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The Utility of Silk-elastin Hydrogel as a New Material for Wound Healing

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Cutaneous ulcers are treated with dressing materials and/or ointments to keep the wound in an appropriately moist environment. However, chronic cutaneous ulcers commonly have bacterial colonization that can cause local infection in such an environment. Therefore, the dressing materials and/or ointments should have antibacterial potency to treat chronic ulcers. Acute cutaneous wounds, by contrast, heal rapidly without local infection. The aim of treating acute cutaneous wounds is therefore not only wound closure but also preventing scar contracture after wound healing. However, no dressing materials or ointments available at present are simultaneously effective for preventing infection in chronic ulcers and reducing wound contracture in acute ulcers. Silk-elastin is a recombinant protein polymer with repeating units of silk-like and elastin-like blocks. Silk-elastin solution can self-assemble from a liquid to a hydrogel. We preliminarily reported that silk-elastin hydrogels have the potential to accelerate wound healing in decubitus ulcers of diabetic mice, which are animal models of severe, intractable cutaneous ulcers. In the present study, we examined the effects of silk-elastin hydrogels in chronic and acute ulcer models in comparison with conventional products (carboxymethyl cellulose gel). Silk-elastin hydrogels resulted in significantly higher epithelialization rates than conventional hydrogels in both the chronic and acute ulcer models and significantly larger areas of granulation tissue in acute ulcer models. These results show that silk-elastin hydrogel is a promising material for promoting the healing of cutaneous wounds, including decubitus ulcers, chronic ulcers, and acute ulcers.
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INTRODUCTION

Chronic cutaneous ulcers, commonly caused by diabetic neuropathy, vascular insufficiency, and connective tissue diseases, are intractable due to bacterial colonization. It is difficult to treat chronic ulcers in medical practice, as the moist environment necessary for wound healing conversely facilitates bacterial infection. In addition, the fibroblasts responsible for wound healing can be defective in chronic ulcers. Indeed, it has been reported that exudates from chronic ulcers inhibit the proliferation of fibroblasts and delay granulation tissue formation and epithelialization.[1,2] Therefore, new materials that not only prevent bacterial infection but also promote wound healing are desired.

Acute cutaneous wounds, in contrast, heal through a combination of epithelial migration from the wound margins and contraction of the granulation tissue to bring the wound margins closer together.[3] However, the wound contraction leads to substantial loss of skin extensibility and a poor aesthetic appearance.[4] Thus, the aim of treating such wounds is not only to promote wound closure but also to prevent wound contracture.

Silk-elastin is a protein produced by recombinant DNA technology with relevant genes of silkworm fibroin and human elastin to generate peptide repeats of the silk fibroin-derived sequence (GAGAGS) and elastin-derived sequence (GVGVP).[5] Previous reports on silk-elastin have shown that the solubility, material strength, immunogenicity, and in vivo degradation profile of silk-elastin can be controlled by varying the composition and sequence of the units.[6,7] Silk-

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elastin has not only biocompatibility but also high elasticity derived from elastin-like blocks, combined with the mechanical and tensile strength derived from silk-like blocks, which are not naturally present in a single molecule.[8]

The concept of moist wound healing is based on the idea that maintaining a moist environment is essential for wound healing; after wound bed preparation, fibroblasts and macrophages migrate and produce extracellular matrix under the moist environment. We previously reported that silk-elastin has the potential to promote the migration of fibroblasts and macrophages and the production of collagen from fibroblasts.[9] In addition, at concentrations of ≥ 4% (w/v), water-soluble silk-elastin at room temperature can form a hydrogel at body temperature.[5] When silk-elastin is applied to a wound in an aqueous condition, it automatically forms a silk-elastin hydrogel that covers the wound and maintains a moist condition without inflammation, leading to increased granulation tissue formation and epithelialization. Our preliminary study showed that silk-elastin hydrogels reduced the bacterial infection and accelerated the wound healing of decubitus ulcers in diabetic mice.[10]

In this study, we evaluated the utility of silk-elastin hydrogels in treating full-thickness skin defects of diabetic mice, which are models of chronic ulcer, and compared them with those of conventional hydrogels. In addition, we also evaluated the utility of silk-elastin hydrogels in treating full-thickness skin defects of healthy guinea pigs, which are models of acute ulcers.

