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Title: Murine Excisional Wound Healing Model and Histological Morphometric Wound Analysis

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen captures provided, do not film

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **29**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Martine Dunnwald**: This protocol uses histological analysis to obtain robust morphometric measurements of mouse excisional wounds **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Martine Dunnwald**: Using this technique, we can analyze the entire wound using a subset of serial sections, allowing us to more accurately detect defects that might otherwise be missed **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.3. **Martine Dunnwald**: Demonstrating the procedure will be Lindsey Rhea, a Research Associate from my laboratory **[1][2]**.

1.3.1. INTERVIEW: Author saying the above
1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Iowa.

Protocol

2. Skin Preparation

2.1. After confirming a lack of response to pedal reflex in an anesthetized, adult mouse **[1-TXT]**, apply ointment to the animal's eyes **[2]** and use an electric razor clipper in a rostral caudal motion to remove the fur on the back of the mouse at the shoulder level **[2]**.

2.1.1. WIDE: Talent pinching toe *Videographer: More Talent than mouse in shot*
TEXT: Anesthesia: 0.5% oxygen -> 1.5% isoflurane

2.1.2. ECU: Ointment being applied

2.1.3. Hair being removed from shoulder

2.2. Use a razor blade held at a 20-degree angle from the back in a caudal rostral motion to remove any remaining hair from the exposed skin **[1]**.

2.2.1. Hair being removed with razor

2.3. Then clean the skin with one povidone-iodine **[1]** and one 70% isopropyl alcohol prep pad wipe **[2]**.

2.3.1. Skin being wiped with povidone-iodine, with povidone-iodine container visible in frame

2.3.2. Skin being wiped with isopropyl alcohol prep pad, with prep pad visible in frame

3. Wound Induction

3.1. For wound induction, drape a clean paper-based towel onto a flat surface covered with dental wax **[1]** and position the mouse onto the towel in the prone position **[2]**.

3.1.1. WIDE: Talent placing towel onto wax

3.1.2. Talent placing mouse onto towel *Videographer: More Talent than mouse in shot*

3.2. Pinch the skin of the mouse between the shoulder blades along the dorsal midline **[1]** and pull the sandwiched skinfold away from the body **[2]**.

3.2.1. Talent pinching skin NOTE: 3.2.1 – 3.3.3 filmed in one shot *Videographer: More Talent than mouse in shot*

3.2.2. Skin being pulled away from body *Videographer: Important step*

3.3. Switching the mouse to a side-lying position **[1]**, place a biopsy punch of the desired wound size as close to the body as possible **[2]** and allow the skin to relax **[3-TXT]**.

3.3.1. Talent placing mouse onto side *Videographer: More Talent than mouse in shot*

3.3.2. Punch being positioned *Videographer: Important/difficult step*

3.3.3. Skin being relaxed *Videographer: Important/difficult step* **TEXT: Caution: Do not stretch skin**

3.4. Press down on the punch, using a rocking motion to puncture all of the layers of the skin **[1]**, and remove the punch biopsies from the wound **[2]**, using sterile scissors and tweezers to free the punch from the surrounding skin as necessary **[3-TXT]**.

3.4.1. Punch being pressed/skin being wounded

3.4.2. Biopsy being removed

3.4.3. Skin being removed with scissors/tweezers **TEXT: Use new punch/animal**

3.5. Then allow the mouse to recover with monitoring and analgesia **[1]**.

3.5.1. Talent placing mouse under heat lamp or similar *Videographer: More Talent than mouse in shot*

4. Morphometric Analysis

4.1. For morphometric analysis of the wound bed, open a digital stained wound image **[1-TXT]**.

4.1.1. WIDE: Talent opening image, with monitor visible in frame

4.2. To set the scale and measurement preferences, under **Analyze**, select **Set Scale** and enter the distance in pixels, the known distance, and the unit of length **[1]**.

4.2.1. SCREEN: 4.2.1: 00:04-00:14

4.3. Select **Global** and **OK** to keep the scale the same for each open image and select **Analyze** and **Set Measurements** to confirm that the **Area** box is checked **[1]**.

4.3.1. SCREEN: 4.2.1.: 00:00-00:13

4.4. To measure the wound length, select **Freehand** and use the tool to trace along the dermo-epidermal junction, starting from the last hair follicle of the uninjured tissue on one side of the wound to the first hair follicle of the uninjured tissue on the other side **[1]**.

4.4.1. SCREEN: 4.4.1 and 4.6.1: 00:00-00:25 *Video Editor: please speed up*

4.5. If the epidermis does not cover the entire wound, follow the dermo-epidermal junction on one side of the wound where the migrating tongue ends and continue following the superior aspect of the granulation tissue or the junction between the granulation tissue and the scab until the migrating tongue and the first hair follicle of the uninjured tissue on the other side of the wound are reached **[1]**.

4.5.1. SCREEN: 4.5.1 and 4.6.1: 00:00-00:19

4.6. Then click **Analyze** and **Measure**. The length of the measurement will appear in the same units as set in the scale **[1]**.

4.6.1. SCREEN: 4.5.1 and 4.6.1: 00:20-00:24

4.7. If the epidermis does not cover the entire wound, measure the distance between each epidermal leading edge following the superior aspect of the granulation tissue or the junction between the granulation tissue and the scab to the first hair follicle **[1]**.

4.7.1. SCREEN: 4.7.1: 00:02-00:08

4.8. To measure the wound area, use the **Freehand** tool to trace along the superior aspect of the epidermis or the superior aspect of the granulation tissue starting from the last hair follicle of the uninjured tissue on one side of the wound to the first hair follicle of the uninjured tissue on the other side **[1]**.

4.8.1. SCREEN: 4.8.1 and 4.9.1: 00:03-00:23 *Video Editor: please speed up*

4.9. Continue to trace vertically along the hair follicle into the granulation tissue. Once the opposite hair follicle and adipose tissue or muscle are reached, follow the inferior border of the granulation tissue to the opposite side of the wound and join the starting point along the hair follicle to close the area **[1]**.

4.9.1. SCREEN: 4.8.1 and 4.9.1: 00:23-00:35 *Video Editor: please can up*

4.10. If the wound is not fully epithelialized, trace along the superior aspect of the epidermis until the leading edge and return to the starting point following the dermo-epidermal junction **[1]**.

4.10.1. SCREEN: 4.10.1: 00:02-00:35 *Video Editor: please speed up*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.2., 3.3.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.3. I make sure that a large enough area on the back is shaved so there is plenty of area to work with even after letting the skin relax.

Results

5. Results: Representative Histological Morphometric Wound Analyses

5.1. As illustrated, a range of measured and calculated morphometric values for wild-type 6-millimeter wounds generated in different mouse strains by multiple surgeons **[1]** can be determined through this analysis **[2]**.

5.1.1. LAB MEDIA: Figure 5 *Video Editor: please emphasize top row of graphs*
5.1.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize bottom row of graphs*

5.2. Here a meta-analysis comparing the percentage of epidermal area among wound area measurements obtained from a "middle" of the wound subset analysis **[1]** exhibited less statistical significance compared to the whole wound analysis **[2]**.

5.2.1. LAB MEDIA: Figure 6A *Video Editor: please emphasize Middle data points*
5.2.2. LAB MEDIA: Figure 6A *Video Editor: please emphasize Whole data points*

5.3. Similarly, the percentage of the epidermis in the wound **[1]** was obtained as the ratio of the epidermal volume over the wound volume **[2]**.

5.3.1. LAB MEDIA: Figure 6B *Video Editor: please emphasize Middle data points*
5.3.2. LAB MEDIA: Figure 6B *Video Editor: please emphasize Whole data points*

5.4. Comparing the average measured wound area, a commonly used stand-alone measurement, from the serial sections of entire wounds **[1]** to that obtained from the middle subset sections revealed no significant difference between experimental groups and between the methods of analysis **[2]**.

5.4.1. LAB MEDIA: Figure 6C *Video Editor: please emphasize Whole data points*
5.4.2. LAB MEDIA: Figure 6C *Video Editor: please emphasize Middle data points*

5.5. The calculated wound volume, however, was significantly different between the experimental groups **[1]**, demonstrating the importance of an in-depth histological analysis of wound healing parameters **[2]**.

5.5.1. LAB MEDIA: Figure 6D *Video Editor: please add/emphasize brackets and asterisks*
5.5.2. LAB MEDIA: Figure 6D

Conclusion

6. Conclusion Interview Statements

6.1. **Martine Dunnwald**: Generating consistently sized wounds for histological analysis and performing rigorous serial sectioning are critical for an accurate morphometric analysis **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (4.1.)

6.2. **Martine Dunnwald**: Unstained sections can be used for immunofluorescence or Masson's Trichrome staining to measure other aspects of wound healing, while flash-frozen wounds can be used alongside morphometry for quantitative protein analyses **[1]**.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera