FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 60949
Scriptwriter Name: Bridget Colvin
Project Page Link: http://www.jove.com/files_upload.php?src=18589493

Title: Performing Colonoscopic-Guided Pinch Biopsies in Mice and Evaluating Subsequent Tissue Changes

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Y**

If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

Enter make and model of microscope.

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

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 by the script return deadline.

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

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **David Montrose**: This method provides an ideal approach for studying colonic wound healing and the molecular and histologic changes that occur following colonic injury, potentially helping to shed light on IBD pathogenesis **[1]**.

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

REQUIRED:

1.2. **David Montrose**: The main advantage of this technique is that it allows a precise control of the location of the injury and the timing of the wound healing process **[1]**.

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

OPTIONAL:

1.3. **Carmen Ferrara**: Inserting the colonoscope into the mouse colon and creating consistent wounds can be tricky. Repeat practice of these techniques should help with their mastery **[1]**.

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

OPTIONAL:

1.4. **Carmen Ferrara**: Given the multiple coordinated steps and nuance involved with this procedure, visual demonstration of this method is critical **[1]**.

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

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Stony Brook University.

Protocol

2. Colonoscopy and Wound Induction

2.1. Before beginning the procedure, insert a 1.9-millimeter rigid bore endoscope into an endoscope sheath **[1]** and attach the assembled endoscope to the light source and video imaging device per the manufacturer's instructions **[2.3.2]**. Use the provided tubing to attach the air pump to the gas valve on the left side of the sheath next to the working channel **[2]**.

2.1.1. WIDE: Talent inserting endoscope into sheath NOTE: 2.3.2 should go between 2.1.1 and 2.1.2

2.1.2. Talent using tubing to attach air pump to gas valve

2.2. Ensure that the working channel is in the open position **[1]** and insert 3-French biopsy forceps through the working channel **[2]**.

2.2.1. Talent checking working channel
2.2.2. Talent inserting forceps through channel NOTE: 2.2.2 and 2.3.1 in one shot

2.3. Advance the forceps to the end of the sheath without protruding out of the sheath **[1]**.

2.3.1. Forceps being advanced
2.3.2. Talent attaching endoscope to light source and/or imaging device NOTE: This should go between 2.1.1 and 2.1.2. It was also split in 2 shots, 2.3.2B is attaching camera head.

2.4. Next, place the anesthetized mouse onto an endoscopic staging platform on its ventral side **[2]** and confirm the appropriate level of sedation by lack of response to pedal reflex **[1-TXT]**.

2.4.1. ECU: Toe being pinched **TEXT: Anesthesia: 2% isoflurane**
2.4.2. Talent placing mouse onto stage NOTE: 2.4.2 should go before 2.4.1
Videographer: More Talent than mouse in shot

2.5. After applying eye ointment, fill a 3-milliliter syringe with an attached rat gavage needle with room temperature PBS **[1]** and insert the needle approximately 1 centimeter into the mouse's anus **[2]**.

2.5.1. Talent filling syringe, with PBS container visible in frame
2.5.2. Needle being inserted

2.6. Gently infuse with PBS until the fecal material has been cleared. Several fecal pellets should exit the mouse along with the PBS that was infused **[1]**.

2.6.1. PBS being infused/pellets being flushed

2.7. Insert the assembled endoscope 0.5 centimeters into the anus **[1]** and advance the biopsy forceps into the cleared lumen of the rectum until the full 'jaws' of the forceps are beyond the end of the sheath **[2]**.

2.7.1. Endoscope being inserted
2.7.2. LAB MEDIA: Supplemental Video 1: 00:00-00:04

2.8. Turn the forceps 90 degrees so that the jaws open in an east-west orientation and open the tips and advance the forceps approximately 1 centimeter, closing and retracting the forceps in one smooth, quick motion to harvest the biopsy **[1-TXT]**.

2.8.1. LAB MEDIA: Supplemental Video 1: 00:04-00:10 **TEXT: Caution: Opening forceps may damage mucosa**

2.9. To avoid fully insufflating the colon while performing the biopsy, leave the right side of the gas valve open **[1-TXT]**.

