A Method to Control Curcumin Release from PELA Fibers by Heat Treatment

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ABSTRACT: Poly(ethylene glycol -co- DL-lactide) (PELA) is a biodegradable polymer, which has attracted considerable interest in the area of drug release due to its potential as drug carrier. Drug-loaded electrospun PELA fibers tend to be a promising candidate for local chemotherapy application in the future. In this study, curcumin was encapsulated into PELA fibers (Cur@PELA) for drug delivery via electrospinning. It was found that the shrinkage ratio, porosity, water absorption and water contact angle of Cur@PELA fiber membrane could be changed by heat treatment with different heating temperature (45°C, 50°C) and heating time (5 min, 10 min, 20 min). Moreover, the release rate of curcumin from Cur@PELA fibers was influenced by the porosity, water absorption and water contact angle of the drug-loaded membrane. Heat treatment is thought to be a novel method to control release of drug from electrospun PELA fibers. © 2016 Wiley Periodicals, Inc. Adv Polym Technol 2018, 37, 21705; View this article online at wileyonlinelibrary.com. DOI 10.1002/adv.21705

KEY WORDS: Control release, Curcumine, Fibers, Poly(ethylene glycol -co- DL-lactide)

Introduction

Drug carriers for controlled release prepared by electrospinning are promising for biomedical applications and have been widely investigated in recent years. There are many outstanding advantages of fibrous carriers compared with the conventional dosage forms (tablets, capsules, powders),[1] such as improving therapeutic efficacy, offering site-specific delivery for drugs to the body and reducing toxicity.[1,2] Electrospun nanofibers have been widely used in drug delivery and tissue engineering, especially postoperative local chemotherapy. For example, electrospun nanofiber membrane has been utilized as anti-adhesion films to prevent postoperative tissue adhesion,[3–5] while drug-loaded electrospun nanofiber membrane has been applied to inhibiting infection and the chronic wound.[6] However, the burst release of drug from the electrospun fibers at initial stage is still one great challenge for researchers. To overcome this problem, different methods have been reported for controlling drug release, such as surface modification,[7,8] surface cross-linking,[8] emulsion, and coaxial electrospinning; some mesoporous silica nanoparticles[9] and hydroxyapatite[10] have been incorporated into electrospun nanofibers to prolong drug release time. However, some issues still exist, such as cross-linking agent may be toxic,[8] coaxial electrospinning need substantial optimization of electrospinning parameters.[10] Emulsifier and mesoporous nanoparticles used may cause toxicity and safety issues as well as the low efficiency of drug delivery.[11] Therefore, development of a nanofiber drug carrier which could possess mitigated burst release and control release of the encapsulated drug still remains a great challenge. To reduce burst release and control

the behavior of drug release from electrospun fibers, a novel method was described in this study.

"Controlled release" refers to the use of polymeric materials to release incorporated drugs at a controlled rate for a desired period of time.[12] PELA is a commonly used material for drug carrier and has received considerable interest in the medical and pharmaceutical field due to their biodegradability. Many researchers have reported electrospun PELA nanofibers as drug carriers.[3,13–16] In this study, we proposed a simple method to control release of drug from electrospun PELA fibers. Curcumin, a yellow pigment present in the Indian spice turmeric, possesses diverse pharmacologic effects including anti-inflammatory, antioxidant, antiproliferative, and antiangiogenic activities,[17,18] was chosen as a model drug in this system.

In this study, a controlable drug release carrier (Cur@PELA) based on semicrystalline polymer (PELA) was developed. The influence of porosity, water absorption and contact angle on the release behavior of curcumin from Cur@PELA nanofibers was studied. Heat treatment could induce the shrinkage of Cur@PELA fiber membrane. The results showed that higher temperature with longer time heat treatment could decrease the porosity and water absorption of Cur@PELA fiber membrane, which lead to a slow down of the release rate. On the other hand, to maintain the geometrical stability of Cur@PELA fiber membrane during heat treatment, fiber membrane was fixed on a flat. Results showed that the water contact angle of fixed fiber membrane became larger by heating for 20 min, and the drug release rate also slowed down. To the best of our knowledge, we firstly described a control release system which can control release of drug from electrospun fibers by varying the porosity, water absorption and contact angle of nanofiber membrane using heat treatment. It is an easier and more effective way to control the release of drug from electrospun PELA fibers.

