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Release characteristics of different diameter ultrafine fibers as antibacterial materials

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Although the electrospinning technique has been devoted to promoting therapeutic purposes as a drug carrier, however, there are still many fundamental problems in this area. This work focuses on a comparison of various diameter polyethersulfone (PES) electrospun ultrafine fibers as antimicrobial materials. The fibrous morphology, antimicrobial agent distribution, thermally property, and biocompatibility evaluation of PES-based ultrafine fibers were systematically investigated. The results demonstrated that the PES-based ultrafine fibers were suitable as antimicrobial material. Furthermore, the drug release behavior and mechanism were studied through total immersion. The release mechanism was confirmed to Fickian diffusion. It was revealed that the drug max release amount (71.5%) and release rate (7.71) are the highest for the smallest diameter ultrafine fibers. Meanwhile, the antimicrobial activity of PES ultrafine fibers is also inversely correlated with the diameter of fiber. The electrospun PES fibers would control their release behavior through the diameter and have a potential application in the wound dressings, such as chronic osteomyelitis and exposure injury.

Keywords: Electrospinning; polyethersulfone; ultrafine fibers; drug release; antimicrobial activity.

1. Introduction

Electrospinning is a simple and most convenient technique to produce polymer ultrafine fibers, their diameter ranging from nanometer to submicrometer.[1–3] In the electrospinning process, a polymer or composite solution is drawn using a high voltage generation. Dry electrospun ultrafine fibers randomly were collected on the collector and formed nonwoven fibrous membranes with the solvent evaporating. Due to the small diameter, high

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surface-area-to-volume ratio, and high porosity, the electrospun ultrafine fibers have been widely applied in separation filter, wound dressing, and food packaging.[4–6] The nature porous of the fibrous membrane is also helpful for the wound exudate drainage and permeation of atmospheric oxygen.[7] Especially, the fibrous morphology, which is a similar structure to the extracellular matrix, can accelerate the cell growth making the electrospun fibrous webs to be excellent functional wound dressing.

The skin wound healing is always limited by the microbial invasion, which would ultimately result in delayed healing. Thus, it is urgent to fabricate the antimicrobial electrospun fibrous membrane. Zhao *et al.* developed sericin blended chitosan nanofiber mats by electrospinning which have huge potential for wound healing.[8] Lili *et al.* also fabricated core/shell chitosan/poly (lactide-co-glycolide) (PLGA)-based fibrous membranes with coaxial electrospinning technology.[9] The chitosan/PLGA membranes have shown good compatibility, cell viability, cell adhesion, and encouraged cells to migrate through the fibrous membranes. The obtained fibrous membranes could be potentially applicable in wound dressings applications. Peng fabricated a multilayer fibrous membrane with cellulose nanofiber as the outer layer and gelatin containing curcumin nanofibers as the inner layer by sequential electrospinning.[10] The multilayer fibrous membrane exhibited good thermal stability, sustained curcumin release, and antioxidant activity. The multilayer fibrous membrane has promising applications for controlled release. From the previous reports, the morphology of fibers will affect the drug release behavior, resulting in the final antioxidant activity.

Although various diameters of electrospun fibers could be controlled according to the preparation process. However, it is not clear whether the size of fiber can cause drug release behavior and antimicrobial effect or not. Thus, this work will focus on the various diameter antimicrobial electrospun fibers and reveal the relation between the morphology and antimicrobial property.

Polyethersulfone (PES), as a synthetic polymer, was usually used in the blood purification or tissues with its good biocompatibility and no-toxic.[11,12] Mansour prepared PES-collagen which is based on PES nanofibers by electrospinning and collagen. Pancreatic genes and proteins on PES-collagen were expressed significantly high, which will be considered as a promising candidate in pancreatic tissue engineering.[13] Wang Rui constructed PES-based membranes containing chitosan-polyurethane coating and Ag nanoparticles (AgNPs) with dual-antibacterial and antifouling properties. The results showed superior broad-spectrum antibacterial activity towards *E. coli* and *S. aureus*. Meanwhile, the PES-based membrane could reload AgNPs to be regenerated as antibacterial. This approach has the potential to fabricate antibacterial coatings on materials surfaces.[14]

Currently, there are many various antimicrobial nanomaterials for the disease treatment,[15] including carbon-based nanomaterials,[16] black phosphorus,[17,18] and two-dimensional nanomaterials.[19] Curcumin, as a yellow powder compound, is a natural polyphenol, which is isolated from the rhizome of turmeric. Curcumin possesses a highly effective bioactive, having excellent properties, such as anticancer, antimicrobial, anti-inflammatory, and antioxidant properties[20–22] Therefore, curcumin has been studied for the treatment of diseases such as cancer, cardiovascular, and Alzheimer's disease. Hieu fabricated polycaprolactone nanofibers-loaded curcumin and polyethyleneglycol by electrospinning.[23] It represented that the treatment using this nanofiber mat had a significant increase for the wound closure rate on day 10, indicating that it can facilitate wound healing with excellent anti-inflammatory properties.

