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Transparent injectable sericin-honey hydrogel with antioxidant and antibacterial activities combined with feeding sericin accelerates diabetic wound healing

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Abstract

Wound healing in diabetics is often impaired or delayed due to the presence of high reactive oxygen species and low antioxidant levels. Here, a sericin-honey semi-interpenetrating network hydrogel with excellent antioxidant activity was prepared. Besides, the sericin-honey hydrogel is transparent, injectable, sticky, highly porous, and has good swelling properties, antibacterial activity, and cell compatibility. Based on its good performance *in vitro*, sericin-honey hydrogel achieved effective *in vivo* treatment on a mouse diabetic wound model, significantly accelerating the wound healing process. Furthermore, the combined effect of feeding sericin solution played a positive role in strengthening the effect of diabetic wound repair.

1. Introduction

The number of adult diabetic patients in the world has reached 450 million, accounting for about 6%. The risk of chronic diabetic ulcers in diabetic patients is 15%, and the prognosis of wounds is poor, the recurrence rate is high, and it often leads to amputation [1]. The medical expenses of patients are high, and the long-term medical burden leads to a decline of the quality of life [2]. The healing of chronic diabetic skin wounds is an extremely complicated procedure. The interactions between different cells are involved in all stages of chronic wound healing, including hemostasis, inflammation inhibition, granulation tissue formation, vascular remodeling, re-epithelialization, and remodeling [1]. The difficulty of diabetic wound healing is mainly due to glucose metabolism caused by hyperglycemia, which causes white blood cells to produce excessive reactive oxygen species (ROS), resulting in reduced cell proliferation, reduced collagen deposition, delayed re-epithelialization, and subsequent bacterial infection [1–4]. These factors render diabetic wounds prone to form long-lasting unhealed chronic wounds [4, 5]. Furthermore, Active dressings with injectable and transparent properties are highly favored in clinical practices. One reason is that injectability facilitates minimally invasive surgery (MIS) and covers irregular wound surfaces [6, 7]; Another is that transparency is favorable for real-time monitoring of wound mending, which could decrease the harm of tearing off the wound dressing frequently [8]. Therefore, to design of injectable transparent wound dressings with effective ROS scavenging and antibacterial activities is of great significance in refractory diabetic wound management.

The phytochemical flavonoids and phenolic acids contained in honey (Hon) play an important role as antioxidants because their free radical scavenging activity can protect cells from free radical damage

and ultimately reduce inflammatory responses. Additionally, honey has anti-inflammatory properties as it obliterates the deleterious and inhibits scarring [9]. Honey contains high levels of glycine, methionine, arginine, and proline, which are essential for collagen formation and fibroblast deposition, thereby beneficial for wound repair [10]. It also promotes wound healing by stimulating tissue regeneration [9]. Moreover, honey has excellent antibacterial activity, which is mainly attributed to its high osmotic pressure, pH value, H_2O_2 production, and the presence of other phytochemical components [11, 12]. The osmotic action of sugar removes water from bacterial cells, which can prevent bacterial growth [13]. In addition, it achieves automatic debridement by absorbing the edema fluid from the wound [14]. The high osmolality of honey provides a moist environment for wound healing and prevents it from attaching to new wound tissue [15]. Some researchers have found that honey can stimulate fibroblast proliferation and angiogenesis [16]. On top of it, honey reduces the pH of the wound, which results in the release of more oxygen from hemoglobin and promotes wound healing [13]. However, honey is difficult to apply directly to the wound because it would flow out of the wound over time and inconvenience the patient. Therefore, incorporation of honey into the hydrogel system seems to be more beneficial and applicable [12].

Sericin (Ser) not only possesses a series of natural biological activities, such as anti-oxidation [17], moisturizing [18], anti-cancer [19], anti-diabetic activity [20], antibacterial [21, 22], anti-freezing [23], anti-inflammatory [24], inhibiting tyrosinase and polyphenol oxidase activities [25], enhancing cell adhesion and proliferation [26] and promoting wound healing [27], etc. Moreover, it has high biocompatibility and no immunogenicity [28, 29]. Sericin is hydrophilic, biodegradable [30], and rich in active groups, making it easy to be modified or cross-linked, including amino groups, hydroxyl groups, and carboxyl groups. At present, sericin has been employed to fabricate various bioactive polymers [27, 31–33], and preliminary studies have been conducted in the repair of injured skin [27], muscle [29], nerve [34, 35], and others. The combination of the merits of sericin with the properties of hydrogels endows the biomaterial with excellent histocompatibility and the capability of creating a moderately moist microenvironment for wounds [36]. Moreover, the porous structure of hydrogel facilitates the loading of cells, drugs, or other biologically active molecules. Therefore, hydrogel attracted great attention in the field of wound repair due to it can protect the damaged wound surface, reduce scar formation, and promote wound healing [8, 37–39]. Sericin-based hydrogel has good biocompatibility and physical–chemical properties, and preliminarily shows good wound repair potential [40–42]. Honey and sericin (sericin hydrogel) have their own advantages and maybe complement each other. However, there are not any publications or reports about pertinent studies.

As shown in Scheme 1, a multifunctional sericin-honey semi-interpenetrating network hydrogel dressing with suitable performance was prepared in the study, and its physical properties, antioxidant activity, antibacterial activity, and cell compatibility were evaluated. Based on the results of the *in vitro* study, the wound healing effects of sericin-honey hydrogel and combined feeding of sericin solution on diabetic wounds were investigated using a mouse diabetic wound model.

2. Materials and methods

2.1. Materials

Natural silk fibroin deficiency mutant cocoons (*140 Nd-s* (white cocoon), *185 Nd-s* (yellow cocoon)) were obtained from silkworm species preserved in our laboratory. H_2O_2 were purchased from Sino pharm Chemical Reagent Co., Ltd (China). Horseradish peroxidase (HRP) was supplied by Biosharp Co., Ltd. Mouse skin fibroblasts (NIH3T3) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Manuka honey (UMF15+) was purchased from Comvita, New Zealand. *Staphylococcus aureus* was purchased from China Center of Industrial Culture Collection (Beijing China). Male C57BL/6 mice (8–10 weeks old) were purchased from Changzhou Cavens Laboratory Animal Co., LTD. Tegaderm transparent dressings were purchased from 3 M (USA).

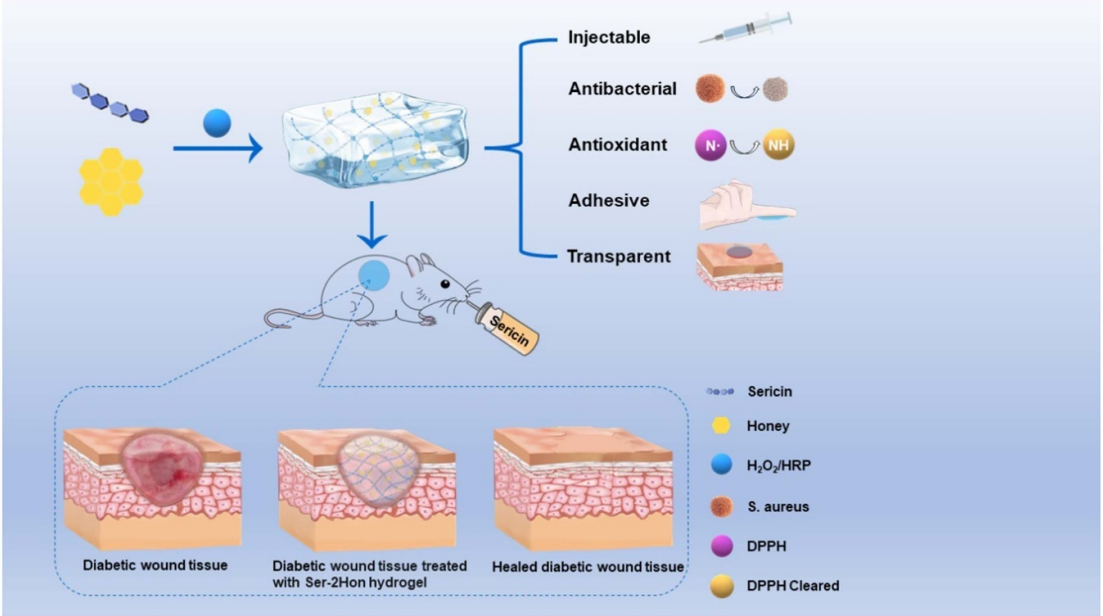
2.2. Isolation of sericin from 140 Nd-s silkworm cocoons

