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Effect of a Silk Sericin and Methylsulfonylmethane (MSM) Blends on Inflammatory Response and Wound Healing

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Abstract: The natural product silk sericin has excellent biological properties, such as biocompatibility, biodegradability, and antioxidant activity, and it also facilitates cell proliferation, collagen type 1 production, and wound healing. Methylsulfonylmethane (MSM) is a natural organic sulfur compound known to relieve pain and inflammation. In this study, we formulated sericin and MSM blends in various ratios, and investigated their effects on the inflammatory response and subsequent healing of a wound in vitro using macrophages and fibroblasts. The experimental findings, derived from cell viability analyses and nuclear factor kappa B (NFκB)/cyclooxygenase-2 (COX-2)/inducible nitric oxide synthase (iNOS) pathway assays, demonstrated that the anti-inflammatory activity of MSM was more effective when it was blended with sericin. In contrast, MSM alone did not enhance wound healing but rather delayed it at higher concentrations. We confirmed that the blend of sericin and MSM exhibited a synergistic effect on suppressing inflammatory responses and promoting subsequent wound healing when the two materials were optimally blended.

Keywords: sericin; methylsulfonylmethane; inflammatory response; wound healing

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1. Introduction

The skin is a barrier that protects the body from various external attacks. When a skin wound occurs, regeneration proceeds through the wound healing process, which involves bleeding, inflammatory response, cell proliferation, and remodeling [1,2]. The inflammatory response protects the body from pathogen invasion and plays an important role in the activation of the immune system, occurring immediately after an injury and lasting for up to 2 days. The activation of blood coagulation, inflammatory pathways, and the immune system at this stage prevents continuous loss of blood and body fluid, eliminates dying tissue, and prevents infection [3]. During the inflammatory process, inflammatory cells, such as macrophages and neutrophils, are mobilized and inflammatory and growth factors are secreted to control the wound healing process [3,4]. However, excessive inflammation and long-term inflammatory responses delay wound healing and increase the difficulty of wound healing due to ulceration; therefore, controlling the inhibition and acceleration of inflammation is critical in wound healing [5,6]. In addition, fibroblasts are important because they migrate to the damaged site in response to cytokines and growth factors that are secreted by platelets and macrophages when the skin is injured and proliferate to help in the wound healing phase [5,7]. In the wound healing phase, nuclear factor kappa B (NFκB) is a transcription factor that regulates the expression of several genes involved in

inflammation, cell migration, differentiation, adhesion, and survival, and is closely related to the wound healing process of many cell types and tissues [8].

Many natural substances have been used as biomaterials for wound healing, and a number of recent studies have focused on the silk protein sericin. Sericin is effective at inhibiting the production of pro-inflammatory cytokines related to the inflammatory response and promoting the activity of anti-inflammatory cytokines [2,9–11]. In addition, silk sericin is a substance that is known to improve cell adhesion, growth, and collagen production, and has been proven to have a wound healing effect [11–14]. Although sericin has not been widely used as a biomaterial because it has been shown to induce an immune response, it displays high physiological activity when used at an appropriate concentration. Therefore, the use of an appropriate sericin concentration is important for its application as a biocompatible material.

Methylsulfonylmethane (MSM) is a natural organic sulfur compound that has been proven to be an effective anti-inflammatory substance. MSM is effective at relieving pain, inflammation, arthritis, allergies, and asthma. Additionally, it can also attenuate the expression of pro-inflammatory cytokines and inhibit nuclear factor kappa B (NFκB) and inducible nitric oxide synthase (iNOS). The latter is known to suppress oxidative stress and IL-6 and TNF-α production by reducing the expression of cyclooxygenase-2 (COX-2) [15]. Recently, MSM's anti-oxidant, anti-inflammation, anti-ketosis, and anti-cancer activities have also been actively investigated [16–18]. Owing to its anti-inflammatory effects, MSM is widely used in health supplements and cosmetics [19–22] and has a high wound healing potential. However, few studies have investigated MSM to determine its wound healing and tissue regeneration capacity.

In this study, we investigated the potential and synergistic effects of sericin blended with MSM in various ratios on anti-inflammatory activity and wound healing efficiency. To evaluate the effect of this new biomaterial on inflammatory response, the expression of COX-2/iNOS and NFκB was analyzed using macrophages via Western blotting. The effect of the blended materials on the wound healing rate was evaluated using fibroblasts and a wound scratch model.

2. Materials and Methods

2.1. Materials

The cells used in this study are the macrophage Raw264.7 (No. TIB-71) and fibroblast L929 (No. CCL-1) cells, both of which were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). MSM was supplied by Sunny Rise Pharmachem Ltd. (Wanchai, Hong Kong). Dulbecco's modified Eagle's medium (DMEM; SH30243.01), fetal bovine serum (FBS; SF0005005), penicillin-streptomycin solution (SH40003.01), and trypsin (SH30042.02) were purchased from Hyclone Laboratories (Longan, UT, USA). Calcein-AM (C3100MP) and Ethidium homodimer-1 (EthD-1, E1169) were purchased from Invitrogen (Carlsbad, CA, USA) Lipopolysaccharide (LPS, purified from Escherichia coli 0111: B4), Griess reagent (G4410), Cell Counting Kit-8 (CCK-8, G4410), ECL prime Western blotting detection reagent (RPN2232), Tween-20 (P9416) and 2× Laemmli sample buffer (S3401) were obtained from Sigma-Aldrich (Merck KGaA, St. Louis, MO, USA). Skim milk (232100) was purchased from Becton, Dickinson and Company (Sparks, MD, USA). Power blotter (PB7320) was received from Thermo Fisher Scientific (Carlsbad, CA, USA). 10× TBS buffer (TR2005-100-74) was obtained from Biosesang (Seongnam-si, Gyenggi-do, Republic of Korea), and 30% acrylamide (A0418-050) was acquired from GenDEPOT (Baker, TX, USA). RIPA buffer (10×) (#9806), PMSF (#8553), COX-2 (#12282), iNOS (#2982), NFκB (#8242), IKKα (#2682), IKKβ (#8943), and IκBα (#9242) were purchased from Cell Signaling Technology (Beverly, MA, USA), and anti-mouse IgG (#7076), anti-rabbit IgG (#7074), and β-actin (sc-47778) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Dulbecco's phosphate-buffered saline (PBS; L0615) was purchased from Biowest (Nuaillé, France).