MATERIALS AND METHODS

Experimental Materials

Silk-elastin is composed of 4 silk fibroin–like blocks, 7 elastin-like blocks, and 1 modified elastin block containing a lysine (K) substitution (MDPVVLQRRDWENPGVTQLNRLAAHPPFASDPMGAGSGAGAGS ([GVGVP] $_4$ GKGVP [GVGVP] $_3$ [GAGAGS] $_4$) $_{12}$ (GVGVP) $_4$ GKGVP (GVGVP) $_3$ (GAGAGS) $_2$ GAGAMDPGRYQDLRSHHHHHH).[6] Silk-elastin was supplied by Sanyo Chemical Co., Ltd., (Kyoto, Japan). We used carboxymethylcellulose (CMC) gels (Granugel; ConvaTec, Inc., Tokyo, Japan; and Intrasite gel system; Smith & Nephew Co., Ltd., Tokyo, Japan) for comparison.

Animals and Operations

Genetically diabetic mice (C57BLKS/J Iar+Lepr db/+Leprdb; CLEA Japan Inc., Osaka, Japan) were maintained at Japan Bio Research Center Co., Ltd. (Hashima, Japan). Guinea pigs were maintained at Sanyo Chemical Industries, Ltd. The number of animals used in this study was kept to a minimum, and all possible efforts were made to reduce their suffering in compliance with the protocols established by the Animal Experiment Committee of Japan Bio Research Center or the Animal Research Committee of Kyoto University.

The Application of Silk-elastin to Full-thickness Skin Defects in Diabetic Mice

We used female diabetic mice at 9 weeks of age (n = 120). Three days before starting this experiment, all mice were shaved and depilated. After anesthetizing the mice using isoflurane (Isoflurane inhalation solution; Pfizer Inc., Tokyo, Japan), all mice were cleansed, and full-thickness wounds measuring 12 mm in diameter were created on the middle of the back of each diabetic mouse as impaired wound healing models (Fig. 1). Aqueous silk-elastin solution [56 μl/animal, 20% (w/v) silk-elastin group: n = 30] or CMC gels (50 μl/animal, Granugel group, n = 30; 50 μl/animal, Intrasite gel system group, n = 30) were implanted into the resulting wounds, which were then covered with polyurethane film (Tegaderm; 3M Healthcare, Ltd., Tokyo, Japan). The control wounds were covered with polyurethane film alone. Thereafter, the wound area was covered with gauze, which was fixed to the skin around the wound areas with a nylon thread. At 14 and 21 days after application, all 60 mice were killed. The specimens were fixed with 20% formalin fluid (Mildfolm; Wako Pure Chemical Industries, Osaka, Japan). The specimens included the surrounding normal skin on the section through the center of the wound. For the histological examination, the sections were stained with hematoxylin and eosin.

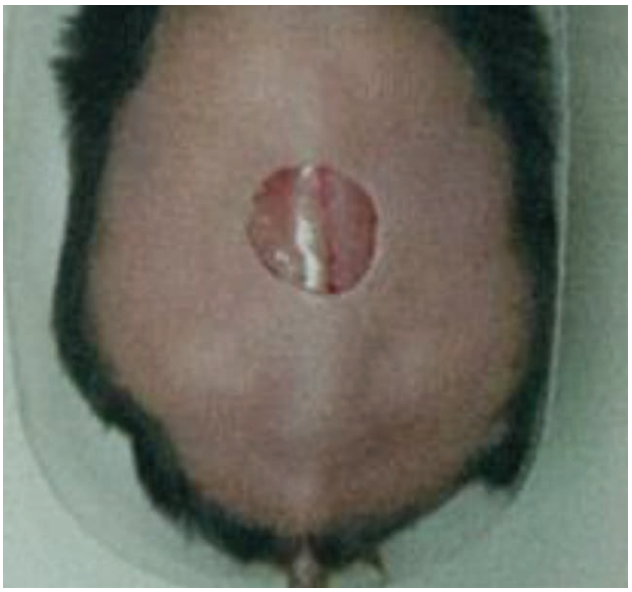

Fig. 1. Full-thickness skin defect on the back of a diabetic mouse. One skin defect (12 mm in diameter) was created on the back of diabetic mouse.