The method for isolating sericin from *140 Nd-s* cocoons was according to our previously established protocols [7, 43]. In short, *140 Nd-s* cocoons were cut up and dissolved in 6 M LiBr solution at 35 °C for 24 h. After dissolution, the supernatant was centrifugalized and transferred to the dialysis membrane (MWCO 3500, Biosharp). At the same time, the supernatant was mixed with 1/4 (v/v) 1M Tris-HCl buffer (pH 9.0) and dialysis was performed for 48 h. After that, the supernatant is concentrated with polyethylene glycol (PEG-6000) solution.

Sericin concentration was detected by the mass method, in which the sericin solution with volume V was taken, and the dry matter weight after drying was M, so the sericin concentration was $M/V \times 100\%$.

2.3. Optimization of formula and preparation of sericin-honey hydrogel

The sericin isolated from of *140 Nd-s* cocoons without pigment, suitable for preparing a transparent and visible hydrogel was employed for fabricating sericin-honey hydrogel. The UV–vis absorption spectra of



Scheme 1. Sericin-honey hydrogel combined with feeding sericin solution to accelerate chronic wound repair of diabetes.

honey solution was measured by UV–vis spectrophotometer (UV-2600, Shimadzu, Japan) with a wavelength range of 250 nm–450 nm. Fluorescence spectra of sericin was determined by an F-4600 fluorophotometer (Hitachi, Japan). According to our previous optimized gelation conditions, 2% (w/v) sericin was employed in this study [8]. A stock solution of 20% (w/v) honey (Hon) was prepared in ultrapure water. A series of mixed solutions were prepared by mixing sericin solution (2%, w/v) with honey solution (20%, w/v) according the mass proportions (sericin content: honey content) of 1:0.5, 1:0.8, 1:1.0, 1:1.5, 1:2.0, 1:2.5, 1:3.0, respectively. Following by that, the fluorescence emission spectra of mixtures was evaluated by F-4600 fluorophotometer with an excitation wavelength of 300 nm. The excitation slit width was set at 10.0 nm and the emission slit width was set at 2.5 nm. The best concentration ratio between sericin and honey was determined by fluorescence spectrum characteristics. Then, the optimized parameters were employed to fabricate sericin-honey hydrogels. It is noted that the ratio of sericin solution, H_2O_2 (0.03%, w/v) and HRP (0.5%, w/v) at a constant ratio of 50:1:1 (v/v/v). Ser and Ser-xHon represented the pure sericin hydrogel and sericin-honey mixture or hydrogel containing sericin to honey (content) at a ratio of 1/*x* (w/w).

2.4. Rheological analysis

The Ser-2Hon hydrogel was subjected to rheological analysis using a hybrid rheometer (Discovery HR10, TA, USA) at 25 °C. G' (Storage modulus) and G'' (Loss modulus) were determined as the strain increased from 1% to 1000%. The frequency was fixed at 1 rad s^{-1} and then a strain scan was performed to assess the critical strain.

2.5. Preparation of sericin solution for feeding

The high-yielding *185 Nd-s* cocoon containing carotenoids was chosen as the raw material for feeding. Fresh cocoons of *185 Nd-s* were washed with ultrapure water, dried at room temperature, crushed, and sifted through a 0.2 mm screen to collect cocoon powder. The 12 g silkworm cocoon powder was dissolved in 200 ml ultrapure water, placed in a pressure steam sterilizer and treated at 121 °C and 0.1 MPa for 30 min. The solution was then centrifuged at 5000 rpm for 10 min to collect the sericin supernatant. Then, the alkaline protease was added to the sericin solution in proportion (1 g enzyme:250 g cocoon powder), and the pH was adjusted to 10. The enzyme was first hydrolyzed in a 45 °C thermostatic water bath for 2 h, and then adjusted to 85 °C for 10 min to inactivate the enzyme. After that, the sericin solution with around pH 7.6 was obtained and its concentration was detected by the mass method. The 2% (w/v) sericin solution, obtained by diluting with water, was used for feeding.

2.6. UV–vis and fluorescence spectrophotometry

The UV–vis absorption spectra of honey solution was measured by UV–vis spectrophotometer (UV-2600, Shimadzu, Japan) over a wavelength range of 250 nm–450 nm.

2.7. Scanning electron microscopy (SEM) analysis

For SEM analysis, the samples were frozen with liquid nitrogen and dried in a vacuum freeze drier. Finally, SEM (JSM-IT300, JEOL Ltd, Japan) was used to

observe the morphology of sericin-honey hydrogel samples. The pore sizes of samples were averaged from 30 randomly selected pores using Image-Pro Plus 6 software.

2.8. Evaluation of porosity

The traditional volume method was adopted for detecting the porosity of sericin-honey hydrogel. The lyophilized sericin-honey hydrogels were placed in an aqueous solution with a known volume of V_a, and the volume of the solution containing the sample was denoted as V_b. One hour later, the lyophilized hydrogel sample was removed after the liquid is completely immersed into the pores of the sample, and the volume of the remaining water is recorded as V_c. The porosity of lyophilized hydrogel was calculated using the following equation:

$$\text{Porosity} = \frac{V_a - V_c}{V_b - V_c} \times 100\%.$$

2.9. Evaluation of swelling ratio

The swelling behavior of sericin-honey hydrogels was assessed by gravimetric methods [8]. Lyophilized hydrogels were immersed in phosphate buffered saline (pH 7.4) at 37 °C and weighted at determined time points. At the predefined time points, the samples were taken out and weighed after removing excess water from the surface. The swelling ratio was calculated using the following equation:

$$\text{Swelling} = \frac{W_s - W_d}{W_d} \times 100\%$$

where swelling is water swelling ratio, W_d and W_s are the dry weight and wet weight of the samples at 37 °C, respectively.

2.10. Evaluation of degradation kinetics

Three samples were randomly taken and sequentially weighted them before and after drying. V_x represents the weight ratio following and before drying. The other hydrogel samples were weighed (the weight is recorded as W_i) and placed in a centrifuge tube, and an appropriate amount of phosphate buffer saline (PBS) with pH 7.4 or pH 3.0 was added. All the samples were placed in the incubator at 37 °C. The degradation solution was changed once a day with fresh PBS, and samples were taken out at preset time points for drying and weighing (the drying weight is recorded as W_p). The degradation ratio was performed by the following equation:

$$\text{Degradation} = \left(1 - \frac{W_p}{W_i \times V_x}\right) \times 100\%.$$

2.11. Fourier transform infrared spectroscopy (FTIR)

FTIR (Nexus, Thermal Nicolet, USA) was employed to analyze the secondary structure of the sericin-honey hydrogel. The spectral region of FTIR was 2400–400 cm^{-1}.

