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Original Article

Effectiveness of Woven Silk Dressing Materials on Full-skin Thickness Burn Wounds in Rat Model

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Abstract

Purpose: This study evaluated woven silk textile for burn wound dressing materials in an animal model.
Methods: Ten rats were used in this experiment. Full-thickness 2×2 cm burn wounds were created on the back of the rats under anesthesia. In the experimental group, the wounds were treated with three different dressing materials from woven silk textile. In the control group, natural healing without any dressing material was set as control. The wound surface area was measured at five days, seven days, and 14 days. Wound healing was evaluated by histologic analysis.
Results: There were no statistically significant differences among groups at five days post injury. The mean defect size at seven days was largest in Group 3 (462.87 mm^2), and smallest in Group 1 (410.89 mm^2), not a significant difference (P=0.341). The mean defect size at 14 days was smallest at the Group 3 (308.28 mm^2) and largest in the control group (388.18 mm^2), not a significant difference (P=0.190). The denuded area was smaller in Group 1 (84.57 mm^2) and Group 2 (82.50 mm^2) compared with the control group (195.93 mm^2), not statistically significant differences (P=0.066, 0.062). The difference between Group 3 and control was also not statistically significant (P=0.136). In histologic analysis, the experimental groups re-epithelialized more than control groups. No evidence was found of severe inflammation.
Conclusion: The healing of burn wounds was faster with silk weave textile more than the control group. There was no atypical inflammation with silk dressing materials. In conclusion, silk dressing materials could be used to treat burn wounds.

Key words: Full thickness burn wound, Wound dressing, Silk weave textile, Rat

Introduction

The wound healing process is very complicated, with many different types of cells and matrices involved to restore damaged tissue in the healing process[1]. Wound dressing is needed to shield injured tissue and to accelerate the healing process without any microbial infection and additional damage[2]. Criteria for dressing materials to speed wound healing include (1) biocompatibility, (2) prevention of dehydration and maintaining a favorable moist environment, (3) protection from foreign bodies and microorganisms, (4) promotion of epithelialization, and (5) non-adhesive properties[1].

Burn wounds are difficult to treat because this type of

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wound produces exudates that can adhere to dressings during healing[3]. Initially, the burn wound appears as painless, charred, with no bleeding. Significant edema often develops within a few hours and may persist[4]. Adhesion can cause secondary trauma when changing dressings. Mesh dressing materials easily damage a burn wound, causing trauma during removal. Wax coated biomaterial can reduce this trauma, but possesses less healing ability than other materials.

Silk has been used medically for many centuries[5]. It is used as surgical suture material and textile fiber. Silk has many advantages for wound dressing materials, such as biocompatibility, minimal inflammatory reaction, capability to promote wound healing, strength, toughness, light weight, and easy chemical modification[6]. Medical research of silk includes its uses as membrane, scaffold, and wound dressing materials[7]. Silk's obvious limitation is the difficulty of mass production.

A woven textile is very easy to mass produce and fabricate silk dressing with various dimensions. In this study, we use woven silk textile as dressing material to cover the burn wound. The purpose of this study was to assess the effectiveness of wound dressing materials made by silk weave textile on full thickness burn wounds on rats.

Materials and Methods

1. Silk dressing materials

We used three different kinds of dressing material obtained from silk weave textiles. The materials were kindly provided by Sangju Myungju Co. (Sangju, Korea). The composition of silk weave textile is silk fibroin, because the silk textile was degummed to remove sericin. The three materials differed in crystallinity index, fiber diameter, and pore size (Fig. 1, Table 1). The crystallinity index was calculated from fourier transform infrared (FT-IR) spectra of silk textile samples following published methods[8,9]. The fiber diameter and pore size were determined by digital optical microscope (Toolis, Daegu, Korea).

2. Experimental animals and housing conditions

Ten Sprague-Dawley rats with body weights of 250 to 300 g were obtained from Samtako Bio Korea Co. (Osan, Korea) at 12 weeks of age. Each rat was housed in separate stainless steel cages and allowed to adapt for 10 days before starting the main experiment. The animals were maintained on a 12 hours dark/light cycle at about 22°C±3°C and allowed free access to standard laboratory diet and tap water ad libitum during experiments. This experiment was approved by the Institutional Animal Care and Use Committee of Gangneung-Wonju National University (GWNU-2014-6).

