FINAL SCRIPT: APPROVED FOR FILMING

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Title: Vascular Casting of Adult and Early Postnatal Mouse Lungs for Micro-CT Imaging

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Y, Zeiss Stemi 508, with an Axiocam 105 or Axiocam Erc5**

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

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. **Beth Kozel**: This protocol gives precise, step-by-step guidance for casting the pulmonary arterial vasculature in both adult and early postnatal mice **[1]**.

1.1.1. LAB MEDIA: IMG_5479.MOV

REQUIRED:

1.2. **Beth Kozel**: This technique enables users to fully cast the entire vascular network for its visualization by a variety of methods, including micro-CT, while maintaining a soft tissue integrity **[1]**.

1.2.1. LAB MEDIA: IMG_5480.MOV

OPTIONAL:

1.3. **Russ Knutsen**: This technique is particularly useful for looking at pulmonary vascular morphology and architecture but can easily be adapted for the targeting of other vascular beds **[1]**.

1.3.1. LAB MEDIA: IMG_5483.MOV

OPTIONAL:

1.4. **Russ Knutsen**: Like many surgeries and dissections, there is no substitute for actually visualizing a procedure and viewers may benefit from seeing some of the more challenging maneuvers being performed **[1]**.

1.4.1. LAB MEDIA: IMG_5484.MOV

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Animal Care and Use Committee (ACUC) at National Heart, Lung, and Blood Institute (NHLBI).

2. Pulmonary Artery (PA) Catheterization and Blood Perfusion

2.0 Revised/Added: Once the xyphoid process is exposed, gently grasp and elevate the ribcage. Carefully make an incision **[1]** in the now exposed semitransparent diaphragm. The lungs will visibly collapse and retract away from the diaphragm. NOTE: Author added this text but as far as I know there are no shots associated with it. I would either skip or add the VO to the 2.1 shots if that is possible.

2.1. After exposing the lung and trachea **[1-TXT]**, thread 15 centimeters of PE-10 tubing onto the hub of a 30-gauge needle **[2]** and attach this unit to a 1-milliliter syringe containing 1 x 10^{-4} -molar sodium nitroprusside in PBS **[3-TXT]**.

- 2.1.1. WIDE: Talent making incision *Videographer: More Talent than mouse in shot* **TEXT: See text for lung and trachea exposure details**
- 2.1.2. at microscope, attaching tubing to needle
- 2.1.3. Talent attaching tubing/needle to syringe **TEXT: Use caution when handling SNP**

2.2. Advance the plunger to prime the tubing until all of the air has been purged **[1]** and use curved, sharp forceps to grasp one end of a 10-centimeter piece of 7-0 silk **[2]**.

- 2.2.1. Plunger being advanced/tubing being filled
- 2.2.2. Silk being grasped

2.3. Penetrate the apex of the heart on one side **[1]** and pass the tips of the forceps through the muscle and out the other side of the tissue **[2]**. NOTE to video editor: Videographer marked some steps, including this one, “AS” and I’m not sure what that means.
Also, author mentioned having a difficult time with the scope kit, so please try to clean up the scope shots if possible.

- 2.3.1. SCOPE: Apex being penetrated NOTE: 2.3.1 – 2.3.2 in one shot *Videographer: Important step*
- 2.3.2. SCOPE: Tips being passed through muscle *Videographer: Important step*

2.4. Using a second set of forceps, pull approximately 2 centimeters of the silk through the tissue **[1]**.

- 2.4.1. SCOPE: Silk being pulled NOTE: 2.4.1 – 2.5.1 in one shot

2.5. After tying off the suture, use the remaining 8 centimeters of suture to pull the heart caudally **[1]** and tape the end of the suture to the surgical board **[2]**. Videographer NOTE: AS

2.5.1. SCOPE: Suture being grasped/heart being pulled
2.5.2. Suture being taped

2.6. Hook the tips of curved forceps under both the ascending aorta and pulmonary artery trunk **[1]** and pull a 3-centimeter length of 7-0 silk back through the opening to create a single-throw loose suture **[2]**.

