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Title: Heterotopic Hindlimb-to-Neck Transplantation in the Mouse: A Supermicrosurgical Model of Vascularized Composite Allotransplantation

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one.**



Interview Statements are read by JoVE's voiceover talent.

Current Protocol Length

Number of Steps: 18

Number of Shots: 24

Introduction

NOTE to VO producer: Please generate VO for interviews

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. This study presents a super-microsurgical mouse hindlimb-to-neck vascularized composite allotransplantation model to study immune mechanisms and graft injury.

1.1.1. B roll: 2.2.1

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

- 1.2. Currently, murine VCA protocols are scarce and difficult to reproduce; this detailed video protocol enhances consistency and reduces technical failures.

1.2.1. B roll: 2.5.1

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. This protocol enables the study of immune tolerance, rejection, ischemia-reperfusion injury, and graft survival in mice.

1.3.1. B roll: 2.9.1

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

- 1.4. Precise supermicrosurgical technique and careful handling of the pedicle are essential for successful graft perfusion.

1.4.1. B roll: 3.1.1

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

- 1.5. Future studies can optimize tolerance induction strategies and translate findings toward clinically applicable immunomodulatory therapies.

1.5.1. B roll: 4.2.1

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) and the Animal Care and Use Review Office at the New York University Grossman School of Medicine

Protocol

2. Donor Right Partial Hindlimb Procurement

Demonstrator: Haruyuki Hirayama

2.1. After making the required incision, elevate the cutaneous flap, beginning from its perforator, including the superficial inferior epigastric artery and vein, and skeletonize as needed [1-TXT].

2.1.1. LAB MEDIA: Movie_39.avi 00:20 – 00:30 and 01:05 – 01:11 **TXT: Anesthesia: Isoflurane; Induction: 3.5%; Maintenance: 3%**

2.2. Transect the inguinal ligament cranially and proceed with dissection caudally [1]. Ensure that branches that are exposed are coagulated with bipolar cautery [2].

2.2.1. LAB MEDIA: Movie_06.avi 00:15 – 00:25

2.2.2. LAB MEDIA: Movie_06.avi 00:35 – 00:45

2.3. Now, coagulate and dissect the surrounding adipose tissue using a larger monopolar cautery to achieve full exposure of the external iliac vessels extending to the level of the common iliac vessels [1]. Dissect the surrounding tissues of the external and common iliac arteries using smaller monopolar cautery [2].

2.3.1. LAB MEDIA: Movie_77.avi 00:25 – 00:45

2.3.2. LAB MEDIA: Movie_77.avi 01:40 – 01:50

2.4. Flex the knee joint to identify its location, and open it using bipolar cautery [1].

2.4.1. LAB MEDIA: Movie_07.avi 00:20 – 00:30

2.5. Extend the knee joint again, and transect the biceps femoris, quadriceps femoris, and other associated muscles with bipolar cautery while coagulating [1].

2.5.1. LAB MEDIA: Movie_07.avi 01:02 – 01:12

2.6. Next, carefully coagulate any remaining branches arising from the external and common iliac vessels, as well as the surrounding muscular tissue, and dissect with

bipolar cautery while maintaining hemostasis [1].

2.6.1. LAB MEDIA: Movie_79.avi 03:55 – 04:08

2.7. Perforate the membrane between the artery and vein at a point distant from the graft [1], then hook an ophthalmic retractor onto the artery [2].

2.7.1. LAB MEDIA: Movie_81.avi 00:15 – 00:23

2.7.2. LAB MEDIA: Movie_81.avi 01:00 – 01:05

2.8. Then, carefully separate the vein and artery as distally as possible using curved Potts scissors [1].

2.8.1. LAB MEDIA: Movie_10.avi 02:59 – 03:17

2.9. Ligate the artery with 8-0 Prolene suture and place a stay suture on the artery as a marker to prevent twisting during vascular anastomosis [1].

2.9.1. LAB MEDIA: Movie_12.avi 04:11 – 04:23

2.10. Next, ligate the vein with 8-0 Prolene [1]. Place stay sutures at two sites on the vein to facilitate its passage through the cuff. Insert a 4-0 nylon into the lumen to facilitate placement of the stay sutures, as the vein is very thin and the vessel wall can occasionally be difficult to distinguish [2].

