Shape Changes and Interaction Mechanism of *Escherichia coli* Cells Treated with Sericin and Use of a Sericin-Based Hydrogel for Wound Healing

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ABSTRACT

To verify the interaction mechanism between sericin and *Escherichia coli*, especially the morphological and structural changes in the bacterial cells, the antimicrobial activity of sericin against *E. coli* as a model for Gram-negative bacteria was investigated. The antibacterial activity of sericin on *E. coli* and the interaction mechanism were investigated in this study by analyzing the growth, integrity, and morphology of the bacterial cells following treatment with sericin. The changes in morphology and cellular compositions of bacterial cells treated with sericin were observed by an inverted fluorescence microscope, scanning electron microscopy, and transmission electron microscopy. Changes in electrical conductivity, total sugar concentration of the broth for the bacteria, and protein expression of the bacteria were determined to investigate the permeability of the cell membrane. A sericin-based hydrogel was prepared for an *in vivo* study of wound dressing. The results showed that the antibacterial activity of the hydrogel increased with the increase in the concentration of sericin from 10 g/liter to 40 g/liter. The introduction of sericin induces membrane blebbing of *E. coli* cells caused by antibiotic action on the cell membrane. The cytoplasm shrinkage phenomenon was accompanied by blurring of the membrane wall boundaries. When *E. coli* cells were treated with sericin, release of intracellular components quickly increased. The electrical conductivity assay indicated that the charged ions are reduced after exposure to sericin so that the integrity of the cell membrane is weakened and metabolism is blocked. In addition, sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated that sercin inhibits the expression of bacterial protein. Sericin may damage the integrity of the bacterial cell membrane, thereby eventually inhibiting the growth and reproduction of *E. coli*. Compared to sterile gauze, the sercin-based hydrogel promoted fibroblast cell proliferation and accelerated the formation of granulation tissues and neovessels.

IMPORTANCE

The specific relationship and interaction mechanism between sericin and *E. coli* cells were investigated and elucidated. The results show that after 12 h of treatment, sericin molecules induce membrane blebbing of *E. coli* cells, and the bacteria show decreases in liquidity and permeability of biological membrane, resulting in alterations in the conductivity of the culture medium and the integrity of the outer membrane. The subsequent *in vivo* results demonstrate that the sericin-poly(N-isopropylacrylamide-N,N'-methylene-bis-acrylamide [NIPAm-MBA]) hydrogel accelerated wound healing compared to that with sterile gauze, which is a beneficial result for future applications in clinical medicine and the textile, food, and coating industries.

Although many bacteria, such as bifidobacteria and lactobacilli, are beneficial for our daily lives, harmful bacteria like *Staphylococcus aureus* and *Escherichia coli* are serious threats to human health due to rapid proliferation and secretion of toxins or other metabolites. Accordingly, disease transmission caused by harmful bacteria has become a huge social problem. Large numbers of natural biological antibacterial agents with no side effects and good biocompatibility, such as chitosan and antimicrobial peptides, have been explored and reported on by No et al. (1) and Casteels et al. (2). However, there are still some disadvantages that have restricted the development of these traditional antibacterial agents; for example, chitosan is insoluble in water, and the extraction and purification processes for natural antimicrobial peptides are rather complex. As a consequence, the development of another natural antibacterial agent as an alternative is highly desirable.

It is well known that the soluble silk glue protein sericin is usually discarded with wastewater in the process of obtaining silk fibroin (SF). But sericin has been proven to have high hydrophobicity and antioxidant, anticancer, and UV-light protecting effects, as well as good antibacterial activity. Sericin is made up of 18 types of amino acids, of which the most abundant are serine (Ser), as-
partic acid (Asp), and glycine (Gly) at 32.3%, 14.5%, and 13.9%, respectively (3). To date, sericin has been used in the fields of food, cosmetics (4), textiles (5), and biomaterials (6–8). However, we still know little about the interaction between sericin and bacteria, and it is believed that sericin has good antibacterial properties. Hence, it is time to further investigate the antibacterial properties of sericin.

Related studies on sericin have been done since 2002 when Zhang (9) reported that sericin protein was antibacterial and could be cross-linked, copolymerized, and blended with other macromolecular material to produce materials with improved properties. Then in 2003, Sarovart et al. (10) prepared an antimicrobial polyester fiber for treatment of polluted air by coating the surface with sericin. In 2009, Senakoon et al. (11) reported the antibacterial actions of eri sericin at the cellular level in E. coli and S. aureus bacteria models. In 2010, Jassim et al. (12) found that cotton fabric coated with a 2% sericin solution exhibited 5 times the tensile strength of an untreated sample, and the zones of inhibition in the diameters of growing Micrococcus luteus reached 9 and 12 mm at concentrations of 10 and 20 g/liter sericin, respectively. Although the antibacterial activity of sericin has been mentioned or discussed previously, to the best of our knowledge, the specific relationship between sericin and bacteria causing concrete shape changes and the interaction mechanism have not been elucidated clearly.

In this work, to analyze the morphological and structural changes in bacterial cells resulting from the interaction between sericin and E. coli and to clarify the interaction mechanism, sericin proteins (40 to 200 kDa) of various concentrations were used as antibacterial agents to treat E. coli cells. The antibacterial activity of sericin against E. coli was first detected by counts of viable cell colonies on agar plates. The shape and size changes of E. coli cells treated with sericin molecules were then observed by an inverted fluorescence microscope (IFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The membrane permeability of E. coli cells was investigated by changes in electrical conductivity and total sugar concentrations. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses of sericin-treated bacteria were carried out to clarify the effect of sericin on protein expression. The expression indicates that, depending on concentrations and interaction times, sericin molecules inhibit E. coli growth by destroying membrane integrity and blocking protein expression in bacteria. Furthermore, due to its excellent hydrophilicity and soft/wet properties, a sericin-based hydrogel has been used to treat minor burns and other skin wounds and to improve skin healing. To further confirm the action of sericin molecules, sericin was incorporated into a traditional hydrogel, which was then used as a wound dressing for the examination of wound closure and histopathology.

This work reveals the process of the interaction between E. coli and sericin, shows the relationship between the shape and membrane integrity of E. coli cells and sericin at various concentrations, and also confirms the beneficial effects of sericin in wound dressing and skin regeneration, which are promising for future applications in clinical medicine and the textile, food, and coating industries.

**MATERIALS AND METHODS**

**Materials.** Sericin (40 to 200 kDa) was purchased from Huzhou XinTiansi Bio-tech Co., Ltd., China. Escherichia coli (ATCC 25922) was a gift from the School of Textiles, Tianjin Polytechnic University. Nutrient agar was obtained from Beijing Aoboxing Bio-tech Co., Ltd. N-Isopropylacrylamide (NIPAm) and N,N’-methylene-bis-acrylamide (MBA) were supplied by Tokyo Chemical Industry Co., Japan, and Tianjin Kemel Chemical Reagent Co., Ltd., respectively. Oxidant ammonium persulfate (APS) and reductant N, N’, N’-tetramethylenediamine (TEMED) were obtained from Tianjin Fengchuan Chemical Reagent Technology Co., Ltd., and Shanghai Chemical Reagent Co., Ltd., respectively. The reagents, bovine serum albumin, prestained marker (14.4 to 97.4 kDa), SDS, glycine (Gly), Tris, and Coomassie brilliant blue (R-250), used in the SDS-PAGE assay were purchased from Beijing Solarbio Co., Ltd., China. All water used in the whole experiment was of Millipore Milli-Q grade (Chongqing Molecular Water System Co. Ltd., Chongqing, China). All of the culture media were sterilized with autoclaving before use.

**Preparation of sericin solution and sericin-based hydrogels.** For the measurement of antibacterial activity, sericin powder was dissolved in 10 ml of sterile distilled water until a transparent yellow solution was obtained. Here, the mass concentrations of sericin were controlled at 10, 20, and 40 g/liter.

According to previous reports (8), the chemical cross-linked sericin-poly(NIPam-MBA) hydrogels were prepared in aqueous solution under the oxidation reduction reaction of APS (0.01 g)-TEMED (100 μl). The percentage of 100% · MBA-NIPam was controlled at 10 wt%. The mass ratios of sericin-(NIPam plus sericin) are 0 and 0.20, and the resultant hydrogels were named HSC00 and HSC20, respectively. For future wound healing experiments and histological examination, all hydrogel samples were UV sterilized after immersion in 75% ethanol for 30 min and washing with phosphate-buffered saline (PBS) (pH 7.2 to 7.4).

**Cultivation of E. coli.** A representative bacterial colony was picked off with a loop and placed in nutrient broth before incubation at 37°C for 12 h. The cultures of E. coli containing ~10^7 CFU/ml were prepared by dilution with sterile nutrient broth and used for further measurement. To avoid the error, the experiment was repeated three times to reevaluate the concentration effect of sericin on the E. coli cells.

**Serum bactericidal assay.** First, 0.1, 0.2, and 0.4 g sericin powder, respectively, was added to 10 g nutrient agar for homogenous mixing. The above mixture solutions were then solidified and slowly poured into sterilized petri dishes to avoid the generation of bubbles. Finally, the plates were subsequently incubated at 37°C for 24 h, and the numbers of viable cells (colonies) were counted manually.

The antibacterial activity against E. coli was expressed in terms of the percentage of bacterial reduction after contact with sericin for 24 h. The percentage of reduction was calculated by equation 1,

\[
R(\%) = \left[\frac{A - B}{A} \times 100\%\right]
\]

where R is the percentage of bacterial reduction and B and A are the surviving bacterial cells (CFU per milliliter) on the sericin-treated and control plates, respectively.

**Inverted fluorescence microscope.** The E. coli cell distribution and cell morphology were acquired by using an inverted fluorescence microscope (IFM). After being treated with 40 g/liter sericin for 12 h, E. coli cells were washed with PBS and stained with acridine orange for 5 min. The excess liquid around the cover glass was removed by filter paper.

**Scanning electron microscopy.** E. coli cells in exponential-phase cultures were incubated with sericin at 37°C and shaken at 200 rpm for 12 h. After centrifugation for 5 min at 3,000 rpm, the resulting pellet was washed twice with sterile saline water and then resuspended in 4 wt% glutaraldehyde for 12 h at 25°C. Stepwise ethanol dehydration with various concentrations of 30, 50, 70, 80, and 90% was performed for 10 min in turn. After centrifugation again, the precipitates were dehydrated by glacial acetic acid and then washed twice with sterile saline water and then resuspended in 4 wt% glutaraldehyde for 12 h at 25°C. Stepwise ethanol dehydration with various concentrations of 30, 50, 70, 80, and 90% was performed for 10 min in turn. After centrifugation again, the precipitates were dehydrated by glacial acetic acid and then washed twice with sterile saline water. Finally, the pellets were embedded in Spurr’s and cured at 60°C for 24 h.

The freeze-dried samples coated with gold vapor were observed by scanning electron microscopy (SEM) (S-4800; Hitachi Ltd., Tokyo, Japan). A cell...
The wounded skin was collected, and wound tissue samples were fixed in a 4% formaldehyde solution. Then the samples were embedded in paraffin, and tissue sections of 4- to 7-μm thickness were cut and positioned on a glass slide, followed by staining using hematoxylin and eosin (H&E). The tissues were visualized using fluorescence microscopy with specific image analysis software from Nikon.

**RESULTS AND DISCUSSION**

**Antibacterial action of sericin at different concentrations.** The reductions in bacteria were detected by counts of viable cell colonies on agar plates. Figure 1 shows the antibacterial activity of sericin against *E. coli* after 24 h of incubation at 37°C. The percentage of *E. coli* reduction with 10 g/liter sericin is about 62% and exceeds 93% in the presence of 40 g/liter sericin. This preliminary result indicates that sericin at higher concentrations is effective in inhibiting *E. coli* proliferation. The tendency is also consistent for the surviving bacterial cells. As shown in the upper left corner of Fig. 1, the no-treatment control shows the maximum number of CFU, which is significantly decreased by treatment with various sericin concentrations from 10 to 40 g/liter. The antibacterial activity of sericin can also be seen in Fig. S1 in the supplemental material. In fact, Senakoon et al. (11) have reported that the Gram-negative bacterium *E. coli* was more susceptible to the 90-min Na2CO3-degummed eri sericin with an initial effective dose of 30 μg, while the effective dose for the 60-min water-degummed eri sericin was 40 μg. They attributed this to the evolutionarily distinct cell wall and/or cell membrane characteristics. On the one hand, Gram-negative bacteria possess an outer membrane outside the peptidoglycan layer that is lacking in Gram-positive organisms. On the other hand, the cell walls of Gram-positive bacteria are thick and strong, while the cell walls of Gram-negative bacteria are thin and loose. Both characteristics give rise to various antimicrobial properties. However, it was found that the increase in the time of incubation at 37°C to more than 3 days accelerates bacterial growth to a certain degree, which will be discussed in another article. Here, to further verify the interaction between sericin and *E. coli*, the morphology and integrity of cell membranes were investigated as follows.

**Observation of shapes and analysis of *E. coli* cells treated with sericin molecules.** To analyze the effect of sericin on the suspension of *E. coli* without the addition of sericin was used as the control.

**Transmission electron microscopy.** Samples containing *E. coli* in nutrient broth were incubated for up to 12 h. Then, culture medium was centrifuged, and the yellowish precipitate was fixed by 2.5% glutaraldehyde (wt/vol). A glutaraldehyde droplet containing the *E. coli* was deposited onto a carbon-coated grid before negative staining with 2% (wt/vol) phosphotungstic acid. The dried grids were observed with transmission electron microscopy (TEM) (H-7650; Hitachi Ltd.). The control experiment was conducted without sericin.

**Bacterial conductivity assay.** *E. coli* cells during a period of logarithmic increasing were washed three times with sterile saline and then diluted in fresh medium to a concentration of 10⁷ CFU/ml. Aliquots of 10 ml of bacterial suspensions were mixed with bacterial suspensions, and the bacterial electrical conductivity was measured every 1 h by using a conductivity meter. Sericin was replaced by sterile water as the control, and the bacterial electrical conductivity was measured every 1 h. Parallel experiments were performed in triplicate, and the average results were used for more accuracy and to minimize possible manual errors.

**Determination of total soluble sugar concentrations in *E. coli*.** To detect the leakage of total sugars through membranes, *E. coli* bacteria and 5-ml aliquots of sericin at different concentrations of 10, 20, and 40 g/liter were mixed in equal volumes. The cultures were incubated at 37 ± 1°C under continuous shaking at 200 rpm, and the cell suspension was used for growing and maintaining the bacterial cultures. The mixture solution treated with sericin for 0, 4, 8, and 12 h was centrifuged at 8,000 rpm. The supernatant liquid was diluted 5-fold. Total sugar concentrations were determined by the anthrone-sulfuric acid method. Control experiments were conducted without sericin.

**SDS-PAGE of whole-cell proteins.** The bacterial pellet was used for SDS-PAGE, and the samples were first boiled before being loaded on the gel. Samples containing *E. coli* in nutrient broth were incubated with 40 g/liter sericin at 37°C. After incubation for 0, 4, 8, and 12 h, 4-ml aliquots were withdrawn, followed by centrifugation at 200 rpm before the supernatant was discarded. Then 50 μl of distilled water and 200 μl of sample buffer were added. The samples were heated at 100°C for 5 min and cooled on ice, and 20 μl of the lysed cell product was run per lane on a 10% SDS gel at 120 V. The sediment was subjected to SDS-PAGE according to the method of Laemmli (13) by means of a vertical gel electrophoresis system (Beijing Liumei Instrument Factory, China). The SDS-PAGE was implemented with a 4% stacking gel and a 12.5% separating gel followed by Coomassie brilliant blue (R-250) staining. A control experiment was run without sericin.