The Application of Silk-elastin to Full-thickness Skin Defects in Guinea Pigs

Because guinea pigs have thicker dermal layer than mice, we prepared full-thickness skin defects of guinea pigs to evaluate granulation tissue formation and wound contraction. Seven-week-old healthy female guinea pigs (std:Hartley; Japan SLC, Inc. Shizuoka, Japan) were anesthetized and depilated. After cleansing, marks 10 mm in diameter were made by tattooing the backs of guinea pigs using a tattoo machine to observe wound contracture (Fig. 2). Inside the tattoo ring, full-thickness wounds were created (8 mm in diameter). After hemostasis and drying, silk-elastin solution [80 μl/animal, 20% (w/v)] or Granugel were applied to the wounds (n = 7–8), which were then

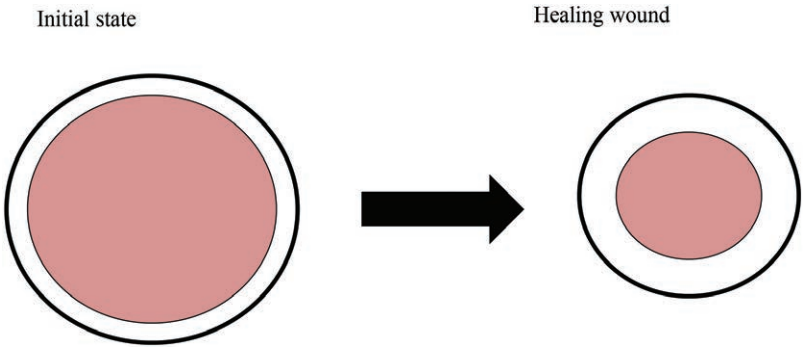

Fig. 2. Full-thickness skin defects on the backs of a guinea pig. Four skin defects were created on the back of a guinea pig. Ten-millimeter diameter circles were marked by tattooing. Inside the marked circles, 8-mm-diameter circles of full-thickness skin defects were created. The marked circles shrank due to the contracture of the healing wounds.

covered with polyurethane film. The control wounds were covered with polyurethane film alone. Thereafter, the wound area was covered with gauze, which was fixed to the skin around the wound areas with a nylon thread. The guinea pigs were killed at 7 or 10 days after wounding, and skin samples from the wounds were taken for histological studies.

Assessments

In the histological examination, the newly formed epithelial length and the area of the granulation tissue formation were measured in the histological sections.

Epidermal Formation

The newly formed epithelial length, from the cut edge of the epidermis to the end of the neoepithelium, was measured on each side of each cross-sectional area using an image analysis software program (Win Roof V7.0; MITANI Corp., Tokyo, Japan). The sum of the lengths of the epithelia was evaluated on both sides as the length of epidermal formation. The epidermal formation rate (%) was calculated as the newly formed epithelial length divided by the full length of the wound.

Area of the Granulation Tissue Formation

The area of granulation tissue formation, from the cut edge of the subcutaneous tissue to the wound floor of the skin defect area, was measured using the image analysis software program mentioned above.

Statistical Analyses

The data are presented as the mean ± SD. All data were statistically analyzed using the Tukey–Kramer paired comparison test, with significance at $P < 0.05$.

RESULTS

The Application of Silk-elastin to Full-thickness Skin Defects in Diabetic Mice

Light microphotographs of the histologic sections (hematoxylin and eosin staining) at 14 and 21 days after implantation are shown in Figures 3 and 4. We evaluated the performance of the silk-elastin for full-thickness skin defects of diabetic mice. The time course of the epidermal formation rate is shown in Figure 5A. At 14 days after administration, the silk-elastin group showed a significantly higher epithelialization rate than all other groups (by about 1.5-fold). At 21 days after administration, the epithelialization rate in the silk-elastin group remained significantly higher than in the control and Intrasite gel system groups but not in the Granugel group. The comparison of the area of newly formed granulation tissue at 14 and 21 days is shown in Figure 5B. At 14 days after administration, the area of the newly formed granulation tissue in the silk-elastin group was significantly larger than in the control and Intrasite Gel System groups, but there was no significant difference in the area compared with that of the Granugel group. At 21 days after administration, there were no significant differences among the groups.

The Application of Silk-elastin to Full-thickness Skin Defects in Guinea Pigs

Macroscopic views of the wound surface and histological sections are shown in Figure 6. We investigated the effect of silk-elastin on the area of granulation tissue formation and the length of epidermal formation (Fig. 7). The length of epidermal formation in the silk-elastin group was significantly higher than in the Granugel group (by about 2-fold) after 7 and 10 days of treatment. The area of the granulation tissue in the silk-elastin group was significantly higher than in the Granugel and control groups (by about 1.5-fold) after 10 days of treatment.

DISCUSSION

In the present study, we examined the effect of silk-elastin compared with conventional hydrogels on skin defects using animal models. In a full-thickness skin defect model in diabetic mice, the newly formed granulation tissue area of the silk-elastin group was not significantly larger than that of the control group at 21 days but was nevertheless reasonably large. The epithelialization rate of the silk-elastin group was significantly higher than that of the other groups, including the control group. In contrast, there were no significant differences in the epi-

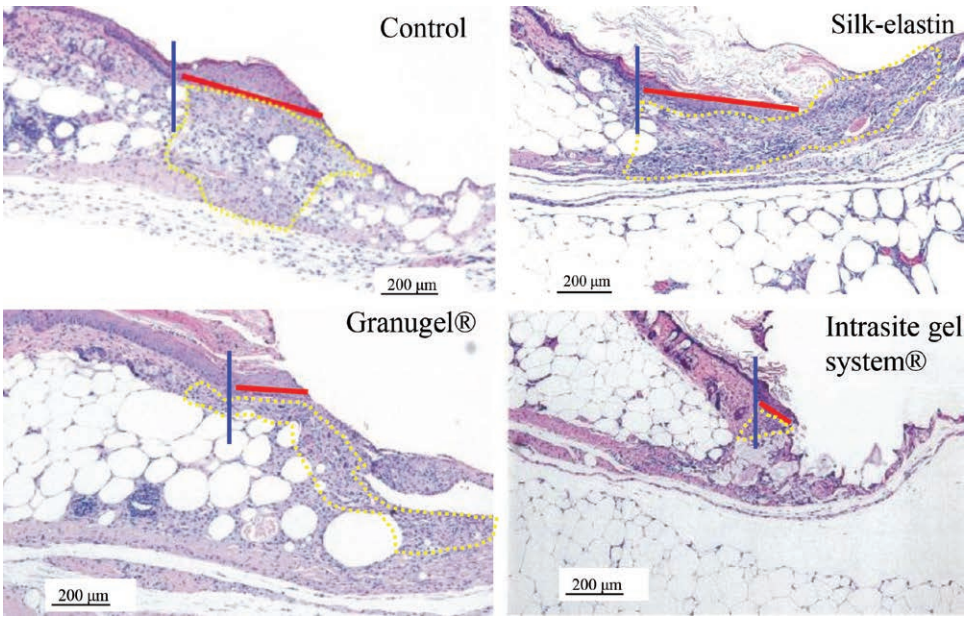

Fig. 3. Histological sections obtained 14 days after wounding in diabetic mice. (A–D) Hematoxylin and eosin staining of the sections of control (A), silk-elastin (B), Granugel (C), and Intrasite gel system (D). Blue lines, wound margin; red lines, newly formed epithelium; yellow broken lines, area of the granulation tissue.