3. Animal experiments

At the beginning of the experiment, general anesthesia

Table 1. Crystallinity index, fiber diameter (μm), pore size (μm^2) of each silk material

Silk	Crystallinity index (%)	Fiber diameter (μm)	Pore size (μm^2)
1	50.9±0.2	217.3±16.1	5,632.0
2	52.9±0.9	337.0±9.1	10,723.2
3	55.7±0.7	422.2±19.2	16,764.3

Values are presented as mean±standard deviation or number only.

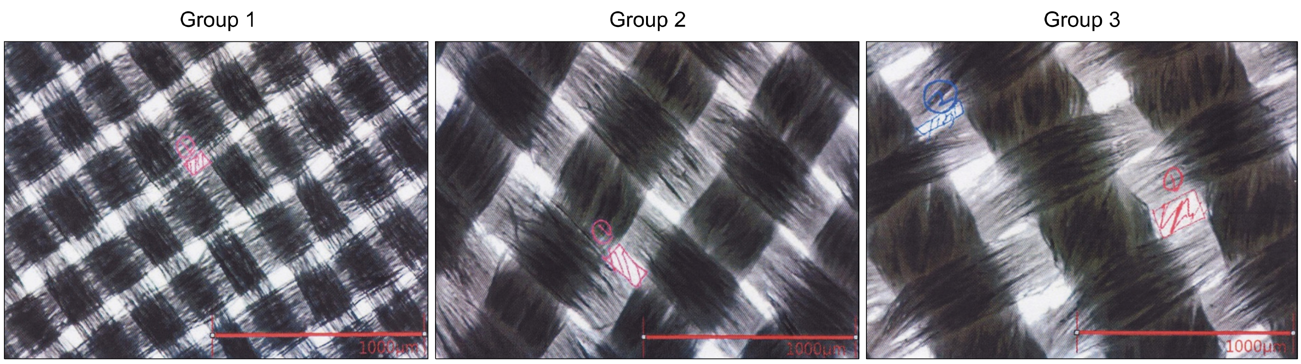

Fig. 1. Scanning electron microscopic image of silk dressing materials made by weaving technique (scale bar: 1,000 μm).

was induced with 0.2 mL Tiletamine and Zolawepam (125 mg/mL; Zoletil; Bayer Korea, Seoul, Korea) and 0.1 mL xylazine (10 mg/kg body weight, Rompun; Bayer Korea). The back zone of each animal was shaved widely, and two 2×2 cm size standard second-degree burns were produced on the back of each rat by hot stamps. Twenty defects were produced and randomly divided into four groups. In the control group, the wounds were dressed simply without any application. In three experimental groups, differing types of dressing materials were applied to each wound. The burn wounds were covered with occlusive dressing (Fig. 2). At five days post-injury, occlusive dressings were removed and clinical signs and healing states were observed. We checked for signs of inflammation, bleeding from secondary trauma, and other clinical abnormalities. We took a picture of the burn wounds at five, seven, and 14 days post-injury and measured residue denuded surface using size measuring software (SigmaScan-Pro; SPSS Inc., Chicago, IL, USA).

4. Histologic analysis

Animals were sacrificed at 14 days post-injury and the remaining scar area was dissected out. H&E staining was carried out to evaluate the infiltration of inflammatory cells