In this work, PES was selected to be the polymer resin to fabricate different diameters ultrafine fibers, and curcumin was used as the antimicrobial drug model to investigate the antimicrobial activity. The ultrafine fibers morphology and the curcumin distribution were first discussed. And then the thermal property and cell viability were studied to confirm the suitability of this material as the wound healings. Finally, the curcumin released behavior and antimicrobial activity were systematically estimated to reveal the relation between the morphology of ultrafine fibers and antimicrobial property.

2. Experimental Section

2.1. *Materials*

Polyethersulfone (PES, Ultrason E2010) was obtained from the BASF chemical company (Germany). Curcumin was purchased from Sigma Aldrich (China). N, N-Dimethylformamide (DMF,

AR) was purchased from Kelong Chemical Co. (China). All the chemicals were applied without further purification.

2.2. *Method*

PES-1 ultrafine fibers were prepared as follows: PES (2.1 g) was dissolved and stirred in DMF (10 mL) at 60°C for 4 h. And then the solution kept pellucid after 10 h for deaeration. The electrospinning Generator (Ucalery, China) was used to supply a high voltage of 16 kV. The inner diameter of the capillary tip was 0.4 mm. Grounded rotating drum (rotation speed 0.9 m/s) was used to be the collector. The distance between the tip and the collector was 20 cm.

PES-2 ultrafine fibers were prepared according to this: PES (2.6 g) was dissolved in DMF (10 mL) at 70°C and kept pellucid after 20 h. A voltage of 15 kV was supplied to this solution. The inner diameter of the capillary tip was 0.6 mm and the grounded rotation speed is 0.6 m/s. The distance remained to be 15 cm between the tip and the collector.

PES-3 ultrafine fibers were fabricated as below, PES (2.8 g) was dissolved in DMF (10 mL) at 90°C and kept pellucid after 15 h. The applied voltage was 12 kV. The inner diameter of the capillary tip was 0.7 mm and the grounded rotation speed is 0.6 m/s. The collection distance remained to 15 cm.

The PES ultrafine fibers containing curcumin is prepared in order to be consistent with the above methods. The curcumin was directly added into the electrospun solutions. The addition amount is the percentage of PES weight.

2.3. *Characterization*

Scanning electron microscopy (SEM, FEI, USA) was used to characterize the surface morphology of the PES-based ultrafine fibers. Au/Pd coating was used to reduce the sample charging.

Thermo-Gravimetric Analysis (TGA) of the PES-based ultrafine fibers was characterized by thermal gravimetric analysis (TGA, TA, USA) from 50°C to 700°C under a nitrogen atmosphere and the heating rate is 10°C/min. A fluorescence microscope (PerkinElmer, USA) was used to measure the curcumin distribution in the curcumin-loaded PES fibrous membranes. 1.5 cm∗1.5 cm-based PES fibrous membranes were measured by maximum emission wavelength of 530 nm and maximum excitation wavelength 425 nm.[24,25]

The biocompatibility evaluation of PES-based ultrafine fibers was studied by cell counting kit-8 (CCK-8) assay using the colon cancer cell HCT116. The HCT116 cell viability was measured using Microplate Reader (iMark, Bio-Rad, USA) at 450 nm.

The release curcumin characteristics from the PES-based ultrafine fibrous membrane were tested with total immersion using a buffer solution. Each specimen (2 cm∗2 cm) was immersed in a 30 mL buffer solution at 37°C. The immersion time is between 0 and 20 h (1200 min). The curcumin release amount was measured by using a UV spectrophotometer (Puxi Co., China) at the wavelength of 426 nm. The results were obtained as average values using the triplicate experiment data.[25]

Antibacterial activity of PES-based fibrous membranes was tested against *E. coli* (ATCC 8739) and *S. aureus* (CMCC 26003). The same size of PES fibrous membranes (20∗20 mm) was autoclaved for 20 min at 120°C to sterilize. They were put on the inoculated agar plates incubating at 37°C for 4 h and 10 h. After incubation, the number of bacteria was counted and used to calculate the antibacterial activity.

3. Results and Discussion

Three different diameters of PES electrospun fibers were fabricated by electrospinning technology. Figures 1(a), 1(d), and 1(g) represented their SEM morphology. The diameter of PES-1, PES-2, and PES-3 ultrafine fibers is 205 ± 58 nm, 2235 ± 423 nm, and 3597 ± 354 nm, respectively. The diameter of PES-1 ultrafine fibers is smallest, implying that the low PES solution will decrease the diameter effectively. Compared to the diameter of PES-2 and PES-3 ultrafine fibers, it becomes apparent that the diameter of PES ultrafine fiber increased with the concentration of electrospun solution increase. Meanwhile, low voltage supply also increases the diameter of fibers with incomplete stretching. Thus, PES-1, PES-2, and PES-3 ultrafine fibers were used to be the final model to study the property of different diameter ultrafine fibers. To study the drug loading, 15% and 40% of

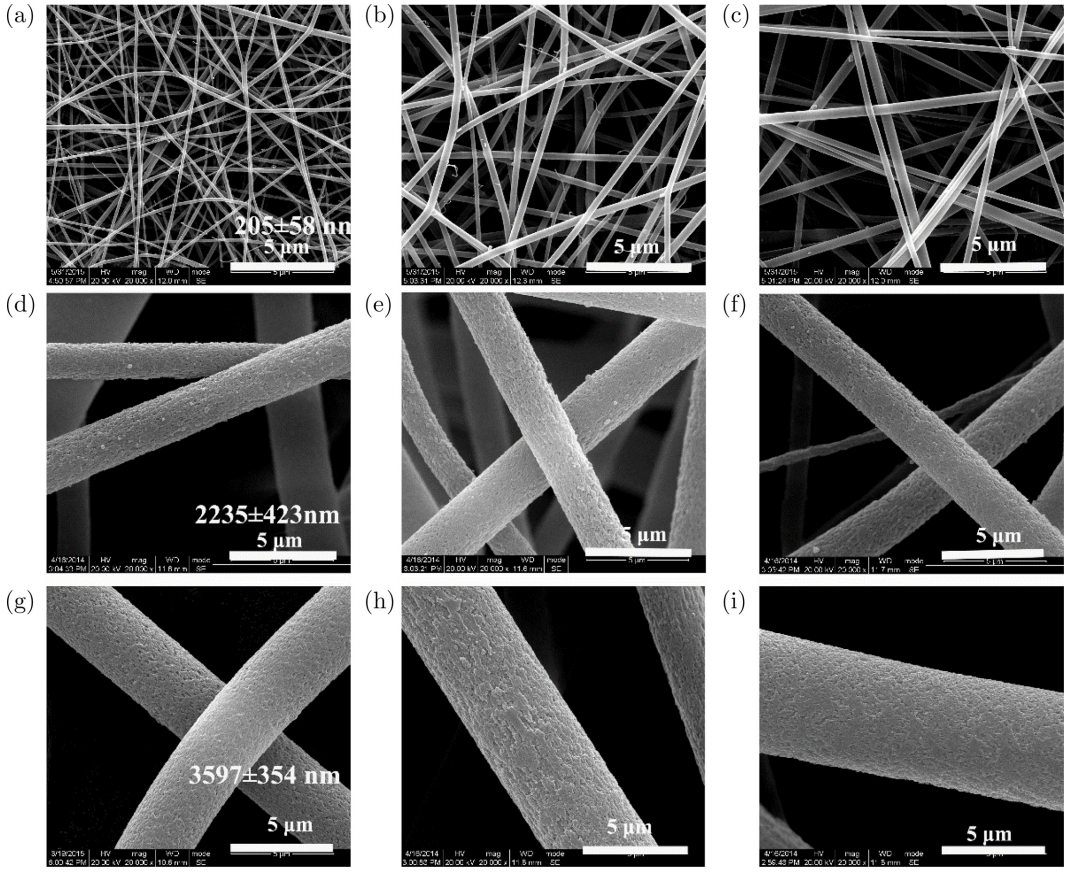


Fig. 1. The SEM morphology of PES-1 (a), PES-1/Cur 15% (b), PES-1/Cur 40% (c), PES-2 (d), PES-2/Cur 15% (e), PES-2/Cur 40% (f), PES-3 (g), PES-3/Cur 15% (h), and PES-3/Cur 40% (i) fibrous membrane.

curcumin was added in the electrospun solution and formed PES based containing drug ultrafine fibers. Figures 1(b), 1(c), 1(e), 1(f), 1(h), and 1(i) show the SEM morphology of PES loading curcumin ultrafine fibers. It is observed that uniform and continuous PES ultrafine fibers were observed. The diameter of the fibers has a slight increase. The main reason is that the curcumin addition will decrease the electrospun solution conductivity, reducing the stretch.

To confirm the curcumin distribution in the PES-based fibers, fluorescence imaging was employed to record PES-based ultrafine fibers containing curcumin because curcumin represents a strong fluorescence display. Figures 2(a)–2(f) represent curcumin fluorescence images of the electrospun containing PES-based curcumin ultrafine fibers. Continuous and uniform ultrafine fibers were observed in all fluorescence images, directly revealing that the curcumin is dispersed in all PES-based containing curcumin ultrafine fibers. For PES-1/Cur 15% and PES-2/Cur 15% ultrafine fibers, the intensity of fluorescence is weak. The intensity of fluorescence increases for PES-1/Cur 40% and PES-2/Cur 40% ultrafine fibers because of the more loaded curcumin amount. Furthermore, the ultrafine fibers could complete the dynamic tracing *in vivo* with the curcumin unique fluorescence.

