FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 62326
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Project Page Link: https://www.jove.com/account/file-uploader?src=19009478

Title: Human Ex Vivo Wound Model and Whole-Mount Staining Approach to Accurately Evaluate Skin Repair

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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 something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as possible.

   Videographer: please film

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? **3 miles**

Current Protocol Length

Number of Steps: 17
Number of Shots: 38

Introduction

Commented [BC1]: Authors: Since at least one Conclusion statement is required, the Optional statement was moved to the Conclusion section.

1. Introductory Interview Statements

REQUIRED:

1.1. **Matthew Hardman**: In this article we present an optimized ex vivo human skin wounding protocol that can provide important efficacy data as part of a clinical translation pipeline **[1]**.

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

REQUIRED:

1.2. **Holly Wilkinson**: The main advantage of this technique is that it allows a high throughput, accurate assessment of wound repair in living human skin **[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. **Matthew Hardman**: Demonstrating the procedure will be Elizabeth Roberts, a postdoctoral researcher, and Alexandria Kidd, a research assistant from our 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.

1.3.3. Maybe added shot? Videographer NOTE: tk3 board not incremented

Ethics Title Card

1.4. Human skin was obtained from patients undergoing reconstructive surgery at Castle Hill Hospital and Hull Royal Infirmary (Hull, UK) under full informed, written patient consent, institutional guidelines, and ethical approval.

Protocol

2. Preparation of Skin for Wounding and Creating Ex Vivo Human Skin Wounds

2.1. To prepare a skin tissue sample for wounding, place the skin dermis side-down in a 90-millimeter Petri dish **[1]** and use sterile scissors to remove the adipose tissue **[2]**.

2.1.1. WIDE: Talent placing skin tissue into Petri dish
2.1.2. Talent removing adipose tissue

2.2. When all of the fat has been removed, place the skin in 25 milliliters of HBSS supplemented with antibiotics **[1]** for 10 minutes at room temperature with intermittent shaking to remove any residual blood and adipose tissue **[2]**.
2.2.1. Talent placing the tissue into HBSS
2.2.2. Sample being shaken

2.3. After 10 minutes, rinse the skin in 25 milliliters of DPBS **[1]**. Before creating the wound, stack two sterile absorbent pads in a 60-millimeter Petri dish **[2]** and add 4 milliliters of human skin medium to the dish **[3-TXT, 4]**.
2.3.1. Talent giving buffer washes
2.3.2. Talent putting pads into Petri dish
2.3.3. Talent adding media to the dish **TEXT: See text for all medium and solution preparation details**
2.3.4. Added shot: CU of end of 2.3.3

2.4. Then place a sterile nylon filter membrane onto the absorbent pad stack **[1]** and dry the dermal side of the skin on sterile gauze in a 90-millimeter Petri dish to remove residual DPBS **[2]**.
2.4.1. Talent keeping the filter membrane onto absorbent pad
2.4.2. Talent drying the skin

2.5. Place the dried skin dermis-side down on a clean 90-millimeter Petri dish lid to remove any residual DPBS **[1]** and dab the epidermis dry with fresh sterile gauze **[2]**. To create a wound, press a 2-millimeter biopsy punch against the skin while holding the skin tight and twisting gently **[3]**.
~~2.5.1. Talent transferring the skin dermis on to Petri dish lid~~ NOTE: 2.5.1 – 2.5.2 are duplicate actions of 2.4.1 – 2.4.2, perhaps try to reuse those?
~~2.5.2. Talent dabbing the epidermis~~
2.5.3. ECU: Punch being pressed against skin while skin is held tightly/twisted NOTE: Audio slate

2.6. Using a curved toothed tissue forceps, pick up each side of the approximately 2 millimeter-diameter wound **[1]** and hook curved iris scissors under the wound **[2]**. Use a 6-millimeter biopsy punch to biopsy around the central 2 millimeter wound to create a 6-millimeter explant with a partial thickness 2-millimeter wound in the center **[4]**.
2.6.1. Wound being grasped NOTE: 2.6.1 – 2.6.3 in one shot
2.6.2. Scissors being hooked under wound
2.6.3. Biopsy being created around wound/uniform explant being created