2.2. Sericin Extraction

We extracted sericin from cocoons at high temperature and pressure according to the method described by Terada et al. [12]. The cocoon pieces were soaked in 99% ethanol to remove impurities and then dried. The pieces were mixed with 0.2% Na_2CO_3 at a ratio of 1:20 (*w*/*v*) and extracted at 95 °C for 5 h using an autoclave (C-AC1, Acculab, USA).

The extraction was filtered through a 0.45 µm syringe filter (Minisarts CA Syringe Filter, Sartorius, Germany) in order to remove the fibroin. The filtrate was dialyzed against deionized water and then lyophilized in a freeze dryer (FD8518, Ilshinbiobase Co., Republic of Korea) to obtain a sericin powder. This extracted sericin ranged in molecular weight from 6 kDa to 67 kDa.

2.3. Sericin and MSM Blends Concentration

To determine the appropriate concentrations of sericin and MSM, respectively, the optimal concentrations of sericin and MSM each were determined using cell proliferation assays. The different concentrations of sericin and MSM are listed in Tables 1 and 2.

Table 1. Sericin and MSM concentrations.

Sample No.	1	2	3	4	5
Sericin (mg/mL)	0.1	0.5	1.0	5.0	10.0
MSM (mg/mL)	0.1	0.5	1.0	5.0	10.0

Table 2. Sericin and MSM blends concentrations.

Sample ID	0.1S + 0.1M	0.1S + 0.5M	0.1S + 1.0M
Sericin (mg/mL)	1.0	1.0	1.0
MSM (mg/mL)	1.0	5.0	10.0

2.4. Cell Proliferation

Fibroblast L929 were seeded at density of 1×10^4 cells/mL into 48-well plates containing DMEM (4.0 mM L-glutamine, 4500 mg/L glucose, sodium pyruvate), supplemented with 10% FBS and 1% penicillin-streptomycin and incubated at 37 °C. After 24 h (about 70–80% confluence), the medium was removed and washed with PBS (Dulbecco's phosphate-buffered saline). Sericin and MSM were processed for each concentration (Table 1) and cultured for 1 and 2 days. Cell proliferation was analyzed using the Cell Counting-8 (CCK-8) reagent, and absorbance was measured at 450 nm.

2.5. Cell Viability

Raw264.7 cells were seeded at a density of 1×10^5 cells/mL into 48-well plates, containing DMEM supplemented with 10% FBS and 1% penicillin-streptomycin, and incubated at 37 °C. After 24 h (about 80–90% confluence), the medium was removed and washed with PBS buffer. LPS (1 µg/mL) and the sample were exposed to each treatment concentration (1.0, 5.0, and 10.0 mg/mL MSM in 1.0 mg/mL sericin) and cultured for 1 day. Cell viability was analyzed using the CCK-8 reagent, and absorbance was measured at 450 nm. And it was visualized through LIVE/DEAD analysis. The cells were treated by dissolving 1 µM Calcein-AM and 2 µM EthD-1 in PBS, and then they were left at room temperature for 20 min. Additionally, results were confirmed with a fluorescence microscope (Leica DMIL LED Fluo, Leica Microsystems, Wetzlar, Germany).

2.6. Nitric Oxide Assay

Raw264.7 cells were seeded at a density of 2.5×10^5 cells/mL into 6-well plates containing DMEM (4.0 mM L-glutamine, 4500 mg/L glucose, sodium pyruvate), supplemented with 10% FBS and 1% penicillin-streptomycin and incubated overnight at 37 °C. After removing the medium, LPS (1 µg/mL) and sample were exposed to each treatment

concentration (Table 2) and cultured for 18–20 h. The culture medium was mixed with the same volume of Griess reagent, and absorbance was measured at 540 nm.

2.7. Western Blotting

Raw264.7 and L929 cells, treated with sericin and MSM, were homogenized in a RIPA buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM β-glycerophosphate, 1 mM Na_3VO_3, 1 µg/mL leupeptin, 1 mM PMSF). The cell lysates were then centrifuged (14,000× *g* for 10 min), and the supernatant was collected. Protein contents were determined using the Lowry Method [23]. The samples were diluted at a ratio of 1:2 with 2× Laemmli sample buffer, incubated for 20 min, and then equal quantities of protein (30 µg) were separated to SDS polyacrylamide gel electrophoresis under reducing conditions on a 10% resolving gel. The resolved proteins were transferred to a pure PVDF membrane and blocked for 60 min with 10% non-fat milk. Membranes were incubated overnight with all primary antibodies (COX-2, iNOS, NFκB, IKKα, IKKβ, IκBα and β-actin), diluted to 1:1000 in T-TBS (24.7 mM Tris, 137 mM NaCl, 2.7 mM KCl, pH 7.4, 0.1% Tween-20)/5% dry milk, washed with T-TBS, incubated with the secondary antibody at a dilution of 1:2000 for 60 min at room temperature in T-TBS/1% non-fat milk, and then washed with T-TBS again. Protein bands were visualized using iBright1500 (Eastman Kodak, Rochester, NY, USA) and the ECL prime Western blotting detection reagent.