The thermal stability of ultrafine fibers is a key factor in the actual applications. Thus, TGA was used to characterize it in this work. As shown in Fig. 3, the PES resin and curcumin decomposition temperatures are 495.8°C and 265.4°C, respectively. For PES–1 loaded curcumin ultrafine fibers, two regions are seen in their weigh lost curves. The front weight loss is due to the curcumin decomposition and the latter one is attributed to PES decomposition. The degradation temperature of PES-1/Cur 15% and PES-1/Cur 40% is 292.91°C and 289.49°C, respectively. The thermal stability of the PES-based containing curcumin ultrafine fibers was lower than the pure PES ultrafine fibers, but it is sufficient for the application as antimicrobial materials. Generally speaking, the degradation

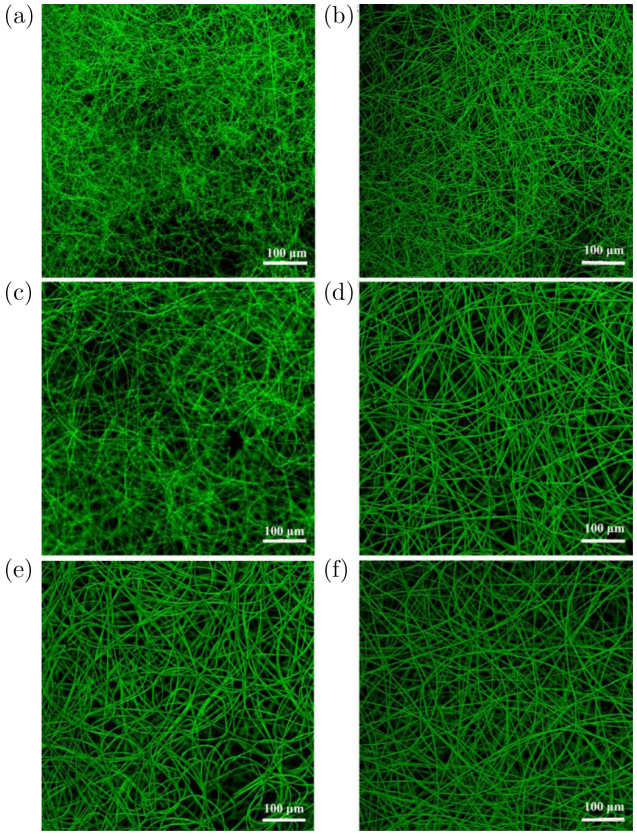


Fig. 2. Fluorescence imaging of the electrospun of PES-1/Cur 15% (a), PES-1/Cur 40% (b), PES-2/Cur 15% (c), PES-2/Cur 40% (d), PES-3/Cur 15% (e), and (f) PES-3/Cur 40% ultrafine fibrous membrane.

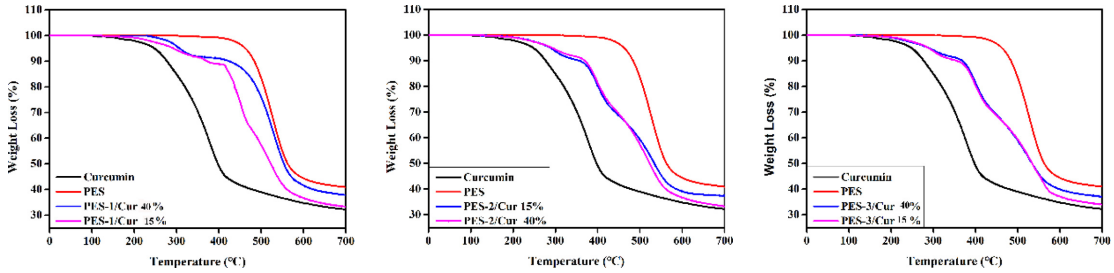
temperature of antibacterial materials is asked to be up 250°C.[26] Meanwhile, the real curcumin weight in PES-based ultrafine fibers is also needed in the following curcumin release study. Thus, the actual curcumin amount was calculated according to the carbon residue at 700°C. The actual curcumin amount is 14.06% and 40.49% for the PES-1/Cur 15% and PES-1/Cur 40% ultrafine fibers, respectively. The trend of thermal stability and the actual curcumin weight percentage of PES-2 and PES-3-based ultrafine fibers is similar to the PES-1 one. They are summarized in Table 1.