2.7. Then place the wound explant epidermis side-up on the nylon filter membrane stack for a 1-7-day incubation at 32-37 degrees Celsius and 5% carbon dioxide **[1-TXT]**.
2.7.1. Talent placing the explants on membrane **TEXT: Replace medium every 2-3 d** NOTE: Audio slate

3. Whole Mount Staining of Ex Vivo Wounds

3.1. For fluorescent staining, collect the wound explants in 1.5-milliliter microcentrifuge tubes containing 500 microliters of skin fixative per tube **[1]** and incubate the explants overnight at 4 degree Celsius **[2]**. The next day, replace the fixative with 1 milliliter of staining wash buffer **[3]**.
3.1.1. Talent collecting the explants into tube Videographer NOTE: take 2, board not incremented
3.1.2. Talent keeping the tubes for incubation
3.1.3. Talent adding wash buffer

3.2. After the wash, cover each sample with approximately 150 microliters of blocking buffer **[1]** and incubate for 1 hour at room temperature **[2]**.
3.2.1. Talent adding blocking solution to the sample
3.2.2. Talent keeping the samples for incubation

3.3. After removing the blocking buffer, add 150 microliters of primary antibody diluted in blocking buffer per tube **[1-TXT]** and incubate the wound explants overnight at 4 degrees Celsius **[2]**.
3.3.1. Talent adding primary antibody **TEXT: See text for Ab suggestion and preparation details**
3.3.2. Talent keeping the samples foe incubation NOTE: action in shot 3.1.2 2nd 1/2

3.4. The next morning, rinse the samples four times with 500 microliters of staining wash buffer containing 0.2% sodium azide for 1 hour at room temperature per wash **[3]**.
3.4.1. Talent rinsing samples

3.5. After the wash, add 150 microliters of an appropriate fluorescence-conjugated secondary antibody diluted in staining wash buffer to each well for a 1-hour incubation

at room temperature **[1]**.
3.5.1. Talend adding secondary antibody

3.6. At the end of the incubation, rinse the explant three times for 30 minutes and 500 microliters of staining wash buffer per wash **[1]** before counterstaining each explant with 150 microliters of DAPI for 10 minutes at room temperature **[2]**. Finally, wash the explant two times for 30 minutes with 500 microliters of washing buffer per wash **[3-TXT]**.
3.6.1. Talent giving washes NOTE: reuse shot 3.4.1
3.6.2. Talent adding counterstain
3.6.3. Talent giving washes. **TEXT: Optional: Store biopsies ≤2 wk at 4 °C protected from light**

4. Explant Imaging

4.1. For explant imaging, place a 60-millimeter Petri dish onto the imaging platform of a confocal microscope **[1]** and add an approximately 1-milliliter layer of DPBS to the dish **[2]**. Use forceps to transfer the wound explants to the dish **[3]**.
4.1.1. Talent placing the dish on imaging platform of microscope
4.1.2. Talent adding buffer to the dish
4.1.3. Talent transferring the explants

4.2. After setting up the imaging software as appropriate for the experiment **[1-TXT]**, position the wound in the center of the imaging plane **[2]** and acquire images of the wound biopsies **[]**.
4.2.1. Talent at microscope, setting imaging parameters, with monitor visible in frame **TEXT: See text for imaging software setup details** NOTE: best action at 00:30 or 01:10
4.2.2. SCREEN: 62326_4.2.2.mp4: 0:05 – 0:15. *Video Editor: Speed this up.*
4.2.3. SCREEN: 62326_4.2.2.mp4: 0:16 – end. *Video Editor: Please create a time lapse of the wound being imaged.*

4.3. To perform wireless digital microscope imaging to obtain high quality images in a cost-effective manner, connect a phone or laptop to a wireless digital microscope **[1]**. Place the explants wound side-up onto a piece of lab tissue **[2]** and remove any residual wash buffer from the sample **[3]**.
4.3.1. Talent connecting microscope to phone or laptop
4.3.2. Talent placing explant onto tissue
4.3.3. Talent removing wash buffer