2.8. Wound Scratch Assay

The wound healing rate was evaluated using a wound scratch assay as per the method described by Gonzalez et al. [24]. L929 cells were seeded at a density of 1.5×10^5 cells/mL in 24-well plates containing DMEM (4.0 mM L-glutamine, 4500 mg/L glucose, sodium pyruvate) supplemented with 10% FBS and 1% penicillin-streptomycin and were incubated at 37 °C for 24 h. A scratch was made in a confluent cell monolayer using a micropipette tip, and the cells were washed with PBS. The sample groups were exposed to DMEM supplemented 1% FBS, sericin and MSM blends concentrations (Table 2). The control group was only treated with fresh DMEM containing 1% FBS. The cells were cultured for 3 days, and images were acquired microscopically at 0, 1 and 3 days. The data were analyzed using Image J.

$$\text{Wound healing rate } (\%) = \frac{\text{Wound area at 0 h} - \text{Wound area at X h}}{\text{Wound area at 0 h}} \times 100$$

2.9. Statistical Analysis

The results are expressed as the mean ± standard deviation (SD). Statistical differences were determined using one-way analysis of variance (AVOVA), followed by the performance of Dunnett's multiple comparisons tests. All statistical procedures were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) at a significance level of 0.05.

3. Results and Discussion

3.1. Cell Proliferation under Various Ratios of the Sericin and MSM Blends

First, the optimal concentration of sericin for cell viability was determined using fibroblasts. On day 1, cell proliferation was highest when the sericin concentration was 10.0 mg/mL. On day 2, cell viability was significantly lower at sericin concentrations of 5.0–10.0 mg/mL, compared with that of the other conditions (0, 0.1 0.5, and 1.0 mg/mL) (Figure 1A, $p < 0.05$). Based on these results, a sericin concentration of 1.0 mg/mL was selected. Subsequently, various concentrations of MSM were blended with 1.0 mg/mL sericin.

In the case of MSM, on day 1, cell proliferation was significantly lower at an MSM concentration of 10.0 mg/mL compared to the control. On day 2, cell proliferation was significantly lower at an MSM concentration of 10.0 mg/mL compared to that of the other conditions (0, 0.1 0.5, 1.0, and 5.0 mg/mL) (Figure 1B, $p < 0.05$). This result is similar to that of Aramwit et al. [10], who also showed that on day 1, a sericin concentration of 1.0 mg/mL

was effective for cell proliferation. However, on day 2, cell proliferation was lower at an MSM concentration of 0.5–1.0 mg/mL compared to that at the other conditions. MSM did not show any cytotoxicity in the concentration range of 0.1–5.0 mg/mL. however, cell viability decreased when the MSM concentration reached 10.0 mg/mL. Therefore, a higher MSM concentration corresponded to greater cytotoxicity.

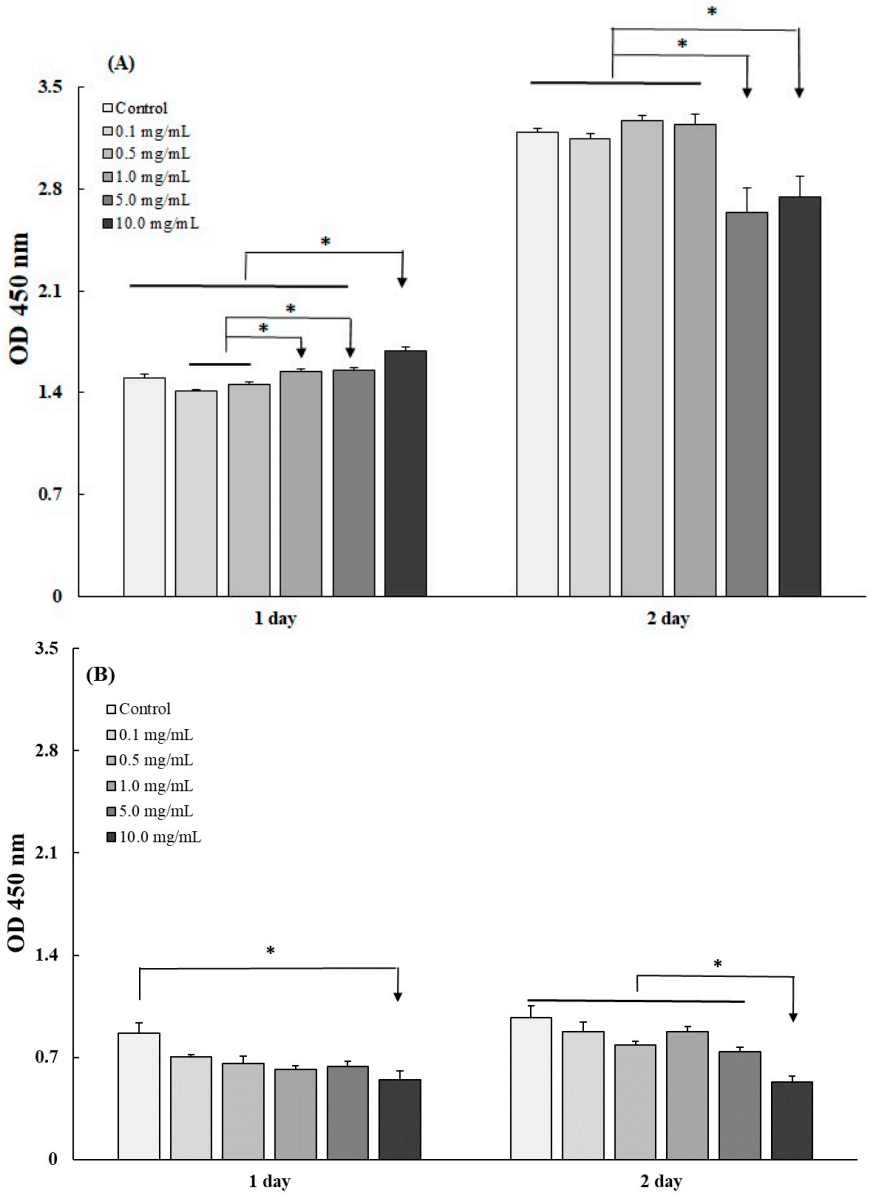


Figure 1. Cell proliferation based on the ratio of sericin to MSM by CCK-8 assay using L929 fibroblast. (**A**) Sericin and (**B**) MSM. All values are presented as the mean ± standard deviation (n = 3). * $p < 0.05$.