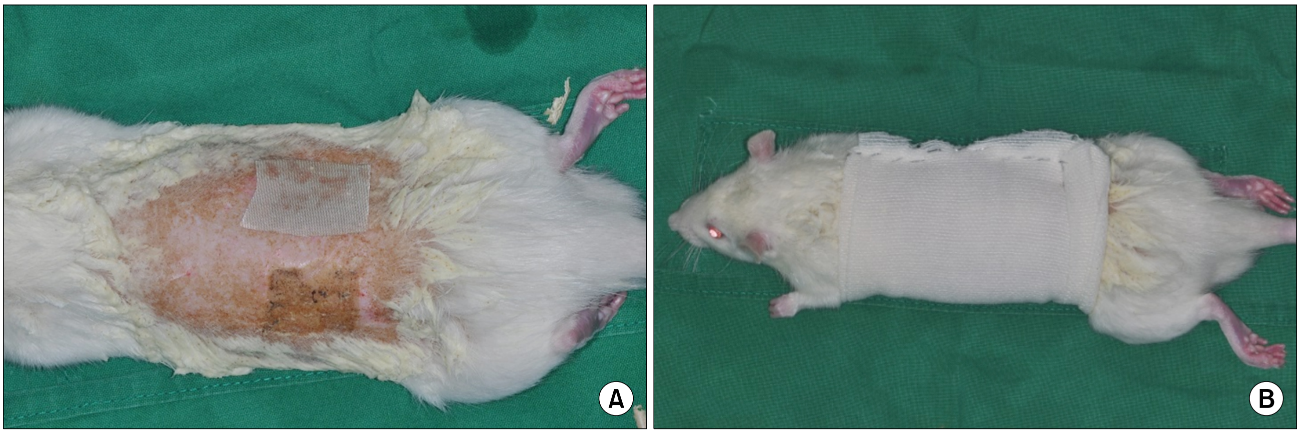

Fig. 2. Burn injured model. (A) A second degree 2×2 cm burn injury was created on the back of the rat using a stamp. We created two defects for each rat. In the experimental groups, silk dressing material was applied to the burn wound. (B) Occlusive dressing was applied to every burn during the five days post-injury.

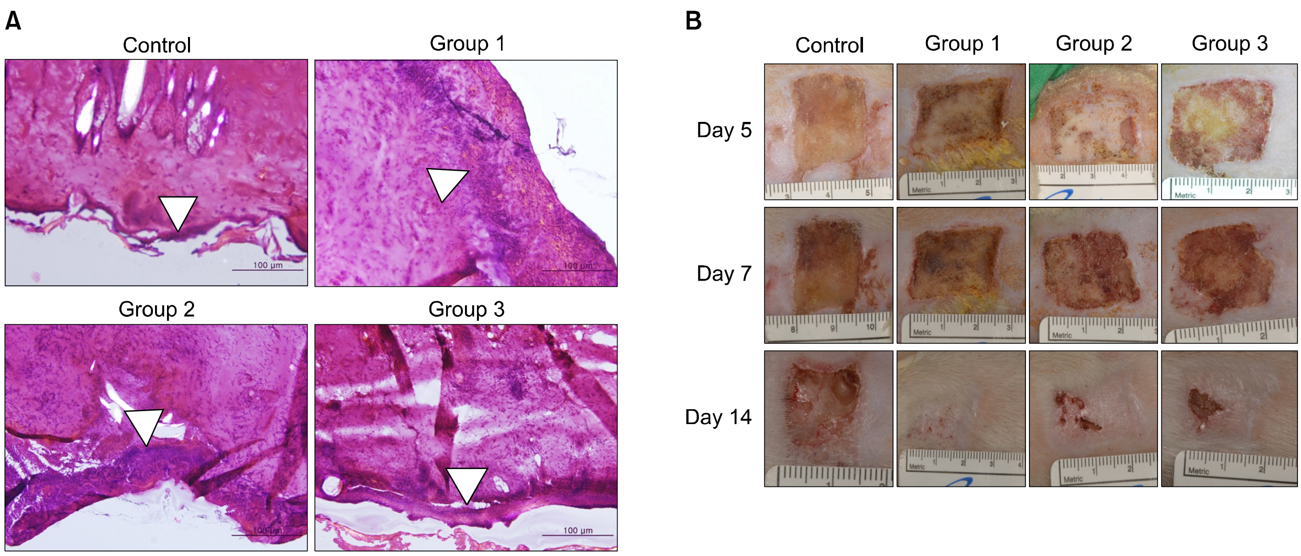

Fig. 3. (A) Histology (H&E, ×400) of each group. Group 1 and 2 and 3 show more re-epithelialization than the control group. There was no evidence of severe inflammation. (B) Clinical photos of healing process. Experimental groups developed smaller residual scars than the control group.

including macrophages and giant cells to assess the extent of foreign body reaction (Fig. 3A).

5. Statistical analysis

Data were statistically analyzed by one-way analysis of variance, followed by post hoc test (least significant difference method). We rejected null hypotheses of no difference if P-values were less than 0.05. Statistical analysis was performed using IBM SPSS Statistics ver. 21 software (IBM Co., Armonk, NY, USA).

Results

In gross observation, there was no infection and severe inflammation in any group up to 14 days post-injury (Fig. 3B). No bleeding tendency was noted when dressings were removed at five days post-injury. All wounds seemed stable and were in varying degrees of healing.

There was no statistically significant difference among groups at five days post-injury (P=0.587). The defects were evenly created.

The mean defect size at seven days was largest in Group 3 (462.87 mm^2), and smallest in Group 1 (410.89 mm^2), not a significant difference (P=0.341). The mean defect size at 14 days was smallest in Group 3 (308.28 mm^2) and largest in the control group (388.18 mm^2), not a significant difference (P=0.190). The denuded area was smaller in Group 1 (84.57 mm^2) and Group 2 (82.50 mm^2) compared with the control group (195.93 mm^2), not statistically significant differences (P=0.066, 0.062). The difference between Group 3 and control was also not statistically significant (P=0.136). The size of denuded area of each group is presented in Fig. 4.

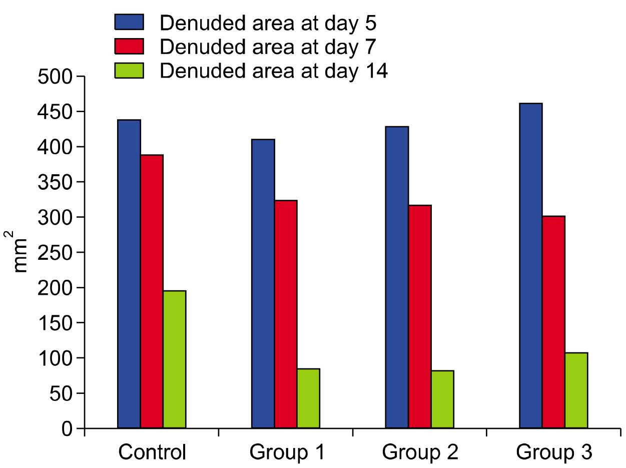

Fig. 4. Denuded area at days five, seven, and 14 post-injury in each group (mm^2).

In histologic analysis, the experimental groups re-epithelialized more than control groups. No evidence was found of severe inflammation, while there were minimal numbers of the inflammatory cells that respond to infection or foreign body reactions (Fig. 3A).

Discussion

We found silk weave textile to be effective for burn wound dressing material. Compared to the control group, the experimental group wounds healed better. There was no typical inflammation and microbial infection. These results indicate that silk weave textile can be a good dressing material for wound healing.

Silk has many advantages for tissue engineering. It has biological and mechanical properties such as strength, light weight, biocompatibility, minimal inflammation reaction, and wound healing promotion[5]. It is easy to modify for different applications[10,11]. Recent research examined clinical applications using silk because of its ability to promote adhesion and proliferation of various cells including keratinocytes and fibroblasts[12,13]. In this study, we found silk dressing materials effective as burn dressing.

Among burn wound dressing materials, mesh type dressing is often used for burn wound treatment[3]. After the wound is healed, the dressing material should be removed. Mesh dressing materials tend to adhere to the wound, inducing secondary injury. To prevent this, wax-coated dressing material is sometimes used[3]. This material reduces risk of secondary injury, but does not have the healing potential of mesh materials. We published a study of a powder dressing material using silk and found acceptable burn wound healing[5]. In this study, although lacking statistical significance, the groups with silk membrane applied healed better than the control group, with no secondary injury by adhesion upon removal. These results suggest that silk membrane is an excellent material for burn wound dressing. Further studies with a larger sample size are necessary to determine the effectiveness of silk weave textile for burn wound dressing.

We tested three kinds of silk textile with different fiber diameters and pore sizes. Regardless of properties, all silk textile samples resulted in better wound healing than the control group. At day 5, the denuded area was higher in the following order: Group 1<Group 2<Group 3. At day 14, Group 1 and Group 2 were similarly denuded, and less than Group 3. Thus the wounds in Group 1 and Group 2 healed better than Group 3. Although the exact reason for these results are beyond the scope of this study, the wound healing effect might be related to the pore size of silk textile. That is, as the pore size is at an optimum value, wound healing improves. When the pore size of silk textile is too high (in case of Group 3), the exudate from the wound is removed too quickly, limiting effective wound healing. Therefore, proper pore size may be necessary for healing, in addition to the healing effect of silk material itself.

There is much active research of silk for medical applications. Kim *et al.*[9] evaluated the biocompatibility of the silk fibroin nanofiber membrane and examined its effect on bone regeneration in a rabbit calvarial model. They observed good biocompatibility with enhanced bone regeneration and no evidence of any inflammatory reaction. Lovett *et al.*[14] studied silk fibroin microtubes for blood vessel engineering. They reported that silk microtubes are an attractive biomaterial for microvascular grafts. Cai *et al.*[15] studied chitosan/silk fibroin composite nanofiber for wound dressing. They noted that chitosan/silk fibroin showed antibacterial activity and proliferation of fibroblasts. They suggested that it could be a good wound healing application. There are numerous studies currently, and there will be more development of silk for medical applications[16].

Conclusion

Woven silk membranes possess good biocompatibility as burn wound dressing materials. Wound healing with silk dressing material was better in all experimental groups, and no silk dressing produced atypical inflammation and only minimal foreign body reactions. In conclusion, woven silk membrane could be used for burn wound dressing material.

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