Video Article

Murine Model of Wound Healing

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Abstract

Wound healing and repair are the most complex biological processes that occur in human life. After injury, multiple biological pathways become activated. Impaired wound healing, which occurs in diabetic patients for example, can lead to severe unfavorable outcomes such as amputation. There is, therefore, an increasing impetus to develop novel agents that promote wound repair. The testing of these has been limited to large animal models such as swine, which are often impractical. Mice represent the ideal preclinical model, as they are economical and amenable to genetic manipulation, which allows for mechanistic investigation. However, wound healing in a mouse is fundamentally different to that of humans as it primarily occurs via contraction. Our murine model overcomes this by incorporating a splint around the wound. By splinting the wound, the repair process is then dependent on epithelialization, cellular proliferation and angiogenesis, which closely mirror the biological processes of human wound healing. Whilst requiring consistency and care, this murine model does not involve complicated surgical techniques and allows for the robust testing of promising agents that may, for example, promote angiogenesis or inhibit inflammation. Furthermore, each mouse acts as its own control as two wounds are prepared, enabling the application of both the test compound and the vehicle control on the same animal. In conclusion, we demonstrate a practical, easy-to-learn, and robust model of wound healing, which is comparable to that of humans.

Video Link

The video component of this article can be found at http://www.jove.com/video/50265/

Introduction

Impaired wound healing is responsible for significant morbidity and mortality worldwide; this is particularly true for sufferers of diabetes mellitus[1,2]. In humans, wound healing is a continuum of processes, in which there is significant overlapping[3]. Immediately following wounding, inflammatory processes are initiated. Inflammatory cells release factors that encourage the processes of cell proliferation, migration and angiogenesis. After re-epithelialization and new tissue formation there is a phase of remodeling that entails both apoptosis and re-organization of matrix proteins such as collagen.

The complexity of wound healing cannot currently be replicated *in vitro* and this necessitates the use of animal models. To date, wound-healing studies have been limited to large animal models, such as swine, to ensure that the healing processes are equivalent and comparable to humans. However, using large animals for such studies can be difficult to house and are not always practical[4]. The laboratory mouse represents an economical animal model that can be easily genetically manipulated for mechanistic investigation[5-7]. However, murine wounds heal differently to human's, primarily due to the process of contraction[8]. This is in part, due to an extensive subcutaneous striated muscle layer called the panniculus carnosus that is largely absent in humans. In mice, this muscle layer allows the skin to move independently of the deeper muscles and is responsible for the rapid contraction of skin following wounding.

To overcome this limitation, murine wound healing can be adapted to replicate human wound healing by use of a splint (**Figure 1**)[8,9]. In this video we demonstrate the splinted murine wound model that eliminates wound contraction and more closely approximates the human processes of re-epithelialization and new tissue formation. In this model two full-thickness excisions that include the panniculus carnosus are created on the dorsum, one on each side of the midline of the mouse. A silicone splint is placed around the wound with the assistance of adhesive and the splint then secured with interrupted sutures. Each mouse acts as its own control, with one wound receiving treatment and the other vehicle control, thereby reducing animal numbers. Following topical applications, a transparent occlusive dressing is applied. The dressing can be removed when required for further topical applications and/or measurement of the wound area[10,11]. At the completion of experiments, wound closure,

morphological architecture and degree of neovascularization can be assessed by immunohistochemistry. This economical and easy to perform model can also be utilized to assess wound healing in the context of diabetes mellitus or other pathophysiologies.

Protocol

1. Preparation of Splints and Occlusive Dressings

1. Outline 10 mm circles on 0.5 mm thick silicone sheeting and use scissors or a biopsy punch to create silicone disks.
2. Centre a 5 mm biopsy punch in the middle of the 10 mm circle and press firmly to create a hole to form a "donut"-like disc that will be used as a splint.
3. Outline 10 mm circles on a transparent occlusive dressing such as Opsite and use scissors to create circular dressings.

2. Experimental Animals

1. Obtain Animal Ethics Committee approval for all experiments that will be performed.
2. Use 8-week old (22-26 grams) male C57BL/6J mice from a commercial breeder (*e.g.* The Jackson Laboratories).
3. Keep mice in standard conditions of 21 °C and a 12 hr light-dark cycle with free access to food and water.
4. (Optional): Diabetes can be induced in 6 to 7 week old mice by a bolus intraperitoneal injection of 165 mg/kg streptozotocin (in citrate buffer, pH 4.5), with hyperglycemia confirmed by regular blood glucose monitoring (AccuChek glucometer). Diabetic mice may have polyuria and so their bedding may need to be changed more frequently to eliminate wetness and their weights should be monitored closely.