3.2. Effect of the Sericin and MSM Blends Treatment on LPS-Stimulated Macrophages

3.2.1. Cell Viability of Raw264.7 Cells Induced by LPS

The viability of Raw264.7 cells induced by LPS was significantly higher than that of the control in all groups, except for the LPS-treated group. In particular, the 0.1S + 0.1M treatment showed significantly higher cell viability than the 0.1S + 0.5M and 0.1S + 1.0M treatments. The 0.1S + 0.5M treatment showed significantly higher cell viability than the 0.1S + 1.0M treatment (Figure 2, $p < 0.05$).

A study by Lee et al. [15] on cell viability under various concentrations of MSM demonstrated that the MSM in *Allium hookeri* root extract was effective at suppressing

inflammation. However, the viability of Raw264.7 cells decreased after the application of 0.01 mg/mL *Allium hookeri* root extract.

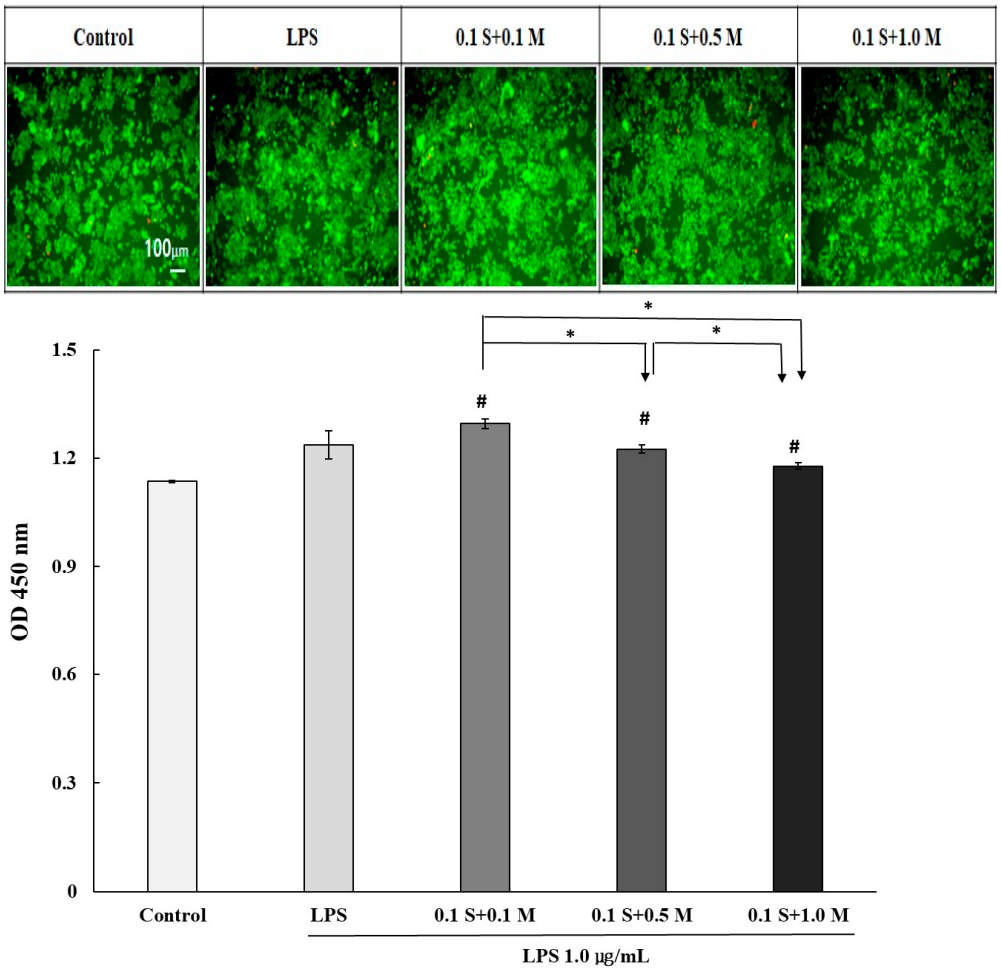


Figure 2. Cell viability based on the ratio of the sericin and MSM blends by CCK-8 assay using Raw 264.7 macrophage. Cell treated with 1.0 (0.1S + 0.1M), 5.0 (0.1S + 0.5M), and 10.0 (0.1S + 1.0M) mg/mL MSM at 1.0 mg/mL sericin, stimulated with 1 μg/mL LPS. All values are presented as the mean ± standard deviation (n = 3). # $p < 0.05$ vs. CON. * $p < 0.05$.

However, our data confirm that an appropriate cell viability was obtained at an MSM concentration of 10.0 mg/mL, which is one thousand times higher than the value of 0.01 mg/mL obtained for the *Allium hookeri* root extract. This result indicates that the toxicity of MSM was dramatically weakened when it was blended with sericin. The strong cell biocompatibility of sericin helped reduce the cytotoxicity of MSM, as supported by the findings of Aramwit et al. [11].

3.2.2. Nitric Oxide of Raw264.7 Induced by LPS

The production of nitric oxide (NO) by Raw264.7 cells, stimulated using LPS and treated with 0.1S + 0.1M, was found to be higher than that observed in the control. However, under conditions of treatment with 0.1S + 0.5M, NO production was significantly lower than that seen with 0.1S + 0.1M and LPS. Under conditions of treatment with 0.1S + 1.0M, NO production was significantly lower than that observed with the other treatments (Figure 3, $p < 0.05$).

