SILK FIBROIN/POLYETHYLENE GLYCOL NANOFIBROUS MEMBRANES LOADED WITH CURCUMIN

by

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In order to improve the stability, utilization ratio and anti-tumor effect of curcumin drug, a set of curcumin-loaded nanofiber membranes with drug releasing property were fabricated using silk fibroin and polyethylene glycol. Various curcumin-loaded silk fibroin nanofiber membranes with different components and drug loading percentages were prepared using electrospinning technology. The morphology structure, mechanical properties, secondary structure, drug release property in vitro, and their interaction effects of the curcumin-loaded silk fibroin nanofiber membranes were examined. The result of in-vitro drug release experiment showed that the curcumin can be released stably up to 350 hours, the drug releasing speed increased with the decrease of the diameter of the fibers. The stability and utilization ratio of curcumin was improved after loading with curcumin-loaded silk fibroin nanofiber membranes. In conclusion, it can be used as a control drug release system alternately in the future.

Key words: curcumin, polyethylene glycol, silk fibroin, nanofibrous membranes

Introduction

Curcumin is an active ingredient extracted from turmeric, which has a wide range of pharmacological performance such as antiviral, antibacterial effect, *etc.* [1]. When combined with the phenol hydroxyl capture free radicals, it can protect human from radiation drug-induced liver injury and oxidative damage [2]. However, it has so many disadvantages such as low water-solubility, instability in neutral to alkaline conditions, easily decomposition in sunshine, and vulnerable metabolism, low bioavailability after entering the body. Hence, its clinical application is limited greatly. Therefore, it is important to develop a novel drug delivery system with combination of drug carrier and curcumin.

Silk fibroin (SF) is a good natural polymer material and widely used in various areas, relying on its nice processing ability, mechanical properties, biocompatibility and biodegradability [3, 4].

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The SF can be processed to SF fibers [5], gel, membrane, micro/nanoparticles, as well as widely used in the field of tissue engineering [6, 7], enzyme immobilization, and drug controlled release system [3, 4]. The SF contains 18 kinds of amino acids, among them leucine can accelerate the metabolism of cells, serine and threonine can prolong the skin aging. Furthermore, SF is harmless to the human body and suitable for development of functional biological materials due to its safe, reliable and good biocompatibility.

Biodegradable polymers as drug carriers in drug release systems can be divided into micro/nanofibers, granule, capsule, latex, micelles, and gel system, *etc.* [8, 9]. Among them, polymer nanofibers with large specific surface area, small size effect, and unique mechanical properties, can improve the carrying capacity of drugs, and meanwhile control the drug releasing properties when using as a medicine material. In addition, a SF fiber-based medicament possesses good physical strength and processing performance. Electrospinning technology is one of the simple, low cost, and fast method for the preparation of drug delivery system (DDS). Nanofibers produced by electrospinning technology can be directly processed into membranes [10, 11], biomedical tubular scaffolds, layered coating on the surface of materials [12-14]. After implantation of these materials, it can achieve the drug delivery function locally. In addition, by adjusting the process parameters can easily adjust the diameter of the fiber and the percentage of drug, *etc.*, can better meet the requirements of DDS.

In order to improve the water solubility, stability of the neutral to alkaline conditions, in sunshine, and *vivo* bioavailability of curcumin drug, so as to enhance antitumor effect and clinical applications of the drug, a drug-loaded nanofiber membrane was developed using SF and polyethylene glycol (PEG) loaded with curcumin in the present work. The PEG is a kind of amphiphilic polymers with high non-immunogenicity and biocompatibility, widely used in medicinal additives and other applications, which can be used to resist non-specific biological interactions. When PEG arrives at the body parts inside which targeted, the temporary transfer of genes can play the role of cracking release of original drugs reversibly under the function of biological enzyme or chemical factors, and therefore it can protect the active functional groups of drugs, avoid failure before arriving target location, so as to effectively solve the water-soluble issues of curcumin.

In the present work, electrospinning technology is adopted to make a set of curcumin-loaded nanofiber membranes with different components, adding ratios and drug loading percentages of SF, PEG, and drug, and explore the morphology structure, mechanical properties, secondary structure, drug release property *in vitro*, as well as analyze their interaction effects of the nanofiber membrane with SF.

Materials and methodologies

Materials

Raw silk fiber was purchased from Shengzhou Concord Silk Co., Ltd., Shengzhou, China; PEG (degree of polymerization is 10,000), was available from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China; Curcumin was available from Sigma-Aldrich; anhydrous carbonate (chemically pure) was available from Sinopharm Chemical Reagent Co., Ltd.; lithium bromide; PBS solution; Tween and methanol were A.R grade. The main equipment: electro-spinning machine NEU, Hitachi s4800 field emission scanning electron microscope (FE-SEM), Thermo Nicolet 5700 Fourier transform infrared (FTIR) spectrometer; UV spectrophotometer (UV- 2550, Shimadzu, Japan).

Preparation of electrospinning nanofiber membrane

The SF solution was purified using a standard protocol [15]. Briefly, a 25 g degummed silk microfibers was dissolved in 100 mL lithium bromide (9.3 M) at 60 \pm 0.5 °C for 4 hours. The solution was dialyzed in distilled water for 36 hours (water changed five times during the dialysis process) to remove the lithium bromide. After dialysis, the solution was centrifuged twice at 12,000 × g, 4 °C for 20 min to remove the impurities. The concentration of SF was calculated using eq. (1):

Concentration of SF [%] =
$$\frac{W_2 - W}{W_1 - W}$$
 100 (1)

where W and W_1 are the plate mass before and after adding of 1 mL SF solution, and W_2 – the plate weight after the solution dried at 60 °C for 4 hours. The purified SF was stored at 4 °C before use.

Different concentrations of pure SF solution (20% and 29%) were defined as the control groups. The PEG mass fraction of 30% and different curcumin content of 0.5, 1, and 1.5 mg/ml were dissolved into the SF solution of 20 wt.% of to prepare three kinds of mixed solution. Similarly, different PEG solutions (3, 30, and 50 wt.%) and 20 wt.% SF solution were added at a curcumin solution (1 mg/ml) to obtain a uniform spinning mixture. Then, the electrospinning nanofiber membranes were fabricated using an electro-spinning machine NEU spinning, the static voltage was 25 kV, the feeding rate was 0.130 mm per minute, and trip-tocollector distance was fixed at 17 cm. A grounded aluminum foil was used as the collector.

Microscopic observations

The surface morphology of the sample was examined using a SEM (Hitachi S-4800, Tokyo, Japan). All specimens were sprayed with gold prior to imaging and observed at a voltage of 3 kV. The length and diameters of fibers were determined using an inverted microscope (Olympus phase contrast microscope TH4 200, Osaka, Japan).

Structure characterization

The secondary structure of the nanofiber membrane was examined using FTIR with a MIRacleTM attenuated total reflection Ge crystal cell in the reflection mode (Nicolet 5700, Thermo Electron Corp, Waltham, Mass., USA). The FTIR wave numbers were set from 4000 to 400 cm⁻¹ during 32 scans, with 2 cm⁻¹ resolution. The major vibration bands were attributed to specific chemical groups.

Drug release properties

The nanofibrous membranes with different drug percentages were treated by water vapor for 6 hours. The SF/PEG/drug fibrous membranes were transferred into the phosphate buffered saline (PBS) solution (92 ml PBS solution, uniformity mixed with 5 ml of 10% tween and 3 ml of methanol). After sealing, put them into thermostatic shaker, set temperature is 37 °C, rotation speed is 100 rpm. At regular intervals at 1, 3, 6, 9, 12, 24, 48, 72, 96, 120, and 300 hours, 0.5 ml of solution was taken from centrifuge tube and its absorbance at 425 nm was measured using Bio-Tek synergy H1 microplate reader. After measuring the absorbance, the solution was returned to the centrifuge tube to ensure constant volume of the release solution. The release and accumulative releasing percentage were calculated using the following eqs. (2) and (3), respectively [12].

$$RPi = \frac{Ci V}{W_0} 100\%$$
⁽²⁾

$$ARi = \sum_{i=1}^{i} RPi \tag{3}$$

where RPi [%] is the release percentage, ARi [%] – the accumulative releasing percentage, C_i – the drug concentration, V – the volume, and W_0 – the total entrapped drug.

Results and discussion

Effect of the PEG concentration on the fiber morphology

The SEM images of nanofiber membranes with different concentrations of PEG are shown in fig. 1. The conglutination phenomenon of fibers within nanofiber membranes decreases with the increase of mass fraction of PEG when SF mass fraction and curcumin content remain the same. Because the adding of PEG increases the charge density on the surface of the jet, so as to the fibers become finer due to larger drafting in the electric, as well as the rapid drying speed leads to the decrease of adhesion between the fibers.