3. Anesthesia and Operative Preparation

1. Induce general anesthesia using 5% isoflurane in 100% oxygen (flow rate 1 L/min) and maintain anesthesia using 1-3% isoflurane.
2. Ensure the deep pedal reflexes of the mouse are suppressed and place the mouse in the prone position.
3. Prepare the operative region by removing fur with clippers from the base of the neck to 3 cm further down the back and between the two shoulder blades.
4. A light application of depilatory cream may be applied for no longer than 2 min. Wet gauze swabs can be used to ensure all cream and remaining fur is removed.
5. Wipe the skin with an alcohol swab and two applications of 10% povidone-iodine (Betadine) and drape the mouse.

4. Excision and Splinting of Wound

1. Diagrammatic representation of the murine wound-healing model is presented in **Figure 1**.
2. Use a sterile 4 mm biopsy punch to outline two circular patterns for the wound on either side of the mouse's midline at the level of the shoulders (see **Figures 2a-2b**).
3. Use serrated forceps to lift the skin in the middle of the outline and iris scissors to create a full-thickness wound that extends through the subcutaneous tissue (**Figures 2c-2d**), including the panniculus carnosus (**Figure 2e**), and excise the circular piece of tissue.
4. Repeat the process for the wound on the other side of the midline (**Figure 2f**).
5. Remove plastic protective coating from each side of the silicone splint.
6. Apply cyanoacrylate adhesive (*e.g.* Super Glue or Krazy Glue) to one side of a silicone splint.
7. Centre the splint over the wound (**Figure 2g**) and anchor the splint with interrupted 6-0 nylon sutures to ensure positioning (**Figure 2h**).
8. Repeat the splinting process on the other wound.
9. If required at this time-point, apply the therapeutic compound to be tested (up to 100 µl) to one wound (**Figure 2i**), and the vehicle control to the other.
10. Cover the wound with a transparent occlusive dressing (for example, OpSite) (**Figure 2j-2k**).
11. A ruler is placed below the splints and a photomicrograph taken (**Figure 2l**).

6. Postoperative Management

1. Carprofen (5 mg/kg) is administered once daily via sub-cutaneous injection for post-operative pain relief.
2. After the surgery animals are individually caged and maintained on heat mats until fully recovered.
3. Monitor animals twice daily for manifestations of pain and weight loss. We observed no gross behavioral displays of pain or weight loss.

7. Wound Measurement and Treatment

1. The wound can be measured daily.
2. Induce general anesthesia using 5% isoflurane gas (flow rate 1 L/min), and then ensure the deep sensory reflexes of the mouse are suppressed using 1-3% isoflurane.
3. Gently peel back the occlusive dressing with forceps.
4. Use surgical calipers to measure the wound diameter. We take the average of three measurements along the X, Y and Z axis (**Figures 2m-2o**) as well as a photomicrograph for future reference (**Figure 2p**).
5. Optional: At this time the animal can be assessed for blood perfusion using a laser Doppler imager (**Figure 2q**).
6. Re-application of the therapeutic compound and the vehicle control can be performed at this point.
7. A clean transparent occlusive dressing is then re-applied and the animals kept warm until fully recovered.

8. **Note:** if splints are not secured properly the wound will readily contract (**Figure 2r**).

8. Histological Analysis

1. Euthanize the mice after 10 days with an overdose of anesthesia.
2. Using forceps and a scalpel blade remove the sutures and carefully peel away the splint.
3. Use iris scissors to create a wide, full excision around and under the wound area and incubate the tissue in 4% paraformaldehyde in phosphate buffered saline (PBS) at 4 °C overnight.
4. Transfer tissue to 17% sucrose in PBS solution for a further 24 hr at 4 °C then remove excess solution by dabbing carefully on tissue and place in O.C.T compound and freeze at -80 °C.
5. Hematoxylin and eosin staining can be used to visualize the wound structure and epithelial gap. Neovascularisation can be assessed by immunohistochemical analyses to determine the number of capillaries (using Von Willebrand factor).