Experimental Section

MATERIALS

PELA (average Mv~180,000) with PEG weight ratios of 20% was provided by Shanghai Divine Medical Technology Co., Ltd. Curcumin was purchased from J&K chemical Co., Ltd. All other chemicals were of analytical grade and were used without any further purification.

PREPARATION OF CUR@PELA FIBER MEMBRANES

Cur@PELA were prepared by electrospinning. Briefly, 1 g PELA and 8 mg curcumin were dissolved in 4 mL Methylene chloride/Trifluoroacetic acid solution (8/2, v/v). The percentage of curcumin to PELA was 0.8%. The electrospinning parameters were set as an applied voltage of 14 kV, a collection distance of 15 cm, and the electrospinning solution flow rate of 1.0 mL/h controlled by a syringe pump.

After electrospinning, Cur@PELA fiber membranes were treated with heating at different temperature and time period. Five different samples were prepared, accordingly, Control (without heat treatment), S-45*5 (45°C, 5 min), S-50*5 (50°C, 5 min), S-50*10 (50°C, 10 min), and F-50*20 (50°C, 20 min). All fiber membranes of five groups were cut into 2 × 3 cm pieces. The process of treatment was different, S-45*5, S-50*5, and S-50*10 were treated by heat without fixation in oven. On the contrary, sample was prepared and treated by heat with fixation in oven, F-50*20 (50°C, 20 min) were cut and fixed on a flat plate using clips. Briefly, the specimen 3 cm long and 2 cm wide dimensions was placed in the center of flat plate, and fixed on it using four clips at four sides of membrane, then heated for 20 min in oven. The F-50*20 nanofibers were collected directly from the flat plate and stored at room temperature. The way of heating treatment for preparing different samples was shown in Table I.

CHARACTERIZATION

Morphology of Fiber Membranes and Diameter of Fibers

The surface morphology of fiber membranes were observed by scanning electron microscope (SEM). The average diameter of fibers was obtained from at least 100 measurements on a SEM image using Image J software.

Fourier Transform Infrared Spectroscopy

Attenuated total reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) was performed by a Nicolet-670 FTIR spectrometer. All spectra were measured in the wavelength range of 500 to 4000/cm.

Thermal Characterization

The thermal behavior of Cur@PELA fiber was characterized by Different Scanning Calorimeter (DSC) in a temperature range from −50 to 150°C at a heating rate of 20°C/min.

SHRINKAGE RATIO

Shrinkage is generally defined as a shortening of fiber membrane length. Small strips (2 × 3 cm) were cut randomly from the fiber membrane. The length of the fiber membrane was measured. The shrinkage ratios of the Cur@PELA fiber membranes were defined as:[19]

TABLE I
Heat Treatment for Preparing Different Samples

Sample	Heating temp (°C)	Heating time (min)	Heating method
Control	0	0	Without fixation
S-45*5	45	5	Without fixation
S-50*5	50	5	Without fixation
S-50*10	50	10	Without fixation
F-50*20	50	20	Fixation[a]

aF-50*20 was fixed on a flat plate for heating.

$$\text{Shrinkage ratio} = (L_1 - L_2)/L_1 \times 100\% \quad (1)$$

Here, L_1 is the length of Cur@PELA fiber membrane before heat treatment, and L_2 is the length of the fiber membrane after heat treated.

POROSITY

The porosity of different Cur@PELA fiber membrane was measured by using a method reported in previous literatures.[10,20] Small strips (2 × 3 cm) were cut randomly from the fiber membrane. The thickness of the fiber membrane was measured with a micrometer. The apparent density (ρa) and porosity (p)of the Cur@PELA fiber membranes were calculated using the following equations:

$$\text{Density}(\rho_a) = m(g)/(\text{d(cm)} \times s(\text{cm}^2)) \quad (2)$$

$$\text{Porosity} = (1 - \rho_a/\rho_b) \times 100\% \quad (3)$$

Here, m, d, and s stand for mass, thickness, and area of the fiber membranes, respectively. The bulk density of curcumin-loaded PELA membrane (ρ_b) is about 1.27 g/cm^3.