The cytotoxic effect of PES ultrafine fibers containing curcumin was evaluated by the CCK-8 assay. For this purpose, the extraction medium containing PES-based ultrafine fibers was cultured with HCT116 cells for 1 day and 3 days. The cell viability for PES-based fibers is represented in Fig. 4. After 1 day incubation, the percentage of HCT116 cells is $94.2 \pm 1.8\%$, $93.3 \pm 2.3\%$, $85.3 \pm 3.4\%$, $88.2 \pm 2.9\%$, $91.7 \pm 2.1\%$, and $91.8 \pm 2.7\%$ for PES-1/Cur 15%, PES-1/Cur 40%, PES-2/Cur 15%, PES-2/Cur 40%, PES-3/Cur 15%, and PES-3/Cur 40% ultrafine fibers, respectively, displaying cells incubated with PES fibrous membrane owning high proliferative activity. When the incubation time increases to three days, it still maintains high activity. The percentage is $96.2 \pm 2.3\%$, $97.8 \pm 2.1\%$, $88.4 \pm 4.5\%$, $90.5 \pm 2.8\%$, $95.9 \pm 1.7\%$, $94.2 \pm 2.1\%$ for PES-1/Cur 15%, PES-1/Cur 40%, PES-2/Cur 15%, PES-2/Cur 40%, PES-3/Cur 15%, and PES-3/Cur 40% ultrafine fibres, respectively. For the longer 10 days, cell activity still keeps stained. The results reveal cells in different incubation times maintained high activity and the cell activity increase with the incubation time, demonstrating that PES-based fibrous membrane exhibited good biocompatibility.

The curcumin release behaviors are displayed in Fig. 5. The max curcumin release amount is w 69.9%, w 71.5% PES-1/Cur 15%, and PES-1/Cur 40%, respectively. For PES PES-2/Cur 15%, PES-2/Cur 40% ultrafine fibers, the max curcumin release amount decreased to w 63.9% and w 65.5%, respectively. There is further decline for PES PES-3/Cur 15%, PES-3/Cur 40% ultrafine fibers, only w 60.9%, and w 61.5%. From the results of the

Table 1. The thermal property and the correlation coefficients and rate parameter of curcumin release in PES-based fibrous membrane.

%	Degradation temperature (°C)	The curcumin actual weight (%)	The max release rate (%)	Rate parameter	R^2
PES-1/Cur 15	292.91	14.06	69.9	7.57	0.999
PES-1/Cur 40	289.49	40.49	71.5	7.71	0.994
PES-2/Cur 15	288.98	18.01	63.9	6.53	0.987
PES-2/Cur 40	285.21	44.57	65.5	6.45	0.972
PES-3/Cur 15	289.99	16.89	60.9	4.47	0.976
PES-3/Cur 40	287.64	43.21	61.5	5.04	0.973



(a) (b) (c)

Fig. 3. TGA curves of the samples PES-1(a), PES-2 (b), and PES-3 (c) based fibrous membrane.

curcumin release behavior, it can be obtained that the max released is highest with the smallest diameter. The main reason is that the small diameter will increase the surface to volume of materials, which would boost the release amount.

To confirm the curcumin release rate and release mechanism of the PES-based ultrafine fibers, the curcumin release kinetics from PES based containing curcumin ultrafine fibers would be determined according to the following equation[27]:

$$\frac{Mt}{M_\infty} = k * t^n \text{ for } \frac{M_t}{M_\infty} < 0.6,$$

where M_t represents the curcumin cumulative released amount at any time t, M_∞ represents curcumin cumulative amount at a final time, k represents the curcumin release rate. For $n = 0.5$, the release mechanism of this material is considered as Fickian diffusion.[28] This diffusion model was used to fitting the collected data for explaining the possible release mechanism. Straight lines can be obtained and shown in Fig. 6. It showed a good correlation. Table 1 lists the correlation coefficients (r^2). The results display that r^2 are 0.999, 0.994, 0.987, 0.972, 0.976, 0.973 for PES-1/Cur 15%, PES-1/Cur 40%, PES-2/Cur 15%, PES-2/Cur 40%, PES-3/Cur 15%, and PES-3/Cur 40% ultrafine fibers, respectively. The curcumin release mechanism of these PES-based containing curcumin ultrafine fibers was conformed to the Fickian diffusion. The release rate parameter k is 7.57, 7.71, 6.53, 6.45, 4.47, 5.04 for PES-1/Cur 15%, PES-1/Cur 40%, PES-2/Cur 15%, PES-2/Cur 40%, PES-3/Cur 15%, and PES-3/Cur 40% ultrafine fibers, respectively. This result represented the curcumin release rate is following this order: PES-1 > PES-2 > PES-3, indicating that the smaller diameter ultrafine fibers will possess a higher release rate.

