PREPARATION AND PROPERTIES OF SILK FIBROIN HYDROGEL FOR BIOLOGICAL DRESSING

by

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As a protective layer of a wound, the medical dressing plays an important role in the healing of the wound. The hydrogel dressing is appeared as a new type of medical dressings and has become a research hotspot. Silk fibroin is a natural polymer protein with excellent biocompatibility, mechanical properties, and various plasticity. In this paper, a drug-loaded silk fibroin hydrogel by the polyethylene glycol was coated on cotton fabrics. The obtained biomedical functional textile dressing had antibacterial properties and biocompatibility.

Key words: *dressing, silk fibroin, hydrogel, antibacterial, biocompatibility*

Introduction

Skin is an important organ of the human body, which has the function of a biological barrier [1]. It plays an important role in maintaining the stability of the internal environment and preventing the invasion of external bacteria and other microorganisms [2]. However, human skin is vulnerable to damage due to trauma, burns, and others. The damaged skin would endanger human health and even human life [3]. Currently, we need to use skin substitutes and medical wound dressings to repair the damaged skin [4]. At present, most of the medical dressing materials were synthetic polymers, which were not degradable, or the degradation products were toxic and easy to cause secondary damage to the damaged skin [5]. Therefore, it is of great significance to develop medical dressing materials with degradability and good biocompatibility for clinical applications.

The hydrogel dressings were developed by Dr. Winter in pig experiments in 1962, and a new concept of *wet healing theory* was put forward. Now hydrogels have been widely studied as a new type of dressings [6]. Hydrogel is a kind of polymer network system with a hydrophilic 3-D network cross-linked structure [7]. It is soft, and can maintain a certain shape, and has good biocompatibility and biodegradability [8]. Since it was first reported in the 1950's, it has been widely used in drug delivery, tissue engineering, 3-D cell culture, and other medical fields [9]. At present, polyacrylonitrile, polyvinyl alcohol, and polyacrylic acid are often used in hydrogel materials. The hydrogel dressing has a smooth surface and could bind tightly to the uneven wound. It is suitable for dry chronic wounds [10]. Thomson showed that in addition to infected wounds and severe drainage wounds, hydrogel dressings were suitable

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for all four stages of wound healing [11]. Anjum and others coated cotton fabric with a mixture of chitosan (CS), polyethylene glycol (PEG), and polyvinylpyrrolidone (PVP), forming a gel layer and then freeze-drying to obtain a potential wound dressing [12]. By adding the tetracycline hydrochloride model drug into the gel, the prepared gel dressing has excellent antibacterial properties. Animal experiments showed that the wound dressing of CPPC-TC made 100% healing within 12 days, and no scar formation was observed in the wound healing. Wound inflammation is a complex process, which is caused by infection or tissue damage [13]. Most of the inflammation is chronic, so anti-inflammatory dressing was needed to prevent or anti-inflammatory. Current studies have found that the anti-inflammatory activity of curcumin is comparable to that of steroidal and non-steroidal drugs, such as indomethacin and butanone, and it is safe in most cases [14].

Silk fibroin (SF), as a kind of natural animal macromolecular protein, contains essential amino acids for the human body [15]. It has good biodegradability, non-toxic and harmless degradation products, and can be absorbed by the human body; furthermore, it has good cell compatibility [16]. It was a relatively safe biomaterial in tissue engineering. The SF has good material plasticity, and can form a variety of material forms, SF hydrogel, as one of the materials, has great potential in the application of hydrogel dressing [17, 18]. Lee *et al.* [19] prepared SF nanoparticle hydrocolloid dressing (SFNHD). The function of SF nanoparticles is to improve the efficacy of dressing. The dressing was used in animal models to treat burns. The results showed that it has good structural stability and biocompatibility. The SFNHD dressing can accelerate wound healing and minimize the size of the wound. It can be concluded that the hydrogel containing SF nanoparticles can be used as a wound dressing.

Considering the mentioned recent study, we hypothesized that cotton fabric can be coated with SF hydrogel. Through selecting and evaluating, a curcumin-loaded SF/PEG hydrogel was usually the best choice. Plain weave cotton fabric was braided, and then the mixed solution containing curcumin and SF was uniformly sprayed on its surface. The morphology, secondary structure, *in vitro* drug release properties, and cytotoxicity of the coated membranes were examined.