2.12. Assessment of antioxidant activity

The antioxidant activity of the sericin-honey hydrogel sample was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay according to the previous protocol [44]. Briefly, the sericin-honey hydrogel sample was cut into pieces of 20 mg and stored at 20 °C with a relative humidity of 65% for 24 h. The samples were placed in the test tube. For the DPPH assay, ultrapure water (0.8 ml) and 0.2 mM ethanolic DPPH solution (1.2 ml) was added to the sericin-honey hydrogel sample in each tube. After uniform oscillation, they were sealed and put into a water bath at 37 °C for 30 min. Subsequently, the sericin-honey hydrogel samples were removed, and the absorbances of samples were measured at 517 nm using a UV–vis spectrophotometer (UV-2600, Shimadzu, Japan), denoted as A_1. The blank sample without sericin-honey hydrogel was a mixture of ultrapure water (0.8 ml) and 0.2 mM ethanolic DPPH solution (1.2 ml), which was recorded as A_0 under the same experimental conditions. Each group was repeated three times to fetch the mean value. The antioxidant activity of sericin hydrogel was detected by the same method. DPPH radical scavenging activity was calculated according to the equation:

$$\text{DPPH radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100\%.$$

2.13. Determination of antibacterial properties

S. aureus was selected as a model for inhibition of bacteria. Firstly, the *S. aureus* freeze-dried powder was added into the nutrient broth medium for resuscitation, and then used after two generations. Then the sterilized liquid medium was poured into three groups of glass test tubes. The first group was used as a blank control group; the second group was added with Ser hydrogel; and the third group was added with Ser-2Hon hydrogel. The activated *S. aureus* was transferred into three groups of glass test tubes and incubated in an air bath constant temperature oscillator for 8 h, followed by OD600 measurement.

2.14. Evaluation of cell compatibility

The sericin-honey hydrogel was cut into pieces, rinsed three times with sterile PBS, and immersed in 75% ethanol for 15 min for disinfection. It was then rinsed with sterile PBS again, and the hydrogel was stored in cell culture medium at room temperature for further use. The cells were seeded in a 96-well cell culture plate (1500 cells per well) and incubated overnight for cell attachment. Thereafter, cells were co-cultured with the pretreated sericin-honey hydrogel, and a piece of sericin-honey hydrogel was placed in each well. Cell culture wells without sericin-honey hydrogel were set as controls. Cells were cultured in dulbecco's modified eagle medium supplemented with 10% fetal bovine serum in a cell culture incubator (37 °C, 5% CO_2, and 100% humidity). After 2 d, the

cell viability was evaluated using a Cell Counting Kit-8 (Dojindo, Gaithersburg, MD).

2.15. Establishment and treatment of diabetic wound model

Streptozotocin (STZ)-induced type-I diabetic model was used in wound healing test according to the previous research method [1, 45]. C57BL/6 mice (8–10 weeks old) fasted for 12 h were injected with STZ dissolved in sterile citrate buffer (0.05 M sodium citrate, pH 4.5, 70 mg kg^{-1}·BW). STZ was administered continuously for 5 d. Then blood glucose was measured every 3 d. When blood glucose remained above 11.1 mmol l^{-1} for 30 d, the diabetes model was successfully established.

The Ser hydrogel and Ser-2Hon hydrogel were rinsed three times with sterile PBS, immersed in 75% ethanol for 1 h, then rinsed with sterile PBS and kept at room temperature for further use. Male C57BL/6 mice were anesthetized with 0.3% pentobarbital sodium (50 mg kg^{-1} BW), and then the hair on the back was shaved, washed with a povidone-iodine solution, and cleaned with an alcohol swab. A diameter of 1.0 cm circular, full-thickness skin wound on the back of mice was surgically created. Then, mice were randomly divided into four groups: (1) Tegaderm was used to cover the wounds of diabetic mice (DM); (2) Ser hydrogel and Tegaderm were used to cover the wounds of DM; (3) the wounds of DM were covered with Ser-2Hon hydrogel; (4) the wounds of DM were covered with Ser-2Hon hydrogel combined with the feeding of sericin solution (the sericin solution prepared in section 2.5 was poured into the drinking bottle of mice). The Tegaderm dressing was administered as the secondary dressing to keep the dressings in place. All the mouse experiments were performed according to the guidelines and approved by the Ethics Committee of Jiangsu University of Science & Technology (Zhenjiang, China).

2.16. Statistical analysis of wound healing rate

Wound sites were photographed on days 0, 7, 14, and 21 post-wounding. Wound areas in each group were measured using the Image pro plus software. The percent of wound area at the indicated days in comparison with the original wound was calculated according to the below equation [1]:

$$\text{The recovery ratio of wound area} = \frac{\text{Wound area}0 - \text{Wound area } x}{\text{Wound area}0} \times 100\%.$$

Wound area x and Wound area 0 are the wound area after treatment on day x and day 0, respectively.

2.17. Statistical analysis

All the experiments were carried out for $n = 3$ samples unless otherwise specified. Data were represented as mean ± standard deviation. Data statistical analysis was performed using GraphPad Prism 8 software (San Diego, CA, USA). The significance level was measured by comparing the data between groups and within groups by performing a one-way analysis of variance (ANOVA) followed by Tukey's test. Microscopic images were analyzed using Image-Pro Plus 6 software.

3. Results and discussions

3.1. Preparation of sericin-honey hydrogels

Sericin hydrogel is promising in wound repair due to its premium properties and biological activity [40, 46]. In this study, H_2O_2/HRP was employed to fabricate sericin hydrogel. H_2O_2 is able to react with phenolic hydroxyl groups catalyzed by HRP [47, 48]. In addition, destyrosine was synthesized by oxidation of sericin with H_2O_2/HRP, which promoted the cross-linking of sericin proteins [49].

According to our previous preparation process of the sericin hydrogel, the sericin solution isolated from *140 Nd-s* cocoons can form into a transparent hydrogel in the presence of H_2O_2/HRP within tens of seconds [48]. In this study, the sericin of *140 Nd-s* cocoons were also employed to prepared sericin-honey hydrogels in view of the visible hydrogels are convenient for observation of wound healing. Honey contains a variety of phenolic compounds, which have biological activities such as scavenging free radicals in the body, delaying aging, preventing cardiovascular diseases, cancer prevention, and anti-radiation [9]. The UV–vis absorption value has an obvious peak at 265 nm, which is a typical characteristic peak of honey, and the absorption value increases along with the higher honey concentration. The standard curve of honey was obtained by UV–vis absorption results of honey at different concentrations (figures 1(a) and (b)). Besides, the fluorescence quenching phenomenon has been understood as a protein/peptide-polyphenol interaction, which is mainly influenced by hydrophobic interactions, electrostatic interactions, van der Waals forces, and hydrogen bonds [50]. With changes in the structure of the protein/peptide and/or the physical and chemical environment surrounding it, the fluorophore could lead to a emission peak shift and/or decrease [51].

To optimize the ratio of honey to sericin, the fluorescence quenching properties of sericin and honey were investigated, and 2.0 (w/v%) sericin was selected for subsequent quenching experiments. In this system, the endogenous fluorescence of sericin mainly depends on tyrosine (Tyr) and phenylalanine (Phe) amino acids [52]. As shown in figure 1(d), the fluorescence peak of sericin gradually was decreased with the increase amount of honey. When the ratio of sericin content to honey content reaches 1:2 (Ser-2Hon), the shift took placed completely, and the fluorescence was not obvious decreased even

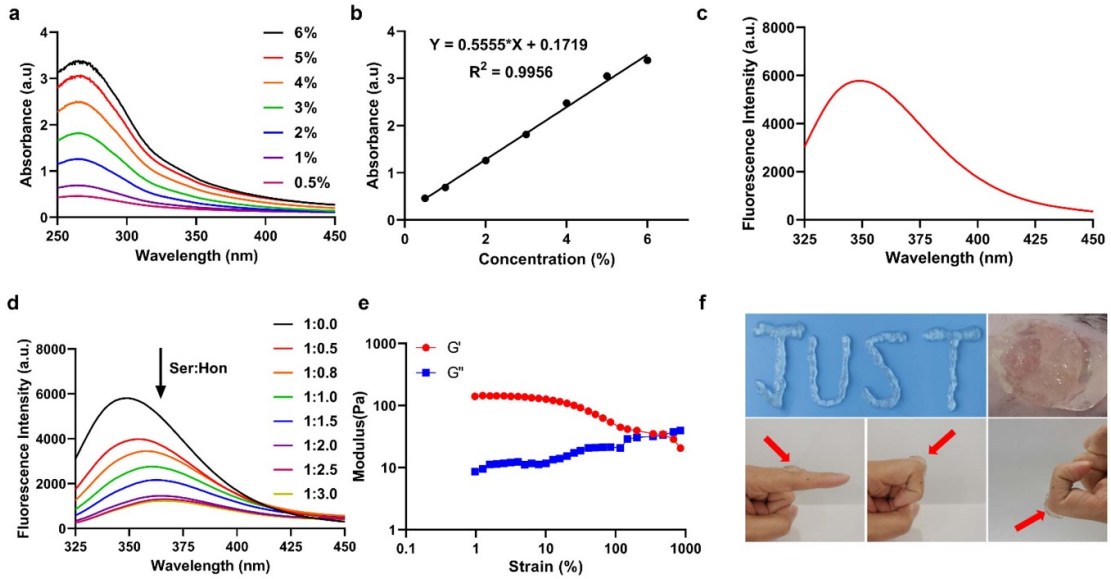


Figure 1. (a) UV–vis absorption results of honey at different concentrations. (b) Standard curve of UV–vis absorption of honey. (c) Fluorescence spectra results of sericin solution (2%, w/v). (d) Fluorescence spectra of sericin mixed with honey under different ratios. (e) Rheometer oscillatory test results of Ser-2Hon hydrogel. (f) The upper left photograph showing the letters of Ser-2Hon hydrogel by injected through the syringe with 25 G needle and the upper right photograph showing the wound of C57BL/6 male mice covered with Ser-2Hon hydrogel. The down photograph showing the bending adaptability and adhesion of the Ser-2Hon hydrogel (The red arrow refers to the hydrogel).

the concentration of honey was further improved. The fluorescence intensity of sericin decreased with red shift of its absorption peak (figures 1(c) and (d)), which suggested a combination had occurred between honey and sericin. Hence, the optimized ratio of sericin to honey was less than or equal to 1:2 (w/w). Furthermore, we found that the gelation was poor when the ratio of sericin content to honey content reached 1:2.5 (Ser-2.5Hon). So, the Ser-2Hon was employed to form sericin-honey hydrogel for the following studies. Meanwhile, the rheological experiment of the hydrogel was carried out, and the results (figure 1(e)) showed that the loss modulus (G'') intersects the storage modulus (G') when the strain was increased to 477%, and then reverses, indicating its transition from the gel state to the solution state and the collapse of the hydrogel network [53].

The Ser-2Hon hydrogel can be injected unimpededly through a syringe with a 25 G needle (inner diameter, 0.51 mm), indicating that it has satisfactory injectable properties [2] (figure 1(f) upper left column, supplementary video S1). Injectable hydrogels not only can easily adapt to irregular wound surfaces, but also convenient for MIS, becoming an attractive candidate for innovative dressing [54]. The Ser-2Hon hydrogel could perfectly cover the mouse wounds and was transparent (figure 1(f) upper right column). Transparent hydrogel dressing is convenient for real-time care of the wound, avoiding the need to uncover the dressing to observe the state of wound healing which is detrimental to wound healing [8]. Adhesive ability is important for hydrogel dressing that can keep the wound dressing from unexpectedly peeling off [53]. We found that the Ser-2Hon hydrogel had good adhesion and was able to stick to the finger joint (figure 1(f) bottom column, supplementary video S2) for repeated bending tests without falling off. These results indicated that the Ser-2Hon hydrogel is suitable for wound repair.

3.2. Microstructure and porosity of sericin-honey hydrogels

The morphology of freeze-dried scaffolds of Ser hydrogel and Ser-2Hon hydrogel were observed by SEM. The Ser hydrogel scaffold had a porous structure with different diameters and distributions, while the pores of the sericin-honey hydrogel scaffold were blocked due to the addition of honey (figure 2(a)). As shown in figure 2(b), the porosity decreased with the increase of honey. The porosity of Ser hydrogel was 93%, while that of Ser-2Hon was 77%. The porous structure with a porosity of 77% provides a breathable environment for the wound and benefits wound healing. Moreover, the pore sizes of Ser hydrogel and Ser-2Hon hydrogel were 15.3 μm and 33.4 μm, respectively (figure 2(c)). On one hand, the Ser-2Hon hydrogel with larger pore size seems more in favor of skin cell migration. On other hand, porous microstructure provides space for cell growth, which also facilitates the exchange of nutrients and the excretion of metabolites.

3.3. Swelling properties of sericin-honey hydrogels

In general, the water absorption properties of hydrogels is expressed by swelling property, which is an imperative parameter because it affects many aspects

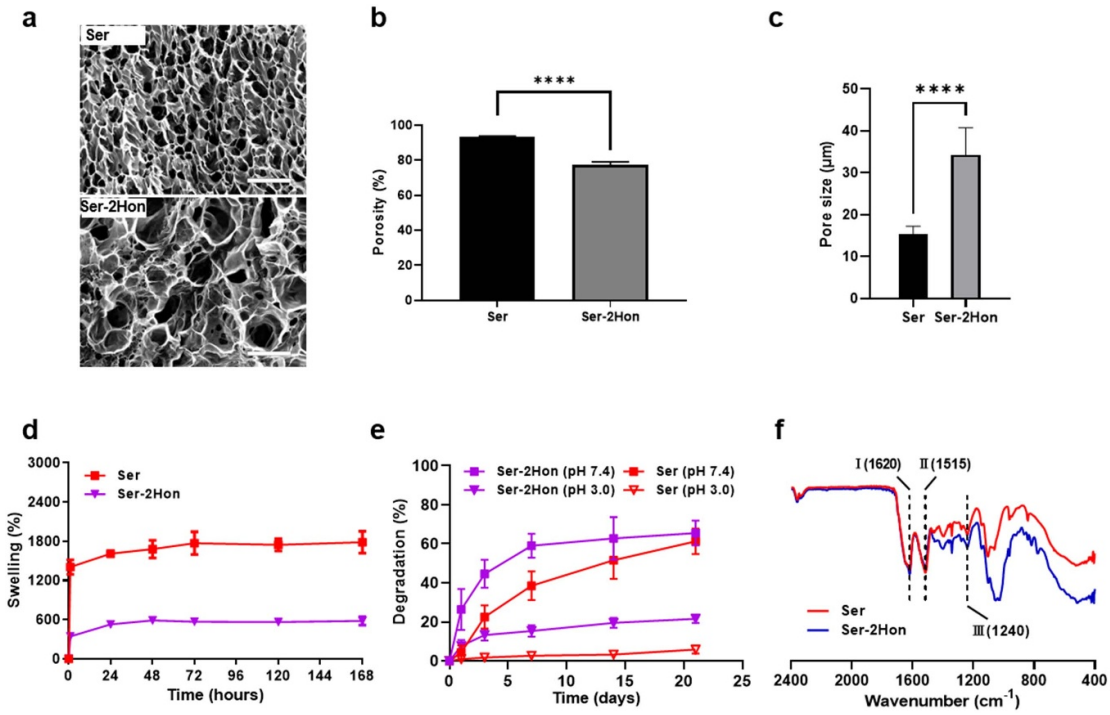


Figure 2. (a) Scanning electron micrographs of Ser hydrogels (up column) and Ser-2Hon hydrogels (low column). Scale bars, 50 μm. (b) Porosity of the Ser hydrogels sand Ser-2Hon hydrogels. (c) Pore size of the Ser hydrogels sand Ser-2Hon hydrogels. (d) Swelling rate of the Ser hydrogels and Ser-2Hon hydrogels from 0 to 168 h at pH 7.4. (e) Degradation rate of the Ser hydrogels and Ser-2Hon hydrogels from 0 to 21 d at pH 7.4 and pH 3.0. (f) FTIR spectra of Ser hydrogels and Ser-2Hon hydrogels. Representative results are presented as the means ± SD; $n = 3$ per group. *P*-values are calculated using one-way ANOVA followed by Tukey's test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

