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Scriptwriter Name: Sulakshana Karkala

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Title: A Standardized Murine Model with a Three-Person Workflow for Studying Acute Cellular and Antibody-Mediated Rejection in Xenotransplantation

Authors and Affiliations:

Xiaofei Zhu^{1,2}, Jian Huang^{2,3}, Yuqi An^{3,4}, Peiyuan Li^{2,3,5}, Kai Xing^{2,3,5}, Yuan Chang^{2,3,5}, Jiangping Song^{2,3,5,6,7,8}

¹Department of Urology, Beijing Jishuitan Hospital Affiliated to Capital Medical University

²Beijing Key Laboratory of Organ Xenotransplantation, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College

³State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College

⁴First Teaching Hospital of Tianjin University of Traditional Chinese Medicine

⁵Department of Cardiac Surgery, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College

⁶Department of Cardiac Surgery, Fuwai Yunnan Hospital, Chinese Academy of Medical Sciences, Affiliated Cardiovascular Hospital of Kunming Medical University

⁷Shenzhen Key Laboratory of Cardiovascular Disease, Fuwai Hospital, Chinese Academy of Medical Sciences

⁸Sanya Institute of China Agricultural University

Corresponding Authors:

Jiangping Song (fwsongjiangping@126.com)

Email Addresses for All Authors:

Xiaofei Zhu	(zhuxiaofei0079@sina.com)
Jian Huang	(huangjian99@sina.cn)
Yuqi An	(yuqian10080@163.com)
Peiyuan Li	(lipyvip@163.com)
Kai Xing	(xingkaibest@163.com)
Yuan Chang	(changyuanxw@163.com)
Jiangping Song	(fwsongjiangping@126.com)

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**

Videographer: Please capture any shots that may potentially require microscope footage using the SCOPE kit. If the authors specifically request microscope capture during filming, please use the SCOPE kit as directed by them.

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? **Yes, 26km**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 24

Number of Shots: 58

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Jiangping Song:** Our research focuses on standardized small-animal xenotransplantation models to study mechanisms of acute cellular and antibody-mediated rejection.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Xiaofei Zhu:** Existing models often suffer from operator variability and long procedures; this protocol provides a rapid, standardized, and reproducible workflow.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Jian Huang:** The main challenge is team coordination; early practice improves workflow synchronization and ensures stable and efficient model establishment.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.4. **Peiyuan Li**: Future studies can use this standardized model to investigate xenograft immune mechanisms and evaluate novel immunosuppressive or tolerance-inducing strategies.

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 Institutional Animal Care and Use Committee (IACUC) at Fuwai Hospital, Chinese Academy of Medical Sciences

Protocol

2. Donor Heart Harvest and Preparation for Heterotopic Cervical Transplantation Using the Cuff Technique

Demonstrator: Peiyuan Li

- 2.1. To begin, obtain neonatal 10 to 12-day old Sprague-Dawley rats and 7 to 8-week old C57BL/6 (*C-Fifty-seven-B-L-bar-6-J*) or Balb/c (*Balb-C*) mice [1]. House all donor rats and recipient mice in a specific pathogen-free environment [2].
 - 2.1.1. WIDE: Shot of Neonatal donor rats and donor mice in labeled cages.
 - 2.1.2. Shot of the rats and mice in the laboratory setup.
- 2.2. With povidone-iodine-soaked cotton balls, disinfect the thoracoabdominal area of the anesthetized rats [1-TXT]. Incise the abdomen with a scalpel blade [2]. Then use a cotton swab to gently move the intestines aside to expose the inferior vena cava [3].
 - 2.2.1. Talent disinfecting the thoracoabdominal area with povidone-iodine-soaked cotton balls. **TXT: Anesthesia: of 30 μ L/ g tribromoethanol (i.p)**
 - 2.2.2. Shot of the abdomen being incised.
 - 2.2.3. Talent gently moving the intestines aside with a cotton swab to expose the inferior vena cava.
- 2.3. Now use an insulin syringe to inject 0.2 to 0.5 milliliters of heparinized saline into the inferior vena cava [1]. Gently compress the injection site with a cotton ball to prevent bleeding [2].
 - 2.3.1. Talent injecting heparinized saline into the inferior vena cava using an insulin syringe.
 - 2.3.2. Talent compressing the injection site with a cotton ball.
- 2.4. Incise the diaphragm [1]. Bilaterally cut the chest wall and retract it to fully expose the heart [2]. Then remove the thymus [3] and sever the superior vena cava to relieve pressure [4].
 - 2.4.1. Talent incising the diaphragm.
 - 2.4.2. Talent cutting and retracting the chest wall to expose the heart.
 - 2.4.3. Talent removing the thymus.
 - 2.4.4. Talent severing the superior vena cava.
- 2.5. Perfuse 1 milliliter of University of Wisconsin solution into the aorta to induce cardiac

