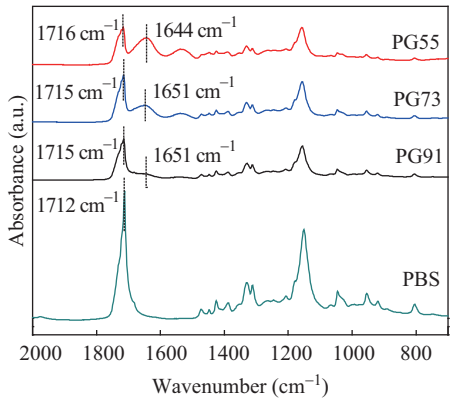

Fig. 3 ATR-FTIR spectra of PBS/gelatin electrospun fiber mats

The DSC curves of PBS/gelatin blend membranes and neat PBS membrane are shown in Fig. 4 and the typical results are summarized in Table 2. The melting temperature and the degree of crystallinity of PBS in neat PBS membrane are similar to the reported data for PBS membranes obtained by electrospinning from different solvents[2]. The melting ranges of PBS in PBS/gelatin blend membranes are much broader than that in neat PBS membrane and the degree of crystallinity of PBS decreases gradually when gelatin content increases, indicating the existence of interaction between PBS and gelatin. It is interesting to note from Table 2 that the crystallization temperature (T_c) of PBS in all the melted electrospun membranes is higher (about 8 °C) than that in melted PBS pellet. This confirms that the PBS molecules in the membranes are orderly arranged even in the static melt state and are more readily crystallized than those in the melted pellet where they are in the disordered state.

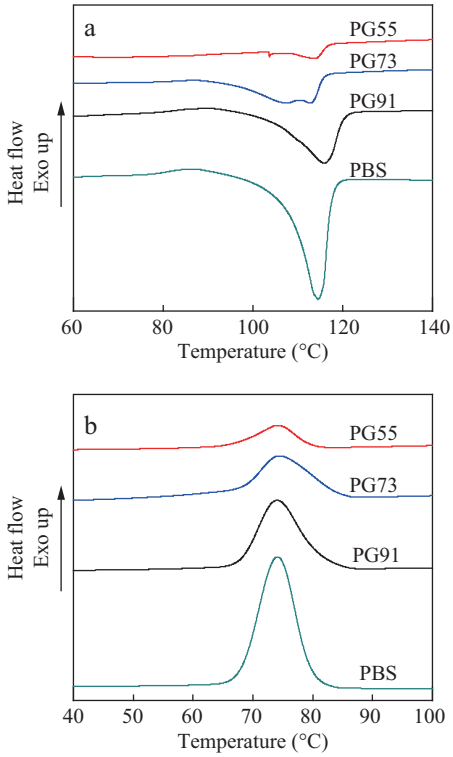

Fig. 4 DSC curves of PBS/gelatin electrospun membranes: (a) first heating and (b) cooling

Table 2 Crystallization properties of PBS/gelatin electrospun membranes

Sample	T_m (°C)	ΔH_m (J/g)	Crystallinity (%)	T_c (°C)
PBS granule	116.0	81.1	40.1	66.8
PBS membrane	114.5	86.1	43.1	74.1
PG91	115.9	71.0	35.5	74.1
PG73	113.0	51.5	25.8	74.5
PG55	114.1	22.1	11.1	74.1

Mechanical Properties

Since the mechanical properties of electrospun membranes of binary polymer blends could be affected by many factors including blend composition, fiber diameter, porosity of the membrane, interaction between the two components, interaction between two adjacent fibers, degree of

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crystallinity, *etc*., it is hard to make a prediction. Thus, the mechanical properties of PBS/gelatin blend membranes in both dry and wet states were evaluated by uniaxial tensile tests. The typical stress-strain curves are shown in Fig. 5, and the tensile strengths and elongations at break are listed in Table 3. In the dry state, the blend membrane with a low gelatin content of 10% (sample PG91) has a 3-times increase in tensile strength while maintaining the good ductility, compared to neat PBS membrane. The membranes exhibit brittle fracture when the gelatin content increases to 30% and 50%. This behavior resembles the effect of rigid filler. In fact, similar variation in mechanical properties has been reported for PLCL/gelatin blend membranes, and some compatibility between PLCL/gelatin and action of gelatin as a rigid filler have been proposed to explain the improved tensile strength with gelatin content of 10%–30%[32]. In this work, the best overall mechanical properties obtained with PBS/gelatin (9/1) membrane can be understood from three major aspects: existence of interaction between the two components PBS/gelatin, little change in crystallinity of PBS, and possible interaction between any adjacent fibers at the intersections. The two formers have been discussed in previous sections. Because there are some gelatin molecules on the fiber surfaces, it is highly possible that there is some interaction between neighboring fibers (at the intersections) due to hydrogen bonding between gelatin/PBS and between gelatin/gelatin, and this would make inter-fiber distance shorter, which can be reflected by the slightly decreased membrane porosity.

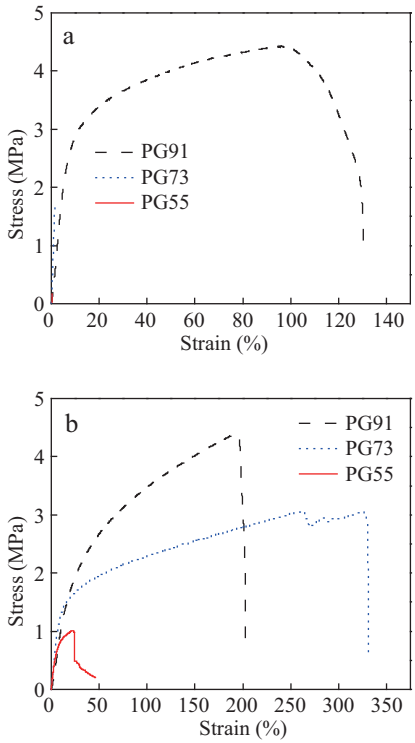

Fig. 5 Typical stress-strain curves of PBS/gelatin electrospun membranes in (a) dry state and (b) wet state

Table 3 Mechanical properties of PBS/gelatin electrospun membranes

Sample	Tensile strength (MPa)	Elongation at break (%)
PG91-dry	3.9 ± 0.3	113.7 ± 11.2
PG73-dry	1.8 ± 0.1	1.6 ± 0.1
PG55-dry	0.3 ± 0.1	2.9 ± 6.7
PG91-wet	4.6 ± 0.2	182.0 ± 22.7
PG73-wet	3.1 ± 0.1	402.0 ± 50.4
PG55-wet	1.0 ± 0.1	46.6 ± 1.7

In the wet state, the mechanical properties of PBS/gelatin (9/1) membrane are as good as those in the dry state, even slightly better in terms of both tensile strength and elongation at break. More surprisingly, the behavior of PBS/gelatin (7/3) membrane changes into ductile fracture, and both the tensile strength and elongation at break have a significant increase. This could be attributed to the gelatin molecules on the fiber surfaces that play a role of binder at the intersections of fibers in the presence of water. The mechanical properties of PBS/gelatin (9/1) and (7/3) membranes in the wet state are much better than gelatin-coated PBS membrane probably because gelatin could not play effectively the role of binder for loosely-packed as-spun fibers[20].

Immobilization of Thrombin onto PBS/Gelatin Membrane

To demonstrate potential applications of PBS/gelatin blend membranes, the clinically-used hemostatic drug thrombin was immobilized onto PBS/gelatin (9/1) blend membrane, which has good mechanical properties in both dry and wet states, by immersing the membrane into thrombin solution, followed by freeze-drying. As shown in Fig. 1(d), the immobilization process does not affect the porous and fibrous structure of the membrane. The initial gelation time t_{gel} of the thrombin-loaded membrane was 90.8 s. The enzyme activity deduced from the calibration curve is 0.16 U/cm^2, meaning that the membrane of a size of 1 cm^2 has the same activity as 0.16 U free thrombin. Because the measuring method involves observation of gelation time of fibrinogen in aqueous solution, it can mimic the hemostatic process. The thrombin-loaded PBS/gelatin blend membrane having detectable enzyme activity means that it can effectively catalyze the conversion of fibrinogen into fibrin and promote platelet aggregation, which is the final step of blood-clotting cascade. Therefore, the thrombin-loaded PBS/gelatin membrane has a great potential in rapid hemostatic applications such as in first aid, and the PBS/gelatin (9/1) blend membrane is a suitable support material for thrombin.

CONCLUSIONS

The mechanical properties of PBS electrospun membranes in both dry and wet states can be significantly improved by co-electrospinning with 10% of gelatin because of the existence of interaction between the two blend components, little change in crystallinity of PBS, and possible interaction between any adjacent fibers. The hydrophilicity of the membrane further increases, as revealed by the accelerated penetration rate of water droplet. The clinically-used hemostatic drug thrombin can be successfully immobilized

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onto PBS/gelatin (9/1) blend membrane, demonstrating that the blend membrane has a great potential in hemostatic application as support materials for hemostatic drugs.

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