2.10.1. LAB MEDIA: Movie_13.avi 03:54 – 04:05

2.10.2. LAB MEDIA: Movie_14.avi 00:19 – 00:30

2.11. Pass the vein through the cuff using stay sutures, and clamp the cuff with a vascular clip [1].

2.11.1. LAB MEDIA: Movie_15.avi 02:01 – 02:18

2.12. Now, evert the vessel wall, and secure it to the cuff with 9-0 nylon [1].

2.12.1. LAB MEDIA: Movie_15.avi 03:27 – 03:30 and Movie_16.avi 03:43 – 03:47

3. Recipient Surgery

3.1. Make a small circular skin incision approximately 10 millimeters wide [1-TXT]. Insert the scissors caudally through the incision and gently open them to dissect the subcutaneous tissue of the chest, thereby creating a pocket for graft placement [2].

3.1.1. LAB MEDIA: Movie_20.avi 00:00 – 00:10 **TXT: Anesthesia: Isoflurane; Induction: 2.5%; Maintenance: 2%**

3.1.2. LAB MEDIA: Movie_20.avi 00:51 – 01:00

3.2. Expose the external jugular vein by cauterizing and dividing its branches with a larger monopolar cautery [1].

3.2.1. LAB MEDIA: Movie_21.avi 00:06 – 00:12 and Movie_21.avi 01:58 – 02:03

3.3. Now, identify and carefully dissect the common carotid artery [1].

3.3.1. LAB MEDIA: Movie_22.avi 00:00 – 00:10

3.4. Perform an end-to-end arterial anastomosis with 11-0 or 12-0 nylon using six interrupted sutures. Begin with four anterior stitches, flip the artery, then complete the posterior wall. Insert 5-0 nylon into both vessels and use it as a stent [1].

3.4.1. LAB MEDIA: Movie_26.avi 00:00 – 00:18

3.5. Insert the cuff into the external jugular vein [1], and secure it with 9-0 nylon, taking care to avoid twisting of the pedicle [2].

3.5.1. LAB MEDIA: Movie_30.avi 02:21 – 02:26

3.5.2. LAB MEDIA: Movie_30.avi 04:06 – 04:13

3.6. Finally, position the graft carefully in the subcutaneous chest pocket, avoiding displacement of the trachea or restriction of forelimb movement and ensure the perforator is untwisted [1].

3.6.1. LAB MEDIA: Movie_70.avi 03:59 – 04:17

Results

4. Results

4.1. The graft was assessed daily after the procedure. On the day of surgery, the graft showed good perfusion [1].

4.1.1. LAB MEDIA: Figure 7A.

4.2. By postoperative day 7, erythema had resolved and the skin color had stabilized [1]. The skin paddle remained viable and showed visible hair regrowth by postoperative day 14 [2].

4.2.1. LAB MEDIA: Figure 7B.

4.2.2. LAB MEDIA: Figure 7C.

4.3. A total of 13 animals completed the study following successful postoperative recovery [1] and the median total operative time was around 169 minutes [2].

4.3.1. LAB MEDIA: Table 1. *Video editor: Highlight the row labeled "Total animals completed the study" with value "13".*

4.3.2. LAB MEDIA: Table 1. *Video editor: Highlight the row labeled "Total operative time (min), median (IQR)" and the value 169*

4.4. One case of graft infection occurred, which was associated with subsequent graft necrosis [1].

4.4.1. LAB MEDIA: Table 1. *Video editor: Highlight the row labeled "Graft infection" with value "1 (7.7%)" and the comment about necrosis.*

4.5. Four cases of partial necrosis of the skin paddle were observed, two of which required a single debridement and resuturing [1].