Representative Results

A wound closure curve is determined by calculating the average diameter of the wound and expressing the results as a percentage, *i.e.* 100 - (Day 0 diameter/Day X diameter). In this experiment a therapeutic compound (or vehicle control) was applied daily to the wound. The therapeutic compound greatly accelerated wound closure (**Figure 3**). It is important to note that the splints must be maintained for the duration of the experiment, as removal of splints will lead to rapid wound contraction (**Figure 2r**) and diverge from the pattern of wound healing observed in humans.

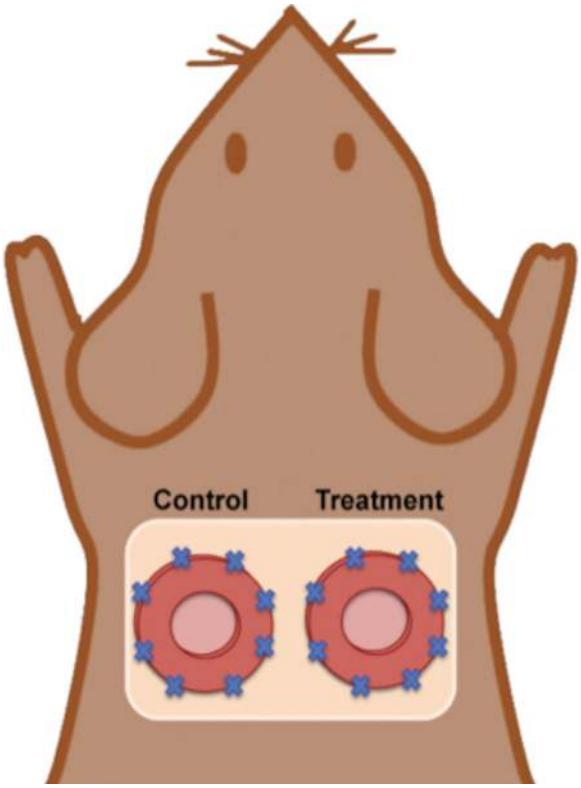

Figure 1. Schematic representation of the murine wound healing model. In this model two full thickness wounds are created on either side of the midline allowing each mouse to serve as their own control. Silicone splints are adhered and sutured to the wound perimeter to prevent wound contraction, providing a model replicable to that of humans.