Experimental

Raw materials

Silk raw fiber and cotton fabric were supplied by Shengzhou Silk Co., Ltd. and Zhejiang Cathaya International Co., Ltd, China. The CUR was purchased from Sigma, USA. Two kinds of polyethylene glycol (PEG, Molecular weights: 400 and 20000) were supplied by Sinopharm chemical reagent Co., Ltd., China. Human dermal fibroblasts (Hs 865.Sk cells) were obtained from ATCC (American Type Culture Collection). Cell culture media were purchased from Wisent Corporation, Canada. All of the chemicals and reagents were analytical grade.

Experimental design

Based on cotton fabric (plain weave, 220 gsm), the best group is SF/PEG gel, which is loaded with curcumin (0.8 mg/mL) and has antibacterial and anti-inflammatory plant extracts. The prepared hydrogel was coated on cotton fabric to prepare antibacterial and anti-inflammatory gel-coated fabric dressings. Then the physicochemical properties, including drug release properties, antibacterial properties, and biocompatibility, were analyzed.

Surface morphology

The surface morphology of the specimens was examined using SEM (Hitachi S-4800, Tokyo, Japan). All specimens were sprayed with gold prior to imaging and observed at a voltage of 3 kV. The fibers in membranes were imaged using an inverted microscope (Olympus phase-contrast microscope TH4 200, Osaka, Japan), and the diameters of the fibers were determined by using Image J software (National Institute of Mental Health, USA).

Air permeability

Set the test parameters according to GB/T5453-1997 standard: set the test pressure difference as 100 Pa and the test sample area as 20 cm^2. During the test, the test area can be adjusted according to the different samples. After the sample is clamped on the sample cone and the suction fan is started, the air passes through the sample, and the flow rate is adjusted so that the pressure gradually approaches the specified value. After waiting for 1 minute, the permeability value is recorded. Under the same test conditions, it is necessary to repeat the test at least 10 times in different parts of each test sample and finally take the average value.

Drug release

Prepared the methanol solution containing curcumin (concentration 1 mg/mL), prepare the release solution: 3 mL methanol, 5 mL tween 80 with 5% mass fraction and 92 mL pH = 7.4 PBS buffer. The mother solution was diluted with the release solution to form 7 equal concentration gradients (the common ratio is 1/2). The absorbance (OD) of 7 diluents was detected at 425 nm with the microplate reader. The standard curve equation of curcumin is $y = 77.854x + 0.0657$, and the fitting degree is $R^2 = 0.9997$. Gelatin coated fabrics with a final concentration of curcumin of 0.8 mg/mL and three silk fibroin concentrations of 0.75%, 1.5%, and 3% were accurately cut 2 cm × 4 cm coated fabric, 6 parallel samples were set for each sample, and then put into 5 mL centrifuge tube. It is transferred to a constant speed shaking table in an oven at 37 ℃. In the set time (1, 3, 6, 12, 24, 48, 72, 96, 120, 156, and 204 hours), take out the centrifuge tube, absorb 1 mL release liquid for testing, then add 1 mL fresh release liquid into the centrifuge tube again, seal it well and put it back on the shaker in the 37 ℃ ovens to continue oscillation. The absorbance value of the released solution was measured at 425 nm, and the release rate of curcumin was calculated through the established curcumin standard curve.

Antimicrobial properties

According to ISO 20645:2004, PEG gel is used as a carrier to prepare SF/PEG/CUR hydrogel biological dressing, the final hydrogel contents to cotton fabric quality are 20 wt.%, 40 wt.%, 60 wt.%, 80 wt.%, 100 wt.%, 120 wt.%, and 150 wt.%, respectively. The experimental method is to prepare the gel material made of circular 1.1 cm diameter, each sample is placed in 1 mL bacteria suspension (bacterial suspension concentration of about 1.0×10^8 CFU/mL) of agar, then put it into a 37 ℃ incubator for 24 hours, and take it out to observe the effect of inhibition zone. The formula of bacteriostatic zone is as follows: $H = (D - d)/2$, H = width of inhibition zone, D = average outer diameter of inhibition zone, d = diameter of sample.

