Skin Wound Healing Model - Excisional Wounding and Assessment of Lesion Area

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[Abstract] This protocol focus on the most common surgical mouse model of cutaneous excisional wound healing used to study the cellular and molecular pathways involved in wound repair and regeneration as well as in translational applications such as the evaluation of new therapeutic modalities. This model allows the monitoring of the wound closure and the tissue collection for histological and molecular analyses. Briefly, full skin thickness excisional wounds are created on the dorsum of the mouse as the excision extends through the *panniculus carnosus*. Wounds larger and minor diameters are then regularly measured and wound closure rate is calculated based on wound area relative to the original size.

Materials and Reagents

1. 5 mm diameter circular biopsy punch (ABC, catalog number: 0418)
2. 10% Ketamine Hydrochloride (Agropecuária Tarumã, catalog number: 8565)
3. 2% Xylazine hydrochloride (Syntec)
4. Potassium chloride (KCl) (Sigma-Aldrich, catalog number: P9541)
5. Potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: P0662)
6. Sodium phosphate dibasic (Na_2HPO_4) (Sigma-Aldrich, catalog number: 255793)
7. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S9888)
8. Saline solution (0.9% sodium chloride injectable solutio) (Equiplex)
9. dH_2O
10. 70% alcohol (see Recipes)
11. Phosphate buffered saline (PBS) (pH 7.2, 10x) (see Recipes)
12. Phosphate buffered saline (PBS) (pH 7.2, 1x) (see Recipes)

Equipment

1. Digital caliper (Mitutoyo, catalog number: 573-661)
2. Hair removal machine (Wahl and Toshiko)

Procedure

A. Creation of skin excisional wounds in mice

1. Anesthetize mice as approved in your animal study proposal. We suggest intraperitoneally injecting a mixture of ketamine 100 mg/kg and xylazine 10 mg/kg, diluted in 100 µl of saline solution. It gives about twenty minutes of surgical anesthesia.

2. Remove hair from the mice dorsum by using a hair removal machine.

3. Prepare the surgical site with an appropriate skin disinfectant. We suggest 70% alcohol.

4. Fold and raise the dorsal skin cranially and caudally at midline using the index fingers and thumbs to form a sandwiched skinfold (Figure 1A). Then, place the animal in a lateral position and press down the 5-mm diameter sterile biopsy punch to completely remove the two skin layers (Figure 1B) and create symmetrical full-thickness excisional wounds (Figure 1C).


Figure 1. Stepwise skin excisional wounding surgery. Fold and raise the dorsal skin cranially and caudally at midline to form a sandwiched skinfold (A). Place the animal in a lateral position and punch through the folded skin (B) to create symmetrical full-thickness excisional wounds (C).

5. After surgery, move the animal to a warm area and monitor its recovery from anesthesia. Return the fully recovered animal to its routine housing. Cage individually.

B. Wound closure monitoring after surgery

1. Animals are anesthetized as described in step A1 and the wound area is assessed every 2-3 days until full closure of the lesions.

2. By using a digital caliper, measure the larger and minor diameters of the lesions (Figure 2) and determine the wound area by applying the following formula: (diameter A/2) x (diameter B/2) x π.

3. Calculate the percentage of wound closure as follow: [(area of original wound-area of actual wound)/area of original wound] x 100.
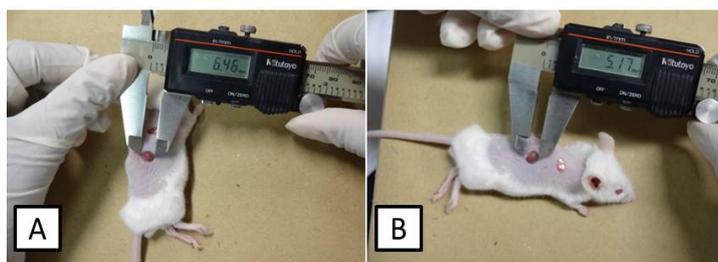
Figure 2. Assessment of the wound area. Measure the larger (A) and the minor (B) diameter of the lesion. Calculate the area as follows: (diameter A/2) x (diameter B/2) x π.

Notes

1. Create wounds with approximately 5 mm apart.

2. Analgesic drugs use depends on the experimental design and the approval by local committee for ethical conduct in the care and use of animals in scientific research. Preemptive use of analgesic drugs is recommended. We suggest Buprenorphine 0.05 mg/kg subcutaneously every 12 h for the first 24 h post-surgery.

3. The actual surface area of a full-thickness cutaneous excisional wound becomes slightly larger than its initial size.

4. For accuracy and reproducibility of the experiments, the area measurement must be performed by a single person throughout the experimental time-course.

5. If the skin is going to be used for histological analysis, after harvesting the wound tissue, lay it on a sandwiched piece of filter paper to avoid tissue folding during the fixation process and place it in a histological cassette for fixation in 10% formalin solution for 24 h. Then, follow for conventional paraffin embedding processing. Otherwise, place the harvested tissue in 1.5 ml microtube and then immediately dip in liquid nitrogen to store the frozen tissue for further tissue analyzes (*e.g.,* biochemical analysis, ELISA, qPCR, *etc.*). Please, see Cassini-Vieira *et al.* (2015) for details on how to harvesting wound tissues for analysis.

Recipes

1. 70% alcohol solution
   700 ml of absolute alcohol
   Complete the volume with distillate water to 1,000 ml

2. Phosphate buffered saline (PBS) (pH 7.2, 10x)
   NaCl   80 g
   Na_2HPO_4 11.05 g or $Na_2HPO_4 \cdot 12H_2O$ 29 g
   KCl   2 g

KH_2PO_4 2.1 g

Add dH_2O to 1,000 ml

3. Phosphate buffered saline (PBS) (pH 7.2, 1x)

50 ml PBS (10x)

450 ml dH_2O

Acknowledgments

References

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2. Cassini-Vieira, P., Moreira, C. F., da Silva, M. F. and Barcelos, L. S. (2015). Estimation of wound tissue neutrophil and macrophage accumulation by measuring myeloperoxidase (MPO) and N-Acetyl-β-D-glucosaminidase (NAG) activities. *Bio-protocol* 5(22): e1662.