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Title: Orthotopic Left Lung Transplantation in Rats

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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? **Yes, all done**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**
3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 27
Number of Shots: 53

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Hailin Liao:** My research focuses on developing a standardized, reproducible rat lung transplantation model to investigate post-transplant complications, particularly chronic rejection, and to better understand mechanisms driving long-term graft failure.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 4.7*

What advantage does your protocol offer compared to other techniques?

1.2. **Hailin Liao:** Our protocol offers a shorter learning curve, single-operator capability, and higher reproducibility compared to existing techniques.

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

How will your findings advance research in your field?

1.3. **Yi Lu:** Our standardized rat lung transplantation model provides a reliable platform for studying complications after lung transplantation and can accelerate translational research to improve graft survival.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.8.2*

What research questions will your laboratory focus on in the future?

1.4. **Yi Lu:** In the future, our laboratory will focus on the study of chronic rejection after lung transplantation, in order to improve the long-term survival rate of lung transplant recipients.

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

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Animal Care and Use Committee at Guangzhou Lai'an Technology Co., Ltd

Protocol

2. Donor Lung Harvest for Syngeneic Lung Transplant in Rats

Demonstrator: Hailin Liao

2.1. To begin, obtain 14-gauge and 16-gauge intravenous catheters and blades **[1].** Trim the catheters into a cuff with a tail with a blade **[2]**. Then divide each cuff into a body and a tail, each approximately 2 to 3 millimeters in length **[3]**.

2.1.1. WIDE: Talent holding 14 and 16 G intravenous catheters and blades.

2.1.2. SCOPE: 2.1.2.Recommended selection 42s-53s and 1min07s-1min52s.mp4 00:42-00:54.

2.1.3. SCOPE: 2.1.2.Recommended selection 42s-53s and 1min07s-1min52s.mp4 01:07-01:22.

2.2. Using a scalpel, create superficial scratches on the cuff body to increase friction for suture fixation **[1]**.

2.2.1. SCOPE: 2.2.1.Recommended selection 1min40s-1min44s.mp4 01:40-01:49

2.3. To harvest the donor lung, retract the tongue of an anesthetized rat outward and upward using forceps **[1-TXT]**. Position a surgical lamp anterior to the neck to illuminate the glottal opening clearly **[2]**.

2.3.1. Talent using forceps to extend the tongue of an anesthetized rat. **TXT: Anesthesia: Pentobarbital injection (i.p) (80 mg/kg)**

2.3.2. Talent adjusting a surgical lamp in front of the rat's neck.

2.4. Now insert a 14-gauge intravenous cannula into the airway through the glottis **[1]**. Set the ventilator to pressure-controlled mode **[2]**. Then input the weight parameters, adjust the pressure to 15 centimeters of water, and connect the ventilator **[3]**.

2.4.1. Talent inserting the cannula carefully into the glottal opening.

2.4.2. Talent inputs weight, adjusts pressure and connects the ventilator.

2.5. Observe the rise and fall of the chest to ensure synchronization with the ventilator frequency **[1]**.

2.5.1. Shot of chest movement aligned with ventilator rhythm.

2.6. Next, fix the limbs and head using tape or restraints **[1]**. Disinfect the chest and abdomen with alcohol **[2]**.

2.6.1. Talent securing limbs and head.

2.6.2. Talent disinfecting the chest and abdomen with alcohol. .

2.7. Then lift the abdominal skin using tweezers **[1]** and cut the skin from the abdomen to the front of the neck with a pair of scissors **[2]**.

2.7.1. Talent lifting abdominal skin with tweezers.

2.7.2. Shot of an incision being made from abdomen up to the neck.

2.8. Make a midline incision along the abdominal wall **[1].** Inject heparin through the exposed peritoneal vein, then allow systemic circulation for 3 minutes to ensure complete heparinization**[2-TXT].**

2.8.1. Talent performing the midline incision.

2.8.2. Talent injecting heparin with a syringe into the vein.**TXT: Heparin: 1000 IU/kg**

2.9. Cut the diaphragm and open the chest cavity from the midline of the sternum **[1]**. Then fix the chest wall on both sides with hemostats **[2].** Remove the thymus to expose the chest cavity organs **[3]**.

