Preparation and Delayed Release of Poly(lactide-*co*-glycolide)/Ketoconazole Composite Fiber Mats

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In this study, slow release materials–poly(lactide-*co*-glycolide) (PLGA) ultrafine fiber mats containing different ketoconazole (KCZ) contents were prepared and their release behaviors were investigated in vitro. PLGA/KCZ ultrafine fiber mats were prepared via electrospinning and characterized by means of scanning electron microscope, Fourier transform infrared, X-ray diffraction (XRD), and thermal gravimetric analysis. The slow release properties of PLGA/KCZ fiber mats in vitro were studied by measuring the concentrations of KCZ dissolved in the phosphate buffered solution (pH = 4.5) at a programmed time. Results indicated that KCZ could be dispersed in PLGA very well in a wide range of KCZ content from 10 to 100% with respect to PLGA. Most KCZ in PLGA fibers were physically dispersed. The thermal decomposition temperature of PLGA was lowered due to the incorporation of KCZ. With increased drug concentration, the release amount would increase in unit time. The two-stage releases would be sustained to achieve the effective utilization of KCZ. POLYM. COMPOS., 34:757–762, 2013. © 2013 Society of Plastics Engineers

INTRODUCTION

As an antimicrobial, ketoconazole (KCZ) is structurally similar to imidazole. The outstanding characteristics of KCZ have broad antimicrobial spectrum and strong antibacterial activity. Currently, it is widely used to treat superficial and internal fungus with significant effects such as tinea corporis, onychomycosis, colpitis, psoriasis, seborrheic dermatitis, hemorrhoid, and so on [1–4]. But the clinical application of KCZ is limited because oral KCZ can easily cause side effects, and most serious and lethal adverse reactions are related to the dose of oral KCZ. There is a lot of research showing that KCZ is closely related to liver metabolism [5, 6]. KCZ as the slow release formulation can not only reduce the side effects and dosage, but also possess a better curative effect. Poly(lactic-*co*-glycolic acid) (PLGA) is one of the biodegradable polymers with wide applications due to excellent biocompatibility, biodegradability, nontoxicity, and good flexibility [7–12]. PLGA fibers can be manufactured by some traditional processing techniques, for example, melting, spinning, and solution spinning. But the ester bond of PLGA is easily hydrolyzed in the process of melting spinning. Furthermore, the molecular mass of PLGA decreases substantially and the quality of fibers is significantly affected. There are many drawbacks such as low spinning speed, difficult solvent recovery, and toxic spinning solvent in the process of solution spinning [13]. Consequently, the best way to fabricate PLGA fibers is electrospinning. Electrospun fibers have a small diameter, high specific area, great draw ratio, and good homogeneity [14–16]. In order to avoid the side effects caused by oral KCZ, a research on the PLGA ultrafine fibers mats containing KCZ via electrospinning was carried out in our work and the slow release formulation of KCZ loaded on PLGA fibers mats was investigated.

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EXPERIMENTAL

Materials

PLGA (LA:GA = 75:25 mol/mol) with molecular weight 1.0×10^5 g/mol was purchased from Jinan Daigang Bio Co., Ltd, China. KCZ (99.7%) was provided by Hubei Hengshuo Chemical Co., Ltd, China. Chloroform and acetone were obtained from Tianjin Fuyu Fine Chemistry Co., Ltd, China.

Preparation of PLGA Spinning Solution

The PLGA spinning solution was prepared at room temperature by dissolving PLGA in an organic solvent mixture of chloroform/acetone (2:1,vol/vol). The concentrations of PLGA were 5 wt%, 7.5 wt%, 10 wt%, 12.5 wt%, 15 wt%, and 17.5 wt% of the chloroform, acetone, and PLGA. In order to dissolve fully, the solution was shaken on the rapid mixing device and placed still for 2 h.

Preparation of PLGA/KCZ Spinning Solution

The concentration of PLGA was 15 wt% of the chloroform, acetone, and PLGA. KCZ accounts for 10%, 25%, 50%, 75%, 100%, and 125% of PLGA in the amount (wt/wt). The solution was shaken on the rapid mixing device to mix uniformly.

Fabrication of PLGA/KCZ Ultrafine Fibers

Using a 10-mL syringe with a 0.9-mm needle, 5 mL solutions of different concentrations were taken. The applied voltage was 16 kV using a high voltage power supply. The ground collection plate of aluminum foil was located at a distance 15 cm from the needle tip. A constant flow rate of 6 mL/h was obtained using a syringe pump. The collected PLGA/KCZ ultrafine fibers mats were dried at 30°C for 2 h.

Preparation of the Phosphate Buffered Solution

About 15.6 g of $NaH_2PO_4 \cdot 2H_2O$ was dissolved in 800 mL distilled water. The solution was then added into a 1,000-mL volumetric flask and additional 200 mL distilled water was added to make it exactly 1,000 mL, which provides the 0.1 mol/L phosphate buffered solution (PBS) solution with pH 4.5.

Release of Ketoconazole

A precisely weighed sample of the PLGA/KCZ ultrafine fibers mats was put in a beaker flask with 100 mL of fresh PBS solution, the sealed flask was placed into a thermostatic oscillator and shaken at 37°C. The accumulated release ratio of KCZ from the PLGA/KCZ ultrafine fibers mats samples was calculated by measuring the concentrations of KCZ dissolved in the PBS solution at a programmed time. To measure the concentrations, 1 mL of mixture was collected from the flask at certain time intervals, and at the same time 1 mL of fresh PBS solution was added in the flask. The mixture was then analyzed by high performance liquid chromatography (HPLC).

Characterization

The morphologies of PLGA/KCZ ultrafine fibers were characterized using scanning electron microscope (SEM) (S4800 type). Samples of SEM were sputter-coated with a thin layer of Au prior to the observations to prevent sample-charging problems. Composition of the fibers were analyzed qualitatively by Fourier transform infrared (FTIR) (Bruker EQUINOX 55 type) for determination of interactions between KCZ and PLGA. The thermogravimetric analysis (TGA) measurements were operated in nitrogen from room temperature to 600°C at a heating rate of 10 °C/min. The crystallographic state of KCZ and the phase composition of the ultrafine fibers were determined by X-ray diffraction (XRD) (D8 advance).

RESULT AND DISCUSSION

PLGA Concentration Selection

PLGA solution concentration was chosen in order to obtain a suitable quantity of PLGA to load KCZ for electrospinning. Figure 1 shows the morphology of fibers obtained from different concentration of PLGA solution, indicating that the morphology of the electrospinning ultrafine fiber depended strongly on the concentration of PLGA solution. When the solution concentration was low (<10 wt%), the fibers could not be obtained. With a higher solution concentration (10 wt%), the fibers could be obtained with some beads. When the concentration was increased further to 12.5 wt% and 15 wt%, the uniform fibers could be electrospun as shown in Fig. 1d and e. When the concentration was increased further to 17.5 wt%, the fibers became heterogeneous due to the high solution viscosity. Therefore, PLGA solution with the concentration of 15 wt% was selected as the optimal one for electrospinning PLGA/KCZ composites.

Fabrication of PLGA/KCZ Fiber Mats

PLGA/KCZ solutions with different KCZ content (relative to PLGA) were electrospun. In this work, the concentration of PLGA solution was fixed at 15 wt%. SEM was used to characterize the surface morphology of PLGA/KCZ ultrafinefibers. Figure 2 shows the SEM images of composite ultrafinefiber samples with different KCZ content. From these SEM images, we observed that a series of KCZ-loaded PLGA ultrafinefibers were prepared, the

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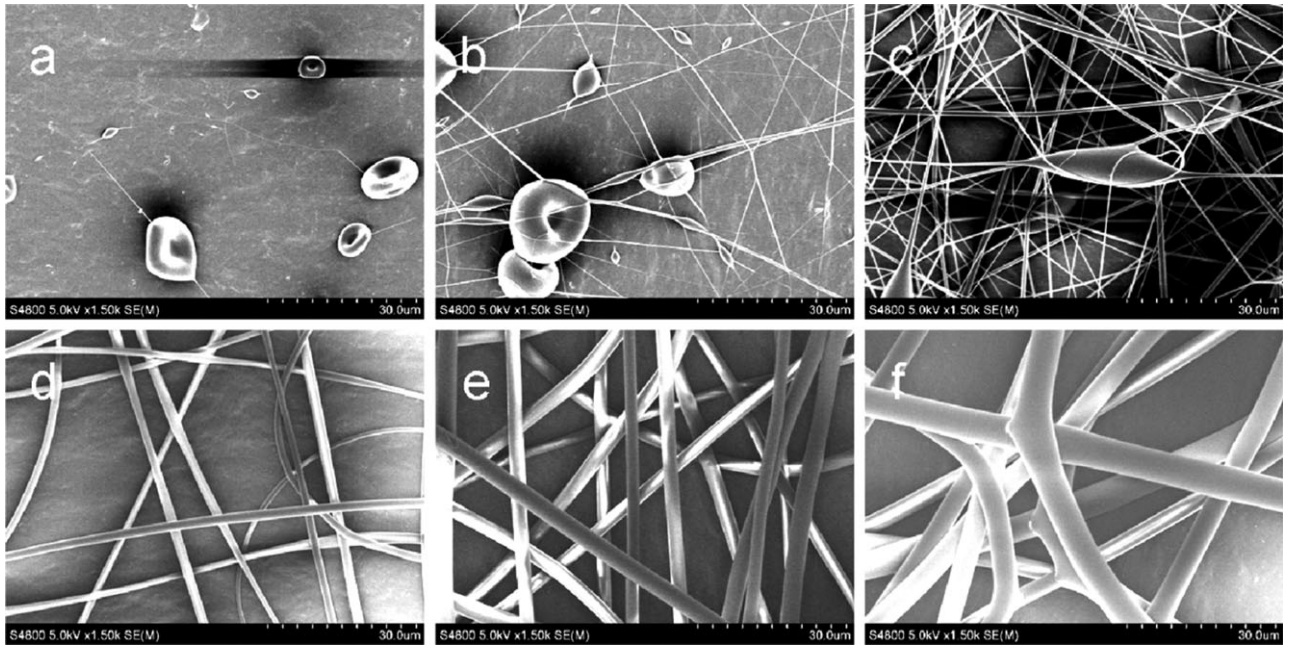

FIG. 1. SEM images of PLGA fibers electospun with different concentrations in chloroform/acetone (2:1, vol/vol) mixed solvent, (a) 5%, (b) 7.5%, (c)10%, (d) 12.5%, (e)15%, (f)17.5%.

composite fibers were smooth and uniform with the variation of KCZ content accounted from 10 to 100% of PLGA in the amount (Fig. 2a–e). The composite fibers became uneven when the content of KCZ was added up to 125% (Fig. 2f), this might be due to KCZ content being too high in PLGA, the properties of PLGA/KCZ solutions were changed with increasing KCZ content as shown in Table 1. The result showed that there was no considerable change although the conductivities of these solutions decreased gradually. The viscosities of these solutions were increased with increasing KCZ content, preventing the jet segment from being stretched by the Coulombic force. This resulted in increasing the diameter of the fibers. The fiber conglutination phenomenon was observed due to the lower solvent volatilization, which occurred because of higher solution viscosity. The test results showed that KCZ could be dispersed in PLGA very well in the wide variation range of KCZ content

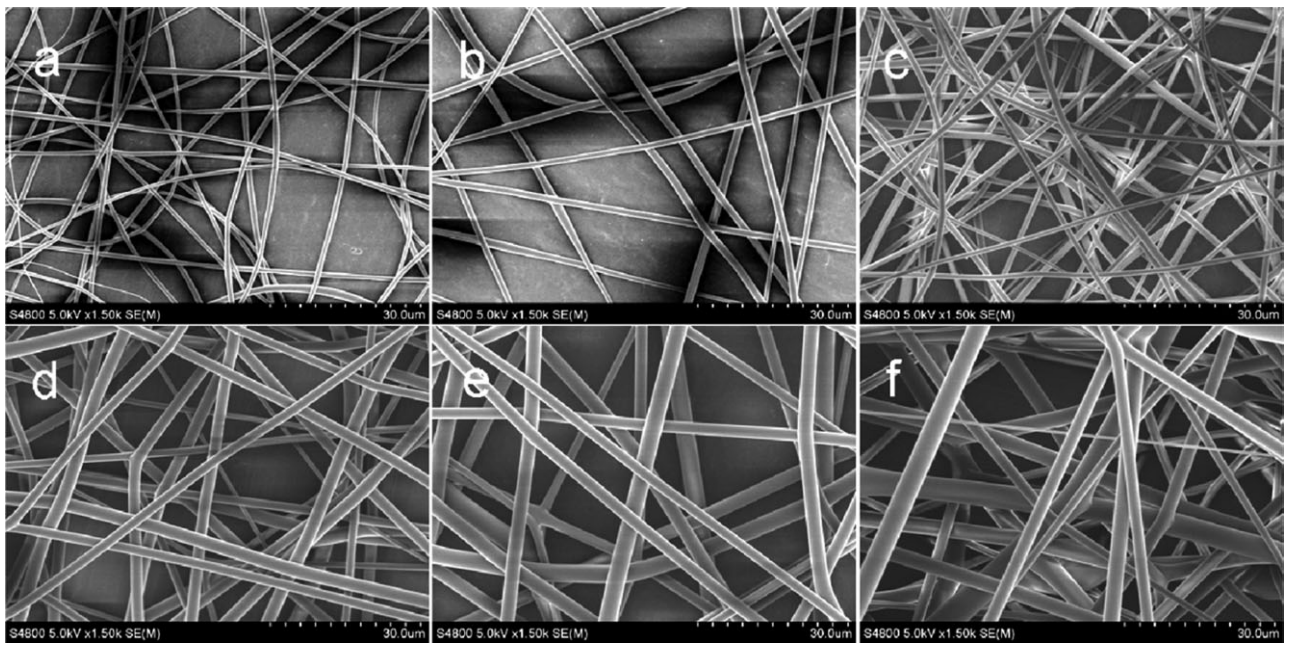

FIG. 2. SEM images of PLGA fibers with different concentrations of KCZ, (a) 10%, (b) 25%, (c) 50%, (d) 75%, (e) 100%, (f) 125%.

TABLE 1. Properties of PLGA/KCZ solutions.

Number	%KCZ[a] (wt/wt)	Conductivity (μs/cm)	Viscosity (cP)
1	10	0.16	573
2	25	0.16	1,130
3	50	0.16	1,437
4	75	0.14	1,853
5	100	0.12	2,340
6	125	0.10	3,133

[a] With respect to the PLGA.

from 10 to 100% with respect to the PLGA. PLGA fibers possessed a strong capability of loading KCZ.

FTIR Analysis

FTIR was used to probe interactions between KCZ and PLGA. The infrared spectra of pure PLGA fibers (a), the PLGA/KCZ composite fibers sample (b), and purchased KCZ (c) were shown in Fig. 3. Characteristic peaks of PLGA at 1,749 cm^{-1} for ester carbonyl (C=O) stretching vibration, 1,190 cm^{-1} for C—O stretching vibration, and 1,086 cm^{-1} for C—O—C group, were found in the spectrum of the PLGA/KCZ composite fibers. The characteristic absorption peaks of KCZ at 1,512 cm^{-1} for C=C stretching vibration of the aromatic groups, 1,458 cm^{-1} for C—H, 1,585 cm^{-1} for N—H stretching vibration, 1,647 cm^{-1} for C=C alkene groups, and 1244 $cm^{-1}$for C=O stretching vibration were also found in the spectrum of the PLGA/KCZ fibers.

The maintenance of the major characteristic peaks of PLGA and KCZ in the IR spectrum of the PLGA/KC composite fibers, and the absence of the new peaks, demonstrated that the drug was only dispersed in the PLGA polymeric matrix. They did not have chemical reaction between the drug and the polymer. Therefore, the KCZ properties would not change after being loaded.

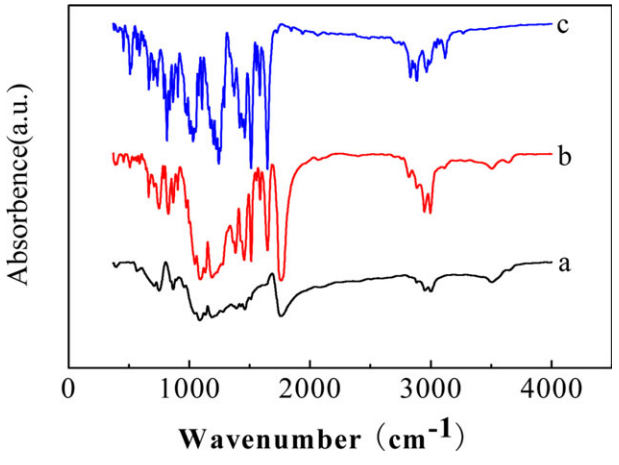

FIG. 3. FTIR spectra of (a) pure PLGA fiber, (b) PLGA/KCZ composite fibers, and (c) pure KCZ. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

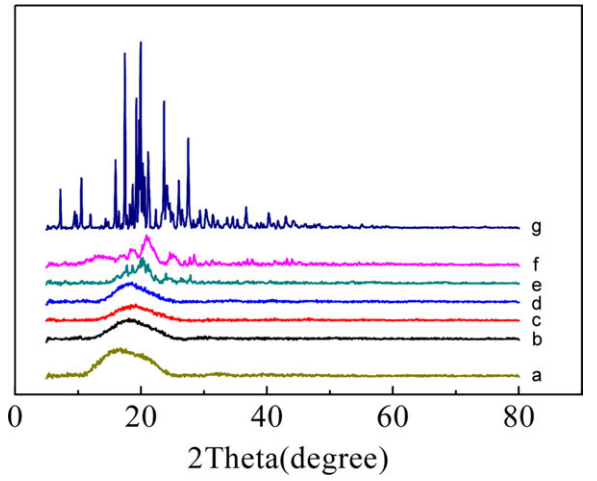

FIG. 4. XRD patterns of PLGA/KCZ composite ultrafine fibers with different content of KCZ (a) 0%, (b) 20%, (c) 40%, (d) 60%, (e) 100%, (f) 200%, and (g) pure KCZ. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

X-Ray Diffraction Analysis

The crystalline state of the purchased PLGA and KCZ and the phase composition of the fibers were further investigated by XRD. For pure PLGA fiber (Fig. 4a), there was a broad diffraction peak at 2θ value of 16.8° which was the characteristic peak of amorphous polymers. Figure 4g shows some narrow and strong diffraction peak, which indicates that the purchased KCZ was crystalline compound.

With the incorporation of KCZ, the diffraction intensity of PLGA/KCZ composite ultrafinefibers was higher than that of pure PLGA fiber sample. Furthermore, the more the content of KCZ, the higher the diffraction intensity of composite ultrafinefibers. This result suggested that KCZ was indeed incorporated into PLGA/KCZ ultrafinefibers, and there might be some interaction between KCZ and PLGA molecules in the composite fibers. If there were no interaction between them, each component would show their own diffraction patterns. It could be predominantly attributed to the following reasons. The interaction between KCZ and PLGA molecules inhibited the crystalline behavior of KCZ molecules, which could hinder the generation of crystal lattice, resulting in the decrease of KCZ crystallization. In the composite fibers, no crystalline peaks like free state KCZ were found. Very few and small crystalline peaks were present, which illustrated that KCZ was dispersed in PLGA fibers in the molecular level. Only a few KCZ was free on surface of fibers and that of internal was formed into crystallization.

Thermogravimetric Analysis of the Ultrafine Fibers

Figure 5 shows the TGA curves of pure PLGA and PLGA/KCZ fibers with 20%, 40%, 60%, and 100% KCZ content with respect to PLGA. Compared to that of pure PLGA fibers, the curves of PLGA/KCZ fibers were

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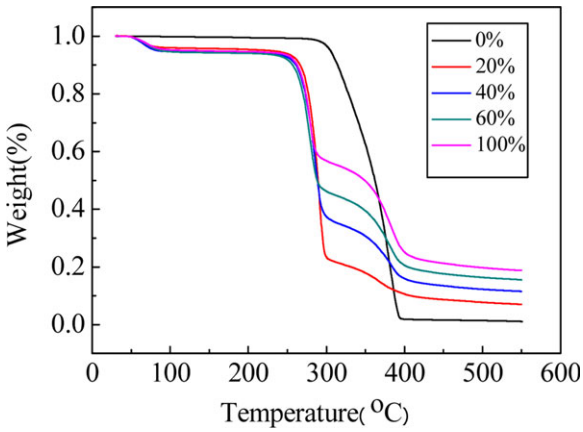

FIG. 5. TG curves of PLGA/KCZ ultrafine fibers with 0%, 20%, 40%, 60%, and 100% of KCZ content. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

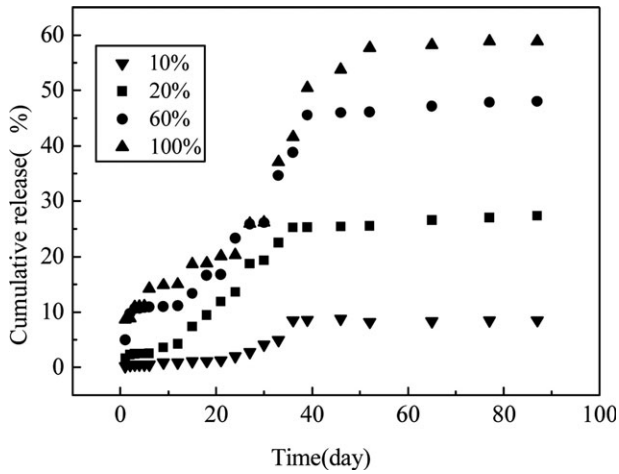

FIG. 6. Release behaviors of PLGA/KCZ composite fibers with different KCZ content.

different, which demonstrated that incorporation of KCZ changed the degradation mechanism of PLGA. From 250°C to 300°C, the weight of PLGA/KCZ fibers was decreased steadily while the process occurred from 332°C to 400°C in PLGA curves. With KCZ increasing, the rate of weight loss was slower. This could be explained as follows: the packing of PLGA molecules on composite fiber mats surface became loose due to the existence of KCZ molecules, which lowered the carbonization temperature of PLGA. The interior PLGA of PLGA/KCZ fibers were covered by KCZ, so hindered the carbonization process of PLGA and declined the rate of degradation in the range of 270–332°C. The situation was different from the pure PLGA in the range of 270–332°C. The rate of weight loss was slower than the pure PLGA ultrafine fibers. When the temperature was about 400°C, most of remainder was KCZ consistent with the calculated value. The PLGA/KCZ ultrafine fibers mats showed two steps on the loss of weight at temperature 250°C and 320°C, corresponding to the thermal decomposition of main chain of PLGA and KCZ, respectively.

Release Behaviors of PLGA/KCZ Composite Fibers

KCZ release behaviors from PLGA/KCZ composite fibers were investigated in PBS at 37°C and pH 4.5. KCZ release from the PLGA/KCZ samples showed two-stage release behaviors, possibly due to the KCZ loading at the different locations on the composite fibers shown in Fig. 6. Because of the dissolution of the KCZ absorbed at the external surface of composite fibers, it would provide a certain amount of KCZ quickly to the environment. KCZ released at a fast velocity in the initial few hours. During the following several hundred hours, the release rate became much slower than that in the first stage, and it was a possible consequence of KCZ diffusion from the internal of fiber to the solution with the degradation of PLGA. The release in the last stage proved to be very slow and would last for a very long time until most of the loaded KCZ was released. The two stages would supply a sustained release to achieve the effective utilization of KCZ. KCZ in composite fibers was gradually released to the environment by diffusion with the degradation of PLGA.

XRD, TGA, and FTIR analysis showed that the interaction between KCZ and PLGA was not chemical but physical in composite fiber we prepared. The molecular structure of PLGA was not changed on addition of KCZ.

CONCLUSIONS

In summary, a homogenous PLGA/KCZ dispersion precursor was prepared and fabricated into continuous composite fibers via electrospinning. The SEM and the XRD analysis showed that the PLGA/KCZ composite fibers were smooth and the KCZ were well dispersed without any agglomeration. FTIR analysis indicated that most KCZ dispersion in PLGA fibers was physical process. Therefore, the KCZ properties would not change after loading. TGA analysis revealed that the thermal property of the fibers was changed due to the incorporation of KCZ. The release content of KCZ would increase in unit time with increasing drug concentration. The two-stage release would be sustained to achieve the effective utilization of KCZ. This ultrafinefibers have potential application against vaginal candidiasis and systemic fungi infection. This composite material will broaden applications of the lactic acid copolymer, improving efficiency in the use of antibacterial agents.

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