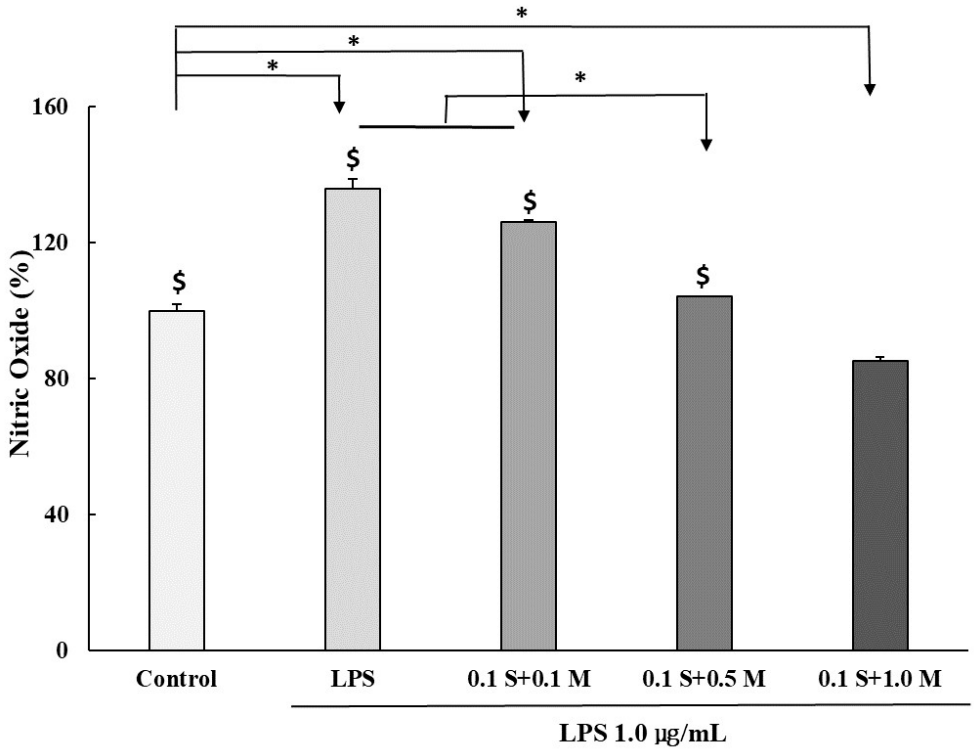


Figure 3. NO assay based on the ratio of the sericin and MSM blends using Raw 264.7 macrophage. Cell treated with 1.0 (0.1S + 0.1M), 5.0 (0.1S + 0.5M), and 10.0 (0.1S + 1.0M) mg/mL MSM at 1.0 mg/mL sericin, stimulated with 1 µg/mL LPS. All values are presented as the mean ± standard deviation (n = 3). * $p < 0.05$. $ $p < 0.05$ vs. 0.1S + 1.0M.

3.2.3. COX-2/iNOS Expression in Raw264.7 Cells Stimulated with LPS

COX-2/iNOS expression at the protein level, in Raw264.7 cells stimulated with LPS, was analyzed. COX-2 expression was higher in all four blends than in the control. However, higher MSM concentrations (5.0 and 10.0 mg/mL) resulted in lower COX-2 expression at the protein level (Figure 4A, $p < 0.05$). NOS expression was significantly higher in LPS treatment than in control. However, 0.1S + 0.1M, 0.1S + 0.5M and 0.1S + 1.0M treatments showed significantly lower than LPS-only treatment. (Figure 4B, $p < 0.05$).

Inflammation is a biological defense mechanism that occurs during wound infection, and it neutralizes wound tissue through an inflammatory response and regenerates damaged tissue [24,25]. It is promoted by oxidative stress generated in the body and the upregulation of certain genes in cells. There are several pathways of inflammation, of which NFκB/COX-2/iNOS pathway is a well-recognized mechanism. In particular, nuclear factor kappa B (NFκB) regulates the inflammatory stage through the regulation of cytokines (IL-1, TNF-α) and several other factors, including inducible nitric oxide synthase (iNOS) [11,26,27].

LPS is an inflammatory mediator that activates NFκB, an intracellular transcription factor in macrophages, and the induced NFκB moves to the nucleus, resulting in the induced expression of inflammatory cytokines, iNOS, and COX-2, which further induce gene expression [28]. NO is an indicator of the inflammatory response and is synthesized by iNOS, while COX-2 generates various mediators of the inflammatory response when inflammation is induced by harmful stimuli, infection, or trauma [28,29]. MSM inhibits nitric oxide prostaglandin E2 production by inhibiting iNOS and COX-2 expression, and is involved in the immune response by inhibiting IL-6 and TNF-α production through transcription factor NFκB [19]. Aramwit et al. [8] reported that COX-2/iNOS expression decreased as the concentration of silk sericin increased, while COX-2/iNOS expression decreased at sericin concentrations of 2.5–10 mg/mL [19].