WATER ABSORPTION TEST

The water absorption of Cur@PELA fiber membranes were tested according to the reported method described in previous literatures.[21,22] Firstly, Cur@PELA fiber membrane was weighted and recorded as the dry weight. Secondly, fiber membrane was placed in tubes containing PBS, and was removed after 2 h. The wet fiber membrane was placed on a paper towel, and the excess water was allowed to drain off. The fiber membrane was then weighed and measured as wet weight. The water content percentage of the fiber membrane was calculated with the following equations:

$$\text{Water content percentage} = (g_1 - g_0)/g_0 \times 100\% \quad (4)$$

g_0 and g_1 are the weight of dry and wet fiber membranes, respectively.

CONTACT ANGLE

Water contact angles of fiber membranes were measured using a contact angle analyzer. The contact angles between water droplets and fiber membrane were measured and recorded by a video monitor.

IN VITRO DRUG RELEASE STUDY

Drug-loaded fiber membrane (about 20 mg) was soaked in a centrifuge tube filled with 4 mL phosphate buffer saline (PBS, pH 7.4) containing 0.1% w/v Tween-80 at 100 rpm in thermostat shaker at 37°C, Tween-80 was employed in the PBS to provide solubility for curcumin in aqueous phase. At appropriate time, 2 mL released media was removed from the centrifuge tube and replaced by an equal volume of fresh media. The curcumin concentration in the released samples was determined at 450 nm using microplate reader.[23]

The drug loading efficiency can be calculated via the following equation:

$$\text{loading efficiency} = M_t/M_0 \times 100\% \quad (5)$$

Here, M_t and M_0 stand for the mass of encapsulated curcumin and the initial total curcumin used for encapsulation, respectively.[11] The mass of encapsulated curcumin in fiber membrane (M_t) were determined with the following method. Briefly, curcumin-loaded nanofiber membrane (about 20 mg) was dissolved in 5 mL dimethyl sulfoxide (DMSO) completely. The curcumin concentration was determined at 450 nm using microplate reader. Then the mass of encapsulated curcumin in fiber membrane (M_t) could be obtained.

STATISTICAL ANALYSIS

All experiments were conducted at least three times. Statistically significant differences were obtained by comparing data using one-way ANOVA. The criteria for statistical significance were *p < 0.05.

Results and Discussion

The surface morphology of the Cur@PELA fiber membranes with different heat treatment was observed via SEM images (Fig. 1). The average diameter of Control (Fig. 1a), S-45*5 (Fig. 1b), S-50*5 (Fig. 1c), and S-50*10 (Fig. 1d) were detected to be 921 ± 268 nm, 1679 ± 329 nm, 1665 ± 341 nm, and 1968 ± 439 nm, respectively. It was observed heat treatment could significantly alter the fibrous morphology of Cur@PELA fibers. Uniform and smooth fibrous fibers could be seen in Control (Fig. 1a), In contrast, the bent and thick fibers could be seen in (Figs. 1b–1d), respectively. Heat treatment with higher temperature for longer time (Fig. 1d) could increase the thickness of fibers. In addition, compared with the other four groups, Fibers in F-50*20 (Fig. 1e) conglutinated together and showed different morphology.

Heat treatment could change the morphology of Cur@PELA fibers, because heat treatment cause the shrinkage of Cur@PELA fiber membrane, which make bend in fibers and increase the diameter of fibers. Moreover, the melting point of Cur@PELA was about 50°C (Fig. 2). Treating Cur@PELA fibers with 50°C for 5 min, 10 min, and 20 min could melt part of the fibers. However, curcumin was relatively stable when it was exposed to temperatures up to 120°C.[24]

Figure 3 shows the FTIR spectra of the free curcumin, PELA fibers, Cur@PELA fibers. From the spectra of Cur@PELA (Fig. 3a), the peak at 1500/cm to 1600/cm could be assigned to the C–C (benzene ring) stretching vibrations of curcumin. The FTIR data verified the loading of curcumin

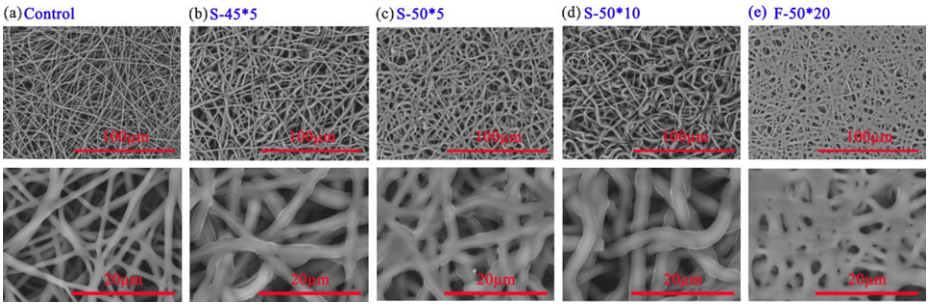

FIGURE 1. SEM images of electrospun Cur@PELA fibers. (a) Control, (b) S-45*5, (c) S-50*5, (d) S-50*10, (e) F-50*20.