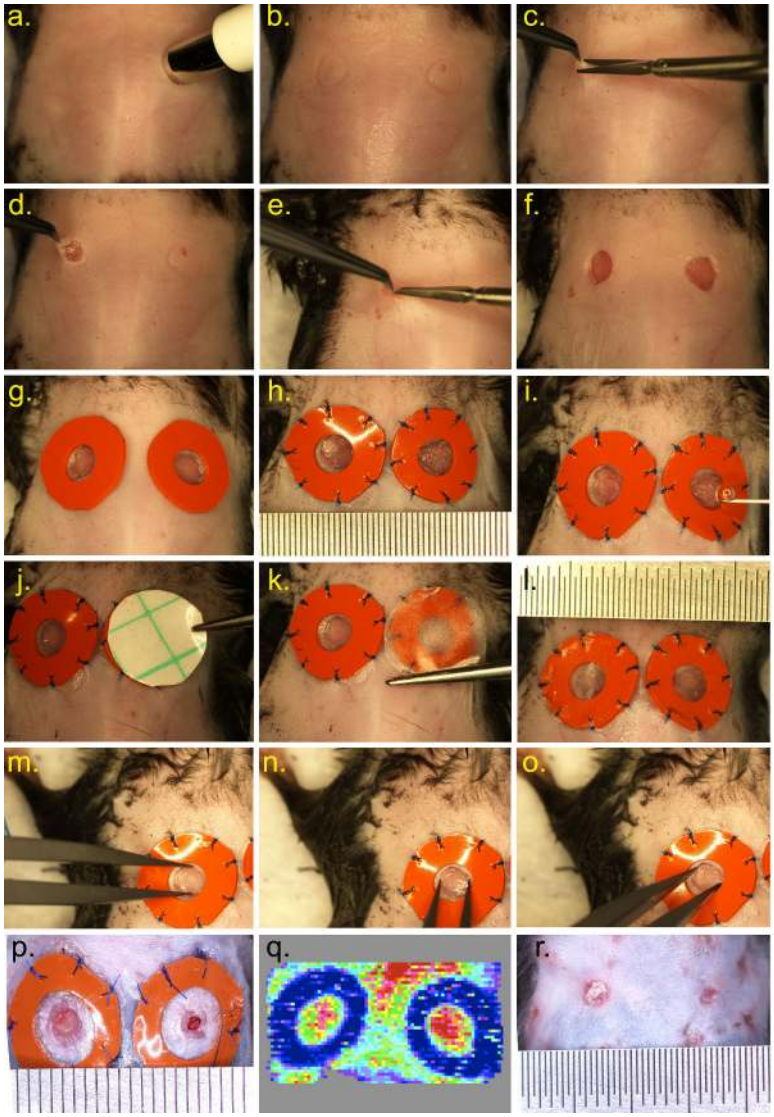

Figure 2. Wound healing surgery and post-surgical measurements. Following hair removal and preparation of the skin with iodine and alcohol **(a-b)** a biopsy punch is gently used to outline two circles on the dorsum, either side of the midline. **(c)** A small incision is then created and **(d)** a circular piece of skin is removed, **(e)** including the panniculosus carnosus, **(f)** to create two full-thickness wounds. **(g)** Adhesive is then applied to the silicone splints and the splints adhered to the wound perimeter. **(h)** Splints are then secured with sutures. **(i)** Treatments are topically applied and **(j-k)** an occlusive, transparent dressing is placed over the wound and adhered to the splints (adhesive can be used if required). **(l)** Photomicrographs are taken daily and wound area is calculated from the average of three diameter measurements on the **(m)** y-axis, **(n)** x-axis and **(o)** z-axis. **(p)** A representative photo of the wounds at day 10, noting the smaller wound on the right that was treated with a therapeutic compound. **(q)** Representative laser Doppler image of blood perfusion of the wound at day 6. **(r)** Example of rapid wound contraction following removal of the silicone splints.

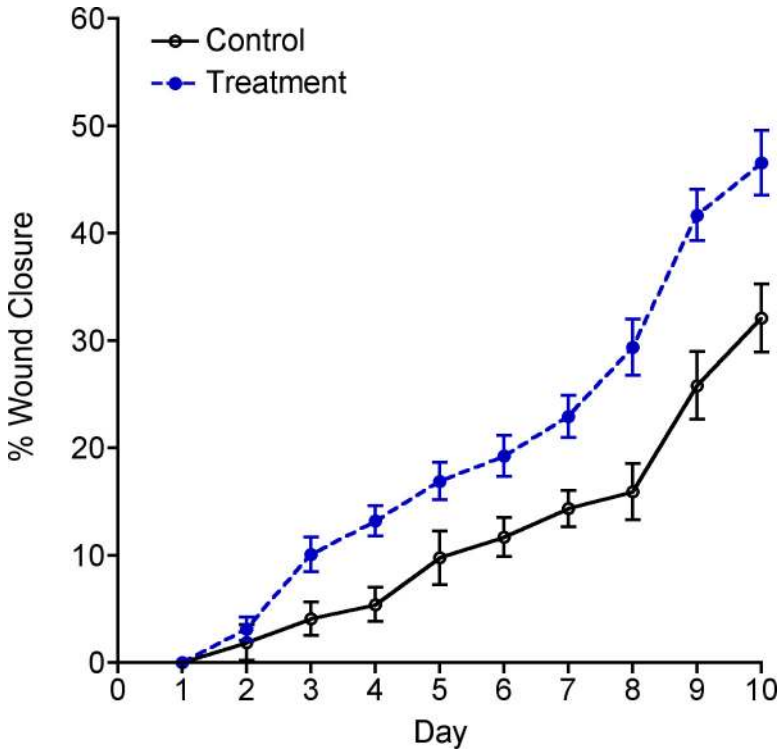

Figure 3. Representative wound closure graph. Wound area is calculated from the average of three daily diameter measurements along the x, y and z-axes. Wound closure is expressed as a percentage of initial wound area at day 0.

Discussion

This is an experimental murine model of cutaneous wound healing. A significant feature of this model is the use of silicone splints to prevent wound contraction so that re-epithelialization and new tissue formation may occur, making it similar to the process that occurs in humans. This model is versatile and can be used to assess wound healing in both physiological and pathophysiological (*e.g.* diabetes mellitus) settings. The model may also be used to assess potential wound healing or angiogenesis therapeutics in an economical setting. With each mouse acting as its own control, animal numbers are minimized. The surgical techniques required for this model are not highly sophisticated, thus this model can be widely used by those with relatively little surgical experience.