**Wound healing test.** A rat model was used to evaluate the wound healing function of sericin-poly(NIPam-MBA) (HSC) hydrogels. Healthy male Sprague-Dawley rats purchased from the Department of Experimental Animals were fed in a controlled environment with constant temperature (25°C) and humidity (50%) for 1 week with food and water available. The dorsal hair of rats was removed by an electric clipper, and then two full-thickness round skin patches (1 cm in diameter) were removed on the upper back area of each rat. Before being wrapped by a hypoallergenic elastic adhesive bandage, one wound on each rat was immediately covered by sterilized HSC hydrogel. Untreated wounds were covered with sterile gauze and served as negative controls. To avoid unexpected problems, the test rats were placed in individual cages. The rats were euthanized 10 days after wounding. The wounded skin was collected, and wound tissue samples were fixed in 4% formaldehyde solution. Then the samples were embedded in paraffin, and tissue sections of 4- to 7-μm thickness were cut and positioned on a glass slide, followed by staining using hematoxylin and eosin (H&E). The tissues were visualized using fluorescence microscopy with specific image analysis software from Nikon.
shape of \( E. coli \) cells, IFM, SEM, and TEM measurements were obtained after treatment of bacterial samples with sericin. Acridine orange, a fluorescent dye, can pass through the normal cell membrane and then combine with DNA or RNA (14). After being dyed with acridine orange, the \( E. coli \) cells showing green or yellow-green colors are the live bacteria. As shown in Fig. 2, the dyed \( E. coli \) cells are rod shaped and green in the IFM image, indicating that they are all alive, but compared to the control cells, the majority of the green matter has disappeared. This suggests that the introduction of sericin molecules at least influenced the growth of \( E. coli \) cells. However, it is hard to clearly see the differences in shapes between the control and \( E. coli \) cells treated with sericin molecules just by using the IFM. Thus, SEM and TEM were employed to further study the interactions between \( E. coli \) cells and sericin molecules.

Figure 3 shows the SEM images of \( E. coli \) cells incubated in Luria-Bertani (LB) medium for 12 h with or without the presence of sericin. As shown in Fig. 3A, the pure \( E. coli \) cells present smooth surfaces and rod shapes with an approximate size of 0.5 by 1.5 \( \mu m \), which is in good agreement with the results of Lee et al. (15). With increases in treatment time from 0 to 12 h, a type of vesicle from the membrane surfaces of \( E. coli \) cells can be found. After treatment with sericin for 4 h, only small quantities of vesicles can be found on the SEM images. However, after treatment for 12 h, as shown in Fig. 3B, C, and D, the membrane surfaces of \( E. coli \) cells have large amounts of vesicles. Based on these results, it can be concluded that there is some interaction between sericin molecules and \( E. coli \) cells. There are two possibilities. The first possibility is the direct adhesion of sericin molecules on the surface of \( E. coli \) cells. The effective diameters of sericin molecules determined by dynamic laser scattering (DLS) are just 536 nm (see Fig. S2 in the supplemental material), which makes it easy for single or assembled sericin molecules to be attached to the surfaces of \( E. coli \) cells. In our previous work, energy-dispersive X-ray (EDX) analysis was adopted to study the contents of the vesicles. It was found that the weight percentages of carbon, nitrogen, and oxygen elements increased to 2.27, 1.89, and 1.05 times those on the blank cover glass (16). However, it was reported that the isoelectric point of...
silk sericin is 3.8 to 4.5 (17), and the sericin molecules are negatively charged as the pH value of the external environment is about 7.2. However, the majority of the bacterial surface is also negatively charged under physiological conditions (18). In general, the positively charged particles will attract bacteria, and the negatively charged particles will repel bacteria.

The second possibility is the antibiotic action on the E. coli cell membrane due to treatment with sericin molecules. Vesicles called blebs are produced, which is a typical mode of action of antimicrobial peptides/proteins. In other words, the treatment with sericin seemed to induce membrane blebbing of E. coli cells. This point was mentioned by Alves et al. (19) and Hayden and Ades (20), and in 1973, Scheie and Ehrenspeck (21) proposed two reasonable mechanisms to account for the bleb formation. One is a ballooning of the outer membrane as it is stretched or unfolded in response to pressure from within, and the other is unbalanced synthesis because of various treatments. Although the synthesis conditions and reaction materials are completely different in our system, the pressure change during the treatment provides a reasonable explanation.

To further study the interaction between sericin and E. coli, TEM was performed (22). Figure 4 shows the TEM images of E. coli cells treated with 40 g/liter sericin for up to 12 h. As seen from Fig. 4a, untreated E. coli cells display smooth and compact surfaces, and the width of the cells is just 0.7 μm. No release of intracellular components and no notable ruptures or pores on the cell surface can be seen. Also, the cellular contents are evenly distributed with a closely integrated membrane. However, as shown in Fig. 4b, after exposure to 40 g/liter sericin for 12 h, the surface of the E. coli cell is covered by obvious vesicles with a thickness of about 200 to 500 nm. It is noteworthy that the cytoplasm shrinkage phenomenon was accompanied by the blurring of the mem-

![FIG 4 TEM images of E. coli cells treated with 40 g/liter sericin for up to 12 h. (a) Untreated E. coli; (b) E. coli treated with sericin for 12 h. Magnification, ×15,000.](http://aem.asm.org/)

![FIG 5 Changes in electric conductivity for E. coli treated with sericin for 8 h.](http://aem.asm.org/)

![FIG 6 Leakage of reducing sugars from E. coli cells treated with various concentrations of sericin. Data from duplicate experiments are averaged. Error bars represent standard deviations of duplicate incubations.](http://aem.asm.org/)
brane wall boundaries. This confirms that sericin molecules containing Ser, Asp, and Gly might induce membrane blebbing of *E. coli* cells after incubation for 12 h. The results of this interaction suggest that, under the induced action of sericin molecules, the normal structure of the cell membrane was distorted and damaged. In other words, the outer appearance of the bacterial cells was barely changed, but leakage of the intracellular contents still occurred. TEM images hint that sericin molecules might induce bacterial cell distortion, as well as deformation.

**Integrity of *E. coli* cell membranes treated with sericin molecules.** Regarding growth of the bacteria, it is known that electrolytes such as organic and amino acids are produced with the digestion of proteins (23). As the cell membrane was affected by electrostatic interactions with sericin, extracellular electrolytes are also changed, leading to different conductivities during the whole treatment period. Figure 5 shows the electrical conductivities of *E. coli* cells treated with sericin for 8 h. It can be seen that with increases in the incubation time from 0 to 8 h, the electrical conductivity of the bacterial medium increased for both the sericin-treated and control groups, which can be attributed to the metabolism of the microorganisms. Generally, the components of the medium are not charged or weakly charged. However, electrically inert substances (carbohydrates, lipids, and proteins) in the medium can be converted into electroactive substances by metabolism of microorganisms. Accordingly, with the growth of microbes, electroactive molecules and ions are gradually replaced by electrically inert ion molecules, resulting in enhanced conductivity of the medium.

In terms of increasing rate, the electrical conductivity increases quickly at 2 h initially and then exhibits slow growth. At 2 h, the electrical conductivities of the bacterial cultures treated with 10,
20, and 40 g/liter sericin significantly increase from 2.64, 2.59, and 2.59 mS/cm to 3.01, 2.96, and 2.94 mS/cm, respectively, while at 8 h, the electrical conductivities only increase to 3.32, 3.18, and 3.17 mS/cm, respectively. Thus, it can be concluded that the metabolic action of sericin on *E. coli* mainly occurs at 2 h. Furthermore, compared to the conductivity of the control, the conductivities of medium solutions treated with 10, 20, and 40 g/liter of sericin are lower. In general, damage to the cell membrane leads to ion leakage and increases in the electrical conductivity of the bacterial culture. It can be found that, with an increase in the sericin concentration from 10 to 40 g/liter, the corresponding conductivity decreases, which suggests that sericin molecules can hamper ion exchange. The decrease in bacterial conductivity can be ascribed to the induced action between sericin molecules containing Ser, Asp, and Gly and the cell membranes of *E. coli* by electrostatic interaction. As a result, the charged ions are reduced by the introduction of sericin, and then the cell membrane permeability decreases and metabolism is partially blocked.

Furthermore, it is well known that carbohydrates are the primary source of carbon and energy substances for microbes. When bacteria are in the normal physiological state, external nutrients will be absorbed for bacterial proliferation. However, because the membrane was affected by its electrostatic interaction with sericin, cell contents like sugars will be leaked from bacterial cytoplasm. Then, by measuring changes in the sugar concentrations in the *E. coli* cultures, the integrity of bacterial membranes was analyzed and judged.

Figure 6 shows the amount of leakage of total sugars from *E. coli* cells treated with sericin. The value for the control group is approximately 24.89 µg/ml, the amount of leakage detected from the bacterial cells at the start time, while the amount of leakage of total sugars from cells treated with 40 g/liter of sericin is 34.45 µg/ml. The sugar initially was detected because of the carbohydrate in the medium. With an increase in the treatment time from 0 to 12 h, the amounts of leakage of total sugars for *E. coli* cells treated with sericin increase. It is clear that after treatment with 40 g/liter of sericin for 12 h, the amount of leakage of total sugars went up to 65.87 µg/ml, which is 1.60 times those of the control and 10-g/liter sericin groups. This suggests that with interaction for a certain time or an increase in the sericin concentration to >20 g/liter, sericin molecules accelerate the membrane leakage of total sugars from the bacterial cytoplasm. In fact, Li et al. (24) pointed out that the turbulence in membranous integrity for leakage of reducing sugars and proteins in cells was an important factor for inhibition of the bacterial growth. Therefore, it is reasonable to assume that sericin molecules might disturb the normal function of cell membranes with interaction for a long time. This result is also consistent with the results of electrical conductivity as shown in Fig. 5.

**Protein expression of *E. coli* treated with sericin.** The bacterial protein profiles are a reflection of the genome of the strain, and, therefore, determination of the whole protein content plays an important role in the comparative studies of bacteria (25). To further confirm the permeabilizing action of sericin, *E. coli* cells were analyzed for soluble proteins by SDS-PAGE. As shown in Fig. 7 (lane 1), the band for all molecular mass proteins was slightly shallow, and the other bands gradually became pale or even disappeared in the test group after 12 h (Fig. 7, lanes 2, 3, and 4). From the figure, it can be concluded that *E. coli* cells grew for 0 to 4 h and then were inhibited by sericin molecules. On the one hand, sericin molecules may block the expression of bacterial protein. On the other hand, considering that sericin molecules accelerated the leakage of total sugars from the bacterial cytoplasm, it was suggested that sericin reduces the content of cellular soluble proteins.

**Interaction mechanism between sericin molecules and *E. coli* cells.** Figure 8 shows the analysis of the interaction between sericin and *E. coli*. It is well known that in the outer membrane of Gram-negative bacteria, porins are the main channel for external molecules moving in and out of the cell body but generally allow molecules of less than 700 Da (26). For almost all of the particles, passage through the cell membrane will be limited by size. As discussed for Fig. 2 to 4, with the increase in treatment time from 0 h to 12 h or in the sericin concentration from 10 to 40 g/liter, more and more sericin molecules were attached to the surface of the *E. coli* cells. Neal (27) suggested that nanoparticles of 1 to 9 nm theoretically can be transported into the cell by porin GspD. By taking into account the molecular mass (40 to 200 kDa) and particle size (536 nm), it is natural to assume that sericin molecules cannot directly enter the cells but induce changes in the outer membrane of *E. coli* cells. Therefore, the mode of action of sericin may be described as antibiotic action on the *E. coli* cell membrane due to treatment with sericin molecules.

Here, bacteria decrease their liquidity and the semipermeabil-
ity of biological membranes, resulting in alterations in the conductivity of the culture medium, as shown in Fig. 5. Furthermore, a break in the permeability of the outer membrane causes the leakage of cellular materials (sugars and proteins), which also affects the extra- and intracellular pressures and cytoplasm shrinkage. Simultaneously, the intracellular environment homeostasis is disrupted. Cell membranes and various enzymes can be induced to lose their intrinsic functions, thus leading to cell death. Therefore, it may be inferred that turbulence in membranous integrity is an important factor for inhibition of the bacterial growth. However, it is still a mystery where the damage takes place, i.e., on the lipopolysaccharide or membrane proteins in the outer membrane, which will be discussed in a future study.

**In vivo wound healing effects and histological examination.** Figure 9 shows macroscopic photographs and closure of wounds treated with HSC00 and HSC20 hydrogels and sterile gauze as the control. In terms of the sericin-poly(NIPAm-MBA) hydrogel, the wound exudates are absorbed, which is beneficial for reducing the risk of dehydration. More importantly, the hydrogels are easily stripped off without any damage to the wound, avoiding secondary harm. In comparison with that covered with sterile gauze alone, the wound area covered with hydrogels decreased significantly. On day 7, as shown in Fig. 9b, the wound treated with gauze is 1.7 times larger than that treated with sericin-poly(NIPAm-MBA) hydrogel. On day 15, the wounds treated with hydrogels were almost completely healed compared with the control. Considering the whole healing process, the use of soft and wet hydrogels can accelerate wound closure and decrease the possibility of infection and death. In fact, Madaghiele et al. (28) reported that hydrogels can absorb and retain wound exudates; in particular, the tight mesh size of hydrogels prevents bacteria from reaching the wound. Conversely, compared to the wound treated with pure poly(NIPAm-MBA) hydrogel, the wound treated with sericin-poly(NIPAm-MBA) hydrogel shows a faster healing rate on day 3. This indicates that sericin molecules in the hydrogel matrix accelerate cell proliferation, migration, and angiogenesis.

Figure 10 shows the histopathological changes in skin samples collected from rats euthanized on day 10 postoperatively. It is seen that after wounds were treated with hydrogel and gauze, no signs of necrotic tissue and infiltrated inflammatory cells were observed. Compared to the control, wounds covered with HSC hydrogels show significant granulation tissue and neovessels on day 10. In particular, in the sericin-poly(NIPAm-MBA) group, the introduction of sericin molecules accelerates fibroblast proliferation and the formation of neovessels and improves the healing of the wounds. Therefore, it is thought that the sericin-based hydrogel accelerates wound healing compared to that with the gauze and
pure poly(NIPAm-MBA) hydrogel, making it a potential candidate for wound dressing and skin regeneration in the future.

In conclusion, sericin solutions with concentrations from 10 to 40 g/liter were used to investigate antibacterial action against *E. coli*. The percentage of *E. coli* reduction with 10 g/liter of sericin is about 62%, while it exceeds 93% in the presence of 40 g/liter sericin. After being dyed with acridine orange, the *E. coli* cells present rod shapes and green color, but the number of *E. coli* cells decreases after treatment with sericin. Pure *E. coli* cells present smooth surfaces and rod shapes with an approximate size of 0.5 μm. With an increase in the sericin concentration from 10 to 40 g/liter, blebs are caused by the antibiotic action on the *E. coli* cell membrane due to treatment with sericin molecules. This is a typical mode of action of antimicrobial peptides/proteins. In other words, the treatment with sericin seemed to induce membrane blebbing of *E. coli* cells. After exposure to 40 g/liter sericin for 12 h, the surface of *E. coli* cells was obviously covered by vesicles with a thickness of about 200 to 500 nm. The metabolism of action of sericin on *E. coli* mainly occurs at 2 h. Higher concentrations of sericin decrease the electrical conductivity of *E. coli* cells. The release of intracellular components (sugars and proteins) by *E. coli* cells quickly increased, destroying the integrity of the bacterial cell membranes. The amount of total sugar leakage after treatment with 40 g/liter sericin is 1.60 times that for the control. Furthermore, sericin molecules may block the expression of bacterial protein and the functions of *E. coli* cell biomembranes disturbed by sericin deposited on the bacterial surface. Compared to the gauze, the prepared sericin-based hydrogel accelerates wound healing, showing promising use in clinical medicine for wound dressings or as a regenerative template.

ACKNOWLEDGMENTS

This work has been supported by the National Nature Science Foundation of China (21104058, 21134004, 31200719, and 21174103), the State Scholarship Fund of China Scholarship Council (201508120037), grants from the Applied Basic Research and Advanced Technology Programs of Science and Technology Commission Foundation of Tianjin (12JCQNJC01400 and 15JCYBIC18300), the Science and Technology Correspondent of Tianjin (14JCTPJC00502 and 15JCPJC62200), and the National Training Programs of Innovation and Entrepreneurship for Undergraduates (201510058005 and 201510058051).

FUNDING INFORMATION

This work, including the efforts of Qingsong Zhang, was funded by State Scholarship Fund of China (201508120037). Applied Basic Research and Advanced Technology Programs of Science and Technology Commission Foundation of Tianjin provided funding to Qingsong Zhang under grant numbers 12JCQNJC01400 and 15JCYBIC18300. This work, including the efforts of Pengfei Liu, was funded by Science and Technology Correspondent of Tianjin (14JCTPJC00502 and 15JCPJC62200). This work, including the efforts of Huazhen Qin, was funded by National Training Programs of Innovation and Entrepreneurship for Undergraduates (201510058005 and 201510058051). This work, including the efforts of Qingsong Zhang, was funded by National Nature Science Foundation of China (NSFC) (21104058, 21134004, 31200719, and 21174103).

REFERENCES


