Silicone Ring Implantation in an Excisional Murine Wound Model

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Abstract: A good animal model for wound healing is indispensable for researchers to study the basic mechanism of tissue repair, and to develop strategies for clinical treatment. Small mammalian wound healing models are the most popular animal models for wound healing research because they are inexpensive, readily obtainable, and easy to handle. One significant challenge of using mice to evaluate wound repair is that wound contraction originates outside of tissue, whereas in humans, re-epithelialization and granulation tissue formation occurs within the wound space. Methods. The present study describes a new excisional skin wound model utilizing an implanted silicone ring on the dorsal side of the mouse for 1 week prior to creating a full-thickness skin defect wound. Results. The results showed that the time required for complete epithelialization of the wound was extended, the re-epithelialization ratio was increased, and more granulation tissue was formed. Conclusion. Permitting the wound to heal mainly through re-epithelialization and granulation tissue formation, this new technique can result in a new excisional murine wound model that closer approximates human wound healing, allowing for more relevant evaluation of molecular signaling and cellular metabolism that occur during skin wound healing.

In the United States, chronic wounds alone affect 5.7 million patients and cost healthcare systems an estimated $20 billion annually. A good animal wound model is indispensable for researchers to study the basic mechanism of tissue repair, to develop strategies for clinical treatment, and to evaluate the product safety and efficacy.

Many different animal models, from rodents to nonhuman primates, have been used to study the wound healing process, e.g., acute wound models, such as incisional or excisional, partial- or full-thickness, burn, contracture, contraction, dead space, and chronic wound models, such as ischemia and vessel ligation. Some impaired healing models have also been devised. These wound models are used for studying various conditions and allow one to observe or harvest a specimen at any time during the healing process for further analysis.
An ideal wound model should reflect the wound pathogenesis and illustrate the clinical situation. However, due to the differences in tissue architecture, physiology, and other healing responses among species, no ideal excisional wound model exists. Small mammalian wound models are popular because they are economical, and easy to house and maintain. However, in an excisional wound in rodents, wound contraction force originates from outside the tissue, whereas in humans, it is the proliferation of granulation tissue and re-epithelialization that occurs within the wound space. While the porcine dorsal wound model or rabbit ear ulcer model can mimic the clinical procedure in human situations, they are expensive to house and maintain, and may not be practical for investigational work. Therefore, exploring an ideal excisional wound model that is less costly, but more effective for researching excisional skin wound repair, is needed.

In the present study, a silicone ring was implanted subcutaneously on the backs of 24 mice 1 week prior to creating a full-thickness skin wound. The effects of this novel technique on wound healing were examined. The new model took more time to reach complete epithelialization, had a higher re-epithelialization ratio, and more granulation tissue formation compared to the other groups in the study. This technique can reduce wound contraction and allow the wound to heal mainly through re-epithelialization and granulation tissue formation.

**Methods**

Animal model. Eleven-week old C57BL/6J mice (n = 24) weighing about 18 g–20 g were used in the study. Each mouse had its own cage post-operation with access to food and tap water ad libitum. The mice were kept on a 12-h light/dark cycle. All of the animals utilized in this experiment received humane care.

Mice were individually anesthetized by using isoflurane (2% isoflurane, 2 L/min oxygen). The dorsal surface was shaved with electric clipper followed by application of a depilatory agent (Nair®, Church & Dwight Co, Princeton, NJ) for 2–3 minutes to remove the remaining hair. Povidone iodine was placed on the exposed skin. A 1.5-cm incisional wound was then created along the caudal midline. Through the incision, the skin and subcutaneous tissue were separated on both sides of the cranial midline, and then a silicone ring (outer diameter 14 mm, inner diameter 10 mm, width 2 mm, thickness 1 mm) was implanted subcutaneously in one side of the cavity. The silicone ring was fixed with three interrupted sutures (5-0 silk) to prevent movement, and the incision was sutured. The animals were allowed to recover from anesthesia and were housed individually in the animal facility.

All sutures were removed on day 7. Half of the implanted silicone rings were fixed with the skin again by interrupted sutures (Fixed group) and half were not fixed (Unfixed group, n = 12 for each group). An 8-mm biopsy punch was used to outline the marker for the wound in the center of the silicone ring. Then an 8-mm diameter, full-thickness skin sample was removed through the panniculus carnosus with scissors. On the other side of midline, the same 8-mm, full-thickness skin sample was removed through the panniculus carnosus (control group, n = 24). The wounds were covered with a transparent film dressing (3M Tegaderm™, 3M Health Care, St. Paul, MN). The animals were allowed to recover from anesthesia and were housed individually in the animal facility. On postoperative days 2–12, the transparent film dressings were changed, and the wounds were cleaned and measured daily.