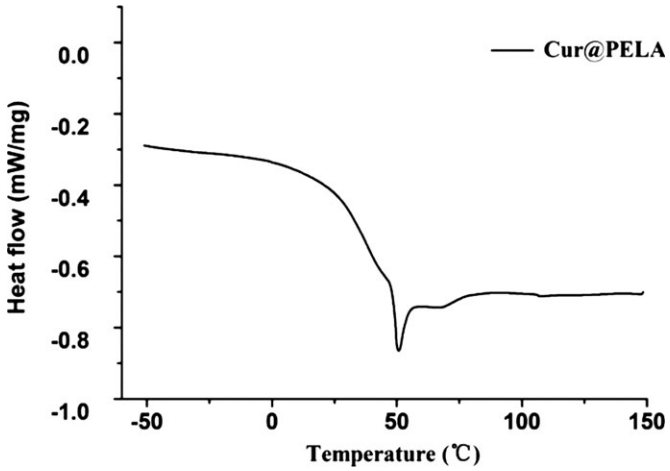

FIGURE 2. DSC profile of Cur@PELA fiber membrane.

onto the PELA fibers. However, from the Fig. 3(b), no difference was observed between Cur@PELA fibers with and without heat treatment. These results suggested that heat treatment would not change the chemical structure of curcurmin.

SHRINKAGE RATIO AND POROSITY

Figure 4 shows the change in shrinkage ratio and porosity of Cur@PELA fibers by varying heating temperature and time. From the Fig. 4(a), it was noted that the shrinkage ratio of Control and F-50*20 were 0%, because F-50*20 was fixed on a flat plate to maintain the dimension. The shrinkage ratio of S-45*5, S-50*5, and S-50*10 increase significantly with increasing heat treatment temperature and time, which were 53%, 59%, and 63%, respectively. It indicated that the stress relaxation of the Cur@PELA fiber membrane could be promoted by heating with the higher temperature and longer time. The porosity of Control, S-45*5, S-50*5, S-50*10, and F-50*20 is presented in Fig. 4(b), which were 80%, 47%, 36%, 24%, and 45%, respectively. It could be found that the porosity of the fibrous membranes decreased with increasing heating temperature and time. The possible reason is that heat treatment made fibers shrink and arrange more closely, which lead to decrease of the porosity of membrane. However, the shrinkage ratio of both control and F-50*20 were similar, although porosity of control was higher than that F-50*20. The possible reason for this is that heat treatment change the morphology of fibers (Fig. 1e), more closely packed to fibers, which lead to the decrease in porosity. As a result, the thickness of F-50*20 decrease after heat treatment, which increase the density of F-50*20.

WATER ABSORPTION

Figure 5 shows the water uptake of different Cur@PELA fiber membranes placed in PBS buffer for 2 h. The water uptake could be attributed to the structure of fibers. It was observed that the water absorption percentage of Control was the highest among samples. The water absorption percentage of Control, S-45*5, S-50*5, and S-50*10 became lower with increasing heating temperature and time. It is possible that the lower porosity of Cur@PELA fiber membrane reduced water uptake. Moreover, the water absorption percentage of F-50*20 was also low. The reason for this was that the porosity of F-50*20 was low, on the other hand, the fibers of F-50*20 were close together, it may decrease the uptake of water.