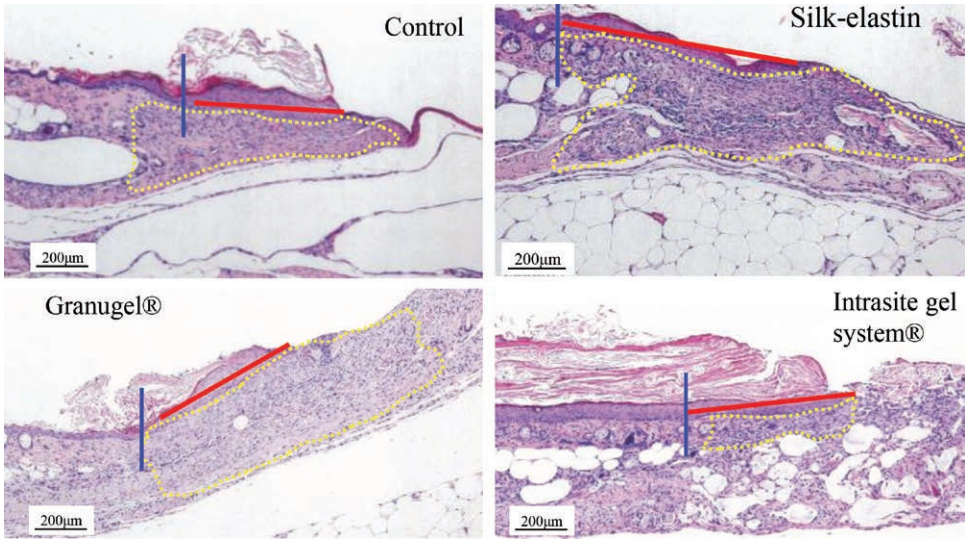

Fig. 4. Histological sections obtained 21 days after wounding in diabetic mice. (A–D) Hematoxylin and eosin staining of the sections of control (A), silk-elastin (B), Granugel (C), and Intrasite gel system (D). Blue lines, wound margin; red lines, newly formed epithelium; yellow broken lines, area of the granulation tissue.

thelialization rate or the newly formed granulation tissue area between the CMC gels (Granugel and Intrasite gel system) and the control group. These results suggest that silk-elastin significantly accelerated wound healing compared with CMC gels. On comparing Granugel with the Intrasite gel system, the newly formed granulation tissue area and the epithelialization rate of the Granugel group were not significantly higher than those of the Intrasite gel system group but were nevertheless reasonably high. This is because Granugel, which contains pectin, has a greater water-absorbing capacity and gel viscosity than the Intrasite gel system.

The newly formed granulation tissue area of the silk-elastin group, however, was not significantly larger than that of the Granugel group. We suspect this was because the dermis of the mice was thinner than that of the guinea pigs. Therefore, we evaluated the granulation formation in guinea pig models of acute cutaneous wounds. For full-thickness skin defects, silk-elastin showed a higher wound healing capacity than the Granugel gel (Fig. 7). This re-