2.9.1. Talent checking right gas valve

3. Wound Bed Visualization and Measurement

3.1. Immediately after the biopsy, depress the foot pedal attached to the colonoscope recording device to initiate the video recording **[1]** and firmly press an index finger against the right side of the gas valve to completely cover the opening, forcing air into the endoscope and thus into the colon **[2]**.

3.1.1. WIDE: Talent depressing pedal
3.1.2. Finger being pressed against gas valve

3.2. Retract the forceps back out of the sheath and into the rectal lumen while in the closed position **[1]** and place the forceps against the rectal wall immediately above the wound until the base of the jaws is aligned with the top edge of the viewing field. Continue fully insufflating the colon until a clear view of the wound can be observed **[2]**.

3.2.1. Forceps being advanced *Videographer: Important/difficult step*
3.2.2. LAB MEDIA: Supplemental Video 1: 00:11-00:16

3.3. **David Montrose**: To obtain an accurate measurement of the wound bed, be patient and place the colonoscope and forceps at a precise angle to achieve the best view of the wound bed **[1]**.

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

3.4. Open the video in an appropriate software program **[1]** and advance the video to a frame showing a point in time at which the wound bed can be easily visualized **[2]** and at which the closed forceps are above the wound bed and against the rectal wall **[3]** and the wall is taut **[4]**.

3.4.1. Talent opening program
3.4.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize wound bed at least Day 2*
3.4.3. LAB MEDIA: Figure 2 *Video Editor: please emphasize forceps at least Day 2*
3.4.4. LAB MEDIA: Figure 2 *Video Editor: please emphasize wall tautness at least Day*

3.5. At each time point, obtain a snapshot of this frame and code the file name to ensure that the measurements of the wound beds are carried out in a blinded manner **[1]**.

3.5.1. SCREEN: Snapshot being acquired, then file being coded *Videographer: please film* NOTE: Use videographer's footage for all SCREEN shots, author had difficulty with OBS.

3.6. To quantify the size of the wound bed, open the images in ImageJ **[1]** and use the **Freehand selections** tool to draw a perimeter around the wound **[2]**.

3.6.1. Talent at computer, opening ImageJ, with monitor visible in frame
3.6.2. SCREEN: Perimeter being drawn around wound *Videographer: please film*

3.7. Under **Analyze**, select **Measure** and the value of that measurement will automatically populate in the **Results** window **[1]**.

3.7.1. SCREEN: Perimeter being measured, Results window appearing *Videographer: please film*

3.8. When all of the measurements have been acquired, calculate the size of the wound on subsequent days relative to the size on day 0 in a spreadsheet **[1]**.

3.8.1. SCREEN: Wound size being calculated for at least one time point in spreadsheet *Videographer: please film*

4. Wound Bed Collection

4.1. At the appropriate experimental end point, open the skin and abdominal muscle layers to expose the body cavity of the experimental animal **[1-TXT]** and place closed scissors under the colon **[2]**.

4.1.1. WIDE: Talent opening abdominal cavity *Videographer: More Talent that mouse in shot* **TEXT: Euthanasia: CO_2 asphyxiation**
4.1.2. Scissors being placed

4.2. Gently lift the colon to release it from the underlying mesentery **[1]** and cut the tissue at its midpoint **[2]** and at the anus to collect it from the mouse **[3]**.

4.2.1. Colon being lifted
4.2.2. Colon being cut at midpoint
4.2.3. Colon being cut at anus

4.3. Use a 20-milliliter syringe filled with ice-cold PBS and equipped with a rat gavage needle to flush out the fecal contents **[1]** and place the cleared colon onto a piece of filter paper **[2]**.

4.3.1. Colon being flushed
4.3.2. Colon being placed onto filter paper

4.4. Open the colon longitudinally, taking care that the mesenteric side is face down against the filter paper **[1]**, and use a Pasteur pipette to cover the mucosa with 0.2% methylene blue **[2]**.

4.4.1. Colon being cut
4.4.2. Colon being stained, with dye container visible in frame

4.5. After a few seconds, drain off the excess stain **[1]** and view the colon under a dissecting microscope to locate the wound bed **[2]**.

4.5.1. Stain being drained off
4.5.2. Talent placing tissue under microscope

4.6. Use 4-inch micro iris scissors to cut around the edge of the bed, being careful not to cut into the muscle layer **[1]**, and use fine point tweezers to transfer the dissected tissue into a tube for snap-freezing and/or storage **[2]**.