- arrest [1]. Continue perfusion until the heart changes from red to pale [2].
- 2.5.1. Talent perfusing University of Wisconsin solution into the aorta.
 - 2.5.2. Shot of the heart changing color from red to pale during perfusion.
- 2.6. Place a gauze pad soaked in ice-cold saline over the heart to lower its temperature and prevent dehydration [2].
- 2.6.1. Talent placing ice-cold saline-soaked gauze over the heart.
- 2.7. Ligate the superior and inferior vena cava with an 8-0 (*eight-Oh*) silk suture [1]. Transect both vessels [2].
- 2.7.1. Talent ligating the superior and inferior vena cava with an 8 to 0 silk suture.
 - 2.7.2. Talent transecting the vessels.
- 2.8. Now cut the aorta at the aortic arch [1]. Sever the pulmonary artery at its bifurcation [2].
- 2.8.1. Talent cutting the aorta at the aortic arch.
 - 2.8.2. Talent severing the pulmonary artery at its bifurcation.
- 2.9. Ligate all pulmonary veins with the 8-0 (*eight-Oh*) silk suture [1]. Then excise the donor heart [2]. Place the harvested heart in ice-cold saline for temporary preservation [3].
- 2.9.1. Talent ligating the pulmonary veins.
 - 2.9.2. Talent excising the donor heart.
 - 2.9.3. Harvested heart placed in ice-cold saline for preservation.

3. Recipient Vessel Preparation and Cervical Heterotopic Heart Transplantation

Demonstrator: Jian Huang

- 3.1. Using a depilatory cream, remove hair from the neck region of the recipient mouse [1]. Disinfect the skin with povidone-iodine [2].
 - 3.1.1. Talent applying depilatory cream to the mouse neck.
 - 3.1.2. Talent disinfecting the skin with povidone-iodine.
- 3.2. Make a 2-centimeter longitudinal incision approximately 1 centimeter to the right of the midline on the neck with ophthalmic scissors [1]. Perform blunt dissection to remove subcutaneous fat and muscle layers [2]. Excise the submandibular gland [3].
 - 3.2.1. Talent making the neck incision with ophthalmic scissors.
 - 3.2.2. Talent performing blunt dissection of subcutaneous fat and muscle layers.
 - 3.2.3. Talent excising the submandibular gland.

- 3.3. After exposing the external jugular vein [1], use an electrocautery pen set to low coagulation mode to individually cauterize the branches [2].
 - 3.3.1. Shot of the exposed jugular vein.
 - 3.3.2. Talent cauterizing branches of the external jugular vein with an electrocautery pen set to low coagulation mode.
- 3.4. Place two 8-0 silk sutures near the distal end of the vein for ligation [1]. Then use microsurgical scissors to transect the external jugular vein between the two ligatures [2].
 - 3.4.1. Talent placing two 8 to 0 silk sutures near the distal end of the vein.
 - 3.4.2. Talent transecting the external jugular vein between the ligatures.
- 3.5. Insert the external jugular vein through the venous cuff [1] and push the cuff to the base of the vein [2]. Then secure it with a vascular clamp [3].
 - 3.5.1. Talent inserting the vein through the venous cuff.
 - 3.5.2. Shot of the cuff being advanced to the base.
 - 3.5.3. Talent securing the cuffed vein with a vascular clamp.
- 3.6. Now use microsurgical scissors to remove the ligatures [1]. Then flush residual blood from the vein using heparinized saline [2].
 - 3.6.1. Talent removing the ligatures with microsurgical scissors.
 - 3.6.2. Talent flushing residual blood from the vein with heparinized saline.
- 3.7. With a pair of microsurgical forceps, evert the external jugular vein over the cuff [1] and secure it with an 8- 0 silk suture [2].
 - 3.7.1. Talent everting the external jugular vein over the cuff using microsurgical forceps.
 - 3.7.2. Talent securing the everted vein with an 8 to 0 silk suture.
- 3.8. Perform blunt dissection posterior to the sternocleidomastoid muscle to locate the carotid sheath [1]. Open the carotid sheath [2]. Carefully separate the common carotid artery from the internal jugular vein and vagus nerve [3].
 - 3.8.1. Talent performing blunt dissection posterior to the sternocleidomastoid muscle.
 - 3.8.2. Talent opening the carotid sheath.
 - 3.8.3. Talent separating the common carotid artery from the internal jugular vein and vagus nerve.
- 3.9. Next, ligate and transect the carotid artery [1]. Then insert the lower end of the transected artery through the arterial cuff [2]. Evert the artery over the cuff and secure it in place [3].