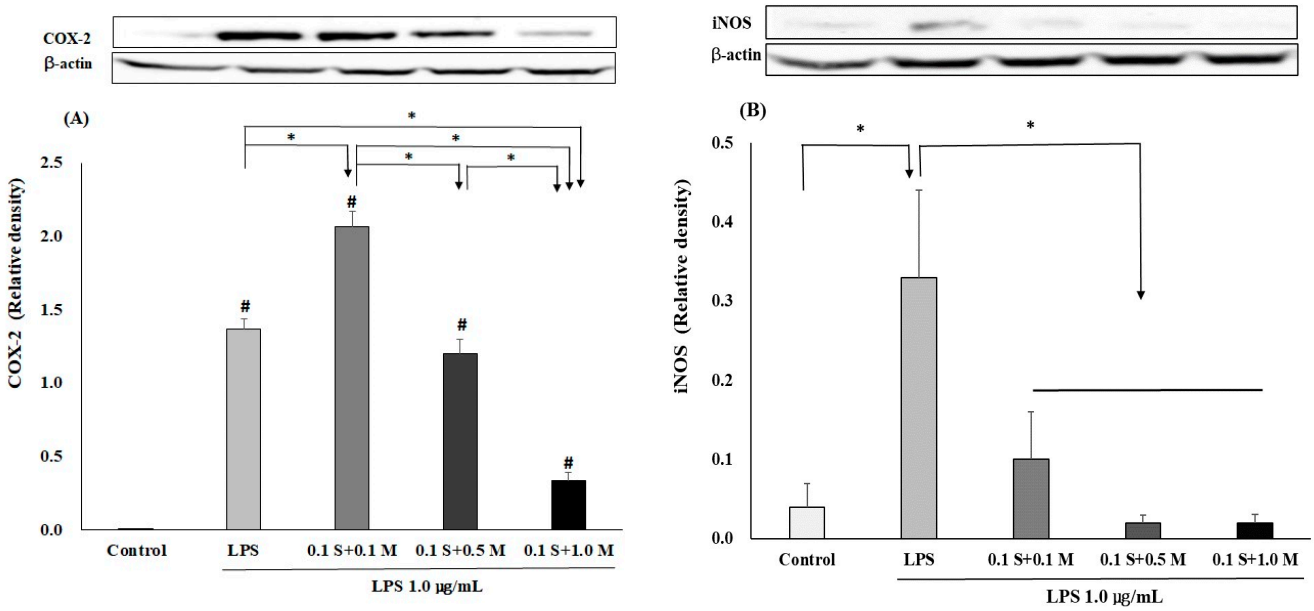


Figure 4. COX-2/iNOS expression based on the ratio of the sericin and MSM blends using Raw 264.7 macrophage. Cells were treated with 1.0 (0.1S + 0.1M), 5.0 (0.1S + 0.5M), and 10.0 (0.1S + 1.0M) mg/mL MSM at 1.0 mg/mL sericin, stimulated with 1 μg/mL LPS. (**A**) COX-2 and (**B**) iNOS. Raw 264.7 lysates were Western blotted with an anti COX-2 or iNOS or β-actin antibody. All values are presented as the mean ± standard deviation (n = 3). # $p < 0.05$ vs. CON. * $p < 0.05$.

In this study, the effect of the sericin and MSM blends on the expression of COX-2/iNOS was investigated by stimulating Raw264.7 macrophages with LPS and then treating the cells with the sericin and MSM blends. Although differences in expression were observed based on the concentration of MSM, NO production and COX-2/iNOS expression were significantly decreased in the group treated with MSM at 5.0–10.0 mg/mL (Figures 3 and 4). Sericin was confirmed to prevent the decrease in cell viability caused by an increase in the concentration of MSM (Figure 1), and it also suppressed the expression of COX-2/iNOS, which likely affected the anti-inflammatory activity of macrophages. These findings indicate that the synergistic effect increased because of the inclusion of sericin in the blends.

3.3. Effects of the Sericin and MSM Blends on Wound Healing in Fibroblasts

3.3.1. Proliferation of L929 Cells

Proliferation analysis of L929 cells on exposure to sericin and MSM blends at days 1, 2, and 4 showed that the wound healing ability of the different groups (0.1S + 0.1M, 0.1S + 0.5M, and 0.1S + 1.0M) was significantly higher than that of the control, and that the differences increased over time. In particular, the 0.1S + 0.1M treatment resulted in significantly higher cell proliferation than the 0.1S + 0.5M and 0.1S + 1.0M treatments (Figure 5, $p < 0.05$).

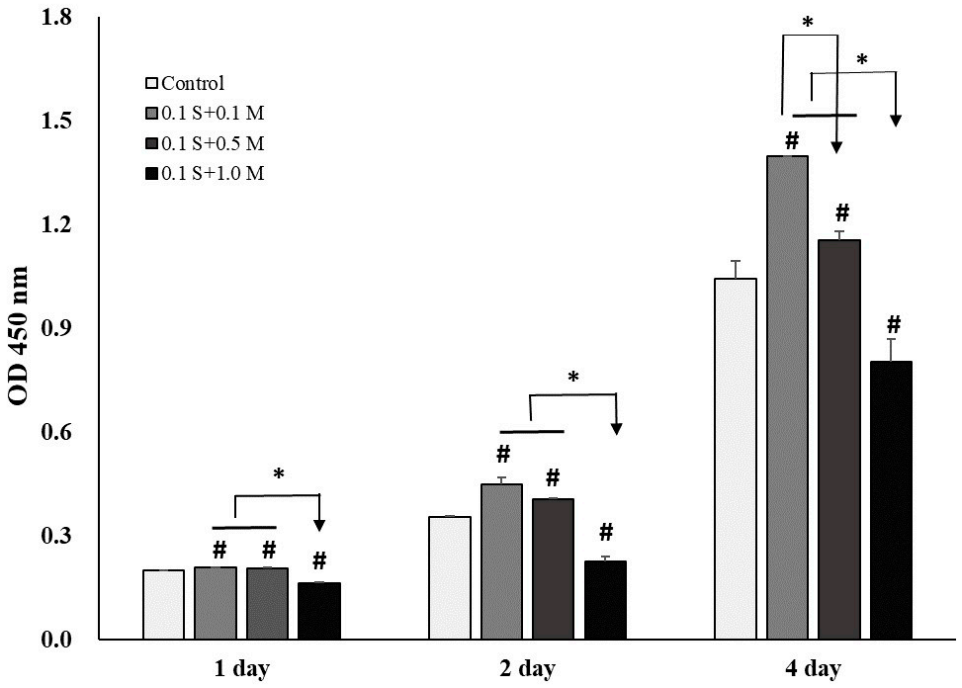


Figure 5. Cell proliferation based on the ratio of the sericin and MSM blends using L929 fibroblast. Cells were treated with 1.0 (0.1S + 0.1M), 5.0 (0.1S + 0.5M), and 10.0 (0.1S + 1.0M) mg/mL MSM at 1.0 mg/mL sericin. All values are presented as the mean ± standard deviation (n = 3). # $p < 0.05$ vs. CON. * $p < 0.05$.

