Silk sericin (SS), a group of glue proteins produced in the middle silk gland of the silkworm, is an essential component of the cocoon filament. It is comprised of granular and high molecular weight proteins with adhesive and gelatin-like characteristics.¹ Our previous study demonstrated that SS can promote reduction in wound size and increase collagen production in rats without causing any allergic reactions.² Several studies also demonstrated the advantages of using SS in wound healing by enhancing the attachment and growth of mouse fibroblasts, human skin fibroblasts, and human and mouse hybridoma in culture media.³⁻⁵ However, there are several reports on immune response that argue against silk sutures containing SS proteins⁶⁻⁸ and assert that the SS proteins are responsible for skin irritation and allergies.⁹ The fact that SS shows no antibacterial activity may also make it less attractive for wound regeneration.

Wound healing is a dynamic process that involves the integrated action of many cell types, the extracellular matrix, and chemical mediators. Wound repair can be divided into three overlapping stages: inflammation, formation
of new tissue, and tissue remodeling. The initial inflammatory phase is characterized by localized activation of innate immune mechanisms. This results in an initial influx of neutrophilic granulocytes into damaged tissue followed by an accumulation of macrophages. An infection that occurs during this stage will impede the regeneration process. The impact of local inflammatory responses on the wound healing process has been debated for decades. Recently, Eming et al demonstrated that the number of macrophages infiltrating the wound tissue mediates accelerated tissue repair and thus is significant. The healing process is characterized by macrophages and fibroblasts initiating repair and deposition of new fibrous tissue above and below the dermal substitutes, which envelopes them under an epidermal layer. Collagen production, an important factor for wound healing, is subsequently stimulated. New blood vessels and capillaries are normally observed within the dermal substitutes and new fibrous tissue is generated beneath the dermal substitutes.

Severe injury of the skin can lead to sepsis, a major cause of high mortality in patients with burns. Various treatments have been used to prevent and control sepsis, but the most successful is the use of topical silver sulfadiazine cream (SSD). Advantages of silver are its broad antimicrobial activities against gram-negative and gram-positive bacteria and minimal development of bacterial resistance. Microscopic evaluation of SSD-treated wounds shows that SSD has the potential to preserve viable dermal tissue. However, epidermal regeneration takes place at a rather slow rate under an easily disintegrating crust, and the newly formed epidermis temporarily has an “irritated” aspect characterized by spongiosis, parakeratosis, and pseudocarcinomatosis. SSD can also induce hemolytic anemia in glucose-6-phosphate dehydrogenase-deficient burn patients, transient leukopenia, which occurs in 3%–60% of patients, and severe hypersensitivity reactions.

Sericin, in combination with SSD cream, may combine the beneficial effect of collagen promotion with antimicrobial properties. However, the silver ion can bind to proteins, including human serum albumin or other substances such as bilirubin, which may inhibit its activity. During the acute inflammatory response, normally there is an increase in the acute-phase proteins, which is usually mediated by proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) or interleukin (e.g., IL-1β). An increase in the amount of these proteins is usually associated with the induction of anorexia, weight loss, sepsis-induced proteolysis, etc. Since both SS and SSD can activate cytokine production, which in turn may affect the inflammatory reaction, it is important to monitor the inflammatory mediators induced by SS cream and compare the levels of these mediators to the levels induced by SS in combination with SSD cream during the healing process.

The aim of this study was to investigate the effectiveness of SS cream when compared to SS in combination with SSD cream by monitoring wound size reduction and the levels of the inflammatory mediators (TNF-α and IL-1β) induced during wound healing in rats.

Materials and Methods
Preparation of silk sericin and silk sericin cream. Fresh cocoons of Bombyx mori were kindly supplied by Chul Thai Silk (Petchaboon province, Thailand). Silkworm cocoons were produced in a controlled environment. After cutting cocoons into pieces (about 5 mm²), SS was extracted with purified water (1-g of dry silk cocoon: 30-mL of water) by autoclaving (SS-320, Tomy Seiko, Tokyo, Japan) at 120°C for 60 minutes using a high temperature and pressure degumming technique. After the membrane was filtered to remove fibroin (a fibrous protein from silkworm), SS powder was obtained by freezing and lyophilizing the SS solution using a Heto LL 3000 lyophilizer (Allrod, Denmark). SS cream was formulated using the combination of white petrolatum, mineral oil, lanolin, glycerin, bisabolol, propylparaben, and methylparaben. Due to the limited solubility of SS, the final concentration of SS in cream was 8% w/w. All chemicals were purchased from Sigma (Singapore) and used without further purification. SSD powder is a commercial product and was purchased from Chemie Trade (Mumbai, India). It was formulated using the same cream base as the SS cream. Sericin with SSD cream was prepared by adding SS and SSD into the cream base using the same formula as other preparations.