Determination of cell morphology and viability

The mouse fibroblasts were removed from liquid nitrogen and thawed in a 37 ℃ water bath. After a period, the thawed cell suspension was inoculated into culture bottles (4×10^6 cells per bottle). The culture flask contained 10% fetal bovine serum culture medium and was then placed in a CO_2 incubator at 37 ℃ for culture. In the process of culture, the culture medium was changed at intervals, and the growth of cells was observed by a light microscope before each change. During the passage of culture, when 80% of the fibroblasts began to aggregate, the cells could be inoculated, cleaned up with trypsin, and added fresh culture medium. Then the cells were inoculated in 24-well plates (before inoculation), and two gel materials were equally distributed into 24-well plates and treated by ultraviolet light. Each gel material was set up in 6 parallel samples. The cells were also inoculated in the orifice plates without gel material, as a blank control, as TCP. The cell density of each well was $1.25 \cdot 10^5$. The fresh culture medium was changed regularly after inoculation. Remove the culture medium from the cultured cells and add 100 ml μL fresh culture medium containing 10% AlamarBlue reagent was added, transferred, and incubated in the incubator containing 5% CO_2 at 37 ℃ for a period, and then 70% AlamarBlue reagent was removed with a pipette gun. The parameters of the microplate reader were set as follows: 560 nm excitation wavelength, 590 nm emission wavelength, and the fluorescence value of the culture plate (TCP) without cell inoculation was used as the background. The activity of the cells to be tested can be calculated as the percentage change in the number of cells. Cell activity (%) = (FCMPD – FO)/(FCTRL – FO) × 100%. The FCMPD was the average fluorescence intensity when cells were added, FO was the average fluorescence intensity of the blank control, FCTRL was the average fluorescence intensity without cells. The cell survival on the membranes was examined after 3 and 7 days in culture using fluorescent microscopy. Briefly, the membrane samples were washed with PBS and incubated in 1×10^{-6} M calcein-AM for 30 minutes. The live cells that were stained in green fluorescence were observed by the fluorescence microscope (Axio Vert.A1, Carl Zeiss, Germany). All samples were run in triplicate.

Results and discussion

Screening and optimization of silk fibroin hydrogel coatings

The SF solution and three kinds of hydrogel could be successfully adhered to cotton fabric, fig. 1(a), and evenly distributed. The coating amount did not increase significantly with the concentration of SF solution increased from 1.5 wt.% to 3 wt.%. While the concentration was 6 wt.%, the coating amount increased significantly, the pores between the fibers were completely covered. Significantly, cotton fabric coated with SF/PEG hydrogel was the most uniform without obvious hardening under low concentration. The SF hydrogel biological dressing of concentration at 1.5 wt.%, 3.0 wt.%, and 6.0 wt.% and research group of SF/PEG/CUR or SF/PEG were tested, tab. 1. Figure 1(b) showed the permeability of different gel-coated fabrics. From the histogram, we could see that the permeability of the fabric after the coating is less than that of the raw cotton fabric, indicating that the existence of the coating leads to the decrease of the permeability of the fabric. The permeability of the same gel material-coated fabric decreased with the increase of silk fibroin concentration. Figure 1(c) showed a broken line diagram of the cumulative release percentage of SF/PEG/CUR hydrogel coated cotton fabric. It could be clearly seen from Curcumin gel-coated fabric that curcumin is released rapidly within 12 hours, and remains unchanged after 24 hours, and the release percentage is also larger than that of hydrogel material, indicating that the release rate of Cur-

cumin gel-coated fabric increases [20]. The reason was that after the functional gel was coated with fabric, the surface of the fabric was covered with a gel layer, which increases the specific surface area of curcumin and the releasing liquid in the gel so that curcumin could be released better and faster [21]. The rapid release of curcumin could highlight the anti-inflammatory effect and give full play to the curative effect [22].