of hydrogels, such as the diffusion of encapsulated biosurfactants [8]. The freeze-dried Ser and Ser-2Hon hydrogels were immersed in PBS with pH 7.4 (neutral) at 37 °C to evaluate their swelling behavior. As shown in figure 2(e), at the beginning of the swelling process, all samples swell rapidly, and the swelling values of all samples reach the maximum after 24 h. The swelling rates of Ser and Ser-2Hon hydrogels are reached 1610% and 528% within 24 h, respectively. As the sericin content increases, the swelling rate of Ser-2Hon hydrogel also increases correspondingly, with that of pure Ser hydrogel being the largest. This phenomenon might be attributed to the fact that more sericin was contained by the higher porosity of hydrogel samples (figure 2(d)). The results showed that the porosity of the sericin-honey hydrogel could be adjusted by manipulating the ratio of sericin to honey. The swelling rate of Ser-2Hon reached more than 500% after 24 h, and the swelling rate increased slowly, followed by the plateau within the subsequent 168 h, indicating that Ser-2Hon hydrogel has the proper swelling properties, fitting for wound dressing.

3.4. Degradation properties of sericin-honey hydrogels

Degradation behaviors of the sericin-honey hydrogels are shown in figure 2(e). In the initial stage, the Ser-2Hon hydrogel began to degrade rapidly, with degradation rates approaching 30%, while the degradation of the Ser hydrogel occurs slightly in a neutral environment. Similarly, the degradation rate of the Ser-2Hon hydrogel was faster than that of the Ser hydrogel under acidic conditions. The degradation rate of Ser-2Hon hydrogel was faster than that of ser hydrogel at the same pH conditions, whether neutral or acidic. Honey contained in the Ser hydrogel is a supersaturation solution of fructose [55], which is rapidly released when encountering the solution in the hydrogel sample. Honey might be distributed amongst sericin chains, reducing contacts between them, which results in a decreased cross-linking ratio as well as entanglement [56]. Or rather, the structure diminishes the stability of the hydrogel, accelerating the degradation rate of the samples. The degradation rate of the hydrogel in the neutral slowed down and leveled off after 7 d, reaching 65% at 21 d. This appropriate degradation ability can avoid secondary injury to the wound when changing the hydrogel dressing, ensure a good wound healing environment, and achieve the desired healing effect. Furthermore, the degradation rate of Ser-2Hon hydrogel or Ser was much slower under acidic condition (pH 3.0) than that of neutral condition (pH 7.4). These phenomena were caused by Ser hydrogel's pH-responsive degradation behaviors which were widely presented in some other sericin hydrogels in previously studies [7, 33, 43, 48].

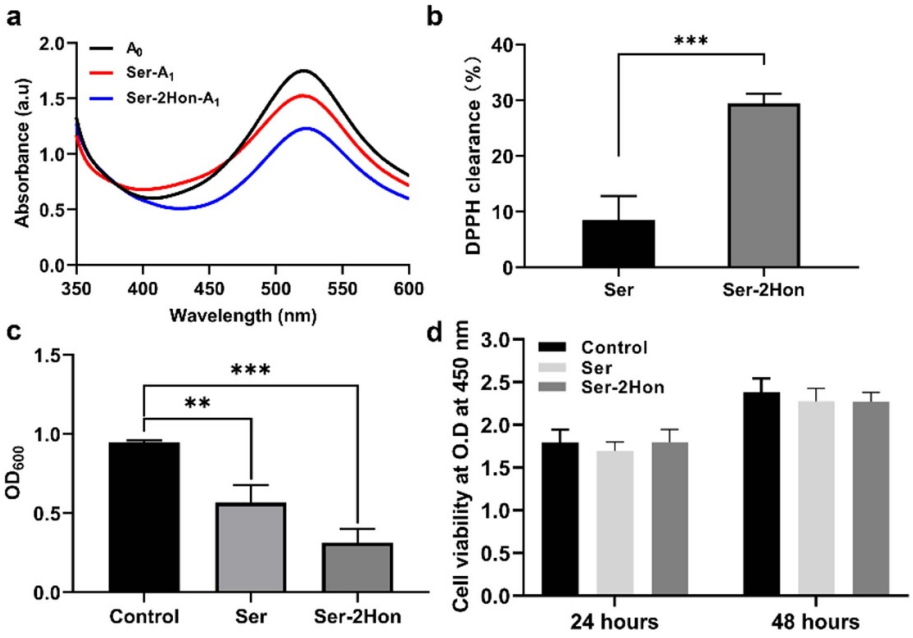


Figure 3. (a) UV–vis absorbance of DPPH: Ser hydrogels and Ser-2Hon hydrogels. (b) Scavenging free radical (DPPH) capability of Ser hydrogels and Ser-2Hon hydrogels. (c) The OD value of bacteria of different groups at 600 nm after co-culture with *S. aureus*. (d) The cell viabilities of mouse skin fibroblasts (NIH3T3) after co-culture with Ser and Ser-2Hon hydrogels for 24 or 48 h. Representative results are presented as the means ± SD; $n = 8$ per group. *P*-values are calculated using one-way ANOVA followed by Tukey's test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.5. Secondary structure of sericin-honey hydrogels

The secondary structure of Ser hydrogel and Ser-2Hon hydrogel freeze-dried scaffolds were analyzed by FTIR. A serial of characteristic infrared absorption bands, produced by polypeptide and protein repeat units, can reveal the secondary structure in the protein. Amide I (1600–1690 cm^{-1}) mainly contributes from the C=O stretching vibrations of the peptide linkages. Amide II (1480–1575 cm^{-1}) mainly produces from N–H bending and the C–N stretching vibration. Amide III (1229–1301 cm^{-1}) primarily arises from the in-phase combination of C–N stretching vibration and in-plane N–H bending vibration [57]. Among them, amide I is widely applied to evaluate secondary structures of sericin because it arises predominantly from the C=O stretching vibration [58]. In the amide I band, the absorption peaks of α-helix, β-sheet, β-turn and random coil were 1658–1650, 1640–1610, 1700–1600 and 1650–1640 cm^{-1}, respectively. As shown in figure 2(f), characteristic absorption bands were observed at 1620 cm^{-1} of amide I, 1515 cm^{-1} of amide II and 1240 cm^{-1} of amide III in Ser hydrogel and Ser-2Hon hydrogel freeze-dried scaffolds. Therefore, it could be safely concluded that the introduction of honey had no significant influence on the secondary structures of sericin.

3.6. Antioxidant activity of sericin-honey hydrogels

The antioxidant activity of sericin-honey hydrogel was evaluated by DPPH radical scavenging experiment. As shown in figure 3(a), the DPPH absorbance of Ser and Ser-2Hon hydrogels decreased after adding DPPH. The DPPH clearance ratio was 8.52% and 29.45%, respectively (figure 3(b)). These results indicated that the antioxidant activity of sericin-honey hydrogels was enhanced with the increase of honey content. Firstly, the rest free amino acids and flavonoids of sericin endow Ser hydrogel with certain antioxidant properties. Honey has strong antioxidant activity, mainly because honey contains a variety of phenolic compounds. These phenolic compounds could function as hydrogen donors, directly combine with oxygen free radicals, scavenge oxygen free radicals, and inhibit the activity of some enzymes, thereby inhibiting the activity of oxygen free radicals. What is more, the proline, flavonoids and superoxide dismutase in honey accounts for the antioxidation of honey [9, 10, 12]. In the process of skin wound repair, the antioxidant activity of sericin-honey hydrogel advanced cell proliferation by reducing the oxidative stress of fibroblasts, thereby promoting the healing process.

3.7. Antibacterial properties of sericin-honey hydrogels

The antibacterial properties of sericin-honey hydrogels were evaluated by detecting the OD values of bacteria after Ser and Ser-2Hon hydrogels were incubated with *S. aureus*. Figure 3(c) reveals that the antibacterial properties of Ser and Ser-2Hon hydrogels were significantly higher than those of the control groups. With the intervention of honey, the antibacterial

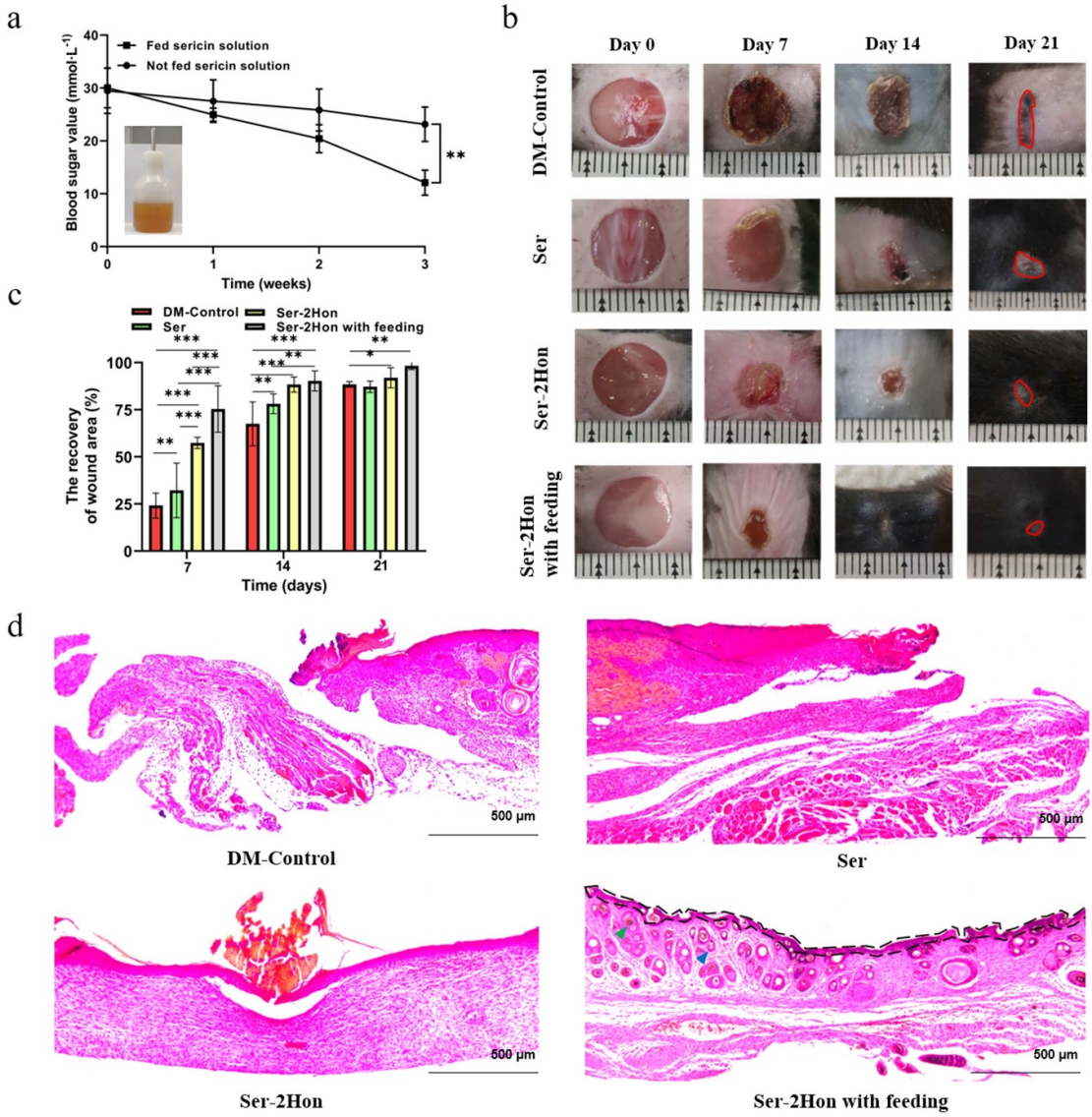


Figure 4. (a) Picture of enzymatically hydrolyzed sericin solution in the mouse water dispenser and changes in fasting blood glucose over 3 weeks in mice fed enzymatically hydrolyzed sericin solution. (b) Representative photographs of the wound healing process were captured during 21 d *in vivo* experiments. (c) Wound closure rates were evaluated at predetermined time points, presented as the percentage of the initial wound area at day 0. (d) Representative images of wound tissue stained with HE on day 14. Black dashed lines indicate intact epithelial tissue, green triangles indicate hair follicles, and blue triangles indicate sebaceous glands. Representative results are presented as the means ± SD; $n = 3$ per group. *P*-values are calculated using one-way ANOVA followed by Tukey's test, $^{*}p < 0.05$, $^{**}p < 0.01$.

activity hydrogel was significantly enhanced. The sericin-honey hydrogels provide a benign microenvironment with less microbes for wound healing.

3.8. Cell compatibility of sericin-honey hydrogels

To assess the cell compatibility of sericin-honey hydrogels, Cell Counting Kit-8 was used to measure the viability of NIH3T3 fibroblasts incubated with sericin-honey hydrogels. The results exhibited no significant difference in proliferation between the cells co-cultured with the sericin-honey hydrogels and the controls (figure 3(d)), confirming that the sericin-honey hydrogels have excellent cytocompatibility.

3.9. Effect of feeding sericin solution on blood glucose in DM

Based on the antioxidant and anti-inflammatory properties of sericin, feeding sericin had a significant hypoglycemic effect. In this study, the enzymatically hydrolyzed sericin solution (2%, w/v), collected from cocoons of a high-yield variety *185 Nd-s* containing carotenoids, was employed for feeding. Carotenoids have antioxidant activity and play a role in lowering blood glucose. At the same time, the cocoon yield of *185 Nd-s* is higher than that of *140 Nd-s*, and the cost of feeding for mice is lower. The blood sugar changes of DM were tested weekly. As shown in figure 4(a), on week 3 after feeding, the parameter of mice was significantly lower than that of mice without feeding with enzymatically hydrolyzed sericin solution,

showing the combination with feeding the solution is promising as an auxiliary treatment.

3.10. *In vivo* evaluation of diabetic wound healing

Sericin is an excellent biocompatibility material without immunogenicity. Simultaneously, sericin has a variety of biological activities in wound repair, such as anti-inflammatory, antioxidant, antibacterial, and promoting cell proliferation [26, 42]. Here, honey is combined with sericin to enhance its effect. Honey has anti-inflammatory properties, which can remove wounds and inhibit scars. It can also promote wound healing by stimulating tissue regeneration [9]. Based on the experiment results above, it is speculated that sericin-honey hydrogel would not cause obvious inflammatory response and immunogenicity during wound healing.

Based on the excellent *in vitro* performance of sericin-honey hydrogel, the most common diabetic full-thickness skin wounds were established in mice to evaluate the healing effect of sericin-honey hydrogel *in vivo*. DM were treated with sericin hydrogel (Ser), sericin-honey hydrogel (Ser-2Hon) and Ser-2Hon hydrogel combined with feeding sericin solution (Ser-2Hon with feeding) every 7 d. Tegaderm dressing was used as a secondary dressing to maintain the position of the dressing. Tegaderm-covered DM (DM-control) was used as controls for *in vivo* healing evaluation. After treatment, the wound healing of each group was monitored for 21 consecutive days.

As shown in figure 4(b), the wound healing rate was slower and the inflammatory period was longer in DM-control group. The rapid proliferation phase did not begin until the inflammatory response weakened on day 14. However, the wound size decreased significantly after Ser-2Hon with feeding treatment, especially in the early stage of wound regeneration, indicating that the wound healing stage accelerated from inflammation to the proliferation and remodeling. As shown in figure 4(c), on day 7 of wound treatment, the wound healing rate of Ser-2Hon with feeding group reached 75.41%, and that of Ser-2Hon group also reached 57.39%, while that of DM-control and Ser groups were only 24.18% and 32.21%. On day 14, the wound healing rate of Ser-2Hon group and Ser-2Hon with feeding group reached 88.38% and 90.28%. These results provided strong evidence that sericin-honey hydrogels promoted diabetic wound healing. In addition, sericin-honey hydrogels combined feeding sericin solution were more effective in wound healing, indicating that the reduction of blood glucose promoted the healing of diabetic wounds.

For histological analysis, wound tissue sections were stained with hematoxylin-eosin (HE) on day 14 after treatment to further evaluate the effect of the hydrogel on wound healing. As shown in figure 4(d), at day 14, the thickness of granulation tissue after treatment in the Ser-2Hon and Ser-2Hon with feeding groups increased compared with the other groups, and the Ser-2Hon with feeding group had significantly intact epithelial tissue. At the same time, significantly more skin appendages (hair follicles, sebaceous glands) were found in the new tissue formed in the Ser-2Hon with feeding group on day 14 than in the other three groups, indicating that reducing blood glucose by feeding sericin protein solution and repairing wounds with sericin honey hydrogel play a combined therapeutic effect on diabetic chronic infection wounds.

4. Conclusion

In this study, a sericin-honey semi-interpenetrating network hydrogel dressing with suitable performance was prepared. The dressing has excellent physicochemical and biological properties, transparency, injectability, stickiness, high porosity, good swelling properties, antioxidant activity, antibacterial activity and biocompatibility. After applied to the diabetic wound model in mice, the wound healing rate was significantly accelerated. In addition, the combined effect of feeding sericin solution played a positive role in strengthening the effect of diabetic wound repair. This study overcame the defects of direct application of honey and broadened the application of sericin-based hydrogels in skin injury repair.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Ethics statement

All the mice experiments were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals and approved by the Ethics Committee of Jiangsu University of Science and Technology (JUST) under ethical ID of G2022SJ01.

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References

[1] Liu S, Zhang Q, Yu J, Shao N, Lu H, Guo J, Qiu X, Zhou D and Huang Y 2020 Absorbable thioether grafted hyaluronic acid nanofibrous hydrogel for synergistic modulation of inflammation microenvironment to accelerate chronic diabetic wound healing *Adv. Healthcare Mater.* **9** e2000198

[2] Wang C, Wang M, Xu T, Zhang X, Lin C, Gao W, Xu H, Lei B and Mao C 2019 Engineering bioactive self-healing antibacterial exosomes hydrogel for promoting chronic diabetic wound healing and complete skin regeneration *Theranostics* **9** 65–76

[3] Dong Y, Rodrigues M, Kwon S H, Li X, A S, Brett E A, Elvassore N, Wang W and Gurtner G C 2018 Acceleration of diabetic wound regeneration using an in situ-formed stem-cell-based skin substitute *Adv. Healthcare Mater.* **7** e1800432

[4] He Y, Liu K, Guo S, Chang R, Zhang C, Guan F and Yao M 2023 Multifunctional hydrogel with reactive oxygen species scavenging and photothermal antibacterial activity accelerates infected diabetic wound healing *Acta Biomater.* **155** 199–217

[5] He J, Li Z, Wang J, Li T, Chen J, Duan X and Guo B 2023 Photothermal antibacterial antioxidant conductive self-healing hydrogel with nitric oxide release accelerates diabetic wound healing *Composites* B **266** 110985

[6] Cha G *et al* 2022 Multifunctional injectable hydrogel for in vivo diagnostic and therapeutic applications *ACS Nano* **16** 554–67

[7] Wang Z, Zhang Y, Zhang J, Huang L, Liu J, Li Y, Zhang G, Kundu S and Wang L 2014 Exploring natural silk protein sericin for regenerative medicine: an injectable, photoluminescent, cell-adhesive 3D hydrogel *Sci. Rep.* **4** 7064

[8] Zhang Y, Chen H, Li Y, Fang A, Wu T, Shen C, Zhao Y and Zhang G 2020 A transparent sericin-polyacrylamide interpenetrating network hydrogel as visualized dressing material *Polym. Test.* **87** 106517

[9] El-Kased R, Amer R, Attia D and Elmazar M 2017 Honey-based hydrogel: in vitro and comparative in vivo evaluation for burn wound healing *Sci. Rep.* **7** 9692

[10] Giusto G, Vercelli C, Comino F, Caramello V, Tursi M and Gandini M 2017 A new, easy-to-make pectin-honey hydrogel enhances wound healing in rats *BMC Complement. Altern. Med.* **17** 266

[11] Molan P and Betts J 2004 Clinical usage of honey as a wound dressing: an update *J. Wound Care* **13** 353–6

[12] Shamloo A, Aghababaie Z, Afjoul H, Jami M, Bidgoli M, Vossoughi M, Ramazani A and Kamyabhesari K 2021 Fabrication and evaluation of chitosan/gelatin/PVA hydrogel incorporating honey for wound healing applications: an in vitro, in vivo study *Int. J. Pharm.* **592** 120068

[13] Al-Waili N, Salom K and Al-Ghamdi A 2011 Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice *Sci. World J.* **11** 766–87

[14] Shukrimi A, Sulaiman A, Halim A and Azril A 2008 A comparative study between honey and povidone iodine as dressing solution for Wagner type II diabetic foot ulcers *Med. J. Malays.* **63** 44–46

[15] Afshari M, Sheikh N and Afarideh H 2015 PVA/CM-chitosan/honey hydrogels prepared by using the combined technique of irradiation followed by freeze-thawing *Radiat. Phys. Chem.* **113** 28–35

[16] Rossiter K, Cooper A, Voegeli D and Lwaleed B 2010 Honey promotes angiogeneic activity in the rat aortic ring assay *J. Wound Care* **19** 442–6

[17] Fan J, Wu L, Chen L, Mao X and Ren F 2009 Antioxidant activities of silk sericin from silkworm bombyx mori *J. Food Biochem.* **33** 74–88

[18] Polaskova J, Pavlackova J and Tlaskova V 2015 Moisturizing effect of cosmetic emulsions with sericin *Int. J. Cosmet. Sci.* **37** 151–2

[19] Zhaorigetu S, Sasaki M and Kato N 2007 Consumption of sericin suppresses colon oxidative stress and aberrant crypt foci in 1, 2-dimethylhydrazine-treated rats by colon undigested sericin *J. Nutr. Sci. Vitaminol.* **53** 297–300

[20] Dong X, Zhao S, Yin X, Wang H, Wei Z and Zhang Y 2020 Silk sericin has significantly hypoglycaemic effect in type 2 diabetic mice via anti-oxidation and anti-inflammation *Int. J. Biol. Macromol.* **150** 1061–71

[21] Ramesan M, Athira V, Jayakrishnan P and Gopinathan C 2016 Preparation, characterization, electrical and antibacterial properties of sericin/poly (vinyl alcohol)/poly (vinyl pyrrolidone) composites *J. Appl. Polym.* **133** 43535

[22] Sapru S, Ghosh A and Kundu S 2016 Antibacterial nanofibrous matrices of nonmulberry silk sericin for wound repair *Tissue Eng.* A **22** S46–S47

[23] Wu J, Rong Y, Wang Z, Zhou Y, Wang S and Zhao B 2015 Isolation and characterisation of sericin antifreeze peptides and molecular dynamics modelling of their ice-binding interaction *Food Chem.* **174** 621–9