The *in vitro* antibacterial activities of the PES-based ultrafine fibrous membranes were confirmed

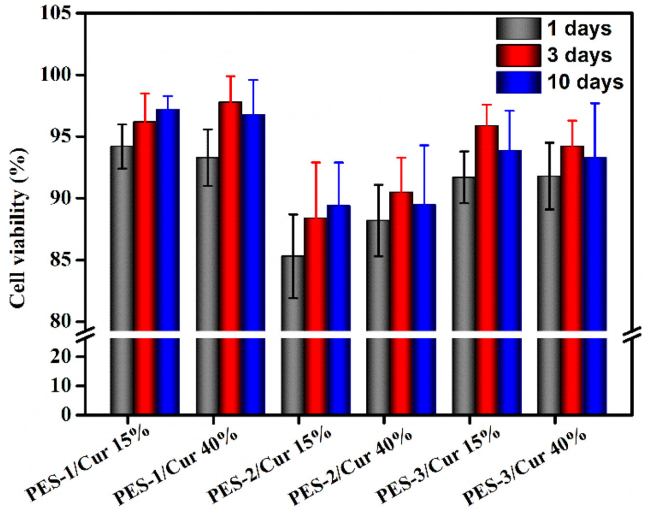


Fig. 4. Cell viability of PES/Cur-based fibrous membranes. The data represent the mean ± standard deviation (SD) from three repeated measurements.

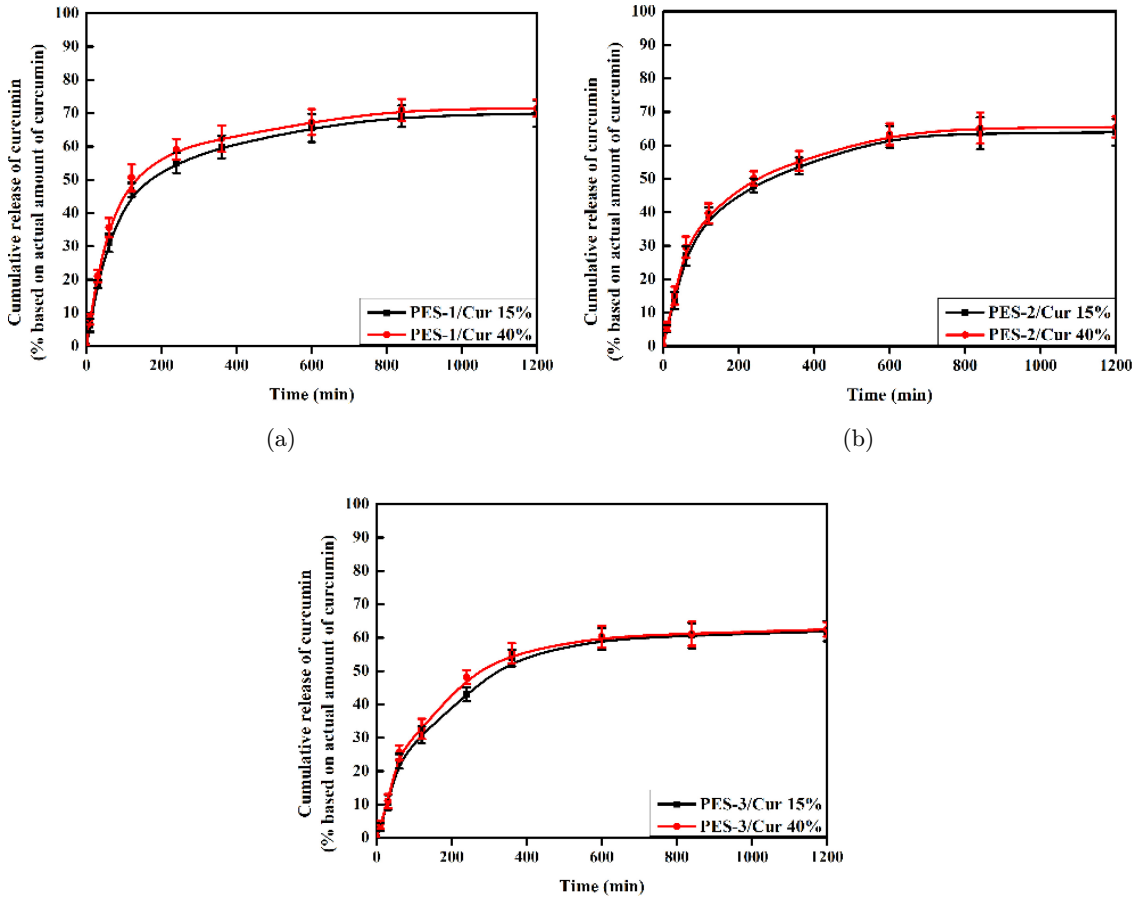


Fig. 5. The curcumin release behavior of PES-1 (a), PES-2 (b), and PES-3 (c) based fibrous membranes.