To ensure reproducibility and accurate quantification it is imperative that the splint is adequately adhered and anchored to the skin with sutures, and that there is minimal delay between creating the two wounds. The propensity of murine wounds to rapidly contract following loosening of the splint or partial removal due to scratching by the mouse requires daily monitoring of the splints. Careful application of adhesive is also required to minimize irritation of healthy skin around the splint that may promote scratching. It is also very important to follow aseptic techniques and thoroughly disinfect equipment, particularly the calipers, between mice. The application of the occlusive dressing must also be considered, especially if wounds are not going to be treated or dressed daily. Opsite and Tegaderm (3M) dressings are comparable [12], and it has been shown that Tegaderm dressings may only remain adhered for 1-2 days. Should longer-term wound dressings be required an alternative approach has been described by Chung and colleagues[13].

Potential weaknesses of this model may include inflammation due to the anchoring of sutures, the diffusion of the treatment or vehicle between the wounds and the entry of the treatment into the systemic circulation. In regards to the sutures inducing local inflammation, the sutures are placed relatively far from the wound and as each wound is created the same, therefore any inflammation that may occur should be similar between wounds. Similarly, the distance between the wounds and a lack of edema between the wounds would minimize diffusion between the two beds. There is some evidence that a treatment may enter the systemic circulation, which may accelerate healing of the control wound [9,10]. To determine the extent of a treatment entering the systemic circulation, littermate mice could be utilized, in which the two wounds are only treated with the vehicle. The differences in rates of wound closure between mice treated with vehicle only, and the mice receiving both vehicle and treatment could then be compared.

In conclusion, we have demonstrated a relatively simple murine model of wound healing that exhibits many of the features observed in human wound healing.

Disclosures

The authors have nothing to disclose.

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References

1. Sen, C.K., *et al.* Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen.* **17**, 763-771 (2009).
2. Sen, C.K. Wound healing essentials: let there be oxygen. *Wound Repair Regen.* **17**, 1-18 (2009).
3. Gurtner, G.C., Werner, S., Barrandon, Y,. & Longaker, M.T. Wound repair and regeneration. *Nature.* **453**, 314-321 (2008).
4. Lindblad, W.J. Considerations for selecting the correct animal model for dermal wound-healing studies. *J. Biomater. Sci. Polym. Ed.* **19**, 1087-1096 (2008).
5. Grose, R. & Werner, S. Wound-healing studies in transgenic and knockout mice. *Mol. Biotechnol.* **28**, 147-166 (2004).
6. Reid, R.R., Said, H.K., Mogford, J.E., & Mustoe, T.A. The future of wound healing: pursuing surgical models in transgenic and knockout mice. *J. Am. Coll. Surg.* **199**, 578-585 (2004).
7. Fang, R.C. & Mustoe, T.A. Animal models of wound healing: utility in transgenic mice. *J. Biomater. Sci. Polym. Ed.* **19**, 989-1005 (2008).
8. Wong, V.W., Sorkin, M., Glotzbach, J.P., Longaker, M.T., & Gurtner, G.C. Surgical approaches to create murine models of human wound healing. *J. Biomed. Biotechnol.* **2011**, 969618 (2011).
9. Galiano, R.D., Michaels, J. t., Dobryansky, M., Levine, J.P., & Gurtner, G.C. Quantitative and reproducible murine model of excisional wound healing. *Wound Repair Regen.* **12**, 485-492 (2004).
10. Galiano, R.D., *et al.* Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. *The American Journal of Pathology.* **164**, 1935-1947, doi:10.1016/S0002-9440(10)63754-6 (2004).
11. Thangarajah, H., *et al.* The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. *Proceedings of the National Academy of Sciences of the United States of America.* **106**, 13505-13510, doi:10.1073/pnas.0906670106 (2009).
12. Raza, A., Bayles, C., & Biebel, D. Investigation of Bacterial Growth and Moisture Handling Properties of Transparent Dressings: 3M Tegaderm Transparent Dressing, 3M Tegaderm HP Transparent Dressing, and Opsite IV3000 Transparent Dressing. *Smith and Nephew Report*, (1998).
13. Chung, T.Y., Peplow, P.V., & Baxter, G.D. Testing photobiomodulatory effects of laser irradiation on wound healing: development of an improved model for dressing wounds in mice. *Photomed. Laser Surg.* **28**, 589-596 (2010).