2.9.1. Talent cutting diaphragm and mid-sternum.

2.9.2. Shot of the chest wall being fixed with hemostats.

2.9.3. Talent removing thymus with forceps.

2.10. Sequentially cut the superior vena cava, inferior vena cava, and the left and right auriculae **[1]**. Now inject cold saline into the root of the pulmonary artery using a 20-milliliter syringe over 1 minute for low-pressure perfusion until the donor lung turns completely white **[2]**.

2.10.1. Talent cutting major vessels.

2.10.2. Talent slowly injecting cold saline at the root of the pulmonary artery until lung turns white.

2.11. Cut tissues connecting the donor lung and extract the donor heart and lung *en bloc* **[1]**. Soak the donor heart-lung block in precooled saline **[2]**.

2.11.1. Talent cutting connecting tissues and removing the organ block.

2.11.2. Talent placing the heart-lung block in a saline-filled container.

3. Donor Lung Modification for Transplant

3.1. Position the heart-lung block on ice under a microscope **[1]**. Grip the trachea with a hemostat and secure it in plasticine **[2]**.

3.1.1. Talent placing organ on ice under microscope.

3.1.2. ~~SCOPE: The trachea is being gripped with a hemostat.~~
AUTHOR'S NOTE: Shot deleted

3.1.3. Shot of the trachea being secured in plasticine.

3.2. Cover the lungs with wet sterile lens paper **[1]**. ~~Separate the lungs to expose the pulmonary hilum fully **[2].**~~

3.2.1. Talent placing wet paper over lungs.

3.2.2. ~~SCOPE: The lungs are being separated until hilum is exposed.~~
AUTHOR'S NOTE: Shot deleted

3.3. Now use forceps to separate the pulmonary artery, bronchus, and pulmonary vein under the microscope **[1]**. Ligate the bronchus close to the lung using 6-0 surgical sutures **[2]**. Use spring scissors to cut the pulmonary artery, bronchus, and pulmonary vein **[3]**.

3.3.1. SCOPE: 3.3.1.Recommended selection 12s-27s and 33s-48s and 1min52s-2min40s.mp4 00:12-00:27, 00:40-00:44, 02:34-02:40

3.3.2. SCOPE: 3.3.2.mp4 00:01-00:18.

Added shot: SCREEN: 3.3.3 add SCOPE.mp4 00:01-00:09, 00:14-00:18, 00:26-00:35

3.4. Extract the pulmonary artery, bronchus, and pulmonary vein from respective cuffs made from 16-gauge, 14-gauge, and 16-gauge indentation catheters **[1]**. Fix the tube walls to the cuff with 8-0 surgical sutures **[2]**.

3.4.1. SCOPE: 3.4.1.mp4 00:01-00:12,.

3.4.2. SCOPE: 3.4.2.mp4 00:40-00:50 .

3.5. Preserve the donor lungs in saline and place them back on ice until implantation **[1]**.

3.5.1. Talent placing donor lungs in saline on an iced platform.

4. Donor Lung Implantation

4.1. Depilate the left thoracodorsal region of the recipient animal **[1].** Position the animal in the right lateral decubitus posture on a thermostatic operating table **[2-TXT]**.

4.1.1. Talent removing fur from the recipient's thoracodorsal region.

4.1.2. Talent positioning recipient in the right lateral decubitus posture. **TXT: Disinfect surgical field with 75% alcohol**

4.2. Now make an incision through the skin and chest wall at the point of the apical impulse in the fourth intercostal space to access the thoracic cavity **[1]**. Use an eyelid retractor to open the thoracic cavity **[2]**. Then gently push the left lung aside using a moist cotton swab to expose and sharply transect the inferior pulmonary ligament **[3]**.

4.2.1. SCOPE: 4.2.1（2）.Recommended selection 8s-1min25s.mp4 00:05-00:14, 01:03-01:25.

4.2.2. SCOPE: 4.2.2.mp4 00:00-00:16.

4.2.3. SCOPE: 4.2.3.mp4 00:01-00:26.

4.3. Next, grasp the left lung with forceps and retract it outside the thoracic cavity **[1]**. Secure the hilum with a hemostat then stabilize it with modeling clay **[2]**.

4.3.1. Talent holding the left lung with forceps and pulling the lung gently outside.

4.3.2. Talent fixing hilum with hemostat and applying modeling clay for stability.

4.4. Now dissect the pulmonary hilum to separate the pulmonary artery, pulmonary vein, and bronchus **[1]**.

4.4.1. SCOPE: 4.4.1.Recommended selection 15s-58s and 1min20s-1min33s.mp4 00:15-00:19, 01:20-01:33, .

4.5. Clamp the proximal ends of the pulmonary artery, pulmonary vein, and bronchus using vascular clamps **[1]**. Pre-tie the recipient's pulmonary artery, bronchus, and pulmonary vein with 8-0 surgical sutures for rapid anastomosis **[2]**.

4.5.1. SCOPE: 4.5.1.mp4 00:00-00:14.

4.5.2. SCOPE: 4.5.2.mp4 00:13-00:36

4.6. Trim a platform on the recipient's clamped left lung to facilitate the placement of the donor lung **[1]**. Make small incisions at the distal ends of the pulmonary artery, pulmonary vein, and bronchus **[2]**.

4.6.1. SCOPE: 4.6.1.mp4 00:00-00:18.

4.6.2. SCOPE: 4.6.2.mp4 00:00-00:10, 00:15-00:23, 00:31-00:35.

4.7. Rinse the pulmonary artery and pulmonary vein with heparinized saline to prevent clot formation **[1]**. Then take the donor lung from ice and position it on the prepared platform of the recipient's left lung **[2]**.

4.7.1. SCOPE: 4.7.1.mp4 00:03-00:26

4.7.2. Shot of the donor lung being removed from ice and being placed on the recipient's left lung.

4.8. Use forceps to lift one side of the cut opening **,** Then sequentially implant and ligate the cuffs of the pulmonary artery, pulmonary vein, and bronchus into the recipient **[1]**.

4.8.1. SCOPE: 4.8.1 (1).mp4 00:16-00:45 .

:

4.9. Open the micro hemostatic clip and observe the donor lung turning from white to red **[1]**. Inspect the anastomotic sites for any signs of bleeding **[2]**.

4.9.1. SCOPE: 4.9.1.mp4 00:02-00:14 .

4.9.2. SCOPE: 4.9.2.mp4 00:00-00:12 .

4.10. Now remove the recipient's left lung and return the donor lung into the thoracic cavity **[1-TXT]**.

4.10.1. SCOPE: 4.10.1(1).mp4 00:03-00:16
. **TXT: Increase ventilator pressure to aid expansion**

4.11. Wipe the chest cavity dry with sterile cotton **[1]**. Then close the thoracic cavity layer by layer **[2]**. Wait for spontaneous breathing recovery before removing the ventilator **[3-TXT]**. After one hour, the recipients were free to eat **[4]**.

4.11.1. SCOPE: 4.11.1.mp4 00:00-00:08.

4.11.2. SCOPE: 4.11.2(3).mp4 00:56-01:10

4.11.3. Talent monitoring breathing and disconnecting ventilator. **TXT: Remove tube with recipient to gradually recover spontaneous breathing**
Added shot: Recipient eating food.

Results

5. Results

5.1. Six months after transplantation, CT*(C-T)* imaging showed that the ventilation of the transplanted lungs was comparable to the sham group, indicating preserved lung function **[1].** The macroscopic appearance of the lungs did not differ visibly between the sham and transplanted groups, showing no signs of deterioration or abnormality **[2].**

5.1.1. LAB MEDIA: Figure 5. *Video editor: Please highlight areas pointed at by the red arrows in the transplantation image of CT*

5.1.2. LAB MEDIA: Figure 5. *Video editor: Highlight both lung images in the "Macroscopic view" column*

5.2. Hematoxylin and Eosin staining of the lung tissues revealed no significant pathological changes in the transplanted lungs compared to the sham group **[1]**, with preserved alveolar structures and absence of inflammatory infiltration **[2].**

5.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the entire stained tissue sections in the "HE staining" column for both groups.*

5.2.2. LAB MEDIA: Figure 5. *Video editor: Zoom in on the magnified inset images*

Pronunciation Guide:

- **Orthotopic**

Pronunciation link:
https://www.howtopronounce.com/orthotopic
IPA: /ˌɔːrθəˈtɑːpɪk/
Phonetic Spelling: or-thuh-tah-pik

- **Anastomosis**

Pronunciation link:
https://www.merriam-webster.com/dictionary/anastomosis
IPA: /əˌnæs.təˈmoʊ.sɪs/
Phonetic Spelling: uh-nass-tuh-moh-sis

- **Auriculae**

Pronunciation link:
https://www.howtopronounce.com/auriculae
IPA: /ɔːˈrɪk.jʊ.li/
Phonetic Spelling: aw-rik-yuh-lee

- **Hilum**

Pronunciation link:
https://www.merriam-webster.com/dictionary/hilum
IPA: /ˈhaɪ.ləm/
Phonetic Spelling: hy-luhm

- **Peristalsis**

Pronunciation link:
https://www.merriam-webster.com/dictionary/peristalsis
IPA: /ˌper.əˈstɔːl.sɪs/
Phonetic Spelling: peh-ruh-stawl-sis

- **Cuff** (as in catheter cuff)

Pronunciation link:
https://www.merriam-webster.com/dictionary/cuff
IPA: /kʌf/
Phonetic Spelling: kuhf

- **Suture**

Pronunciation link:
https://www.merriam-webster.com/dictionary/suture
IPA: /ˈsuː.tʃɚ/
Phonetic Spelling: soo-chur

- **Trachea**

Pronunciation link:
https://www.merriam-webster.com/dictionary/trachea
IPA: /ˈtreɪ.ki.ə/
Phonetic Spelling: tray-kee-uh

- **Thymus**

Pronunciation link:

https://www.merriam-webster.com/dictionary/thymus
IPA: /ˈθaɪ.məs/
Phonetic Spelling: thigh-muhs

- **Hemostats**

Pronunciation link:
https://www.howtopronounce.com/hemostats
IPA: /ˈhiː.mə.stæts/
Phonetic Spelling: hee-muh-stats

- **Decubitus** (as in “right lateral decubitus”)

Pronunciation link:
https://www.howtopronounce.com/decubitus
IPA: /dɪˈkjuː.bɪ.təs/
Phonetic Spelling: dih-kyoo-buh-tuhs

- **Eosin** (from “Hematoxylin and Eosin staining”)

Pronunciation link:
https://www.merriam-webster.com/dictionary/eosin
IPA: /ˈiː.ə.sɪn/
Phonetic Spelling: ee-uh-sin

- **Alveolar**

Pronunciation link:
https://www.merriam-webster.com/dictionary/alveolar
IPA: /ælˈviː.ə.lɚ/
Phonetic Spelling: al-vee-uh-ler

- **Ligation**

Pronunciation link:
https://www.merriam-webster.com/dictionary/ligation
IPA: /laɪˈgeɪ.ʃən/
Phonetic Spelling: ly-gay-shuhn

- **Cannula**

Pronunciation link:
https://www.merriam-webster.com/dictionary/cannula
IPA: /ˈkæn.jə.lə/
Phonetic Spelling: kan-yuh-luh