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Title: A Permanent Window for Investigating Cancer Metastasis to the Lung

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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? **NO**

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? **NO**

Current Protocol Length

Number of Steps: 16
Number of Shots: 41

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Lucia Borriello:** Important questions in cancer metastasis field like tumor cell colonization and dormancy in the lungs and factors activating the dormant cells to form metastasis can be studied using this protocol.

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

1.2. **Lucia Borriello:** The main advantage of this protocol is that the dynamic metastatic process can be directly studied in vivo, in real-time, and in a real microenvironment.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.8.1*

OPTIONAL:

1.3. **Brian Traub:** This method can provide insight into several pulmonary diseases but is particularly useful for understanding the behavior of individual tumor cells disseminating to the lung, a site of metastasis.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the Albert Einstein College of Medicine.

Protocol

2. Lung Window Surgery

2.1. To begin with, use a 2-0 silk suture to tie a knot at the base of a 22-gauge catheter leaving 2-inch long tails **[2]**.

2.1.1. WIDE: Talent placing the anesthetized mouse on the table.

NOTE: Shot number 2.1.1. and its VO was moved and is placed before 2.2.1.

2.1.2. CLOSE UP: Talent tying the suture to the catheter.

NOTE: Footage from shot 2.1.1A should be used for shot 2.1.2.

2.2. Once the mouse is completely anesthetized, move the mouse to the operation table, intubate the mouse, and then secure the intubation catheter by tying the 2-0 silk suture around the mouse's snout **[1-TXT]**.

Added shot: WIDE: Talent placing the anesthetized mouse on the table

NOTE: For the added shot, use the VO – "Once the mouse is completely anesthetized, move the mouse to the operation table".

2.2.1. Talent securing the intubation catheter around the mouse's snout **TEXT: Anesthesia: 5% isoflurane in the anesthesia chamber.**

2.3. Connect the ventilator to the intubation catheter **[1]**. Using paper tape secure the front cranially **[2]** and hind limbs caudally to the heated surgical stage **[3]**. Place a paper tape along the length of the mouse's back to maximize exposure to the surgical field **[4]**.

2.3.1. Talent connecting the ventilator to the intubation catheter.

2.3.2. Talent securing the front cranially with paper tape to the heated surgical stage.

2.3.3. Talent securing the hind limbs caudally with paper tape to the heated surgical stage.

2.3.4. Talent placing paper tape to the back of the mouse.

NOTE: Shots 2.3.2. to 2.3.4. were combined and filmed together as one continuous shot.

2.4. Lift the skin with the forceps **[1]** and make an approximately 10-millimeter circular incision at about 7 millimeters to the left of the sternum and about 7 millimeters superior to the subcostal margin **[2]**. Excise the soft tissue overlying the ribs **[3]**.

2.4.1. Talent lifting the skin with forceps.

2.4.2. Talent making a circular incision.

2.4.3. Talent excising the soft tissue.

NOTE: Shots 2.4.1. to 2.4.3. were combined and filmed together as one continuous shot.

2.5. Elevate the sixth and seventh rib using forceps **[1]**. Use a single blade of the blunt micro-dissecting scissors with the rounded side towards the lung to carefully pierce the intercostal muscle between the sixth and seventh ribs for entering the intrathoracic space **[2]**. *Videographer: This step is important!*

2.5.1. Talent elevating the ribs.

2.5.2. Talent cutting the intercostal muscle between the sixth and seventh ribs.

NOTE: Shots 2.5.1. and 2.5.2. were combined and filmed together as one shot.

2.6. Delicately discharge the compressed air canister at the defect to collapse the lung **[1]** and separate the lung from the chest wall **[2]**. Fire the compressed air in short bursts to prevent iatrogenic lung injury **[3]**. *Videographer: This step is important!*

2.6.1. Talent discharging the compressed air canister.

2.6.2. Talent separating the lung from the chest wall.

2.6.3. Talent operating the canister and the compressed air entering the lung.

2.7. Place the biopsy punch over the cutting tool **[1]** and carefully maneuver the cutting tool's base through the intercostal incision **[2]**. Orient the cutting tool base parallel with the chest wall **[3]** and punch a 5-millimeter circular hole through the rib cage **[4]**. *Videographer: This step is important!*

2.7.1. Talent placing the biopsy punch over the cutting tool.

2.7.2. Talent moving the cutting tool's base through the intercostal incision.

2.7.3. Talent arranging the base of the cutting tool parallel with the chest wall.

2.7.4. The rib cage being punched.

NOTE: Shots 2.7.2. to 2.7.4. were combined and filmed together as one continuous shot.

2.8. Position the window frame into the chest wall with the edges of the circular defect captured within the window's groove **[1]**. Tightly tie the 5-0 silk suture to securely lock

the implanted window **[2]**. Apply a steady and gentle stream of compressed air for about 10 to 20 seconds to dry the lung **[3]**.

2.8.1. Talent positioning the window frame.

2.8.2. Talent tying the suture to lock the implanted window.

NOTE: Shots 2.8.1. and 2.8.2. were combined and filmed together as one shot.

2.8.3. Talent applying compressed air to the lungs.

2.9. Use the forceps to grip the window frame by its outside edge and gently lift **[1]** to ensure separation of the lung from the undersurface of the window frame **[2]**. Dispense a thin layer of cyanoacrylate adhesive along the undersurface of the optical window frame **[3]**.

2.9.1. Talent lifting the window frame.

2.9.2. The lung getting separated from the undersurface of the window frame.

2.9.3. Talent applying adhesive to the undersurface of the optical window frame.

2.10. Gently but firmly press the optical window frame onto the lung tissue for 10 to 20 seconds for attachment **[1]**. Dispense a 5 millimeter drop of adhesive on a rectangular coverslip **[2]**. Use the vacuum pickup tool to pick up a 5-millimeter coverslip **[3]**. *Videographer: This step is important!*

NOTE: Shots 2.10.1. to 2.13.3. were all combined and filmed together as one continuous shot. Audio slates were spoken as each shot took place.

2.10.1. Talent pressing the window frame on the lungs.

2.10.2. Adhesive being dispensed on a rectangular coverslip.

2.10.3. The coverslip being picked up by the vacuum pickup tool.

2.11. Dip the undersurface of the coverslip into the adhesive **[1]** and then scrape off the excess adhesive three times against the side of the rectangular coverslip to make a very thin layer of adhesive on the coverslip **[2]**. *Videographer: This step is important!*

2.11.1. Talent dipping the undersurface of the coverslip into the adhesive.

2.11.2. Talent scrapping the excess adhesive.

2.12. Carefully position the coverslip at an angle inside the recess at the center of the optical window frame, just above the lung tissue **[1]**. Briefly clamp the ventilator to generate positive pressure, hyper-inflating the lung **[2]**. *Videographer: This step is important!*

2.12.1. Talent positioning the coverslip.

2.12.2. Talent clamping the ventilator to the lungs.

2.13. Orient the coverslip parallel to the lung tissue using the rotating motion to create direct apposition between the lung's surface and the undersurface of the coverslip **[1]**. Maintain gentle pressure for approximately 25 seconds to set the cyanoacrylate adhesive **[2]**. Separate the coverslip from the vacuum pickup tool with forceps **[3]**. *Videographer: This step is important!*

2.13.1. Talent positioning the coverslip parallel to the lung tissue.

2.13.2. Talent gently pressing the coverslip.

2.13.3. Talent separating the coverslip from the vacuum pickup tool.

NOTE: Shots 2.10.1. to 2.13.3. were all combined and filmed together as one continuous shot. Audio slates were spoken as each shot took place.

2.14. Create a purse-string stitch circumferentially less than 1 millimeter from the cut edge of the skin incision using 5-0 silk suture **[1]**. Tuck any excess skin underneath the outer rim of the window frame before tying the window tightly with locking knots **[2]**.

2.14.1. Talent creating a purse-string stitch.

2.14.2. Talent tucking the skin inside.

NOTE: Shots 2.14.1. and 2.14.2. were combined and filmed together as one shot.

2.15. Dispense a small amount of adhesive at the metal-glass interface to ensure an air-tight seal between the coverslip and window frame **[1]**.

2.15.1. Talent applying a small amount of adhesive at the metal-glass interface.

2.16. Attach the sterile needle to a 1-milliliter insulin syringe **[1]**. Insert the needle below the xiphoid process, advancing toward the left shoulder, entering the thoracic cavity through the diaphragm **[2]**. Gently draw back the syringe to remove any residual air from the thoracic cavity **[3]**.

2.16.1. Talent attaching a sterile needle to a 1 mL insulin syringe.

2.16.2. Talent inserting the needle below the xiphoid process and advancing the needle towards the thoracic cavity.

2.16.3. Talent pulling the plunger of the syringe.

NOTE: Shots 2.16.1. to 2.16.3. were combined and filmed together as one continuos shot.

NOTE: An added angle of the entire procedure was filmed showing the mouse from a different angle - this was filmed at the end of the day in one continuous take but can easily be intercut with slate shots.

Results

3. Results: Assessment of Tumor Cell Migration

3.1. The multiphoton intravital imaging of a single lung region showed that the microvasculature relocated over 3 consecutive days **[1]**.

3.1.1. LAB MEDIA: Figure 2

3.2. The definable branch point from a single vessel was identified each consecutive day **[1]**. The unlabeled erythrocytes created shadows when flowing in larger vessels. The angle of the branch points relative to the vessel can be used to calculate the erythrocyte flow rates **[2]**.

3.2.1. LAB MEDIA: Figure 2 *Video editor: Please emphasize the yellow arrows.*

3.2.2. LAB MEDIA: Figure 2 *Video editor: Please emphasize 4 yellow lines in Day 3 image.*

3.3. The cell speed was quantified by injecting the mouse with the fluorescent microspheres, and the length of the tracks was measured, divided by the frame acquisition time **[1].** The stationary **[2]** and flowing microspheres were distinguishable **[3]**.

3.3.1. LAB MEDIA: Figure 3

3.3.2. LAB MEDIA: Figure 3 *Video editor: Please emphasize the yellow arrows.*

3.3.3. LAB MEDIA: Figure 3 *Video editor: Please emphasize the yellow bracketed lines.*

3.4. The fate of the disseminated tumor cells was tracked with serial imaging over several days using the window for high-resolution imaging of the lung **[1]**.

3.4.1. LAB MEDIA: Figure 4

3.5. The tumor cell arrived at and lodged in the lung vasculature on day 1 **[1]**. However, the cell was not detected in the lung vasculature on the second and third days, indicating recirculation or extravasation **[2]**.

3.5.1. LAB MEDIA: Figure 4A *Video editor: Please emphasize the green-colored circle in the Day 1 image.*

3.5.2. LAB MEDIA: Figure 4A *Video editor: Please emphasize the green dotted circles in the Day 2 and Day 3 images.*

3.6. The culminating steps of metastatic progression in the lung were visualized starting with the tumor cell arrival **[1]**, the extravasation of tumor cell into the lung parenchyma **[2]**, and proliferation to form macro-metastasis **[3]**.

3.6.1. LAB MEDIA: Figure 4B *Video editor: Please emphasize the green circle in the center of the image.*

3.6.2. LAB MEDIA: Figure 4C *Video editor: Please emphasize the small green patch.*

3.6.3. LAB MEDIA: Figure 4D *Video editor: Please emphasize the large green patches.*

Conclusion

4. Conclusion Interview Statements

4.1. **Brian Traub:** Take care to avoid trauma to the lung during the optical window frame placement. The lung is prone to bleeding, which will later impede coverslip adherence during placement.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.7.3 and 2.7.4*

4.2. **Brian Trub:** Other methods that can be performed following this procedure include intravital microscopy, employed with a modified approach to microcartography, thereby allowing precise relocalization of areas of interest for repeated imaging.

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