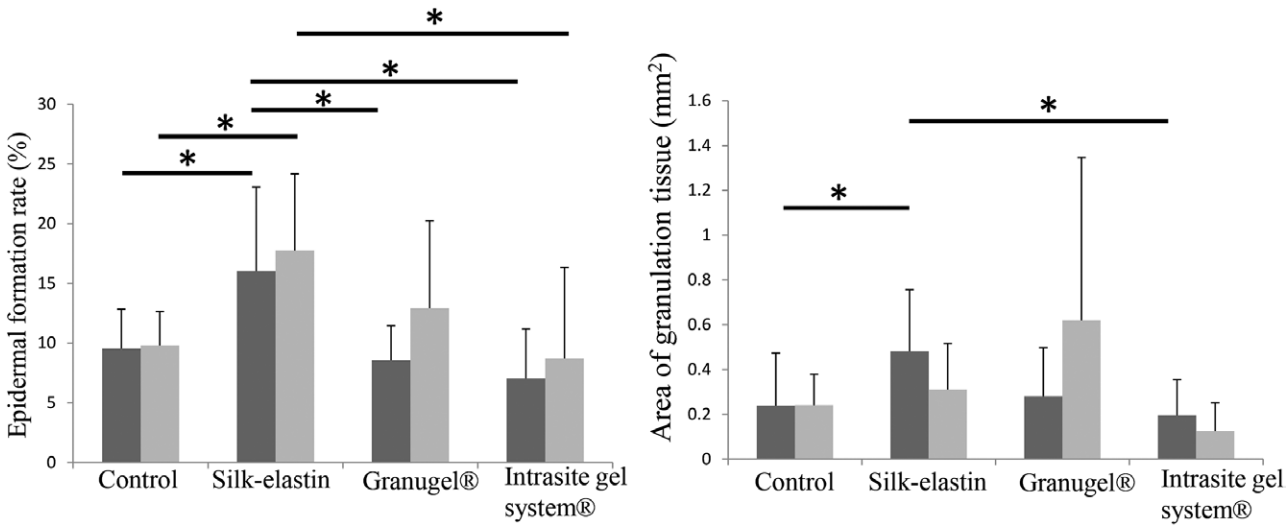

Fig. 5. The evaluation of the wound healing in diabetic mice. (A) Epidermal formation rate and (B) the area of the granulation tissue. The black bars show the results at 14 days after wounding. The gray bars show the results at 21 days after wounding. *$P < 0.05$.

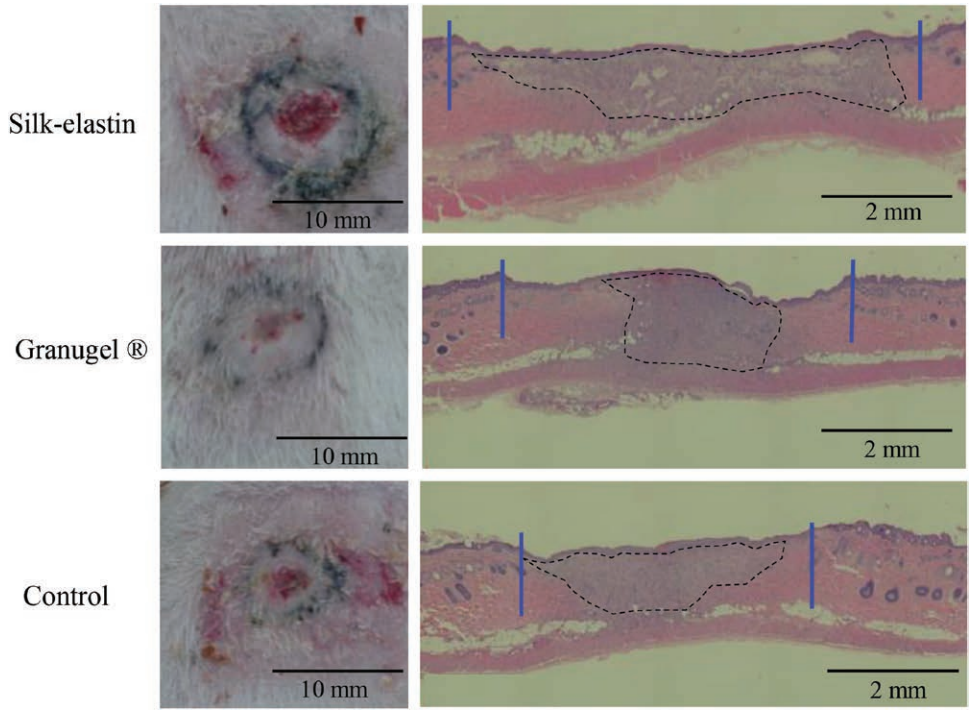

Fig. 6. Macroscopic views of the wound and histological sections obtained 10 days after wounding. (A–C) Macroscopic views of the wound of silk-elastin (A), Granugel (B), and control (C). (D–F) Hematoxylin and eosin staining of the sections of silk-elastin (D), Granugel (E), and control (F). Blue bars, locations of tattooing; broken lines, area of the granulation tissue.

sult may be attributable to the fact that the silk-elastin hydrogels became a scaffold for fibroblasts and promoted granulation tissue formation. Indeed, on a microscopic observation of the wound surface, the silk-elastin hydrogels were found to be incorporated in the granulation tissue, and fibroblasts were localized in the surrounding area (Fig. 6).

