

## Research Article

# Intelligent Drug Delivery System Based on Silk Fibroin/Wool Keratin

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It is critical to develop drug carriers for intelligent drug delivery systems. Normally, the drug carriers of the intelligent drug delivery system are stimulus-responsive polymers. However, as drug carriers, the biocompatibility of the materials should be the primary consideration. In this work, two smart drug systems were prepared using natural protein materials (silk and wool) as drug carriers and poly(N-isopropylacrylamide) (PNIPAM) as a functional additive material. (1) Silk fibroin (SF)/wool keratin (WK) composite films: the differences in drug release efficiency of composite films are based on the different crystallinity of composite films. Analysis of the XRD results showed that the content of the  $\beta$ -crystallite structure in different proportions of the composite films changed from 38.3% to 19.5%. The percentage of drugs released of the mixed films with different proportions ranged from 28% to 58% after eight hours of release, showing a gradient release. (2) SF/WK and PNIPAM composite films: the particle size of the PNIPAM microspheres changed with the temperature. The particles' size of the prepared PNIPAM microspheres was reduced from 700 nm to 300 nm from 25°C to 35°C. After eight hours of release, the composite films with temperature-sensitive microspheres increased the percentage of drug released by up to 20% compared with the composite films without microspheres when the temperature was ranged from 25°C to 37°C. The result of drug release showed the amount of drug released could be changed with temperature stimulation, which could satisfy people's controllable requirements for the time, location, and dose of drug release.

## 1. Introduction

Oral administration is the most widely used route of administration by which the drug is absorbed rapidly. Adverse drug reactions and poor patient compliance with the drug may appear with the fluctuations in the level of the drug in the body. The development of a controllable drug delivery system can improve the pharmacokinetic properties of drug molecules in the human body, thereby significantly improving the utilization of drugs and reducing the high-concentration toxic side effects that may occur when the drug is uncontrolled. One of the main requirements of a controllable drug release system is the choice of the drug carrier.

Stimuli-sensitive drug delivery systems or “smart drug delivery systems” have been the focus of research. Chemical

stimuli, physical stimuli, and biological stimuli have been used for the design of smart drug delivery systems. Chemical stimuli include pH and redox, physical stimuli include temperature and light, and biological stimuli include enzymes. Temperature is the most widely used stimulus to trigger structural changes in drug carriers.

Silk fibroin is a natural macromolecular protein with excellent biocompatibility that has a wide range of applications in many fields such as textiles and medical treatment. There is a model that silk protein materials have a hierarchical structure from micro to macroscale [1], which believes that silk protein fibers have five levels of structure. The macroscopic properties of silk protein and its hierarchical structure exhibit a close connection. Silk fibroin can be prepared into nanoparticle, microparticle [2], hydrogel [3], film [2], fibrous material, and sponge-like material. The

application of SF can be extended to many fields because it changes in forms. Wool keratin is relatively easy to obtain among all keratin materials. A widely accepted wool keratin model is a microfibrillar repeating unit model based on wool microfibers. Similar to silk fibroin, keratin-based materials can also be prepared into protein hydrogel [4], keratin fiber [5], keratin film [6], and keratin sponge-like material [7]. Because of its unique molecular composition and biological features, it is used in bone regeneration, hemostasis, and biomedical fields such as nerve regeneration, angiogenesis, wound healing, and drug dispensing, which has attracted a great deal of attention. Poly(*N*-isopropylacrylamide) is the most common type of temperature-sensitive polymer due to good temperature sensitivity, easy to chemically alter, and low critical solubility temperature, which is close to the biological temperature. The low critical dissolution temperature of this temperature-sensitive polymer is 32°C. The solution is a homogeneous system when the temperature is below 32°C because the molecular chains expand. The inner hydrophilic group and the outer water molecules have a hydrogen binding. The solution is phase separated when the temperature reaches 32°C. The molecular chains shrink, and the hydrogen bonds between the amide bonds and the water molecules are broken. The molecular chains agglomerate into a spherical shape under the action of the hydrophobic group.

## 2. Experimental Part

Lithium bromide (LiBr) was purchased from Aladdin Reagent (Shanghai) Co., Ltd. Cellulose tubing was purchased from Beijing Solarbio Science & Technology Co., Ltd. Diclofenac sodium ( $C_{14}H_{10}Cl_2NNaO_2$ ), urea ( $CH_4N_2O$ ), sodium dodecyl sulfate (SDS), sodium sulfide ( $Na_2S$ ), and sodium bicarbonate ( $NaHCO_3$ ) were obtained from Shanghai Maclean Biochemical Technology Co., Ltd. The wool was purchased from Tongxiang Dushi Wool Products Co., Ltd. Bombyx mori cocoon was obtained from Guangxi Sericulture Research Institute. *N*-Isopropylacrylamide ( $C_6H_{11}NO$ ), hexyl hydride ( $C_6H_{14}$ ), methylene-bis-acrylamide ( $C_7H_{10}N_2O_2$ ), and potassium persulfate ( $K_2S_2O_8$ ) were purchased from Aladdin Reagent (Shanghai) Co., Ltd. Gelatin was purchased from Xilong Chemical Co., Ltd. Papain was purchased from Sinopharm Chemical Reagent Co., Ltd. Methacrylic anhydride was purchased from Shanghai Maclean Biochemical Technology Co., Ltd. Phenyl(2,4,6-trimethylbenzoyl) phosphate lithium salt (LAP) was purchased from Shanghai Yinchang New Material Co., Ltd.