4.4. Then position the explant in the center of the field of view of the microscope **[1]** and acquire images using the connected camera **[2-TXT]**.
4.4.1. Talent positioning explant

4.4.2. SCREEN: 62326_4.4.2.mp4.

Results

5. Results: Wound Closure Evaluation by Whole-Mount Tissue Staining

5.1. Immunoperoxidase **[1]** and immunofluorescence can be used for whole mount tissue staining of fresh tissue **[2]**.
- 5.1.1. LAB MEDIA: Figure 1C *Video Editor: please emphasize open wound staining in Immunoperoxidase image*
- 5.1.2. LAB MEDIA: Figure 1C *Video Editor: please emphasize merged signal Immunofluorescence image*

5.2. The tissues can also be successfully imaged after fixation **[1]**.
- 5.2.1. LAB MEDIA: Figure 1D *Video Editor: please emphasize signal in Imaging Post Fixation image*

5.3. Whole mount staining of wounds can be used to determine wound closure rates in a reproducible manner **[1]**.
- 5.3.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize formula box*

5.4. In this representative analysis, healthy skin wound closure was observed after 4 to 5 days **[1]**, while diabetic skin wounds failed to close fully within the 7 day analysis period **[2]**.
- 5.4.1. LAB MEDIA: Figure 2B *Video Editor: please emphasize days 4-5 healthy images*
- 5.4.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize day 7 Diabetic image*

5.5. K14 (K-fourteen) expression peaked on day 2 in healthy skin wounds **[1]** before rapidly declining with increased epidermal differentiation **[2]**. This re-epithelialization **[3]** and subsequent epidermal differentiation was delayed in the diabetic skin wounds **[4]**.
- 5.5.1. LAB MEDIA: Figure 2D *Video Editor: please emphasize blue data line from Day 1 to Day 2 peak*
- 5.5.2. LAB MEDIA: Figure 2D *Video Editor: please emphasize blue data line from Day 2 peak to end of graph*
- 5.5.3. LAB MEDIA: Figure 2D *Video Editor: please emphasize red data line from Day 1 to Day 4 peak*
- 5.5.4. LAB MEDIA: Figure 2D *Video Editor: please emphasize red data line from Day 5 peak to end of graph*

5.6. The re-formation of the early epidermal barrier **[1]** excludes K14 antibody penetration within the differentiated epidermal layers **[2]**.

5.6.1. LAB MEDIA: Figure 2E graphic *Video Editor: please emphasize on basal layer from figure*

5.6.2. LAB MEDIA: Figure 2E graphic *Video Editor: please emphasize Spinous, Granular, and Cornified layers*

5.7. Early in the re-epithelialization process, K14-positive basal layer keratinocytes migrate inwards over the open wound, such that the epidermis closer to the outer wound edge forms earlier than the epidermis closer to the inner wound edge **[1]**.

5.7.1. LAB MEDIA: Figure 2E images *Video Editor: please emphasize on arrows in early closure image*

5.8. Later in the repair stage, K14 staining is lost as the epidermis differentiates from the outside inwards **[1]**.

5.8.1. LAB MEDIA: Figure 2E *Video Editor: please sequentially emphasize green signal in Later Healing image then lack of signal in Closed Wound image*

5.9. Whole-mount staining can also be used to study the expression and localization of other wound-relevant markers in non-diabetic skin **[1]** as well as the presence of immune cells of interest at the wound site at different stages of healing **[2]**.

5.9.1. LAB MEDIA: Figure 3A *Video Editor: please sequentially emphasize green signal in top right image and red signal in bottom right image*

5.9.2. LAB MEDIA: Figure 3C *Video Editor: please emphasize green signal in magnified image*

Conclusion

6. Conclusion Interview Statements

6.1. **Holly Wilkinson**: Generating reproducible excisional wounds can be challenging at first. We would advise practicing the wounding procedure using surplus skin **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.6.3.)