**Wound Analysis**

The wound areas were traced and measured immediately after surgery and daily from days 2–12. Wound areas were determined by tracing the wound area onto transparent plastic paper, measuring the weight of the plastic paper, and counting the percentage to that of the original area.

Relative wound size = weight of wound area on xx day/weight of wound area on day 0 x 100

The total number of days required for complete epithelialization was recorded. A wound was considered as

**Keypoints**

- An ideal wound model should reflect the wound pathogenesis and illustrate the clinical situation. However, due to the differences in tissue architecture, physiology, and other healing responses among species, no ideal excisional wound model exists.
- A 1.5-cm incisional wound was created along the caudal midline of the mice. Through the incision, the skin and subcutaneous tissue were separated on both sides of the cranial midline, and then a silicone ring was implanted subcutaneously in one side of the cavity. The silicone ring was fixed with three interrupted sutures (5-0 silk) to prevent movement, and the incision was sutured.
completely closed when moist granulation tissue was no longer visible and was covered with epithelium. The percentage of the weight of re-epithelialization area to that of the original area was recorded as the re-epithelialization ratio.

**Histology and Immunohistochemistry**

The tissue in the re-epithelialization area, scar tissue, and some normal skin, were harvested and fixed with 10% formalin on day 12. Formalin-fixed, paraffin-embedded tissues were sectioned at 5 µm. The samples underwent routine histological processing of hematoxylin and eosin (H&E) and Masson's trichrome staining. Cell proliferation was assessed through staining with rabbit anti-mouse Ki67 antibody ([1:200 dilution], Thermo Scientific, Fremont, CA). Sections were mounted on positively charged glass slides, autoclaved in a water bath in citrate buffer to unmask antigenicity, immersed in 0.3% H2O2 to block endogenous peroxidase activity, and then incubated with horse serum, primary antibody, and secondary antibody. The avidin biotinylated enzyme complex system (Vectorstain Elite ABC, Vector, Burlingame, CA) and 3,3'-Diaminobenzidine substrate chromogen solution (Dako, Carpinteria, CA) were used for tissue staining.

Epithelial regeneration, cell proliferation, tissue architecture, and collagen deposition were analyzed. Epithelial regeneration was quantified by measuring the epithelium thickness using ImageJ, and dermal repair was quantified by counting the number of Ki-67 positive cells per power field at 40x magnification in the wound field. Histologi-
cal morphology was used to analyze collagen deposition. The investigators who performed the histological evaluation were blinded to the experimental data. Two independent investigators measured the epithelium thickness and counted Ki-67 positive cells, and their observations were averaged.

**Statistical Analysis**

Statistical analysis was performed with SPSS 17.0 software based on analysis of variances followed by Bonferroni test. A significant difference was accepted at $P < 0.05$.

**Results**

Effects of fixed silicone ring on wound healing in C57BL/6J mice. The wounds in the Fixed group were significantly larger than that of the Control and Unfixed groups at nearly all time points except on day 0 ($P < 0.05$; Figures 2A, B). The relative wound size on days 3 and 6 were $85.62\% \pm 2.00\%$ vs. $44.31\% \pm 1.63\%$ in the Fixed group, compared to $68.61\% \pm 2.88\%$ vs. $28.43\% \pm 1.83\%$ in the Unfixed group, and $64.12\% \pm 4.27\%$ vs. $21.18\% \pm 1.90\%$ in the Control group. The time required for complete epithelialization in the Fixed group (10.08 ± 0.08 days) was also significantly longer than that of the Unfixed group (8.58 ± 0.15 days) and Control group (8.25 ± 0.13 days; $P < 0.05$). There was no statistically significant difference between the Unfixed group and the Control group ($P > 0.05$).

The re-epithelialization ratio (Figure 2C) in the Fixed group (32.04% ± 1.92%) was also significantly higher than that of the Control and Unfixed groups at nearly all time points except on day 0 ($P < 0.05$; Figures 2A, B). The re-epithelialization ratio in the Fixed group was significantly higher than that of Unfixed group and Control group ($P < 0.05$). Data represent mean ± SEM (n = 12 or 24).