Preparation of SF solution: Bombyx mori cocoon (20 g) was boiled in 2.5 L of 0.5% (w/v)  $NaHO_3$  for 30 min and rinsed with distilled water as part of the degumming process. SF solution was obtained by dissolving 1 g of digummed silk into 6 mL of 9.3 mol/L LiBr at 60°C for 4 h and then dialyzed against deionized water for 3 days using cellulose tubing (molecular weight cut off of 3500D). The SF solution was diluted to 50 mg mL<sup>-1</sup> for the following process.

Preparation of WK solution: wool fibers (5 g) were dissolved in 100 ml aqueous solution containing 42 g urea,

1.9 g  $Na_2S$ , and 0.8 g of SDS and heated at 60°C for 8 hours and then filtered and dialyzed against deionized water, using cellulose tubing (molecular weight cutoff of 3500D) for 3 d. The solution was finally concentrated to 50 mg mL<sup>-1</sup> for the following process.

Preparation of SF/WK composite films: the SF/WK composite films were prepared by the solution casting method. Keratin solution (5 wt%) was mixed with SF solution (5 wt%) at volume ratios of 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, respectively. Then, 1 mL of the mixture solution was added in a 24-well cell culture plate and dried at a temperature of 25°C and a relative humidity of 75% for 48 h to form a film.

Preparation of PNIPAM microspheres: 50 mL deionized water containing 1.136 g *N*-isopropyl acrylamide, 0.0616 g *N,N'*-methylene bisacrylamide (MBA), and 0.027 g KPS was heated at a temperature of 70°C under the condition of argon for 4 h. Then, the solution was centrifuged and lyophilized to obtain powders.

Preparation of SF microneedles: the SF microneedles were prepared by the microtemplate method. The polydimethylsiloxane (PDMS) was poured into the silicon microneedle template (200 μm) and heated at 60°C for 4 h. Then, the PDMS template was peeled from the silicon microneedle, and 3 mL of SF solution (6 wt %) and 64 μL of glutaraldehyde (1 wt %) were mixed and poured into the PDMS-negative template. The SF microneedles were dried at a temperature of 25°C and a relative humidity of 50% and were posttreated with water vapor.

*In vivo* simulation of drug release: 200 μL of mixed solutions containing different proportions of drug-loaded (0.6 mg mL<sup>-1</sup>) and temperature-sensitive molecular microspheres (50 mg mL<sup>-1</sup>) was dripped on the surface of the microneedles and dried at a temperature of 25°C and a relative humidity of 75%. The skin-like gel is made of gelatin.

The gelatin was prepared by dissolving gelatin and methacrylic anhydride in deionized water in a mass-to-volume ratio of 1:10 and then dialyzing in deionized water for 3 days (molecular weight cutoff of 3500D). Then, the solution was lyophilized. The lyophilized powder of the solution was dissolved in deionized water to obtain a solution with a mass fraction of 20%. 2 mg of phenyl (2,4,6-trimethylbenzoyl) phosphate lithium salt (LAP) was added in 1 ml solution, and then the solution was irradiated with an ultraviolet lamp for 3 minutes. The microneedle was pierced into the gel and then placed in a constant temperature and humidity box to release for 1 h. Then, the gel was dissolved by papain and the characteristic absorption peak intensity of the solution was measured with a microplate reader.

*In vitro* drug release: the drug was added to the solution before the mixed solution was dried to form a film, and the concentration of the drug was 0.6 mg mL<sup>-1</sup>. 1 ml of deionized water was added into the hole of the 24-well plate, which contained composite films with drugs, and then the 24-well plate was placed at a temperature of 25°C and 37°C and a humidity of 75% to release for different time periods. To calculate the amount of the drug released, the microplate reader was used to measure the absorption peak intensity of the sample at 290 nm. The UV-vis spectrometer (lambda750,

PETKL in Elmer) was used to measure the characteristic absorption peak intensity of different concentrations of diclofenac sodium solution at a wavelength of 290 nm. The microplate reader (M 200, Swiss Tecan Group) was used to measure the characteristic absorption peak intensity of the drug solution.

The drug release experiment process of the composite film containing PNIPAM was similar to the above process, but the PNIPAM microspheres were added before the mixed solution was dried to form a film at a concentration of 50 mg ml<sup>-1</sup>.

Structural analysis of the composite membrane: the XRD (Bruker D8 ADVANCE) pattern of the composite film was obtained in the range of 2°–50° with a beam size of 0.5 mm and a scan rate of 2° min<sup>-1</sup>.

Images and particle size measurement of temperature-sensitive polymers: the SEM (Hitachi SU70, Tokyo, Japan) was used to observe the apparent morphology of the temperature-sensitive polymer microspheres and the SF film containing the temperature-sensitive polymer microspheres. The potentiometric particle size analyzer (Mastersizer2000, Malvern Instruments Shanghai Spectacle Instrument System, China) was used to measure the particle size of temperature-sensitive polymer microspheres in solution at different temperatures.

### 3. Results and Discussion

**3.1. The Secondary Structure of SF/WK.** The SF/WK composite films with macro-adjustable properties were prepared by adjusting the microstructure of the composite films. Figure 1 shows the preparation process of the SF/WK composite films. The microstructure of the composite films was changed by changing the secondary structure of the composite films. The configuration of SF includes random coil,  $\alpha$ -helix,  $\beta$ -turn, and  $\beta$ -sheet. The  $\alpha$ -helix and  $\beta$ -turn structures are called the SF type I structure, and the  $\beta$ -sheet structure is called the SF type II structure. Posttreatments such as methanol and ethanol treatment and water vapor annealing of the SF film can promote the transition of SF from the Silk I structure to the Silk II structure. WK material contains  $\alpha$ -helix, intramolecular  $\beta$ -sheet, and random coil structure. To quantify the  $\beta$ -crystallite content in the SF/WK composite films, the XRD experiment was performed and the results are shown in Figure 2(a). It can be seen that the pure WK film has a relatively smooth and broad dispersion peak at  $2\theta = 22.6^\circ$ . With the increase of the SF content, the crystallization peak at  $2\theta = 22.6^\circ$  is obvious and becomes sharper and stronger, and a new peak position gradually appears at  $2\theta = 15.2^\circ$ . It can be seen that  $2\theta = 22.6^\circ$  is the SF type II structure ( $\beta$ -crystalline) and  $2\theta = 15.2^\circ$  is the SF type I structure corresponding to the literature. The result shows that as the proportion of SF increases, the content of  $\beta$ -crystalline increases and the content of  $\beta$ -crystalline in different ratios of SF/WK film is different. The  $\beta$ -crystalline content of different samples is shown in Figure 2(b). It can be seen that as the content of WK increases, the  $\beta$ -crystalline content in the composite film shows a gradient decreasing trend, starting from 34% dropped to 18%. The keratin/SF

composite films were termed 1, 2, 3, 4, 5, and 6 on the basis of keratin and SF content, and sample 1 had the highest SF content. A simple simulated-solubility test for the films was conducted in the deionized water. The solubility of the composite films was evaluated by weighing the residual mass of SF/WK after immersing in deionized water (temperature 37°C and relative humidity 75%) for different time periods. The results are shown in Figure 2(c). It can be seen that, in the first 1 hour, the dissolution of all composite films was relatively high, which may be due to the existence of soluble molecules and free small molecular chains in the composite films. With the decrease of the SF content, the dissolved amount of the composite films showed an increasing trend at different time periods.

**3.2. Selection of Model Drugs and Changes in the Particle Size of Temperature-Sensitive Polymers.** Diclofenac sodium is a well-known anti-inflammatory drug with obvious analgesic, anti-inflammatory, and antipyretic effects. It is often used for musculoskeletal and systemic inflammation and has a characteristic absorption peak at a wavelength of 290 nm. The absorption peak intensity of diclofenac sodium solution of different concentrations is different, as shown in Figure 3(a). We prepared PNIPAM microspheres whose particle size changed with temperature, as shown in Figure 3(b). It can be seen that when the temperature increases from 25°C to 35°C, the average particle size of the microspheres decreases from 700 nm to 300 nm, and when the temperature is higher than 35°C, the average particle size of the microspheres remains unchanged. Figure 3(c) shows an SEM image of PNIPAM microspheres. As shown in the image, the shape of PNIPAM microspheres is relatively regular spherical with a relatively uniform size. Figures 3(d)–3(f) are SEM images of PNIPAM microspheres dispersed on SF film. We notice that the microspheres are scattered on the surface of the film, and film appears uneven. Some of the microspheres form agglomerations. Figure 3(g) shows the schematic diagram of the effect of the change in the particle size of the PNIPAM microspheres on the drug release of the composite films. The microspheres are embedded in the composite films, and when the temperature increases, the particle size of the PNIPAM microspheres decreases. The surface area increases and the solubility of the films also increases at the same time, so the release of small-molecule drugs wrapped in the composite films will also increase.

**3.3. Gradient Drug Release and Temperature-Sensitive Release of SF/WK Composite Film.** Figures 4(a) and 4(b) show the quality of drug release at different time periods of different composite films without PNIPAM microspheres at 25°C and 37°C, respectively. Figure 4(c) shows the quality of drug release at different time periods of different composite films with PNIPAM microspheres at 37°C. It can be seen that no matter what the temperature is, the different composite films release the most drugs in the first 1 hour, and as time increases, the drug release rate slows down. We noticed that the released drug would follow a gradient increase trend with the decrease of the SF content in the composite films.

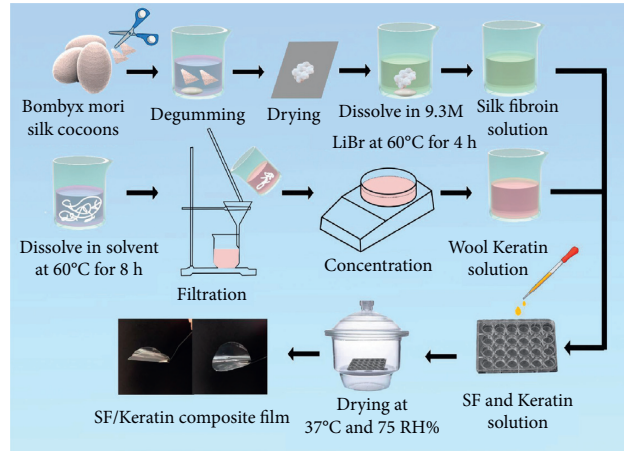


FIGURE 1: Schematic illustration for the fabrication process of SF/WK composite films.

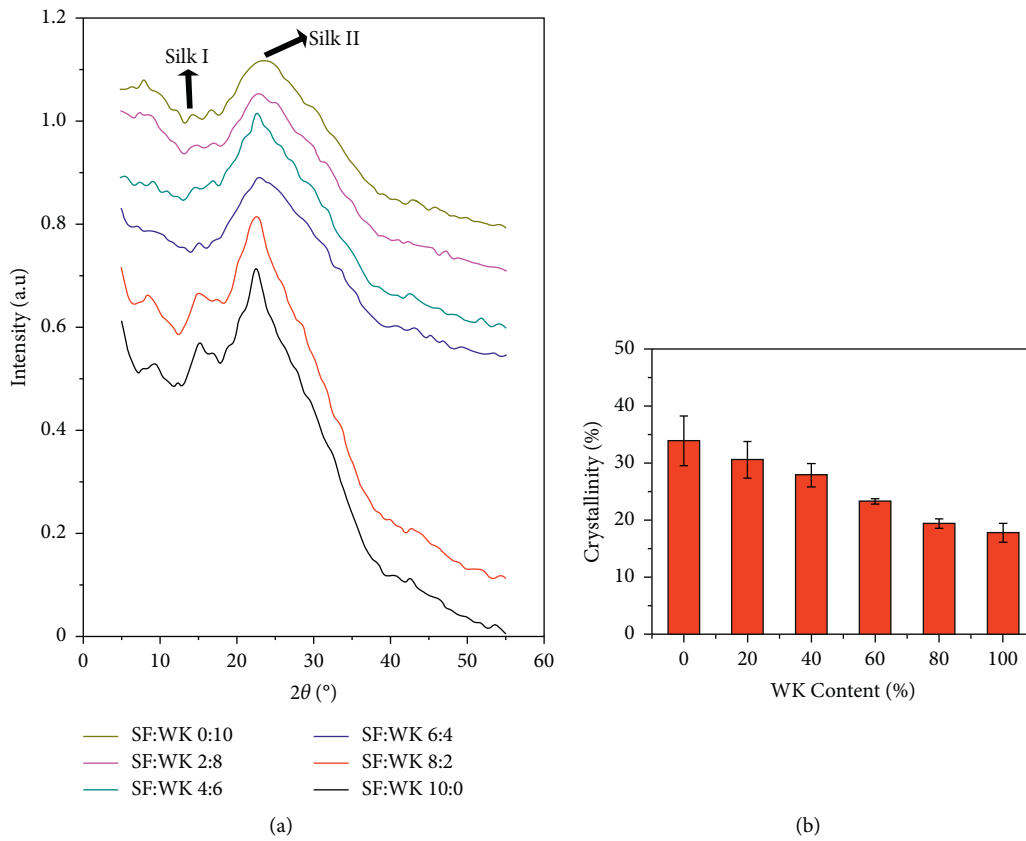


FIGURE 2: Continued.

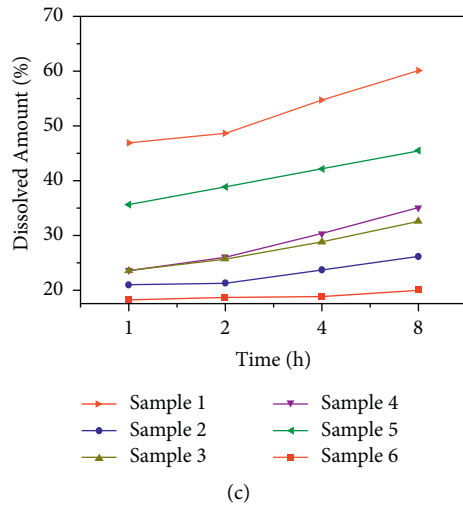


FIGURE 2: (a) XRD spectra of composite films with different ratios, (b) crystallinity of composite films with different WK contents, and (c) soluble percentage of composite films with different ratios at different time periods.

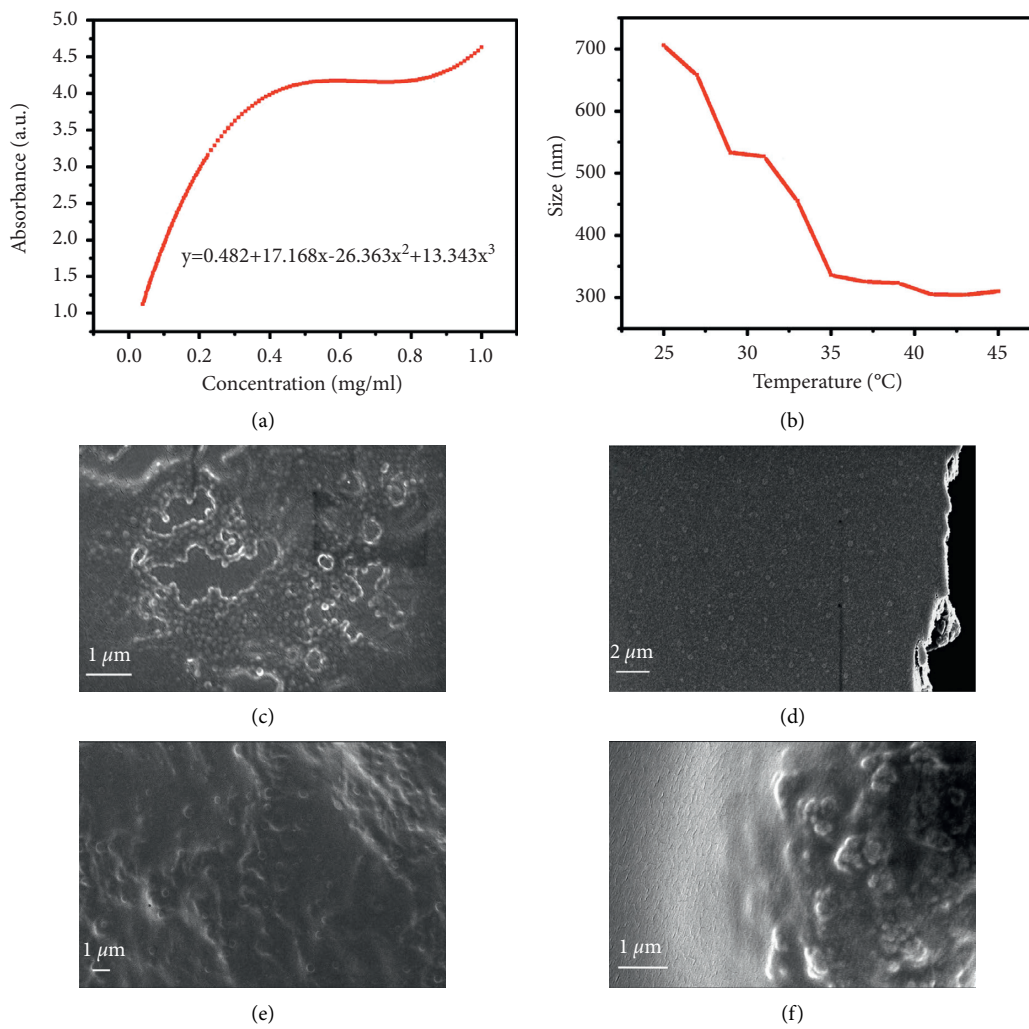


FIGURE 3: Continued.

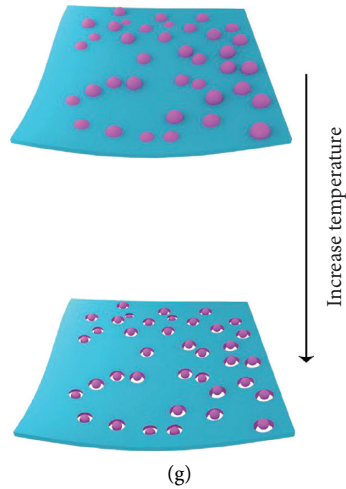


FIGURE 3: (a) Standard curve of diclofenac sodium, (b) change of PNIPAM microsphere particle size with temperature, (c) image of PNIPAM microsphere, (d-f) image of PNIPAM microspheres on the surface of composite films, and (g) mechanism of the effect of PNIPAM microspheres on the efficiency of drug release.