4.5.1. LAB MEDIA: Table 1. *Video editor: Highlight the row labeled "Partial skin paddle necrosis" with value "4 (30.8%)" and the comment about debridement and resuturing.*

4.6. One case of wound dehiscence occurred, requiring resuturing three times before acute rejection developed [1].

4.6.1. LAB MEDIA: Table 1. *Video editor: Highlight the row labeled “Wound dehiscence” with value “1 (7.7%)” and the comment about repeated resuturing and rejection.*

1. Cutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/cutaneous>
IPA: /kyu:'temiəs/
Phonetic Spelling: kyoo·tay·nee·uhs
2. Perforator
Pronunciation link: <https://www.merriam-webster.com/dictionary/perforator>
IPA: /'pɜ:fə'reɪtər/
Phonetic Spelling: pur·fuh·ray·ter
3. Epigastric
Pronunciation link: <https://www.merriam-webster.com/dictionary/epigastric>
IPA: /,epə'gæstri:k/
Phonetic Spelling: eh·puh·gas·trik
4. Skeletonize
Pronunciation link: <https://www.merriam-webster.com/dictionary/skeletonize>
IPA: /'skelətn,aɪz/
Phonetic Spelling: skel·uh·tuh·nyze
5. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /,aɪsoʊ'florem/
Phonetic Spelling: eye·soh·floor·ayn
6. Inguinal
Pronunciation link: <https://www.merriam-webster.com/dictionary/inguinal>
IPA: /'ɪŋgwənəl/
Phonetic Spelling: ing·gwuh·nuhl
7. Cranially
Pronunciation link: <https://www.merriam-webster.com/dictionary/cranial>
IPA: /'kreɪniəli/
Phonetic Spelling: kray·nee·uh·lee
8. Caudally
Pronunciation link: <https://www.merriam-webster.com/dictionary/caudal>
IPA: /'kɔ:dəli/
Phonetic Spelling: kaw·duh·lee
9. Coagulate
Pronunciation link: <https://www.merriam-webster.com/dictionary/coagulate>
IPA: /koʊ'ægjə'leɪt/
Phonetic Spelling: koh·ag·yuh·layt
10. Bipolar
Pronunciation link: <https://www.merriam-webster.com/dictionary/bipolar>
IPA: /baɪ'poʊləɹ/
Phonetic Spelling: bye·poh·ler

11. Monopolar
 Pronunciation link: <https://www.merriam-webster.com/dictionary/monopolar>
 IPA: /ˌmɑːnəˈpoulər/
 Phonetic Spelling: mon·uh·poh·ler
12. Adipose
 Pronunciation link: <https://www.merriam-webster.com/dictionary/adipose>
 IPA: /ˈædəˌpous/
 Phonetic Spelling: ad·uh·pohs
13. Iliac
 Pronunciation link: <https://www.merriam-webster.com/dictionary/iliac>
 IPA: /ˈɪliˌæk/
 Phonetic Spelling: il·ee·ak
14. Hemostasis
 Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostasis>
 IPA: /ˌhiːmoʊˈsteɪsɪs/
 Phonetic Spelling: hee·moh·stay·suhs
15. Ophthalmic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
 IPA: /ɑːfˈθælmɪk/
 Phonetic Spelling: ahf·thal·mik
16. Retractor
 Pronunciation link: <https://www.merriam-webster.com/dictionary/retractor>
 IPA: /rɪˈtræktər/
 Phonetic Spelling: ree·trak·ter
17. Distally
 Pronunciation link: <https://www.merriam-webster.com/dictionary/distal>
 IPA: /ˈdɪstəli/
 Phonetic Spelling: dis·tuh·lee
18. Prolene
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Prolene>
 IPA: /ˈproʊliːn/
 Phonetic Spelling: ɹɹoh·leen
19. Anastomosis
 Pronunciation link: <https://www.merriam-webster.com/dictionary/anastomosis>
 IPA: /əˌnæstəˈmoʊsɪs/
 Phonetic Spelling: uh·nas·tuh·moh·suhs
20. Lumen
 Pronunciation link: <https://www.merriam-webster.com/dictionary/lumen>
 IPA: /ˈluːmən/
 Phonetic Spelling: loo·muhn
21. Evert
 Pronunciation link: <https://www.merriam-webster.com/dictionary/evert>
 IPA: /ɪˈvɜːt/
 Phonetic Spelling: ih·vert

22. Jugular
Pronunciation link: <https://www.merriam-webster.com/dictionary/jugular>
IPA: /ˈdʒʌɡjələr/
Phonetic Spelling: jug·yuh·ler
23. Carotid
Pronunciation link: <https://www.merriam-webster.com/dictionary/carotid>
IPA: /kəˈrