Published in final edited form as:

Wound Repair Regen. 2015 ; 23(6): 874–877. doi:10.1111/wrr.12338.

The murine excisional wound model: Contraction revisited

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Abstract

Rodent models of healing are considered limited because of the perception that rodent wounds heal by contraction while humans heal by re-epithelialization. The purpose of this report is to present evidence that simple murine excisional wounds provide a valid and reproducible wound model that heals by both contraction and re-epithelialization. Previous studies have shown that, although rodent wounds contract by up to 80%, much of this contraction occurs only after epithelial closure. To confirm these previous findings, we measured re-epithelialization and contraction in three separate mouse strains, (BALB/c, db/+ and db/db); re-epithelialization and contraction each accounted for ~40-60% of the initial closure of full thickness excisional wounds. After closure, the wound continues to contract and this provides the impression of dominant closure by contraction. In conclusion, the simple excisional rodent wound model produces a well-defined and readily identifiable wound bed over which the process of re-epithelialization is clearly measurable.

Keywords

wound healing; contraction; mouse; skin

Wound healing consists of overlapping phases of hemostasis, inflammation, tissue formation and remodeling (1). The rodent excisional model of wound healing has been widely used to study each phase of healing; however, rodent models of healing are often considered limited because of the perception that rodent wounds heal primarily by contraction while humans heal by re-epithelialization. The amount of contraction that occurs in rodent excisional wounds has been carefully quantified in multiple published studies dating from the late 1950s (2-5). These prior studies suggest that in rodents, the final contribution of contraction to wound closure is around 80%. This level of contraction has created concern about the relevance of the model to human wound healing. In considering the influence of contraction, what has not been well appreciated is the rate and timing of contraction in excisional rodent wounds. Prior studies suggest that the majority of contraction in rodent wounds occurs only

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Disclosure statement: The authors have no conflicts of interest.

after epithelial closure (2), and a lag phase in contraction has been described in rats (5). A large number of studies demonstrate that rodent wounds exhibit a well-defined and readily identifiable wound bed over which the process of re-epithelialization is clearly measurable (e.g., (6, 7)). In this context, rodent models might be considered highly appropriate for the studies of the earlier phases of healing such as inflammation, re-epithelialization, collagen formation, and angiogenesis.

Given the controversy over the whether contraction abrogates the utility of rodent wound models, we recently re-evaluated the contribution of wound contraction to closure in excisional wounds of mice by two different methods. First we used external measurements to examine the contributions of wound contraction and re-epithelialization to wound closure (Figure 1). Two 8 mm diameter excisional wounds were made on the dorsum of anesthetized BALB/c and db/+ and db/db mice on a C57Bl/6 background (Jackson Laboratories, Bar Harbor, ME), and a permanent marker was used to draw an approximately 1 cm square around the wound. Using digital images and Image J software, we monitored the areas of the wound and the external square daily until day 10 post-injury. Using these measurements, we calculated the percent total wound closure (%TWC) as: $[(WA_0 - WA_T) / WA_0] \times 100\%$ where WA_0 = wound area at day 0 and WA_T = wound area at time point T. We then estimated the percent of wound closure attributable to contraction (%WC_C) as: $[(MA_0 - MA_T) / WA_0] \times 100\%$ where MA_0 = measured area of the square at day 0 and MA_T = measured area of the square at time point T. Finally we estimated the percent of wound closure attributable to re-epithelialization (%WC_R) as: %TWC - %WC_C. The results demonstrate that in this model, the percent of wound closure attributable to contraction was typically less than 40% in each mouse strain and at each time point examined (Figure 1). When compared to the amount of closure attributable to contraction, the amount wound closure that was derived from re-epithelialization was greater at each time point that was examined (Figure 1). These results indicate that a sizeable portion of closure in normal murine excisional wounds involves re-epithelialization.

To assess the relative contributions of contraction and re-epithelialization by histological analysis, we next examined these parameters in db/db mice and db/+ controls. Full thickness 8mm diameter excisional wounds were prepared and wound contraction and re-epithelialization were measured by morphometric analysis of H&E stained cryosections taken from the center of the wound (found by serial sectioning through the entire wound) (7). Closure by contraction was estimated as the percentage reduction of the original wound diameter (measured as the distance between each edge of the wound, where wound edges were identified by the appearance of hair follicles and collagen in the dermis). Closure by epithelialization was estimated as the sum of the lengths of the epithelial tongues extending beyond the wound edges divided by the original wound diameter (Figure 2). Of note, and as reported by others (8), external measurements appear to underestimate actual wound closure assessed by histology (compare Figures 1 and 2), with the caveat that data in Figures 1 and 2 were generated from different groups of mice. This may occur due to the presence of an external residual fibrin clot overlying the restored epithelium; visually this situation would suggest the wound is still open when viewed from the surface even though the wound is closed by histologic assessment.