3.3.2. Expression If NFκB Pathway Has Intermediaries in L929 Cells

NFκB pathway intermediary expression after treatment with the sericin and MSM blends was analyzed. The expression of NFκB, IκBα, and IKKα was significantly higher in the treatment groups (0.1S + 0.1M, 0.1S + 0.5M, and 0.1S + 1.0M) than in the control (Figure 6A–C, $p < 0.05$).

IKKβ expression was significantly higher at 0.1S + 0.1M and 0.1S + 0.5M than the control, although significant differences were not observed between the 0.1S + 1.0M treatment and the control (Figure 6D, $p < 0.05$).

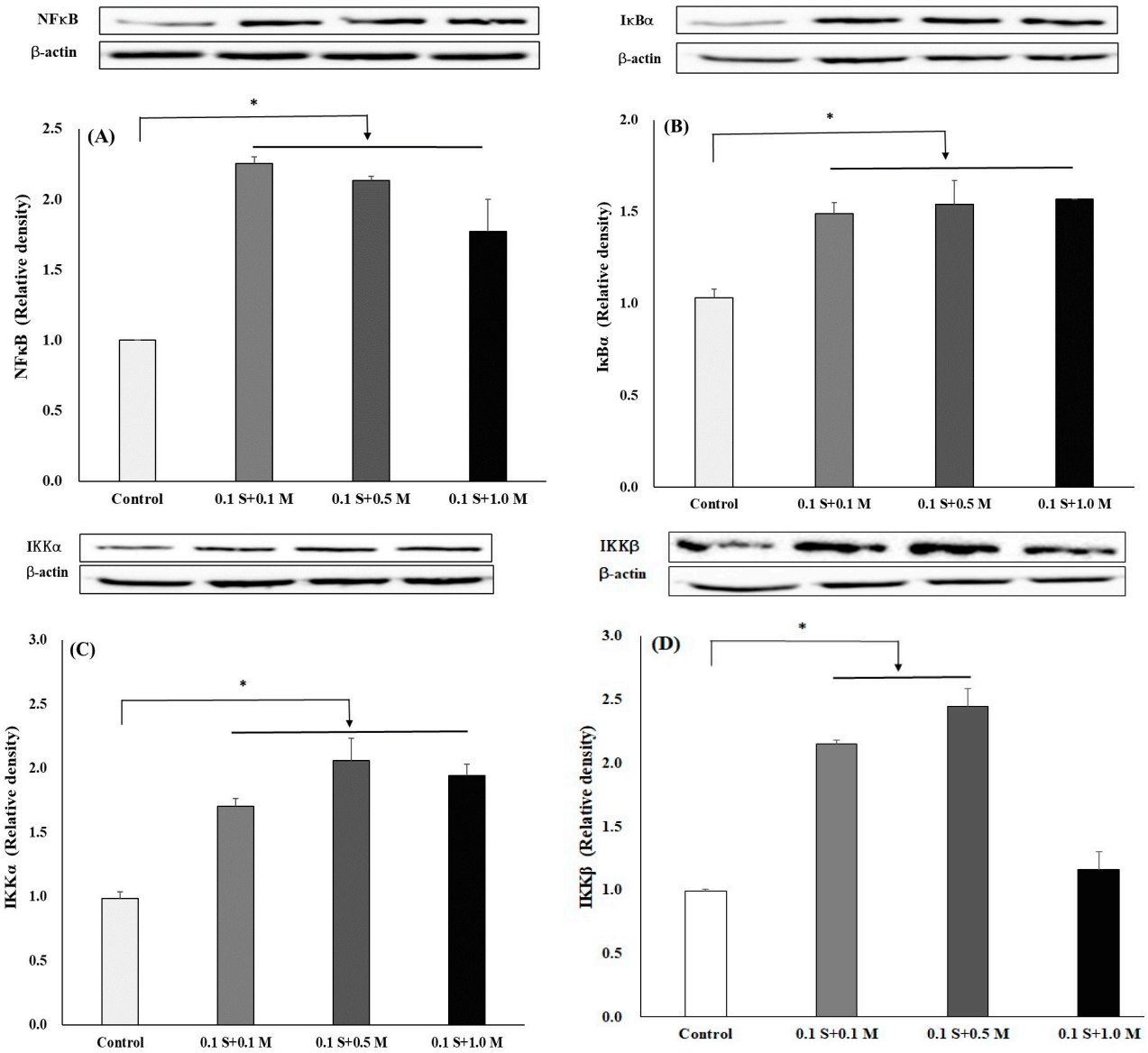


Figure 6. NFκB pathway expression based on the ratio of the sericin and MSM blends using L929 fibroblast. Cells were treated with 1.0 (0.1S + 0.1M), 5.0 (0.1S + 0.5M), and 10.0 (0.1S + 1.0M) mg/mL MSM at 1.0 mg/mL sericin. (**A**) NFκB, (**B**) IκBα, (**C**) IKKα and (**D**) IKKβ. L929 lysates were Western blotted with an anti NFκB or IκBα or IKKα or IKKβ or β-actin antibody. All values are presented as the mean ± standard deviation (n = 3). * $p < 0.05$.

3.3.3. Wound Healing Rate in L929 Cells

The wound healing rate at different concentrations of the sericin and MSM blends was analyzed using fibroblasts. The wound healing rate was significantly higher on days 1 and 3 for the sericin and MSM blends groups than for the control group. However, on day 3, the 0.1S + 1.0M group showed a significantly lower wound healing rate than the 0.1S + 0.1M group (Figure 7, $p < 0.05$).

Healthy skin fibroblasts are responsible for connective tissue regeneration and tissue remodeling [4,30,31]. Sericin promotes fibroblast proliferation, collagen (type 1) generation, wound healing, and skin regeneration [2,32], and MSM is known to be effective in treating pain and inflammation [18–21]. These substances are also involved in the NFκB pathway [19,33]. In the wound healing process, NFκB is a transcription factor that regulates the expression of several genes involved in cell migration, differentiation, adhesion,

and survival, and it has been reported to be involved in fibroblast migration and wound healing [4,34,35].

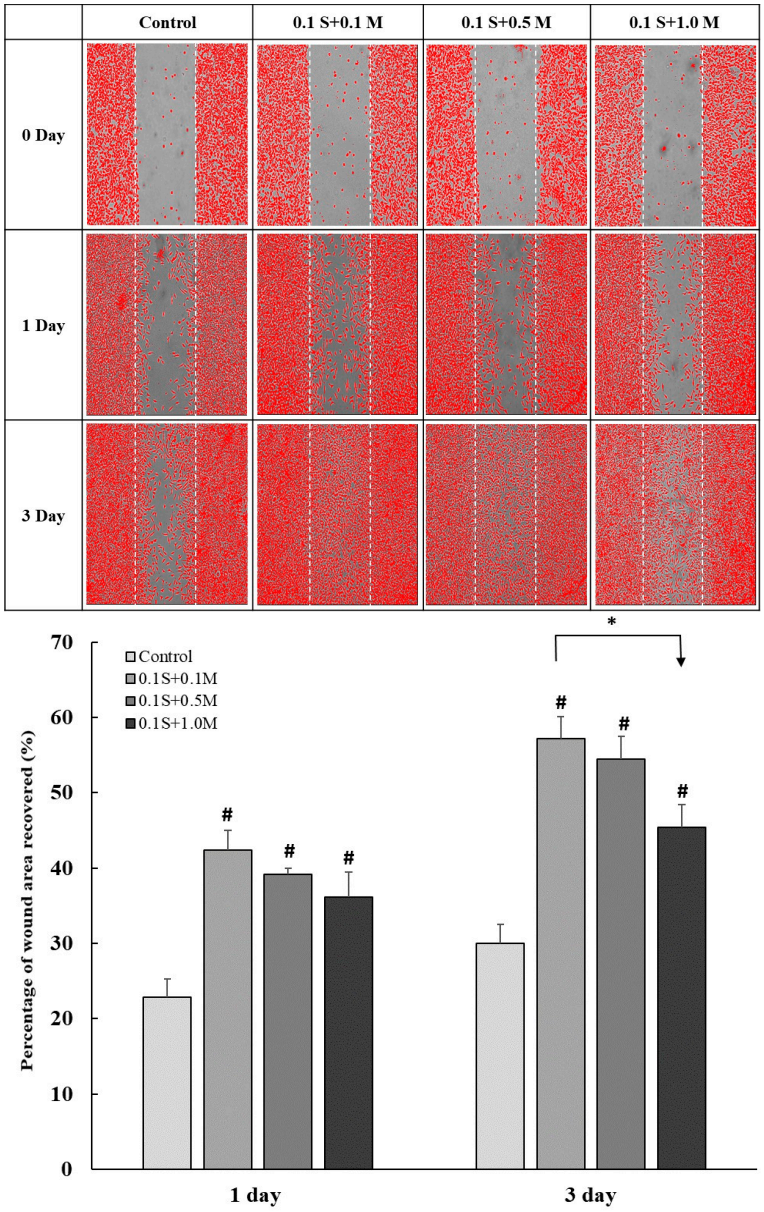


Figure 7. Wound healing rate of the sericin and MSM blends according to an in vitro scratch assay using L929 fibroblast (Upper: Image. Lower: Quantitative Analysis). Cells were treated with 1.0 (0.1S + 0.1M), 5.0 (0.1S + 0.5M), and 10.0 (0.1S + 1.0M) mg/mL MSM at 1.0 mg/mL sericin. All values are presented as the mean ± standard deviation (n = 3). # $p < 0.05$ vs. CON. * $p < 0.05$.

Park et al. [4] reported that silk fibroin increased NFκB, IKKα, and IKKβ expression, and Eidet et al. [33] treated human retinal pigment epithelial (hRPE) cells with 1% sericin for 12 days and reported that the expression of NFκB was increased. Other studies demonstrated that silk sericin-based cream and hydrogel have a positive effect on wound healing in a rat model [2,9,32].

In this study, the anti-inflammatory effects of the sericin and MSM blends were evaluated based on NFκB pathway protein expression and wound healing rates using a wound scratch model. The results indicated that, when L929 cells were mixed with sericin at 1.0 mg/mL and MSM at 1.0, 5.0, and 10.0 mg/mL, the protein expression of NFκB, IKKα, IKKβ, and IκBα was increased compared to that of the control (Figure 6). In

addition, although differences in wound healing were observed based on the concentration of MSM in the sericin and MSM blends, wound healing was increased overall (Figure 7). Therefore, the sericin and MSM blends represent good biomaterial that exerts effective anti-inflammatory action and promotes wound healing.

4. Conclusions

The results of this study showed that a higher concentration of MSM in the sericin and MSM blends led to more effective anti-inflammatory activity. In addition, all of the sericin and MSM blends could enhance cell proliferation and subsequent wound healing efficiency, which indicated the excellent biological activity of sericin at the proper concentrations.

In conclusion, the natural products sericin and MSM have great potential as biomaterials for effective wound healing. However, further in vivo studies are necessary to confirm the findings presented here.

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