Effect of fiber morphology from the curcumin percentage

The SEM images of nanofiber membranes with different concentration of curcumin and fiber diameter distribution are shown in fig. 2. The fiber diameter decreases with the increase of curcumin content when SF mass fraction and PEG content remain the same. The results showed that the fiber diameter become fine and uniform after adding of curcumin, which increases the charge density on the surface of the jet, thus it carries more charge and which receives larger drafting in the electric.

The secondary structure of the fibers

As shown in curve a of fig. 3, pure silk nanofiber membrane appears significant peaks near the 1650.0 cm⁻¹ and 1540.0 cm⁻¹, which results from vibration of C = O and N-H in the samples. Hydroxy and amino peaks both increase, and the change of peak is almost the same with the ones after adding a certain of PEG and curcumin. It happens because adding of PEG and curcumin can impact on the vibration and structure of the original chemical bonds. At the same time, curves (b), (c), and (d) near 1120 cm⁻¹ have new peak compared to pure silk nanofiber membrane curve. It is probably caused by the produced C-O after adding PEG and curcumin [5, 6].

Drug release properties

The drug release profile of the curcumin/SF/PEG fibrous membrane at 37 °C was characterized in PBS pH = 7.4 ± 0.2 as the release medium, fig. 4. The drug release curves showed that the drug release rate and accumulative release rate increase while the drug loading percentage increases. The experimental result of *in vitro* drug release showed a stable and effective drug releasing property. As shown in fig. 4, there is a steep growth ability of sustained drug release with the maximum slope in 0-24 hours, so it has the maximum reaction rate. After 24 hours the slope of the curve rise becomes small. The content of curcumin for curves of nanofiber membrane 0.5 mg/ml and 1.0 mg/ml in 0-125 hours are almost the same after the release to 125 hours, the content of curcumin for 0.5 mg/ml curve almost did not rise.

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Figure 1. The SEM images of nanofiber membranes with different concentrations of PEG; (A)-(C) are 3, 30, and 50%, respectively

The content of curcumin for 1.0 mg/ml curve increased slowly and the curcumin content of 1.5 mg/ml is still in the continuous release, and shows longer release time. Because the drug nanofiber release process is a series of actions from the outside to the inside of the release process, first with the fiber surface part of the SF blend in bentonite dissolved in water, the medicinal fibroin also dissolved in water lead to early rapid release. Then some swelling of SF get forming material coated on fiber dimension, slow down the water into the fiber, increase the time of fiber internal medicine into solution, lead to the drug release rate slow down. With the increase of the content of curcumin in nanofiber membranes, its release rate also increased. This is because the curcumin reduced the diameter of the nanofiber

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Figure 2. The SEM images of nanofiber with different concentration of curcumin and fiber diameter distribution; A, B, and C are 0.5, 1.0, and 1.5 mg/ml, respectively

membranes, the distance that drug diffuse into the fiber surface is reduced, and drug are more likely to release, with the faster release speed. At the same time, content of 0.5 mg/ml, 1.0 mg/ml of the cumulative release amount of curcumin around only 40%, while the content of 1.5 mg/ml nanofiber membranes can reach 80%. This may be because the less the amount of curcumin added, failed to make the electro-spun fiber more fully when pumping long attenuated, the fiber diameter is larger, leading to incomplete release of curcumin is not released completely.

Conclusion

In the present work, we successfully fabricated the drug-loaded SF fibrous membranes using electrospinning. The morphology, secondary structure, drug release and their

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Figure 3. The FTIR spectrums of different nanofiber membranes; (a), (b), (c), and (d) are the pure SF, 1.5, 0.5, and 1.0 mg/ml drug membranes, respectively



Figure 4. Drug release curves of different percentages of curcumin nanofiber membranes; (a), (b), and (c) are drug loaded membranes with curcumin percentages of 0.5, 1.0, and 1.5 mg/ml, respectively

interaction effects of fibrous films were examined. Random coil and silk conformation were predominantly found in the drug-loaded SF fibrous membranes. The experimental result of *in vitro* drug release showed a stable and effective drug releasing property. The stability and utilization ratio of curcumin were improved by loading them into SF/PEG films. In conclusion, it can be used as a novel tissue engineering material in the future.

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