against *S. aureus* and *E. coli*, which are commonly found in diabetic wounds. The released curcumin was able to inhibit the growth of bacteria. As shown in Figs. 7(a) and 7(b), the PES ultrafine fibers containing curcumin showed obvious antibacterial activities against both *S. aureus* and *E. coli*. A general trend could be obtained that all PES/Cur 40% of fibrous membranes possess higher

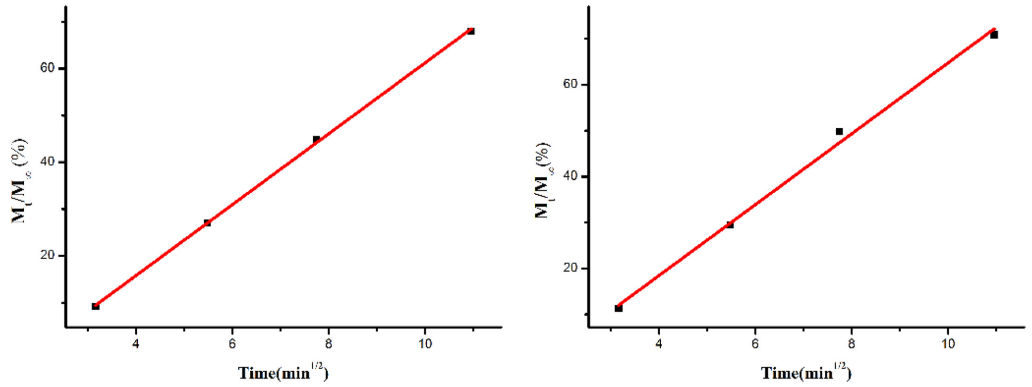


Fig. 6. The curcumin release mechanism of PES-1/Cur 15% (a), PES-1/Cur 40% (b), PES-2/Cur 15% (c), PES-2/Cur 40% (d), PES-3/Cur 15% (e) andPES-3/Cur 40%, (f) ultrafine fibers.

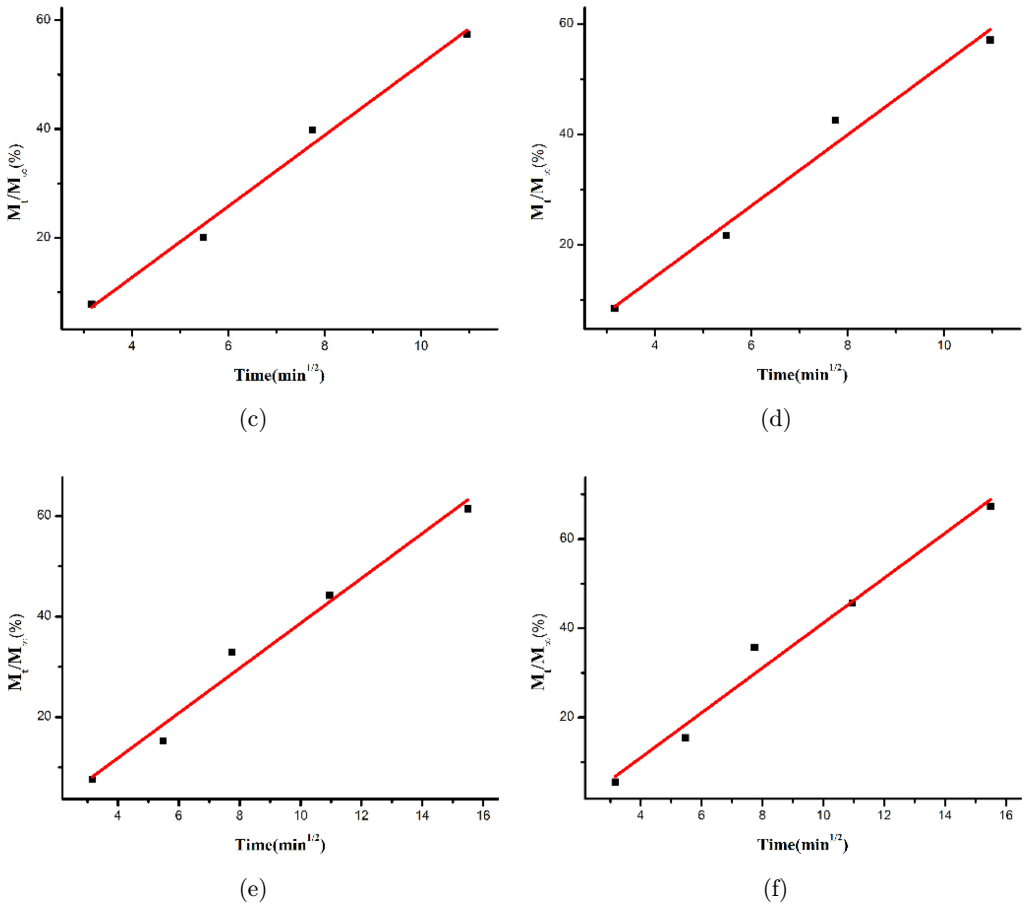


Fig. 6. (*Continued*)