![Figure 2. Fixed silicone ring prevented wound contraction in C57BL/6J mice. A) Simple, gross observation of wound healing on postoperative days 0, 3, 6, and 9 in Fixed group, Unfixed group, and Control group (the wound on the right side). The wound in the Fixed group was larger than that of Control and Unfixed groups ($P < 0.05$). B) Wound healing speed among the three groups. Fixed silicone ring prolonged the healing time in C57BL/6J almost 2 days comparing to that of Unfixed and Control group ($P < 0.05$). Each point represents the mean of the relative wound size. C) The re-epithelialization ratio of wound in different groups. The re-epithelialization ratio in the Fixed group was significantly higher than that of Unfixed group and Control group ($P < 0.05$). Data represent mean ± SEM (n = 12 or 24).](image-url)
than that of Unfixed group (12.35% ± 2.01%) and Control group (11.23% ± 1.49%; \(P < 0.05\)). No significant difference was found between the Control group and the Unfixed group \(P > 0.05\).

Effects of fixed silicone ring on epithelial regeneration and dermal repair in vivo. On day 12, Masson’s trichrome staining showed more collagen fibers were formed and arranged parallel to the surface in the wound bed in the Fixed group, but less collagen was deposited in the Unfixed group and Control group. According to H&E stains, the new epithelium in the wound beds were 54 ± 11 µm, 31 ± 7 µm, and 29 ± 8 µm thick, in the Fixed group, Unfixed group, and Control group, respectively, on postoperative day 12. Immunohistochemical staining of Ki-67 showed the number of Ki-67 positive cells were 72 ± 14, 51 ± 8, 54 ± 12 per power field at 40x magnification, in the Fixed group, Unfixed group, and Control group, respectively. The new method produced an increase in re-epithelialization, collagen formation, and cell proliferation in the wound area. Data represent mean ± SEM (n = 12 or 24).

Discussion

In the United States, chronic wounds affect 5.7 million patients and cost healthcare systems an estimated $20 billion annually.\(^1\) Animal models are important tools for studying and resolving these problems.\(^2,3,12\)

Many different animal wound-healing models, such as acute wound models, chronic wound models, and impaired wound healing models, have been utilized. These models are used to study various conditions of wounds and have improved researchers’ ability to study mecha-

**Figure 3.** Effects of fixed silicone ring on epithelial regeneration and dermal repair in vivo. A) H&E (20x), Masson’s trichrome staining (20X) and immunohistochemical staining of Ki67 (40x) of the wound bed on day 12. B) The thickness of the epithelium in different groups on day 12 post-operation. C) The number of positive Ki67 cells per power field on day 12 post-operation in the wound bed at 40x magnification. More collagen formed, more epithelial regeneration occurred, and more positive Ki67 cells were found in the Fixed group than in the other two groups. The new method increased re-epithelialization, collagen formation, and cell proliferation in the wound area. Data represent mean ± SEM (n = 12 or 24).
nisms of human disease. Among these animal models, the rodent wound model is the most popular because of its low cost and ease of use. However, in an excisional rodent wound model, the wound contraction force originates from outside the wound, whereas in humans, it is the proliferation of granulation tissue and re-epithelialization that occurs within the wound space. Most of these animal wound models cannot reflect actual human pathophysiologic processes. The porcine wound model was thought to be ideal for studying cutaneous disease since its architecture is very similar to human skin; however, they are expensive to house and maintain, as are rabbit ear ulcer models. A low-cost animal model that can reflect real-world clinical procedures is needed.

In 2004, Galiano et al. reported a new technique to resolve the problem of wound contraction in mice. In this model, a full-thickness skin wound on the backs of mice were created, and then a silicone ring was fixed with an immediate-bonding adhesive and interrupted sutures around the wound to reduce wound contraction. This technique was repeated in the present study, and it was found that wound contraction was notably reduced in the first few days. However, the bonding adhesive had changed into dry eschar with reduced adhesive ability in a few postoperative days. Most of the sutures had detached from the skin and lost their fixing ability around the fifth postoperative day. Furthermore, it was difficult to resuture them due to the wound contraction that had already occurred.

The novel technique described in the present study resolves the problems that were encountered with Galiano et al. technique. By subcutaneously implanting the silicone ring first and then creating the excisional skin wound 7 days later, the sutures did not detach from the skin, and the skin remained properly fixed until the end of the experiment. The wound healing process was not affected by the silicone ring, as the skin and subcutaneous tissue healed very well by day 7 after the first implanting operation. No significant difference was found between the Unfixed group and the Control group in regard to wound healing speed, re-epithelialization ratio, cell proliferation, and histological morphology.

**Conclusion**

This new technique can effectively reduce wound contraction and allow the wound healing mainly through re-epithelialization and granulation tissue formation. Therefore, it might be a better choice for the assessment of molecular and cellular mechanisms within the wound healing procedure, and also facilitates the evaluation of drug effects on wound healing.

**Acknowledgement**

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**References**