2.6.1. SCOPE: Tips being hooked *Videographer: Important step*
2.6.2. SCOPE: Suture being pulled/knot being created *Videographer: Important step*

2.7. Use scissors to make a 1-2-millimeter incision toward the apex of the heart, penetrating the thin-walled right ventricle **[1]**, and introduce the primed tubing into the right ventricle, gently advancing the catheter into the semitransparent, thin-walled pulmonary artery trunk **[2-TXT]**.

2.7.1. SCOPE: Incision being made
2.7.2. SCOPE: Tubing being inserted **TEXT: Check tubing for air before connecting**

2.8. Visually verify that the catheter has not advanced into either the left or right pulmonary branches and does not abut the pulmonary artery branchpoint **[1]**.

2.8.1. SCOPE: Shot of tubing *Video Editor: please indicate lack of catheter in branches and not abutting branchpoint*

2.9. Tape the distal portion of the tubing to the surgical board **[1]** and gently tighten the loose suture around both of the great vessels **[2]**. NOTE: in the recorded shot of the first animal the tubing is taped directly to the board. The second animal the tubing is elevated and taped differently. If this is problematic that might require cropping

2.9.1. SCOPE: Tubing being taped
2.9.2. SCOPE: Suture being tightened

2.10. Cut the 8-centimeter piece of suture to return the heart to a natural resting position **[1]** and clip the left auricle of the heart to allow the perfusate to exit the vasculature **[2]**. Videographer NOTE: AS

2.10.1. Suture being cut
2.10.2. Auricle being clipped

2.11. Then use a syringe pump to infuse the sodium nitroprusside at a 0.05 milliliter/minute flow rate to both flush the blood and to maximally dilate the vasculature until the perfusate runs clear **[1]**. Videographer NOTE: AS

2.11.1. Solution being perfused

3. Tracheostomy and Lung Inflation

3.1. To construct the lung inflation unit, cut the flexible plastic 24-gauge intravenous catheter from its hub and connect to the needle of a butterfly infusion set **[1]**. NOTE: Shot of cutting the plastic is available and can be added before 3.0.1.

3.1.1. WIDE: Talent at microscope, connecting plastic 24-gauge IV cath to the needle

3.2. Attach this unit to the stopcock **[1]** and attach the stopcock to an open, 50-milliliter syringe **[2]**.

3.2.1. Talent connecting butterfly infusion set to stopcock Videographer NOTE: Use 3.2.1 take 2.
3.2.2. Talent attaching stopcock to syringe Videographer NOTE: Use 3.2.1 take 1.

3.3. After loading the syringe with formalin and priming the catheter **[1]**, with the stopcock closed, position the syringe in a ring stand to the point at which the meniscus is 20 centimeters above the trachea **[2]**.

3.3.1. Talent loading syringe with formalin
3.3.2. Talent adjusting syringe height

3.4. Place two loose sutures 2-4 millimeters apart inferior to the cricoid cartilage **[1]** and use scissors to make a small incision in the cricothyroid ligament superior to the sutures **[2]**. Videographer NOTE: AS

3.4.1. SCOPE: Suture(s) being placed
3.4.2. SCOPE: Incision being made

3.5. Insert the catheter into the opening **[1]** and advance the tip beyond the two loose sutures **[2]**. Videographer NOTE: AS

3.5.1. SCOPE: Catheter being inserted
3.5.2. SCOPE: Tip being advanced

Commented [KR([1]: note: we filmed this and could add here but it's not essential

3.6. Tighten the sutures around the trachea **[1]** and open the stopcock to allow ~~the~~ formalin to enter the lungs by gravity **[2]**. Videographer NOTE: AS

3.6.1. SCOPE: Suture(s) being tightened
3.6.2. Stopcock being opened

3.7. Wait for 5 minutes for the lungs to fully inflate **[1]**. If the lungs adhere to the ribcage during inflation, use blunt tipped forceps to grasp the outside of the ribcage **[2]** and move the ribs in all directions to assist in freeing the lobes without making direct contact with the lungs **[3]**. Videographer NOTE: AS

3.7.1. Lungs inflating
3.7.2. Ribcage being grasped
3.7.3. Ribs being moved

3.8. After 5 minutes, retract the catheter **[1]** behind the first suture and ligate **[2]**. Then retract the catheter **[3]** behind the second suture and ligate **[4].** Videographer NOTE: AS

3.8.1. Catheter being retracted
3.8.2. First suture being ligated
3.8.3. Catheter being retracted
3.8.4. Second suture being ligated

3.9. The lungs should now be inflated in a closed, pressurized state **[1]**.

3.9.1. Shot of inflated lungs

4. Casting the Vasculature

4.1. To cast the vasculature, load 1 milliliter of an 8:1:1 solution of polymer-diluent-curing agent into a 1-milliliter syringe **[1]** and carefully insert the plunger **[2]**.

4.1.1. WIDE: Talent holding syringe with tip covered, then compound being loaded into syringe *Videographer: Important step* NOTE: 4.1.1 – 4.2.1 in one shot
4.1.2. Plunger being placed into base of syringe *Videographer: Important step*

4.2. With the syringe inverted, depress the plunger to remove the air and to allow the formation of a meniscus at the tip of the syringe **[1]**.

4.2.1. Shot of ~~inverter~~ inverted syringe, then plunger being depressed *Videographer: Important step*

4.3. Remove the sodium nitroprusside syringe from the hub of the needle **[1]** and drip additional PBS into the hub to create a meniscus **[2-TXT]**.

4.3.1. Syringe being removed *Videographer: Important/difficult step*
4.3.2. PBS being dripped into hub *Videographer: Important/difficult step* **TEXT: Dislodge trapped air as necessary**

4.4. Join the polymer compound syringe to the hub **[1]** and start the infusion at 0.02 milliliters/minute **[2]**.

4.4.1. Syringe being attached to hub
4.4.2. Solution being infused

4.5. Monitor the compound as it moves freely through the tubing **[1]**, noting the syringe volume as it enters the pulmonary artery trunk **[2]**.

4.5.1. SCOPE: Compound moving through tubing *Videographer: Important/difficult step*
4.5.2. SCOPE: Shot of syringe volume *Videographer: Important/difficult step*

4.6. Continue the perfusion until all lobes are completely filled down to the smallest vessel **[1]**. Stop the pump **[2]** and, once again, note the syringe volume **[3]**.

4.6.1. SCOPE: Shot of lobes filled completely to smallest vessel NOTE: Shot with 4.5.1
4.6.2. Talent stopping pump
4.6.3. Shot of syringe volume

4.7. Cover the lungs with a fiber optic cleaning wipe **[1]** and liberally apply PBS **[2]**.

4.7.1. Lungs being covered
4.7.2. PBS being applied

4.8. Allow the specimen to sit undisturbed for 30-40 minutes at room temperature **[1]**.

4.8.1. Talent setting timer *Videographer: More Talent than mouse in shot*

4.9. ~~When~~ Once the polymer compound has cured and hardened **[1]**, sever the limbs and lower half of the mouse **[2]**, and place the head and thorax into a 50-milliliter conical filled with 10% buffered formalin overnight **[3]**.

4.9.1. Shot of cured compound

4.9.2. Shot of scissors being placed onto limb *Videographer: Do not show severance*

4.9.3. Talent placing mouse into tube

4.10. The next morning, grasp the trachea to gently separate the heart and lung unit from the remaining rib cage and thorax **[1]**. ~~and~~ Place the harvested tissue block in a formalin-filled scintillation vial **[2]**.