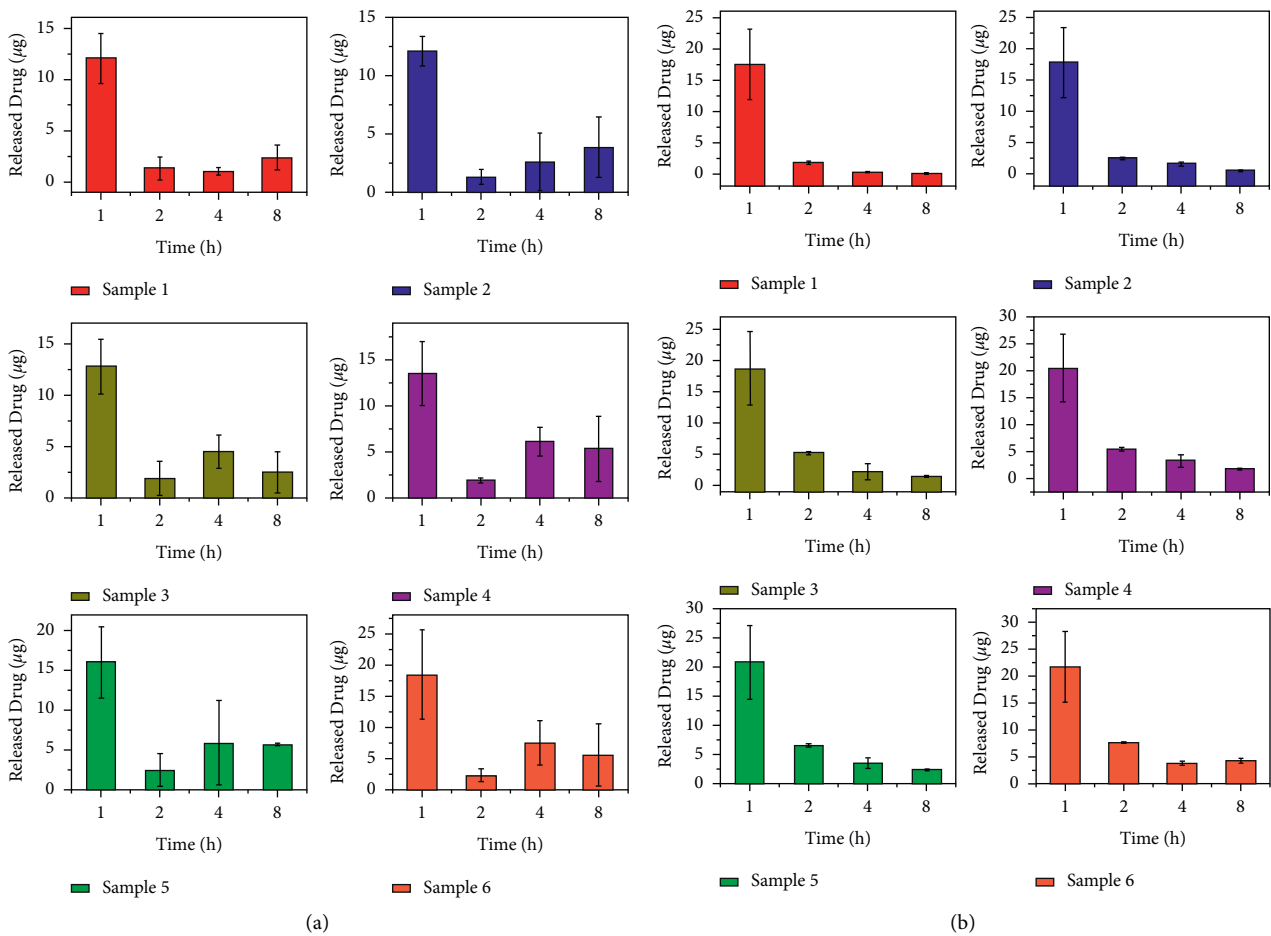
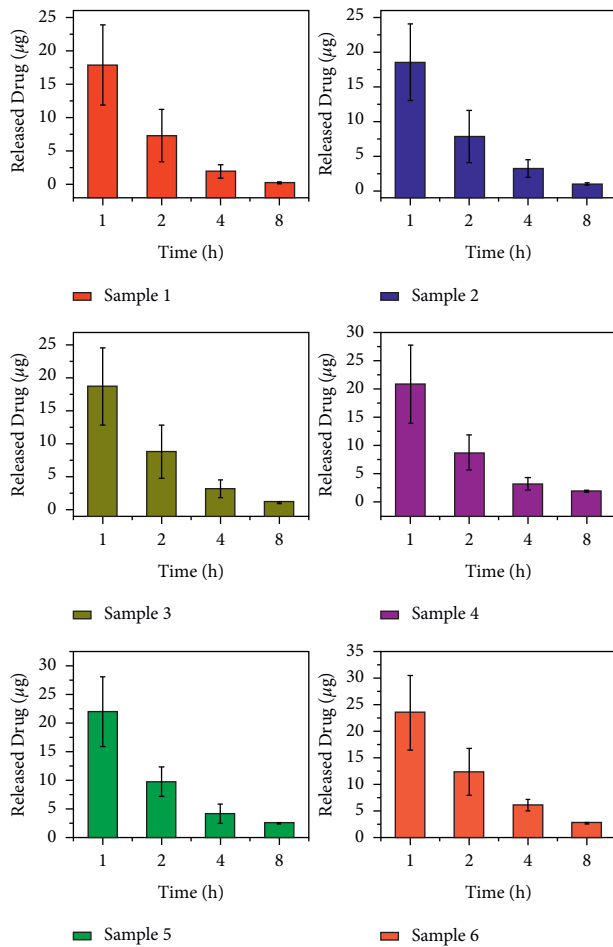
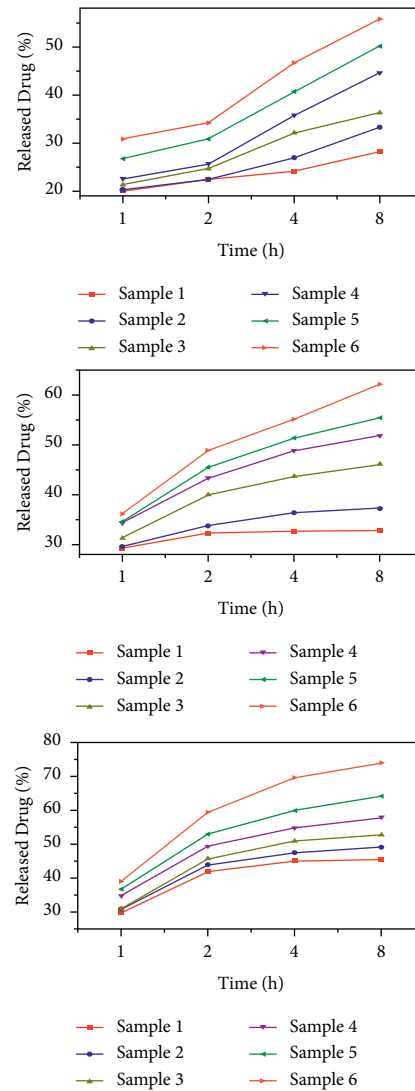


FIGURE 4: Continued.



(c)



(d)

FIGURE 4: (a, b) The quality of the drug released of SF/WK composite films without PNIPAM microspheres at 25°C and 37°C at different time periods, (c) the quality of the drug released of SF/WK composite films with PNIPAM microspheres at 37°C. (d) The comparison of the cumulative percentage of released drug of the SF/WK composite films without PNIPAM microspheres at 25°C and 37°C at different time periods and the comparison of the cumulative percentage of released drug of the SF/WK composite films with PNIPAM microspheres at 37°C.

Figure 4(d) shows the comparison of the drug release percentages of different composite films at different time periods. It can be seen that as the SF content decreases, the slope of the drug release curve increases, which indicates that the rate of drug release is increasing. As mentioned above, the rate of drug release can be determined by the content of SF in the composite films. Figure 5 shows the comparison of the cumulative drug release percentages of different samples at different temperatures in 1 h, 2 h, 4 h, and 8 h. In the first 1 hour, a temperature increase will lead to an increase in the amount of drug released because when the temperature is high, the movement of small molecules and free segments accelerates. However, after the small molecules and free segments are completely dissolved as the drug release time

increases, the large molecular chains are no longer dissolved, and the influence of the temperature on the drug release is reduced when the SF content in the composite films is high. It can be seen that all the composite films without PNIPAM increased the amount of drugs released by about 10%. There is almost no difference in the amount of drug released of samples with the WK content of 0 at different temperatures after 8 hours of release, while the the amount of drug released of samples with a WK content of 100% increases by about 15% as the temperature rises. The composite films with PNIPAM do not show a significant difference in the amount of drug released in the first 1 hour compared with the composite films without PNIPAM at 37°C. The composite films containing PNIPAM gradually

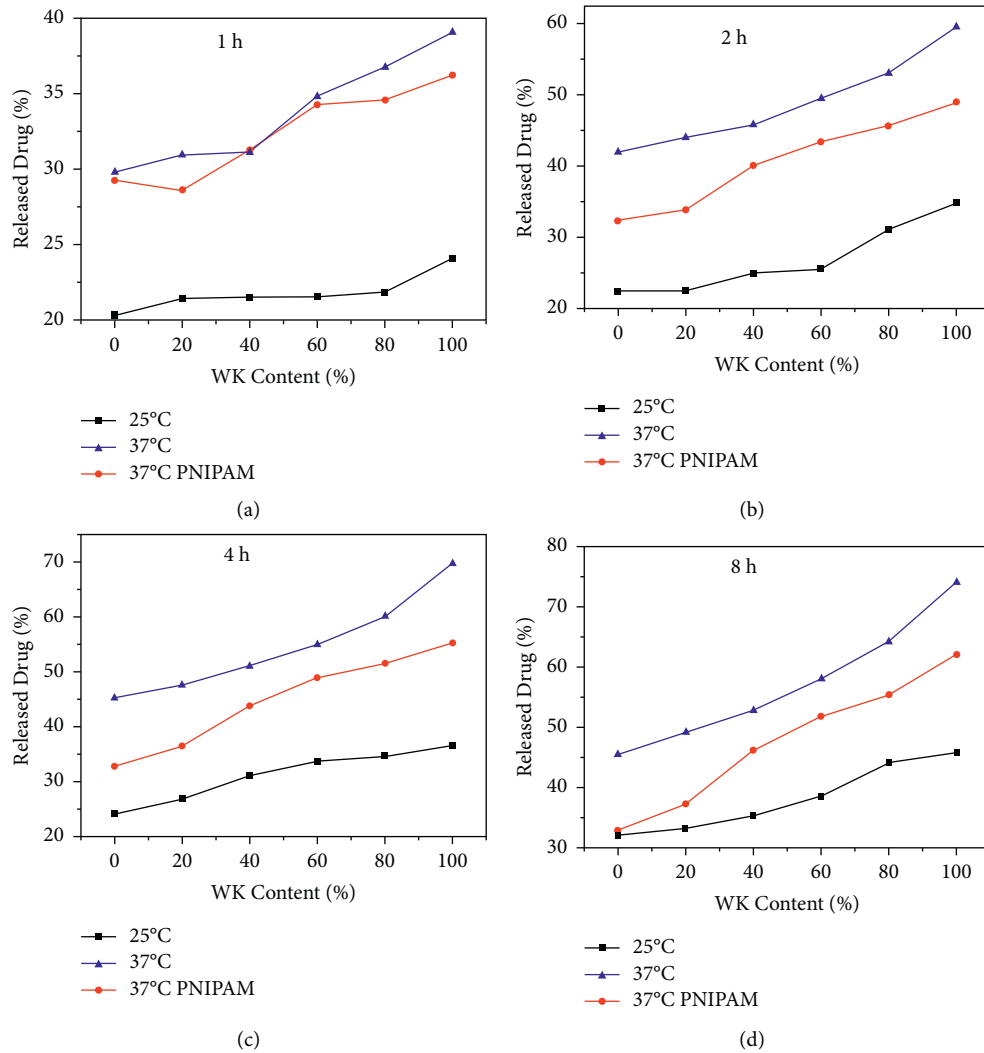


FIGURE 5: (a–d) The comparison of the cumulative percentages of released drug of different samples at different temperatures in 1 h, 2 h, 4 h, and 8 h.

exhibit the advantage of drug release under high temperatures as the release time increases. It can be seen that the composite films containing PNIPAM increase the amount of drug released by more than 10% at 37°C compared with the composite films without PNIPAM after 8 hours of release.

**3.4. Simulated In Vivo Drug Release Experiment.** Both silk material and WK material are biomaterials with good biocompatibility and low cytotoxicity. They can play an important role in the transportation of some macromolecular drugs, such as insulin and antibodies. These drugs generally cannot be administered orally. They are administered transdermally. To verify the effect of the temperature-sensitive composite films on the amount of released drug in the human body under the influence of temperature, gelatin was used to prepare a skin-like gel and the prepared SF microneedles were dropped and loaded with a drop coating method. Figure 6 shows the

preparation process of SF microneedles. The image of the microneedle that we finally get is shown in Figure 7(a). Figure 7(b) shows the image of a skin-like gel. Rhodamine solution was dripped on the SF microneedles, and the microneedle patch was attached to the gel, as shown in Figure 7(c). It can be seen that the dye gradually spreads around after half an hour, as shown in Figure 7(d). Figure 7(e) shows the difference in the amount of drug released by microneedles in the skin-like gel for 1 h at 25°C and 37°C. It can be seen that when the WK content in the composite films is 0, the amount of drug released by the microneedles is about 8%, and when the WK content in the composite films is 100%, the amount of drug released by the microneedles is 23% at 25°C. As the content of WK in the composite films increases, the amount of drug released by the microneedles increases more with an increase in temperature. When the WK content in the composite film is 100%, the drug released by the microneedle increases by 5% as the release temperature increases from 25°C to 37°C.