Animals. Eight-week-old male Sprague-Dawley rats, purchased from National Laboratory Animal Center (Mahidol University, Thailand) weighing 250 g ± 5 g were used for these experiments. Each rat was caged alone at 25°C ± 2°C and subjected to a 12:12-hour light-dark cycle (standard fluorescent light) and had access to food and water ad libitum. Animals were acclimatized for 1 week before experimental use. The animals were maintained according to the Guide for the Care and Use of Laboratory Animals established by the National...
Laboratory Animal Center (Mahidol University, Thailand).

**Inhibitory effect of silk sericin on silver sulfadiazine.** Antibacterial assays of SS solution were performed using a filter disk method. Filter disks (diameter 6 mm) made from AA grade filter paper (Whatman, Maidstone, England) were placed on nutrient agar plates seeded with various strains of bacteria, including *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Micrococcus luteus*. SS solutions, 2-1 of 1%, 5%, and 8% were pipetted onto the center of each of the test disks. The organisms were incubated at 37°C ± 0.2°C for 24 hours.

The antibacterial activities of various concentrations of SS cream, 1% w/w SSD cream, and various concentrations of SS in combination with 1% SSD cream were evaluated against the previously mentioned five strains of aerobic bacteria using the standard cup-plate method. Nutrient agar medium was used for culturing and the bacteria were incubated for 24 hours at 37°C ± 0.2°C. Inhibition zone diameters were measured using a zone reader.

**In-vivo animal tests.** Forty-five rats were divided into three groups of 15. The skin over the dorsal area was shaved completely; application fields were outlined with a marking pen. Two full-thickness skin wounds were prepared by making (1.5 cm × 1.5 cm) on the left and right side of the dorsum of each rat at least 3 cm apart to ensure no interference between each wound. Then, each wound was exposed to water at 90°C for 7 seconds in order to produce a full-thickness burn.
bation, each well was again washed five times with buffer (400 µL) and 100-µL of substrate solution was added. The plates were incubated for 30 minutes at room temperature, and then 100-µL of stop solution was added. The reaction was measured quantitatively at 570 nm based on a standard curve of TNF-α and IL-1β.

**Statistical Analysis**

All data were expressed as mean ± SD. Evaluation of statistical significance was determined by paired and unpaired Student’s t-test; P < 0.05 was considered significant.

**Results**

**Inhibitory effect of silk sericin on silver sulfadiazine.** Sericin solution extracted by the high temperature and pressure technique showed no inhibitory effect against any of the bacteria tested, while SSD cream inhibited all strains of bacteria even at the low concentration of 0.25% w/w (data not shown). SS in combination with SSD cream showed an antimicrobial effect but at a lower degree of inhibition than SSD cream alone, as shown by the smaller inhibition zone (Table 1). This indicates that the microbial inhibition effect of SS in combination with SSD cream came only from SSD. The cream containing 1% SSD in combination with 1% SS inhibited the test bacteria better than cream containing 1% SSD in combination with 8% SS, as indicated by larger clear zones in inhibition disk assays (Table 1). There was a significant difference in the size of the clear zone when comparing 1% SSD cream, 1% SSD in combination with 1% SS cream, and 1% SSD in combination with 8% SS cream for all of the test organisms.

**Wound measurement.** Size reductions of the rat wounds treated with cream base, SS cream, SSD cream, and SSD in combination with SS cream are shown in Table 2. Compared to the cream base-treated wounds, the SS-treated wounds showed a significant difference in wound size on days 3 through 15. Wounds treated with 8% SS cream also showed a significant difference in size reduction when compared with wounds treated with 1% SSD for 7 days of treatment. However, 1% SSD in combination with 8% SS-treated wounds showed a significant difference in the size reduction only at the initial stage (day 3) when compared to wounds treated only with 8% SS cream. All wounds except the one treated with the cream

![Figure 1. The percent area fraction of collagen per field from all wounds treated with different creams on day 15.](image)

**Table 1.** Inhibition zone (mm) on agar plates treated with 1% SSD, 1% SSD + 1% SS, and 1% SSD + 8% SS cream (n = 6).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>1% SSD cream</th>
<th>1% SSD cream + 1% SS cream</th>
<th>1% SSD + 8% SS cream</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>18.7 ± 1.1*</td>
<td>13.1 ± 1.3*</td>
<td>11.9 ± 0.1*</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>12.3 ± 0.3*</td>
<td>11.8 ± 0.2*</td>
<td>10.8 ± 0.1*</td>
</tr>
<tr>
<td><em>M. luteus</em></td>
<td>25.3 ± 0.6*</td>
<td>20.1 ± 0.5*</td>
<td>19.3 ± 0.2*</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>14.0 ± 0.4*</td>
<td>12.6 ± 0.1*</td>
<td>11.6 ± 0.1*</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>11.8 ± 0.3*</td>
<td>11.7 ± 0.2*</td>
<td>10.7 ± 0.2*</td>
</tr>
</tbody>
</table>

*Indicates significant difference (P < 0.05).

**Table 2.** Percent reduction in size of rat wounds treated with cream base, SS cream, SSD cream, and SSD in combination with SS cream.

<table>
<thead>
<tr>
<th>% wound size reduction</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td></td>
<td>Cream base</td>
</tr>
<tr>
<td>Day 3</td>
<td>6.04 ± 3.12</td>
</tr>
<tr>
<td>Day 7</td>
<td>53.77 ± 5.49</td>
</tr>
<tr>
<td>Day 15</td>
<td>76.13 ± 5.20</td>
</tr>
</tbody>
</table>

*Indicates significant difference when compared within the group (P < 0.05).
Collagen content in wounds. Collagen content in wounds was assessed by the percent area fraction of collagen per field from all rats’ wounds treated with different creams on day 15. There was a significant difference in percent area fraction of collagen per field on day 15 between the cream base and the 8% SS-treated wounds, between the 8% SS-treated and the 1% SSD-treated wounds, and between the 1% SSD-treated and the 1% SSD in combination with 8% SS-treated wounds (Figure 1; \( P < 0.05 \)). However, the fraction of collagen per field showed no significant difference between the cream base and the 1% SSD treated wounds or between the 8% SS-treated wounds and the 1% SSD in combination with 8% SS-treated wounds. This indicates that mainly SS, as reported by the fraction of collagen per field, generates the amount of collagen in wounds.

The percent area fraction of collagen per field and the histological appearance from group 3 wounds treated with 8% SS and 1% SSD in combination with 8% SS on days 3, 7, and 15 are compared in Figure 2. The results indicate that there is no significant statistical difference in area fraction of collagen in wounds treated with SSD cream when compared to SSD in combination with SS cream on either days 3, 7, or 15.

TNF-\( \alpha \), IL-1\( \beta \) production from rat tissues. Figure 3 shows the amount of inflammatory cytokines generated from rat tissues after 7 days of treatment. There was no significant difference in IL-1\( \beta \) levels generated from wounds treated with either cream base, 8% SS cream, 1% SSD cream, or 1% SSD in combination with 8% SS cream. Within the same group, the 8% SS cream-treated wounds generated slightly lower levels of IL-1\( \beta \) compared to wounds treated with the other treatments. Regarding TNF-\( \alpha \) levels, again there was no significant difference in the TNF-\( \alpha \) levels generated from wounds treated with either cream base, 8% SS cream, 1% SSD cream, or 1% SSD in combination with 8% SS cream. Within the same group, the 8% SS cream-treated wounds generated lower TNF-\( \alpha \) levels compared to wounds treated with other treatments, but the level of change was not significant.

Discussion

Our previous study demonstrated that SS, a globular protein from silkworm, has biological activities associated with wound healing and good biocompatibility and degradability. Some of these biological activities may be due to the fact that most amino acids in SS have strongly polar side groups such as hydroxyl, carboxyl, and amino groups, which can covalently bind with each other or with active groups of other compounds. Zhang reported that SS also has antibacterial activity. However, the results of the present study indicate that this is not the
case and that SS solution at high concentrations (up to 8%) has a negative effect on the antibacterial activity across the five bacterial strains tested. This may be due to the protein extraction method used, as the extracted portion may not contain the fraction that confers antibacterial activity. Other researchers have reported that the antimicrobial property of SS was derived from a low molecular weight protein, seroin, from *B. mori*.[39-40] Seroin is not involved in silk fiber construction and/or coating, and may play a role in protecting silk against microbial degradation.[40] Zurovec et al.[39] reported that seroin polypeptides are present in silk as 22.5 kDa and 23 kDa molecules, and that these polypeptides are liberated from other silk proteins when silk components are dissolved. The procedure in the present study to prepare SS used high temperatures and pressures to dissolve the silk proteins may have caused the release of seroins and the loss of antimicrobial activity in the SS preparations. The molecular weight of SS extracted by high temperature and pressure methods similar to those employed here is in the range of 35 kDa–150 kDa,[41] which is consistent with seroin not being included in our SS preparations. Other SS extraction methods that contain seroin together with other lower molecular weight fractions should be used in future studies to better understand the effects of SS on wound healing, and may show dramatically different results, especially in its antimicrobial activity.

Silver has a long and intriguing history as an antibiotic, especially for burn wounds. The antimicrobial effect of silver and silver compounds is proportional to the bioactive silver ion (Ag⁺) released and its availability to interact with bacterial or fungal cell membranes.[42] Silver ion is biologically active and readily interacts with proteins, amino acid residues, free anions, and receptors. However, upon binding with other biological molecules, silver no longer retains its antimicrobial activity. Topical antimicrobial agents are thought to promote normal healing by protecting the wound from infection. Since SS can promote wound healing but lacks antimicrobial activity, combining SS with SSD cream may benefit the healing process. However, if silver molecules dominantly bind with SS, the silver compounds may be inactivated. The authors speculate that the decrease in antimicrobial activity at higher concentrations of SS in cream is due to SSD partially binding with SS. Zhao et al.[43] reported similar results showing that silver can interact with other proteins such as collagen and DNA, resulting in a controlled release property.[43] The binding of a bioactive silver ion with SS is possible because SS has a strong negative molecular charge. At low SS concentrations, more unbound and bioactive silver ions are available compared to what is available at high SS concentrations. Nevertheless, we found that SS (as high as 8% w/w) did not totally refrain the activity of SSD. Since the present study was conducted in vitro, similar studies should be performed in vivo to validate these findings.

SSD in combination with SS cream did not show better healing properties with respect to wound size reduction when compared to SS cream only. This may be due to the uninfected conditions of the wounds tested, as there was no need for wound healing improvement via the antimicrobial action of silver. A study, with a bacterial challenge to the wounds, should be attempted to determine the effects of SS with and without SSD in the presence of infection, and without SSD in the presence of infection. However, SS cream still reduced wound size when compared with the cream base and SSD cream alone. Although the cream-containing silver did not accelerate the healing process in the present study, antimicrobial agents will remain a necessity for daily burn wound care under non-sterile conditions.

The present results further indicate that SS promotes collagen production even in the presence of SSD. Lee et al.[44] reported a similar outcome; SSD did not inhibit collagen synthesis but improved dermal wound healing when coated onto a collagen membrane. SS in combination with SSD accelerated collagen deposition; however, there was not a significant reduction in wound size when compared to the other treatments. These data indicate that collagen is not the most important factor for wound healing, especially at the initial stage, which corresponds to the healing mechanism that collagen production normally occurs during formation of new tissue and tissue remodeling—the last stage of wound repair. Moreover, histological samples from wounds treated with 8% SS cream and 1% SSD in combination with 8% SS cream revealed closely packed, thick fibrils of collagen fibers and a collagen fibril organization with fibril bundles that were parallel to each other, which may indicate wound contraction and hypertrophic scar formation.[45-46] Further development for other forms of SS delivery, such as in a scaffold, may be beneficial to reducing wound contraction and result in a lower level of scar formation.

IL-1β and TNF-α are proinflammatory cytokines that are involved in a variety of immunological functions. Their levels are shown to be strongly upregulated during the inflammatory phase of healing and normally reach their peak levels on day 7 after injury.[47-48] IL-1β is a very
important inflammatory mediator in the skin and a key factor in initiation of the inflammatory response,\(^{49,50}\) while TNF-\(\alpha\) induces the expression of cutaneous and endothelial adhesion molecules, causing the development of skin irritation and inflammatory responses.\(^{51}\) Goldberg et al\(^{52}\) showed that TNF-\(\alpha\) also suppresses the tissue growth factor-\(\beta\) (TGF-\(\beta\))-induced fibroproliferative phenotypic genes such as collagen type I A and fibronectin at the mRNA level. SS itself can also activate IL-1\(\beta\) and TNF-\(\alpha\) production from fibroblast cells, but not at significant level.\(^{53}\) The present study showed that wounds treated with SS cream did not yield significantly high levels of IL-1\(\beta\) and TNF-\(\alpha\) on day 7, which suggests SS did not induce an inflammatory or immunological response. However, these proinflammatory cytokine levels may vary depending on the response of each individual subject. In order to avoid that variable, the formulas being compared need to be tested on the same subject. SSD itself causes significantly profound inflammatory responses when used in severely burned children.\(^{54}\) From our results, wounds treated with SSD in combination with SS demonstrated elevated levels of both proinflammatory cytokines when compared to SS-treated wounds—similar to results reported by other researchers.\(^{54,56}\)

**Conclusion**

Adding SS to an SSD cream formulation can partially inhibit SSD action, as was shown by the smaller microbial inhibition zones, but does not significantly reduce wound size when compared to other treatments. Sericin can promote collagen production in wounds—even in the presence of SSD—without generating significantly higher levels of inflammatory cytokines. Although wounds treated with SSD in combination with SS show elevated levels of both proinflammatory cytokines when compared to SS-treated wounds, the levels are not significant and should not affect its clinical use. However, since there was no untreated control to compare proinflammatory cytokines levels, it cannot be concluded that the SS-treated wounds alone decreased this response.

**Acknowledgement**

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**References**

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