As expected, diabetic db/db mice show impaired closure (Figure 2). Importantly, on day 5 post-injury, the percentage of closure due to re-epithelialization exceeded that due to contraction in both db/db and db/+ mice. On day 10, around the time of wound closure, re-epithelialization and contraction each accounted for about half of the total wound closure. These latter values are similar to the results we obtained using external measurements (Figure 1). Contraction continued to increase from day 10 to 20, and by day 20, the apparent contribution of contraction to total wound closure increased relative to that of re-epithelialization.

Our results suggest that the murine excisional wound does exhibit contraction, but that the level of contraction is limited at time points up until complete epithelial closure. Our studies show that in three separate strains, (BALB/c, db/+ and db/db), re-epithelialization and contraction each accounted for ~40-50% of the initial closure of full thickness excisional wounds as measured using either external or histological of assessments. After closure, the wound continues to contract and this provides the impression of dominant closure by contraction. Our data is consistent with prior published data that indicates a contribution of ~50% by re-epithelialization during the time period of wound closure (4, 7, 9, 10, 11).

The perception that mouse wounds heal nearly entirely by contraction has led to development of models to limit contraction of mouse wounds, including splints secured to the skin with adhesives and/or sutures (12, 13) and the dorsal skinfold chamber, a model in which the dorsal skin is compressed within an external steel frame(14). Although these models appear to reduce contraction, they do so by opposing the intrinsic contractile forces developed in the wound area. Such manipulations seem certain to create undefined mechanical and biochemical signaling in the wound that does not occur in unsplinted wounds (15). In side by side comparisons, splinted wounds take about 25% longer to close than unsplinted ones (16). Yet whether the healing process induced by these models is actually closer to that of humans is not clear. Splinting has been suggested to provide an element of stress shielding, and therefore to alter the healing process (17). Stress shielding has been shown to modify both porcine and human wound healing, and to result in reduced scar formation in these species (18). Therefore, the use of a splint, while reducing contraction, may add a confounder that creates an altered healing pattern. When using a splinted wound, conclusions about effect of any pharmacologic treatment may need to consider the tandem effect of the splint itself when predicting clinical utility.

While no single model will be ideal for all studies, the data here provide additional reassurance that simple murine excisional wounds provide a valid and reproducible wound model that heals by both contraction and re-epithelialization. The element of contraction does not negate the validity of the model, but is simply an additional consideration in model choice. Our results, along with those of prior groups, suggest that the unsplinted rodent wound model can be very useful for the analysis of early wound healing, as a defined wound bed and re-epithelialization can both be easily located and measured. For many studies, then, confidence in unsplinted rodent excisional wound models is warranted.

Acknowledgments

We thank Drs. Wendy Cerny, Corrie Galant-Behm, and Margaret Novak for their review of the manuscript and critical comments.

Source of funding: Research reported in this publication was supported by National Institute of Health Awards R01GM50875 (LAD) and R01GM092850 (TJK), and the Schour Scholar Fund at the University of Illinois. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

List of Abbreviations

H&E	hematoxylin and eosin
TWC	total wound closure
WC_C	wound closure attributable to contraction
WC_R	wound closure attributable to reepithelialization
WA	wound area
MA	measured area

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Figure 1. Percent of wound closure attributable to contraction and re-epithelialization in normal and diabetic mice

Wound closure and wound contraction of 8mm circular full thickness wound were assessed by image analysis of photographs of wounds as well as the surrounding demarcated square daily. a) Representation of measurements used for calculations, b) Formulas used to calculate % of wound closure attributable to contraction and % of wound closure attributed to re-epithelialization. c, e, g) Representative photographs of wounds for BALB/c, db/+, and db/db mice, respectively. Mean percent of wound closure attributable to contraction (black bars) or re-epithelialization (gray bars) for BALB/c, db/+, and db/db mice, respectively, at each time point. The sum of the two stacked bars represents the percent wound closure (total) at each time point. Lines = SD for contraction and re-epithelialization values.

Figure 2. Wound closure and contraction in db/db and db/+ mice

Full thickness excisional wounds of 8 mm were prepared and covered with Tegaderm. a) Representative photomicrograph of wound histology, cryosection of wound center stained with hematoxylin and eosin, scale bar = 0.5mm, b) Diagram demonstrating how histologic measurements were made, c) Formulas used to calculate % of wound closure attributable to contraction and % of wound closure attributed to re-epithelialization, d, e) Mean percent of wound closure attributable to contraction (black bars) or re-epithelialization (gray bars) in db/+ and db/db mice, respectively, at each time point. The sum of the two stacked bars represents the percent wound closure (total) at each time point. Lines = SD for contraction and re-epithelialization values.