[24] Aramwit P, Towiwat P and Srichana T 2013 Anti-inflammatory potential of silk sericin *Nat. Prod. Commun.* **8** 501–4

[25] Chlapanidas T *et al* 2013 Sericins exhibit ROS-scavenging, anti-tyrosinase, anti-elastase, and in vitro immunomodulatory activities *Int. J. Biol. Macromol.* **58** 47–56

[26] Cao T and Zhang Y 2015 Viability and proliferation of L929, tumour and hybridoma cells in the culture media containing sericin protein as a supplement or serum substitute *Appl. Microbiol. Biotechnol.* **99** 7219–28

[27] Li Y, Wu T, Zhang G, Fang A, Li Y, Wang S, Yan H, Liang P, Lian J and Zhang Y 2023 A native sericin wound dressing spun directly from silkworms enhances wound healing *Colloids Surf.* B **225** 113228

[28] Aramwit P, Ekasit S and Yamdech R 2015 The development of non-toxic ionic-crosslinked chitosan-based microspheres as carriers for the controlled release of silk sericin *Biomed. Microdevices* **17** 84

[29] Song Y, Zhang C, Zhang J, Sun N, Huang K, Li H, Wang Z, Huang K and Wang L 2016 An injectable silk sericin hydrogel promotes cardiac functional recovery after ischemic myocardial infarction *Acta Biomater.* **41** 210–23

[30] Fan J, Zheng L, Wang F, Guo H, Jiang L and Ren F 2010 Enzymatic hydrolysis of silk sericin by proteases and antioxidant activities of the hydrolysates *J. Food Biochem.* **34** 382–98

[31] Li Y, Wei Y, Zhang G and Zhang Y 2023 Sericin from fibroin-deficient silkworms served as a promising resource for biomedicine *Polymers* **15** 2941

[32] Zhang Y, Wu T, Shen C, Xu G, Chen H, Yan H, Xiong M and Zhang G 2022 A robust sericin hydrogel formed by a native sericin from silkworm bodies *Fibers Polym.* **23** 1826–33

[33] Zhang Y, Wang S, Li Y, Li X, Du Z, Liu S, Song Y, Li Y and Zhang G 2023 A sterile, injectable, and robust sericin hydrogel prepared by degraded sericin *Gels* **9** 948

[34] Xie H *et al* 2015 A silk sericin/silicone nerve guidance conduit promotes regeneration of a transected sciatic nerve *Adv. Healthcare Mater.* **4** 2195–205

[35] Zhang L *et al* 2017 Sustained local release of NGF from a chitosan-sericin composite scaffold for treating chronic nerve compression *ACS Appl. Mater. Interfaces* **9** 3432–44

[36] Li Y *et al* 2023 Resveratrol loaded native silk fiber-sericin hydrogel double interpenetrating bioactive wound dressing facilitates full-thickness skin wound healing *Biomed. Mater.* **18** 045007

[37] Xu Z, Han S, Gu Z and Wu J 2020 Advances and impact of antioxidant hydrogel in chronic wound healing *Adv. Healthcare Mater.* **9** e1901502
[38] Zhang Y *et al* 2019 A sterile self-assembled sericin hydrogel via a simple two-step process *Polym. Test.* **80** 106016
[39] Ye R, Liu S, Zhu W, Li Y, Huang L, Zhang G and Zhang Y 2023 Synthesis, characterization, properties, and biomedical application of chitosan-based hydrogels *Polymers* **15** 2482
[40] Chen C, Zeng F, Xiao X, Wang Z, Li X, Tan R, Liu W, Zhang Y, She Z and Li S 2018 Three-dimensionally printed silk-sericin-based hydrogel scaffold: a promising visualized dressing material for real-time monitoring of wounds *ACS Appl. Mater. Interfaces* **10** 33879–90
[41] Yang C, Yao L and Zhang L 2023 Silk sericin-based biomaterials shine in food and pharmaceutical industries *Smart Mater. Med.* **4** 447–59
[42] Liu J, Shi L, Deng Y, Zou M, Cai B, Song Y, Wang Z and Wang L 2022 Silk sericin-based materials for biomedical applications *Biomaterials* **287** 121638
[43] Zhang Y, Jiang R, Fang A, Zhao Y, Wu F, Cao T, Liang S, Xia G and Zhang Z 2019 A highly transparent, elastic, injectable sericin hydrogel induced by ultrasound *Polym. Test.* **77** 105890
[44] Ma M, Hussain M, Memon H and Zhou W 2015 Structure of pigment compositions and radical scavenging activity of naturally green-colored cotton fiber *Cellulose* **23** 955–63
[45] Yang Y, Zhao X, Yu J, Chen X, Wang R, Zhang M, Zhang Q, Zhang Y, Wang S and Cheng Y 2021 Bioactive skin-mimicking hydrogel band-aids for diabetic wound healing and infectious skin incision treatment *Bioact. Mater.* **6** 3962–75
[46] Nardini M *et al* 2020 Growth factors delivery system for skin regeneration: an advanced wound dressing *Pharmaceutics* **12** 120
[47] Baek N, Fan X, Yuan J, Xu J and Wang Q 2021 Polymerization and dyeing properties of gallic acid on silk fabric catalyzed by horseradish peroxidase *Fibers Polym.* **22** 2145–55
[48] Zhang Y, Cao X, Zhang J, Zhang G, Zhu M, Yan H and Li Y 2023 A novel injectable sericin hydrogel with strong fluorescence for tracing *Int. J. Biol. Macromol.* **258** 129000
[49] Sahoo J, Xu D, Falcucci T, Choi J, Hasturk O, Clark D and Kaplan D 2023 Horseradish peroxidase catalyzed silk–prefoldin composite hydrogel networks *ACS Appl. Bio Mater.* **6** 203–8
[50] Guerrero P, Kerry J and de la Caba K 2014 FTIR characterization of protein–polysaccharide interactions in extruded blends *Carbohydrate Polym.* **111** 598–605
[51] Qin X, Yuan D, Wang Q, Hu Z, Wu Y, Cai J, Huang Q, Li S and Liu G 2018 Maillard-reacted whey protein isolates enhance thermal stability of anthocyanins over a wide pH range *J. Agric. Food Chem.* **66** 9556–64
[52] Wang Y and Zhang Y Q 2011 Three-layered sericins around the silk fibroin fiber from Bombyx mori cocoon and their amino acid composition *Adv. Mater. Res.* **175–176** 158–63
[53] Guo B, Liang Y and Dong R 2023 Physical dynamic double-network hydrogels as dressings to facilitate tissue repair *Nat. Protocols* **18** 3322–54
[54] Zhou C, Sheng C, Chen J, Liang Y, Liu Q, Li P, Huang X and Liu B 2022 Gradual hydrogel degradation for programable repairing full-thickness skin defect wound *Chem. Eng. J.* **450** 138200
[55] Khan R, Naz S and Abudabos A 2017 Towards a better understanding of the therapeutic applications and corresponding mechanisms of action of honey *Environ. Sci. Pollut. Res.* **24** 27755–66
[56] El-Refaie K, Elbadawy A, Mohamed S and Mahmoud A 2014 Physically crosslinked poly(vinyl alcohol)-hydroxyethyl starch blend hydrogel membranes: synthesis and characterization for biomedical applications *Arab. J. Chem.* **7** 372–80
[57] Kim J, Lee J, Jung H, Cho J, Heo J and Chang Y 2007 Preparation and properties of collagen/modified hyaluronic acid hydrogel for biomedical application *J. Nanosci. Nanotechnol.* **7** 3852–6
[58] Teramoto H and Miyazawa M 2005 Molecular orientation behavior of silk sericin film as revealed by ATR infrared spectroscopy *Biomacromolecules* **6** 2049–57