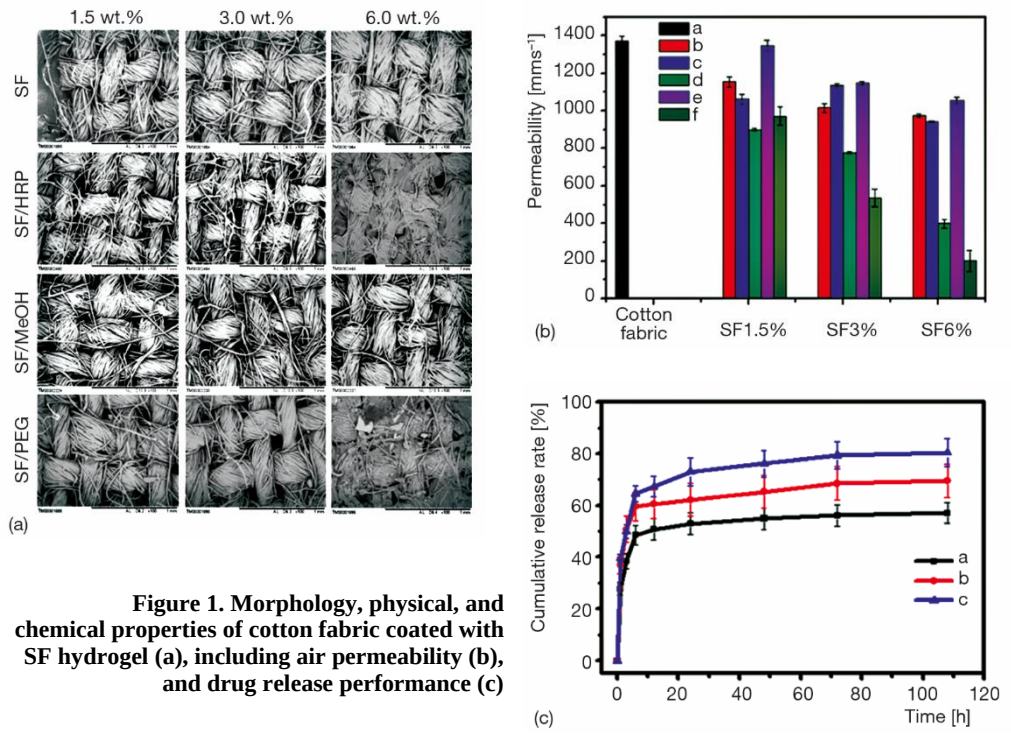

Figure 1. Morphology, physical, and chemical properties of cotton fabric coated with SF hydrogel (a), including air permeability (b), and drug release performance (c)

Figure 2(a) showed an oscillating bacteriostatic rate experiment. It could be seen from the graph that with the increase of SF/PEG/CUR hydrogel content, the inhibition rate of *S. aureus* and *E. coli* would also increase, and the effect of inhibiting *S. aureus* was stronger than that of Escherichia coli in the same content. This was consistent with the result of the gel inhibition zone, and the content of SF/PEG/CUR hydrogel was 80 wt.%. The inhibition rate of *S. aureus* and *E. coli* was 100 % and 95%, respectively. Figure 2(b) was a statistical chart of the bacteriostatic circle size of the prepared SF/PEG/CUR hydrogel. The size of the bacteriostatic ring is measured by IMAGEJ software [23]. From the chart, we could clearly see that the inhibition zone width of *S. aureus* was obviously larger than that of the *E. coli* bacteriostatic circle, and with the content of SF/PEG/CUR hydrogel from 20 wt.% to 80 wt.%, the size of inhibition zone also increased, when the content increased from 80 wt.% to 150 wt.%, the results showed that there was no significant change in the size of inhibition zone, and the same trend was observed in both bacteria. Therefore, the optimal inhibition content of SF/PEG/CUR hydrogel was 80 wt.%, the inhibition zone width to *S. aureus* and *E. col* was 1.45 ± 0.14 mm and 2. 10 ± 0.21 mm, respectively.

The morphology of mouse fibroblasts grows well, and the number of mouse fibroblasts increased from 3 days to 7 days, fig. 3(a). Three kinds of hydrogel materials with

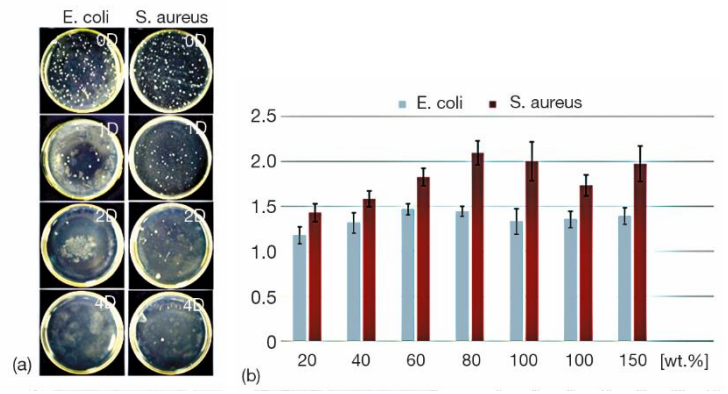

Figure 2. Antibacterial effect of SF/PEG/CUR hydrogel coated cotton fabric (a) and statistical chart of bacteriostatic zone size (b) for *S. aureus* and *E. coli* in a period of four days, while content increased from 20 wt.% to 150 wt.%

different SF concentrations showed increased cell numbers and showed good cell viability, indicating that they were suitable for fibroblast growth and good biocompatibility. The number and morphology of the cells are consistent with the results of the cell viability diagram in fig. 3(b). It showed the fluorescence detection results of cells cultured on the gel of different silk fibroin concentrations under Al-B indicator [24]. From the chart, we could see that the fluorescence detection value of three gel materials changed little in the first three days, and the reduction rate of Al-B increased rapidly from third days to seventh days later, 43.85% and 46.25%, respectively, lower than a group (47.03%). The difference was not obvious. From this result, we could see that mouse fibroblasts have a high metabolism and cell activity in SF/PEG/CUR hydrogel coated cotton fabric.

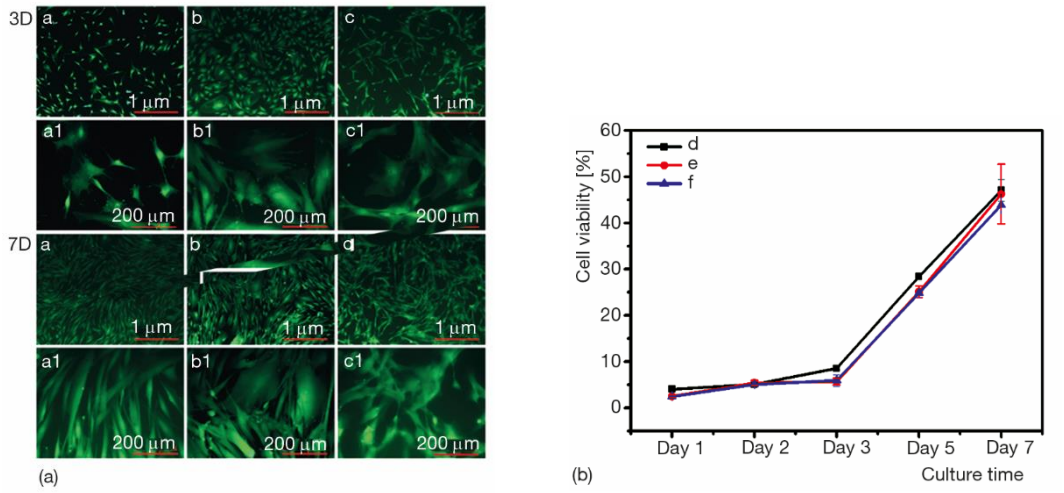

Figure 3. Fluorescence microscope images (a) and cell viability (b) of live fibroblasts cultured on SF/PEG/CUR hydrogel coated cotton fabric with different SF concentration for 7 days

Conclusion

Three kinds of silk fibroin materials, SF/PEG gel, SF/HRP gel, and SF/methanol gel, were prepared and coated with silk fibroin solution as a control. The physicochemical properties of silk fibroin gels were screened by scanning electron microscopy and infrared spectroscopy. It is considered that SF/PEG gel is suitable for coating on the surface of base cotton fabric so as to prepare biological wound dressing. The inhibition zone width to *S. aureus* and *E. col* was 1.45 ±0.14 mm and 2. 10 ±0.21 mm, respectively, and the antibacterial rate was above 95%. The results of the mouse fibroblast compatibility test showed that the cell compatibility was good. It showed that the gel-coated fabric could be a potential anti-inflammatory and antibacterial wound dressing.

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