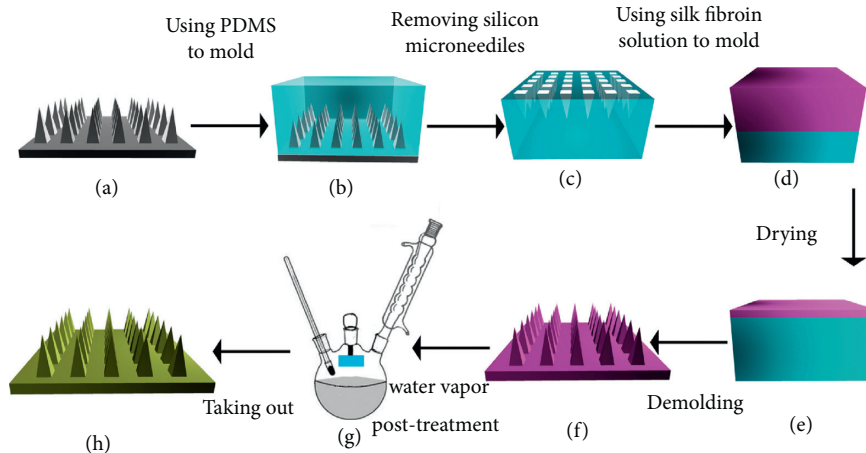


FIGURE 6: Schematic illustration for the fabrication process of SF microneedle.

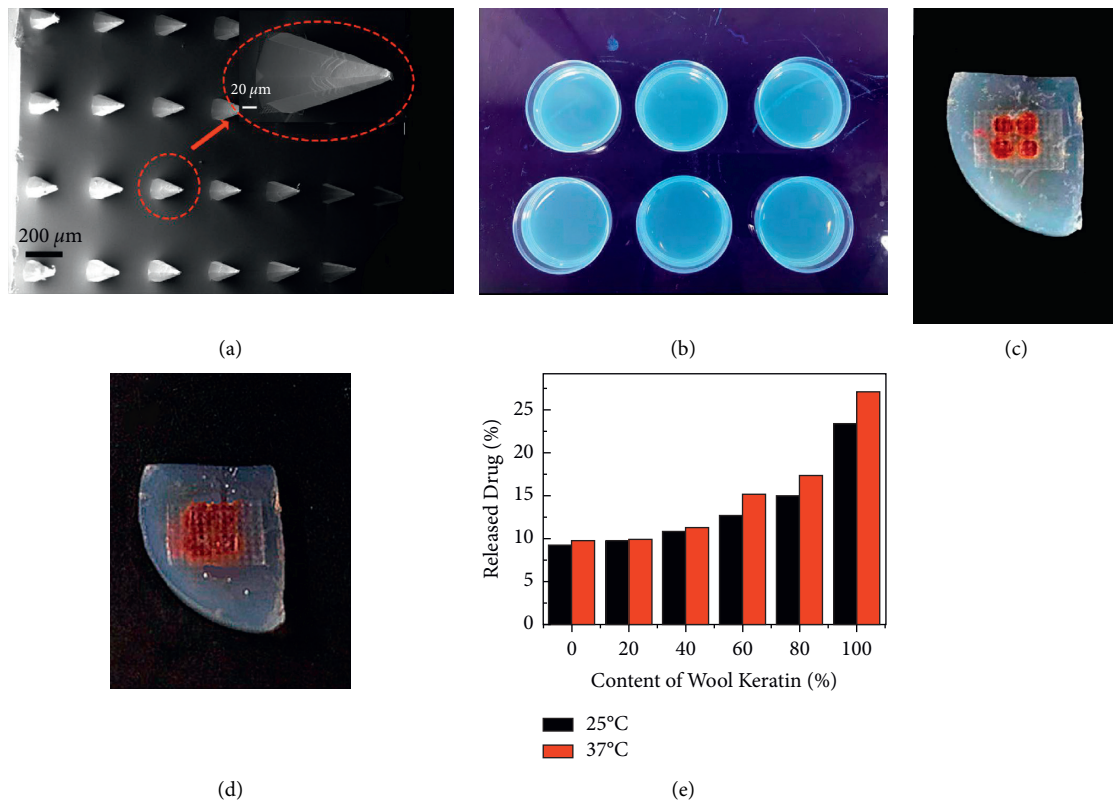


FIGURE 7: (a) The morphology of SF microneedles, (b) the image of skin-like gel, (c) the state of rhodamine-stained SF microneedles just pierced into the skin-like gel, (d) Rhodamine 30 minutes after the dyed SF microneedles were pierced into the skin-like gel, and (e) the comparison of the drug release percentage of the composite film containing PNIPAM microspheres with different WK contents at different temperatures.

#### 4. Conclusion

In this work, we use natural macromolecular protein materials to prepare different ratios of SF/WK composite films. We also combine temperature-sensitive polymers with SF/WK to prepare temperature-sensitive films. The composite films of different proportions of WK/SF show a trend of gradient dissolution from 22% to 60% after 8 h

with the content of  $\beta$ -crystallite from 34% to 18%. The drug release efficiency of different composite films also shows a trend of gradient. The amount of released drug ranges from 28% to 58% after 8 hours at 25°C. The drug release efficiency of the composite films with temperature-sensitive microspheres has a significant response to temperature changes compared to the composite films without temperature-sensitive microspheres. When the

temperature increases from 25°C to 37°C, the amount of released drug increases by at least 13% after 8 hours of release. As the content of WK increases, the amount of the released drug will also increase. Therefore, the temperature can be a variable to adjust the drug release of the composite films. As the content of WK in the composite films is different, the change in temperature will also change the amount of released drug. The drug release efficiency will also increase with the increase of temperature in the biomimetic skin drug experiment. In summary, we have successfully prepared protein composite films with self-regulating drug release efficiency, and the composite films with the temperature-sensitive polymer can also control the drug release efficiency by temperature.

### Data Availability

The experimental data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare that they have no conflicts of interest regarding this work.

### Acknowledgments

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