- 3.9.1. Talent ligating the carotid artery with an 8 to 0 silk suture then transecting the carotid artery.
- 3.9.2. Talent inserting the lower end of the artery through the arterial cuff.
- 3.9.3. Talent everting and securing the artery over the cuff.

4. Cervical Heterotopic Heart Transplantation

Demonstrator: Xiaofei Zhu

- 4.1. After trimming the donor heart, place it in the recipient mouse's neck [1]. Using an 8-0 silk suture, create a loose knot loop around the donor aorta [2].
 - 4.1.1. Talent positioning the trimmed donor heart in the recipient neck.
 - 4.1.2. Talent creating a loose knot loop around the donor aorta.
- 4.2. Slip the donor aorta over the arterial cuff on the recipient carotid artery [1]. Secure it with the 8-0 silk ligature [2].
 - 4.2.1. Talent slipping the donor aorta over the arterial cuff.
 - 4.2.2. Talent securing the aorta with an 8 to 0 silk ligature.
- 4.3. Now create a loose knot loop around the donor pulmonary artery [1]. Slip the pulmonary artery over the venous cuff on the recipient external jugular vein [2]. Then secure it with the silk ligature [3].
 - 4.3.1. Talent creating a loose knot loop around the donor pulmonary artery.
 - 4.3.2. Talent slipping the pulmonary artery over the venous cuff.
 - 4.3.3. Talent securing the pulmonary artery with an 8 to 0 silk ligature.
- 4.4. Release the vascular clamps on the recipient carotid artery and external jugular vein to restore blood flow [1]. Observe the donor heart resume strong contractions and rapidly turn red within 10 seconds [2].
 - 4.4.1. Talent releasing the vascular clamps.
 - 4.4.2. Shot of the donor heart resuming strong contractions and turning red.
- 4.5. Once the donor heart regains a stable rhythm, adjust its position [1]. Using 4-0 sutures, close the skin incision on the neck [2]. Disinfect the surgical site with povidone-iodine [3].
 - 4.5.1. Talent adjusting the position of the donor heart.
 - 4.5.2. Talent closing the neck incision with 4 to 0 sutures.
 - 4.5.3. Talent disinfecting the surgical site with povidone-iodine.
- 4.6. Place the mouse on a warm, soft pad maintained at 37 to 38 degrees Celsius to preserve

body temperature [1]. Monitor the mouse until it regains consciousness [2]. House the mouse individually in a cage with adequate access to food and water once fully awake [3].

4.6.1. Talent placing the mouse on a warm soft pad.

4.6.2. Talent monitoring the mouse during recovery.

4.6.3. Shot of the mouse housed individually in a cage with food and water available.

Results

5. Results

- 5.1. The median graft survival time in BALB/c recipients was 4 days [1], whereas in C57BL/6 recipients it was significantly prolonged to 6.5 days [2].
 - 5.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the green survival curve labeled "SD-to-BALB/c"*
 - 5.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the orange survival curve labeled "SD-to-C57BL/6"*
- 5.2. In the SD-to-BALB/c (*S-D-to-Balb-C*) group, hematoxylin and eosin staining showed severe myocardial destruction with widespread erythrocyte extravasation [1]. In the SD-to-C57BL/6 group, hematoxylin and eosin staining showed myocardial damage characterized predominantly by inflammatory infiltrates [2].
 - 5.2.1. LAB MEDIA: Figure 3A. *Video editor: Show the top row labeled "SD-to-BALB/c".*
 - 5.2.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the bottom row labeled "SD-to-C57BL/6"*
- 5.3. In the SD-to-BALB/c group, immunofluorescence staining showed minimal CD3(*C-D-Three*)-positive T cell infiltration [1], minimal CD68 (*C-D-Sixty-Eight*)-positive macrophage infiltration [2], and intense widespread C4d (*C-Four-D*) deposition [3].
 - 5.3.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the top row image labeled "CD3" under "SD-to-BALB/c"*
 - 5.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the top row image labeled "CD68" under "SD-to-BALB/c"*
 - 5.3.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the top row image labeled "C4D" under "SD-to-BALB/c"*
- 5.4. In the SD-to-C57BL/6 group, immunofluorescence staining showed prominent CD3-positive T cell infiltration [1], moderate CD68-positive macrophage presence [2], and moderate C4d deposition [3].
 - 5.4.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the bottom row image labeled "CD3" under "SD-to-C57BL/6"*
 - 5.4.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the bottom row image labeled "CD68" under "SD-to-C57BL/6"*
 - 5.4.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the bottom row image labeled "C4D" under "SD-to-C57BL/6"*

5.5. The average success rate was 93.30% in the three-person group [1], compared with 86.70% in the single-person group [2].

5.5.1. LAB MEDIA: Table 1. *Video editor: Highlight the value “93.30%” in the row “Success rate” under the column “Three-person workflow (n = 30)”.*

5.5.2. LAB MEDIA: Table 1. *Video editor: Highlight the value “86.70%” in the row “Success rate” under the column “Single-person workflow (n = 30)”.*

1. Thoracoabdominal
Pronunciation link: <https://www.merriam-webster.com/dictionary/thoracoabdominal>
IPA: /ˌθɔːr.ə.koʊ.æbˈdɑː.mə.nəl/
Phonetic Spelling: thor·uh·koh·ab·dom·uh·nuhl
2. Povidone-iodine
Pronunciation link: <https://www.merriam-webster.com/medical/povidone-iodine>
IPA: /ˌpoʊ.vəˌdoʊn ˈaɪ.əˌdaɪn/
Phonetic Spelling: poh·vuh·dohn eye·uh·dyne
3. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /əˈnes.θəˌtaɪz/
Phonetic Spelling: uh·ness·thuh·tyze
4. Tribromoethanol
Pronunciation link: <https://www.howtopronounce.com/tribromoethanol>
IPA: /ˌtraɪˌbroʊ.moʊˈeθ.əˌnɔːl/
Phonetic Spelling: try·broh·moh·eth·uh·nawl
5. Scalpel
Pronunciation link: <https://www.merriam-webster.com/dictionary/scalpel>
IPA: /ˈskæl.pəl/
Phonetic Spelling: skal·puhl
6. Inferior vena cava
Pronunciation link: <https://www.merriam-webster.com/medical/inferior%20vena%20cava>
IPA: /ɪnˈfɪr.i.ər ˈviː.nə ˈkeɪ.və/
Phonetic Spelling: in·feer·ee·er vee·nuh kay·vuh

7. Heparinized
Pronunciation link: <https://www.merriam-webster.com/medical/heparinize>
IPA: /'hep.ə.rəˌnaɪz/
Phonetic Spelling: hep·uh·ruh·nyze
8. Diaphragm
Pronunciation link: <https://www.merriam-webster.com/dictionary/diaphragm>
IPA: /'daɪ.əˌfræm/
Phonetic Spelling: dye·uh·fram
9. Thymus
Pronunciation link: <https://www.merriam-webster.com/medical/thymus>
IPA: /'θaɪ.məs/
Phonetic Spelling: thy·muhs
10. Perfuse
Pronunciation link: <https://www.merriam-webster.com/medical/perfuse>
IPA: /pər'fjuːz/
Phonetic Spelling: per·fyooz
11. Aorta
Pronunciation link: <https://www.merriam-webster.com/dictionary/aorta>
IPA: /eɪ'ɔːr.tə/
Phonetic Spelling: ay·or·tuh
12. Ligate
Pronunciation link: <https://www.merriam-webster.com/medical/ligate>
IPA: /'laɪˌɡeɪt/
Phonetic Spelling: ly·gate
13. Transect
Pronunciation link: <https://www.merriam-webster.com/dictionary/transect>
IPA: /træn'sekt/
Phonetic Spelling: tran·sekt
14. Pulmonary
Pronunciation link: <https://www.merriam-webster.com/dictionary/pulmonary>
IPA: /'pʊl.məˌner.i/
Phonetic Spelling: pull·muh·nair·ee
15. Heterotopic
Pronunciation link: <https://www.merriam-webster.com/medical/heterotopic>
IPA: /ˌhet.ə.roʊ'tɑː.pɪk/
Phonetic Spelling: het·uh·roh·top·ik
16. Transplantation
Pronunciation link: <https://www.merriam-webster.com/dictionary/transplantation>
IPA: /ˌtræns.plæn'teɪˌʃən/
Phonetic Spelling: trans·plan·tay·shuhn
17. Depilatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/depilatory>
IPA: /dɪ'pɪl.əˌtɔːr.i/
Phonetic Spelling: dih·pill·uh·tor·ee

18. Ophthalmic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
 IPA: /ɑːfˈθælmɪk/
 Phonetic Spelling: off·thal·mik
19. Submandibular
 Pronunciation link: <https://www.merriam-webster.com/medical/submandibular>
 IPA: /ˌsʌb.mænˈdɪb.jə.lər/
 Phonetic Spelling: sub·man·dib·yuh·ler
20. Electrocautery
 Pronunciation link: <https://www.merriam-webster.com/medical/electrocautery>
 IPA: /ɪ.lek.troʊˈkɑː.tə.ri/
 Phonetic Spelling: ih·lek·troh·kaw·tuh·ree
21. Sternocleidomastoid
 Pronunciation link: <https://www.merriam-webster.com/medical/sternocleidomastoid>
 IPA: /ˌstɜː.noʊ.klaɪ.doʊˈmæs.tɔɪd/
 Phonetic Spelling: ster·noh·kly·doh·mass·toyd
22. Carotid
 Pronunciation link: <https://www.merriam-webster.com/dictionary/carotid>
 IPA: /kəˈrɑː.tɪd/
 Phonetic Spelling: kuh·rah·tid
23. Vagus
 Pronunciation link: <https://www.merriam-webster.com/medical/vagus>
 IPA: /ˈveɪ.gəs/
 Phonetic Spelling: vay·guhs
24. Hematoxylin
 Pronunciation link: <https://www.merriam-webster.com/medical/hematoxylin>
 IPA: /ˌhiː.məˈtɑːk.sə.lɪn/
 Phonetic Spelling: hee·muh·tok·suh·lin
25. Eosin
 Pronunciation link: <https://www.merriam-webster.com/medical/eosin>
 IPA: /ˈiː.oo.sɪn/
 Phonetic Spelling: ee·oh·sin
26. Myocardial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/myocardial>
 IPA: /ˌmaɪ.oʊˈkɑːr.di.əl/
 Phonetic Spelling: my·oh·kar·dee·uhl
27. Erythrocyte
 Pronunciation link: <https://www.merriam-webster.com/medical/erythrocyte>
 IPA: /ɪˈrɪθ.rə.sɑɪt/
 Phonetic Spelling: ih·rith·ruh·syte
28. Extravasation
 Pronunciation link: <https://www.merriam-webster.com/medical/extravasation>
 IPA: /ɪkˌstræv.əˈseɪ.ʃən/
 Phonetic Spelling: ek·strav·uh·say·shuhn

29. Immunofluorescence

Pronunciation link: <https://www.merriam-webster.com/medical/immunofluorescence>

IPA: /ɪˌmjuːˌnoʊˌflʊˈres.əns/

Phonetic Spelling: ih·myoo·noh·flor·ess·uhns

30. Macrophage

Pronunciation link: <https://www.merriam-webster.com/medical/macrophage>

IPA: /ˈmæk.rəˌfeɪdʒ/

Phonetic Spelling: mak·ruh·fayj

31. Infiltration

Pronunciation link: <https://www.merriam-webster.com/dictionary/infiltration>

IPA: /ˌɪn.filˈtreɪ.ʃən/

Phonetic Spelling: in·fil·tray·shuhn

32. Deposition

Pronunciation link: <https://www.merriam-webster.com/dictionary/deposition>

IPA: /ˌdep.əˈziʃ.ən/

Phonetic Spelling: dep·uh·zish·uhn