4.10.1. Heart and lungs being separated from ribs/thorax NOTE: 4.10.1 – 4.10.2 in one shot

4.10.2. Talent placing tissue into vial

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.3., 2.6., 4.1.-4.3., 4.5.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?

4.3., 4.5. The most difficult aspect of this procedure is identifying the appropriate vascular endpoints and stopping the cast at the right moment. Filling the vasculature at a slow, constant rate helps make this possible.

Results

5. **Results: Representative Successful Vascular Casting and Common Errors during Polymer Compound Infusion**

5.1. A successful cast will result in a uniform filling of the entire pulmonary arterial network **[1]**.

5.1.1. LAB MEDIA: Figure 4 *Video Editor: please add P90, P30, P7, and P1 labels over Figures 4A-4D, respectively*

5.2. If there is damage to the lung or lung airways **[1]**, small leaks will prevent the lungs from holding pressure **[2]**.

5.2.1. LAB MEDIA: Figure 5B *Video Editor: please emphasize white outline*
5.2.2. LAB MEDIA: Figure 5C *Video Editor: please black outline*

5.3. Patchy **[1]** or incomplete filling can arise from an "airlock" **[2]** as a result of air being introduced into the vascular system via the catheter, blocking downstream flow of the compound **[3]**.

5.3.1. LAB MEDIA: Figures 5D and 5E *Video Editor: please emphasize right lung image in Figure 5D*
5.3.2. LAB MEDIA: Figures 5D and 5E *Video Editor: please emphasize empty lung tissue in bottom of Figure 5E*
5.3.3. LAB MEDIA: Figures 5D and 5E

5.4. Underfilling occurs when too little compound is introduced into the vasculature **[1]**.

5.4.1. LAB MEDIA: Figure 5F

5.5. Alternatively, overfilling or introducing too much polymer compound too rapidly **[1]** can cause arterial rupture **[2]** or, more commonly, venous transit **[3]**.

5.5.1. LAB MEDIA: Figures 5G and 5H
5.5.2. LAB MEDIA: Figures 5G and 5H *Video Editor: please emphasize arrow/tissue indicated by arrow in Figure 5G*
5.5.3. LAB MEDIA: Figures 5G and 5H *Video Editor: please emphasize arrow/tissue indicated by arrow in Figure 5H*

5.6. Advancing the catheter too far down the pulmonary trunk can cause the tip to wedge into one pulmonary artery branch **[1]**, creating an imbalance in flow and causing one side to fill faster than the other **[2]**.

5.6.1. LAB MEDIA: Figure 5I
5.6.2. LAB MEDIA: Figure 5I *Video Editor: please emphasize left lobe*

5.7. Careful performance of the technique as demonstrated using the appropriate direct monitoring of distal vascular endpoints and standard infusion rates can result in optimal filling of pulmonary vasculature **[1]**.

5.7.1. LAB MEDIA: Figure 5A

5.8. Furthermore, adapting this technique to the systemic vasculature can yield equally favorable results in other vascular beds **[1]**.

5.8.1. LAB MEDIA: Figure 6

5.9. After casting, samples can be processed for micro-computed tomography scanning **[1]**.

5.9.1. LAB MEDIA: Figures 7A and 7B

5.10. For post-processing, a commercial software package can be used to generate a 3D volume rendering of the pulmonary vascular tree **[1]**.

5.10.1. LAB MEDIA: Figure 7C

Conclusion

6. Conclusion Interview Statements

6.1. **Russ Knutsen**: When attempting this technique for the first time, be especially careful around the fragile lungs and the pulmonary artery. Most importantly, avoid introducing air into the vascular circuit **[1]**.

6.1.1. LAB MEDIA: IMG_5487.MOV

6.2. **Russ Knutsen**: Following a successful cast, the sample can be scanned by micro-CT and questions involving vascular morphometry and architecture can be explored **[1]**.

6.2.1. LAB MEDIA: IMG_5490.MOV