CONTACT ANGLE

The contact angle of Control, S-45*5, S-50*5, S-50*10, and F-50*20 fiber membranes were presented in Fig. 6. After water droplet placed on the fiber membranes at 30 s, the contact angle of Control, S-45*5, and S-50*5 were all 0°, showed hydrophilic properties. However, the contact angles of S-50*10 and F-50*20 were 47° and 124°, respectively. It can be clearly seen that F-50*20 showed hydrophobic properties. The video image of water droplet in Fig. 6(b) indicates the same results as Fig. 6(a). The previous studies indicated that the contact angle could be influenced by the membrane surface composition, pore size, and roughness.[19] In this study, heat treatment can influence the hydrophilic properties of Cur@PELA fiber membranes. This may be due to the fact that heat treatment could influence the porosity and morphology of fiber membrane, which lead to the change in hydrophilic properties. There was a little differences on fibers morphology among S-50*10, S-45*5, and S-50*5. But the porosity of S-50*10 was lower than S-45*5 and S-50*5, which indicated the porosity of fiber membranes could influence the hydrophilic properties, lower porosity of fiber membrane was more hydrophobic. Moreover, compared S-50*10 with F-50*20, the porosity of S-50*10 was lower than F-50*20, however, the contact angle of

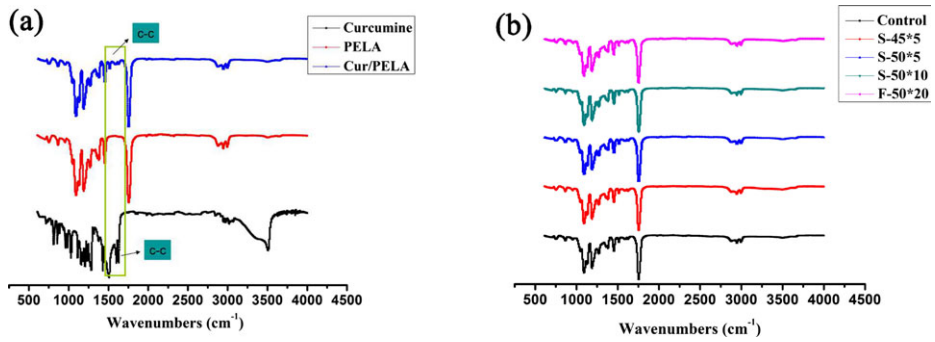

FIGURE 3. FTIR spectra of free curcumin, PELA fibers and different Cur@PELA fibers.

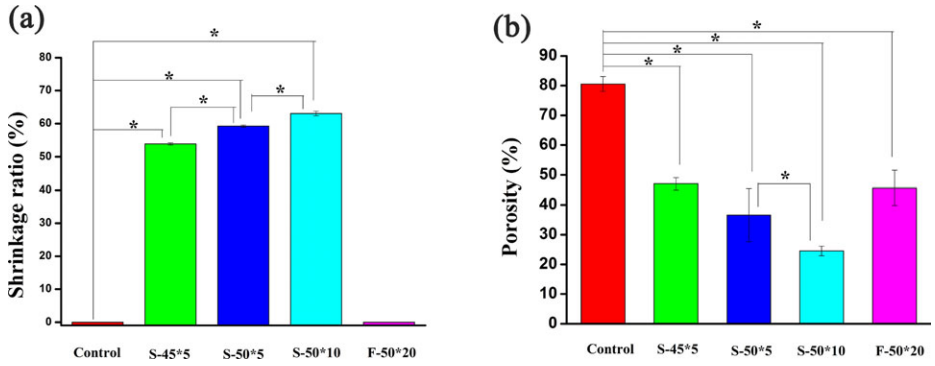

FIGURE 4. (a) Shrinkage ratio and (b) porosity of different Cur@PELA fiber membranes. Significant difference between groups is indicated (*$p < 0.05$).

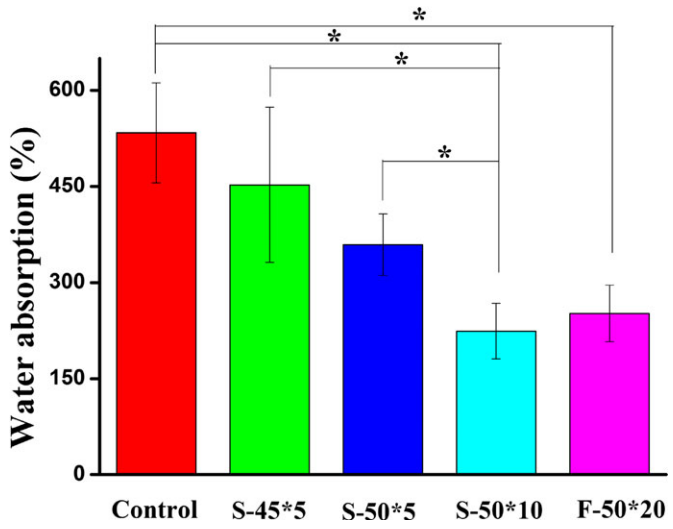

FIGURE 5. Water absorption percentages of different Cur@PELA fiber membrane after placed in PBS in 2 h. Significant difference between groups is indicated (*$p < 0.05$).

S-50*10 was smaller than F-50*20. That indicated the fibers morphology could also influence the hydrophilic properties of fiber membrane. Comparing with the other four groups, F-50*20 (Fig. 1e) would conglutinate together in the membrane which showed different morphology. It can be observed that melting nanofibers were more closely packed, that may be the reason why F-50*20 was more hydrophobic than others.

RELEASE BEHAVIOR STUDY

The release profiles of curcumin from Control, S-45*5, S-50*5, S-50*10, and F-50*20 nanofibers were shown in Fig. 7. It is clear noted that heat treatment can influence the release behavior of curcumin from Cur@PELA fibers. As shown in Figs. 7(a) and 7(c), the release profiles for Control can be illustrated by two stages. In the first 24 h, approximate 16% of curcumin was released, showed an initial burst release. On the second stage, a relatively stable release behavior was observed. However, S-45*5, S-50*5, and S-50*10 showed a slower release rate compared to the Control. In the first 24 h, 9.4%, 6.6%, and 4.4% of curcumin were released from S-45*5, S-50*5, and S-50*10, respectively. After 30 days of incubation, around 60%, 53%, 46%, and 36% of curcumin were released from Control, S-45*5, S-50*5, and S-50*10, respectively. It is important to note that F-50*20 (Figs. 7b and 7d) showed a constant stable release behavior and almost no burst release. As shown in Fig. 7(c), the release of curcumin from S-45*5 fibers was slower than Control, but faster than S-50*5. It indicates that the slower drug release profiles may be obtained by increasing heating temperature at the same treating time (5 min). As shown in Fig. 7(c), the drug release result suggested that the slower drug release profiles could be obtained by prolonging heating time at the same treating temperature (50°C). Briefly, slower release

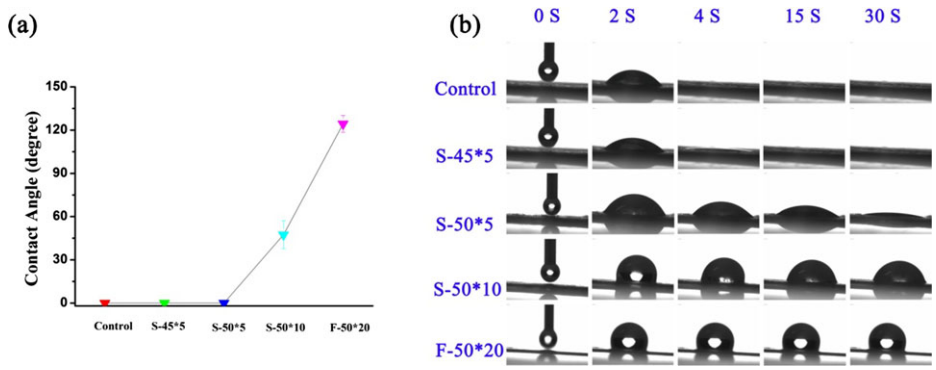

FIGURE 6. (a) Water contact angles of different Cur@PELA fiber membrane 30 s after water droplets were placed on the fibrous membrane. (b) The video images of water droplets after placed on the fibrous membrane at 0 s, 2 s, 4 s, 15 s, 30 s.

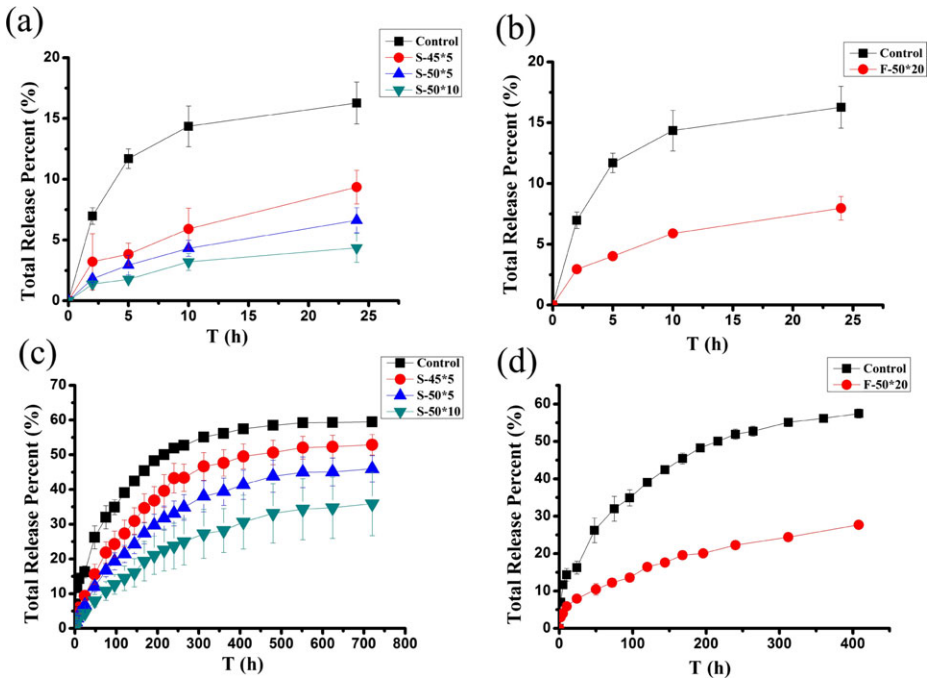

FIGURE 7. In vitro release behavior of curcumin from Control, S-45*5, S-50*5, S-50*10, and F-50*20 fibers.

rate of curcumin from Cur@PELA fibers can be achieved by increasing heating temperature or prolonging heating time.

The burst release of drug from the electrospun fibers at the initial stage was a key problem for researchers to solve.[1] Drug which loaded on the surface of fibers dissolved into buffer quickly, so it is easy to understand the burst release of drug from Control. However, in this study, drug release rate could be slow down by heat treatment with different heating temperature and time. It was observed that that the porosity and water absorption of drug-loaded nanofiber membrane was changed using different heating temperature and treatment time. The results of porosity, water absorption and drug release profiles of control, S-45*5, S-50*5, and S-50*10, suggested that the lower porosity and water absorption of fiber membranes, the slower release rate of drug from them. The drug release rate was influenced by different factors, there are two possible ways to control drug release from electrospun PELA fibers: (i) transport trough water-filled pores, (ii) transport through the polymer.[25] Some literatures also revealed that water absorption[25,26] and porosity[25,27] influence in rate-controlling processes of drug release, as drug will diffuse through water-filled pores formed by water absorption and low porosity decreases the surface area for drug dissolution, which could slow down the drug release rate.

In addition, as shown in Figs. 7(b) and 7(d), the release rate of drug from F-50*20 was slower than Control, S-45*5 and S-50*5. Although the porosity of F-50*20 was not higher than S-45*5 and S-50*5. The water contact angle of F-50*20 was larger than S-45*5 and S-50*5, and the water absorption F-50*20 was lower than S-45*5 and S-50*5. The release rate of curcumin from F-50*20 fibers was slower because of the hydrophobic nature. That might be the reason for the slow drug release from F-50*20.

Conclusions

In this study, we have discussed the influence of heat treatment in the release profile of curcumin from Cur@PELA fibers. Due to the fact that the fibers morphology, porosity, water absorption, and water contact angle of drug-loaded fibers were changed by heat treatment. The slower release of curcumin from fibers could be obtained by increasing the heating temperature (from 45°C to 50°C) and heating time (from 5 min to 10 min). Heat treatment could make Cur@-PELA fiber membrane be shrinkage, which cause the low porosity and low water absorption. That slowed down the release rate of curcumin. Moreover, Cur@PELA was fixed on a flat plate for heat treatment to prevent membrane shrink, the slower release profile may be obtained by choosing heating temperature (50°C) and heating time (20 min). In this condition, due to the larger water contact angle of fiber membrane, the slower release rate of curcumin was noted. Heat treatment is an easier and more effective way to control drug release from electrospun PELA fibers.

Acknowledgments

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References

1. Zeng, J.; Xu, X.; Chen, X.; Liang, Q.; Bian, X.; Yang, L.; Jing, X. J Control Release 2003, 92, 227–231.
2. Kenawy, E.-R.; Abdel-Hay, F. I.; El-Newehy, M. H.; Wnek, G. E. Mater Chem Phys 2009, 113, 296–302.
3. Liu, S.; Hu, C.; Li, F.; Li, X.-J.; Cui, W.; Fan, C. Tissue Eng Part A 2012, 19, 529–537.
4. Hu, C.; Liu, S.; Zhang, Y.; Li, B.; Yang, H.; Fan, C.; Cui, W. Acta Biomater 2013, 9, 7381–7388.
5. Wang, H.; Li, M.; Hu, J.; Wang, C.; Xu, S.; Han, C. C. Biomacromolecules 2013, 14, 954–961.
6. Abrigo, M.; McArthur, S. L.; Kingshott, P. Macromol Biosci 2014, 14, 772–792.
7. Huang, X.; Brazel, C. S. J Control Release 2001, 73, 121–136.
8. Wu, L.; Brazel, C. S. Int J Pharm 2008, 349, 144–151.
9. Qiu, K.; He, C.; Feng, W.; Wang, W.; Zhou, X.; Yin, Z.; Chen, L.; Wang, H.; Mo, X. J Mater Chem B 2013, 1, 4601–4611.
10. Zheng, F.; Wang, S.; Wen, S.; Shen, M.; Zhu, M.; Shi, X. Biomaterials 2013, 34, 1402–1412.
11. Wang, S.; Zheng, F.; Huang, Y.; Fang, Y.; Shen, M.; Zhu, M.; Shi, X. ACS Appl Mater Interfaces 2012, 4, 6393–6401.
12. Alexis, F. Polym Int 2005, 54, 36–46.
13. Yang, D. J.; Zhang, L. F.; Xu, L.; Xiong, C. D.; Ding, J.; Wang, Y. Z. J Biomed Mate Res A 2007, 82, 680–688.
14. Yang, Y.; Xia, T.; Chen, F.; Wei, W.; Liu, C.; He, S.; Li, X. Mol Pharm 2011, 9, 48–58.
15. Yang, D.-J.; Chen, F.; Xiong, Z.-C.; Xiong, C.-D.; Wang, Y.-Z. Acta Biomater 2009, 5, 2467–2474.
16. Yang, Y.; Xia, T.; Zhi, W.; Wei, L.; Weng, J.; Zhang, C.; Li, X. Biomaterials 2011, 32, 4243–4254.
17. Anand, P.; Kunnumakkara, A. B.; Newman, R. A.; Aggarwal, B. B. Mol Pharm 2007, 4, 807–818.
18. Anand, P.; Thomas, S. G.; Kunnumakkara, A. B.; Sundaram, C.; Harikumar, K. B.; Sung, B.; Tharakan, S. T.; Misra, K.; Priyadarsini, I. K.; Rajasekharan, K. N. Biochem Pharmacol 2008, 76, 1590–1611.
19. Wang, H.; Ding, S.; Zhu, H.; Wang, F.; Guo, Y.; Zhang, H.; Chen, J. Sep Purif Technol 2014, 126, 82–94.
20. Ma, Z.; Kotaki, M.; Yong, T.; He, W.; Ramakrishna, S. Biomaterials 2005, 26, 2527–2536.
21. Wharram, S. E.; Zhang, X.; Kaplan, D. L.; McCarthy, S. P. Macromol Biosci 2010, 10, 246–257.
22. Zong, X.; Ran, S.; Kim, K.-S.; Fang, D.; Hsiao, B. S.; Chu, B. Biomacromolecules 2003, 4, 416–423.
23. Yallapu, M. M.; Gupta, B. K.; Jaggi, M.; Chauhan, S. C. J Colloid Interface Sci 2010, 351, 19–29.
24. Paramera, E. I.; Konteles, S. J.; Karathanos, V. T. Food Chem 2011, 125, 913–922.
25. Fredenberg, S.; Wahlgren, M.; Reslow, M.; Axelsson, A. Int J Pharm 2011, 415, 34–52.
26. Chung, T.-W.; Tsai, Y.-L.; Hsieh, J.-H.; Tsai, W.-J. J Microencapsul 2006, 23, 15–27.
27. Fredenberg, S.; Wahlgren, M.; Reslow, M.; Axelsson, A. J Control Release 2011, 150, 142–149.