antibacterial activity than PES/Cur 15%. The main reason is that more amounts of curcumin were loaded in the PES/Cur 40% fibrous membranes with the same size. And the larger amounts of curcumin will be released from these membranes, which increase the antibacterial activity. On the other hand, three different diameter ultrafine fibers exhibited different antibacterial activity at 4 h. PES-1-based ultrafine fibers have the best antibacterial activity. The antibacterial activity is up to

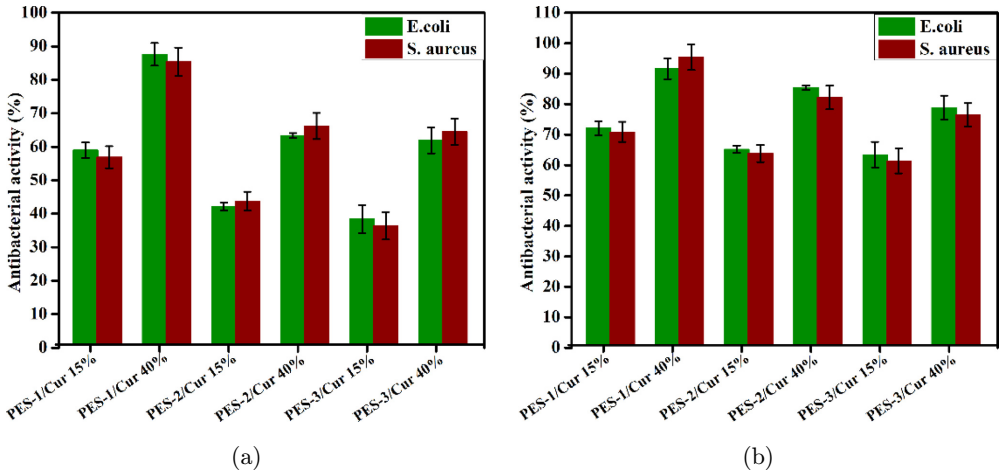


Fig. 7. The antibacterial activity of PES-based fibrous membrane at 4 h (a) and 10 h (b) for *S. aureus* and *E. coli*.

$59.2 \pm 2.3\%$ and $87.6 \pm 3.4\%$ for PES-1/Cur 15% and PES-1/Cur 40%, respectively. However, the antibacterial activity decreased to $42.2 \pm 1.2\%$ and $63.4 \pm 0.7\%$ for PES-2-based fibrous membrane. For the PES-3-based fibrous membrane, the antibacterial activity decreased furthermore, only 38.4 $\pm 4.2\%$ and $61.9 \pm 3.9\%$. It can be explained that the curcumin release rate of PES-1 is larger than the others and the larger amount curcumin released from the PES-1-based fibers at 4 h. There is still a little distinction among ultrafine fibers at 10 h (shown in Fig. 7(b)), the main reason is that curcumin max release amounts are different among three ultrafine fibers at 10 h. For the PES-1 ultrafine fibers, the antibacterial activity is largest, the main reason is that the curcumin release amount is the largest. All in all, the antibacterial activity of the fibrous membrane is different at a unique time and fiber diameter.

4. Conclusion

Three different diameters of PES ultrafine fibers, namely PES-1, PES-2, and PES-3 were fabricated via electrospinning and subsequently used as a drug carrier in wound dressings applications. Curcumin, as the antibacterial agents, was loaded in these ultrafine fibers and its distribution was investigated. The thermal property and cell activity were also characterized and the results represent that the PES-based ultrafine fibers are suitable to be used as wound dressings. The curcumin release characteristics showed that the max release amount is up to 71.5% for PES-1/Cur 40% ultrafine fibers, which is higher than the PES-3/Cur 40% one (61.5 %), indicating that the small diameter of ultrafine fibers possesses high release amounts. Meanwhile, the curcumin release mechanism was confirmed to the Fickian diffusion. Finally, the antibacterial activity of PES ultrafine fibers was explored and the antibacterial activity is up to $87.6 \pm 3.4\%$. And various diameter ultrafine fibers have different antibacterial activities. These results showed that the drug release behavior and antibacterial activity could be controlled by the diameter of fibers. It is highly important that beyond the potential application of electrospun antibacterial fibers for the treatment of wounds with various etiologies, more and more methods for accurate control drug release should be developed.

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

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