4.6.1. SCOPE: Shot of wound bed, then tissue being cut *Videographer: Important step*
4.6.2. Talent placing tissue into tube *Videographer: Important step*

5. Histological Analysis Preparation

5.1. At the appropriate experimental end point, open the harvested colon longitudinally on a piece of filter paper, mesenteric side down **[1]**, and gently cover the tissues with 4% paraformaldehyde **[2]**.

5.1.1. WIDE: Talent cutting colon
5.1.2. PFA being added to tissue, with PFA container visible in frame

5.2. Cover the tissue with parafilm in a sealed container for 4-6 hours **[1]** before storage in 70% ethanol **[2]**.

5.2.1. Tissue being covered
5.2.2. Talent adding tissue to ethanol, with ethanol container visible in frame

5.3. On the day of processing, remove the parafilm **[1]** and add 0.2% methylene blue to the tissue as demonstrated **[2]**.

5.3.1. Film being removed
5.3.2. Dye being added

5.4. After draining off the excess stain, place the tissue under a dissecting microscope to locate the wound bed **[1]**.

5.4.1. Talent placing tissue under microscope

5.5. Using a scalpel with a number 10 blade, cut directly through the center of the wound bed **[1]** and continue cutting through the remainder of the colon in a straight line, such that the colon is cut in half, lengthwise **[2]**.

5.5.1. SCOPE: Center of wound being cut *Videographer: Important step*
5.5.2. SCOPE: Remainder of wound being cut *Videographer: Important step*

5.6. After processing the colon in a standard tissue processor, paraffin embed the samples such that the side that was cut by the scalpel is face down in the paraffin **[1]**.

5.6.1. ~~Tissue being embedded in paraffin~~ *Videographer: Important step*

5.7. For cryosectioning, embed the colon pieces such that the side that was cut by the scalpel is face down in a base mold half-filled with tissue freezing medium **[1]**.

5.7.1. Tissue being embedded in tissue freezing medium

5.8. Secure the tissue in place with fine tweezers **[1]** and place the base mold onto a metal plate or thick aluminum foil on top of dry ice to harden the freezing medium **[2]**.

5.8.1. Tissue being secured NOTE: 5.8.1 and 5.8.2 merged
5.8.2. Mold being placed onto dry ice

5.9. Once the bottom portion of the medium is frozen **[1]**, fill the remaining volume of the base mold with freezing medium at room temperature **[2]** and return the mold to the dry ice until the entire tissue block is frozen **[3]**.

5.9.1. Shot of frozen bottom
5.9.2. Medium being added to mold on dry ice

5.10. Then transfer the mold to a minus 80-degeree Celsius freezer until sectioning **[1]**.

5.10.1. Talent placing mold into freezer

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.2., 4.6., 5.5., 5.6.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.2. Patience and proper maneuvering of the colonoscope and forceps

Results

6. Results: Representative Wound Bed Visualization

6.1. Here representative images of acceptable views of the wound bed for an accurate quantification of the size of the wound bed and closure rate of the wound are shown **[1]**.

6.1.1. LAB MEDIA: Figure 2 *Video Editor: please sequentially trace/emphasize blue regions from top left to bottom right of Figure*

6.2. In this ex vivo view of a wound bed, indicators of the perimeter of the wound bed **[1]** and where to cut the tissue to enable visualization of the wound bed upon sectioning can be observed **[2]**.

6.2.1. LAB MEDIA: Figure 3A *Video Editor: please add dotted line as in original Figure 3A/indicate wound bed perimeter*

6.2.2. LAB MEDIA: Figure 3A *Video Editor: please add black line/indicate incision line as in original Figure 3A*

6.3. In this representative image, an H&E-stained section of a wound bed can be clearly observed **[1]**.

6.3.1. LAB MEDIA: Figure 3B

Conclusion

7. Conclusion Interview Statements

7.1. **David Montrose**: It is important to acquire consistent images of the wound bed over all of the wound healing time points to ensure the most accurate measurements of the healing rate **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (Step: 3.2.-3.4.)

7.2. **David Montrose**: Following the pinch biopsy, different agents of interest can be injected directly into the wound bed to test their effects on the wound healing rate **[1]**.

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