Our preliminary study in decubitus models showed that silk-elastin reduced the rate of infection and accelerated wound healing.[10] Ozaki et al[9] reported that silk-elastin promoted the migration of fibroblasts and production of collagen. In the present study, we similarly observed the invasion of fibroblasts into the silk-elastin hydrogels. The fibroblasts were presumed to have been activated by the silk-elastin and migrated from the wound bed into the silk-elastin hydrogels, subsequently producing collagen.

No significant differences were observed among the groups in the rate of bacterial infections in this experi-

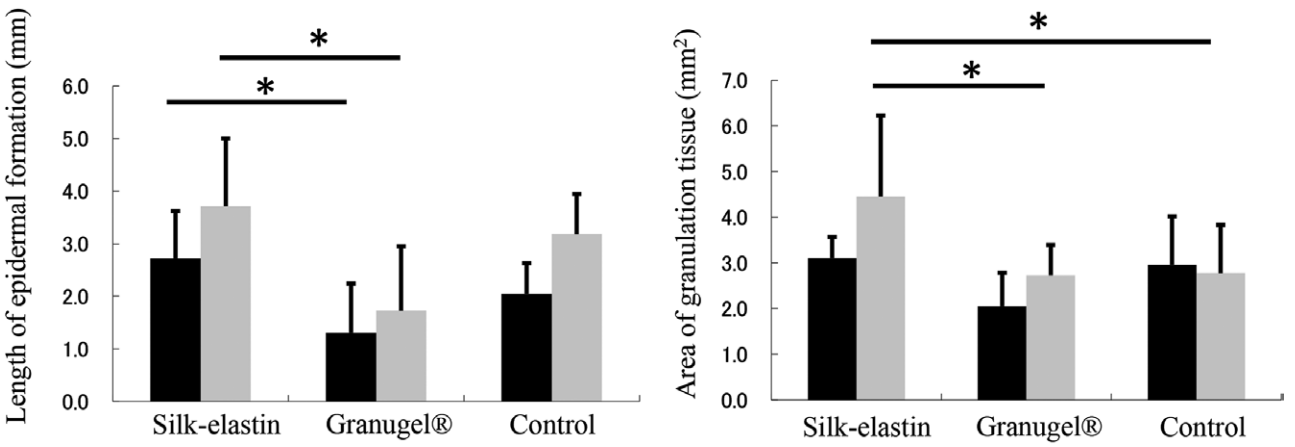

Fig. 7. The evaluation of wound healing in guinea pigs. (A) Length of epidermal formation and (B) the area of the granulation tissue. The black bars show the results at 7 days after wounding. The gray bars show the results at 10 days after wounding. *$P < 0.05$.

ment (data not shown). Silk-elastin is administered as a solution, spreads onto the wound, and forms a hydrogel, which is in close contact with the wound surface. Therefore, the silk-elastin hydrogel not only protects the wound from exposure to bacteria outside the body but also traps any bacteria as well. It has also been reported that silk-elastin promotes the migration of macrophages.[9] Our preliminary study showed that the silk-elastin hydrogels reduced the rate of infection in diabetic mice with pressure sores as an animal model of intractable ulcer.[10] CMC gels may also exert protective effects against external bacteria similar to the silk-elastin hydrogels. However, bacteria may still proliferate inside the wound, as CMC gels have poor adhesion to the surface of uneven wounds.

In the present study, we showed that silk-elastin is a novel material meeting the therapeutic concept of cutaneous ulcers, such as wound bed preparation and moist wound healing.

CONCLUSIONS

Silk-elastin is a useful new material for promoting wound healing. Silk-elastin will be a promising candidate for application in clinical practice in the near future.

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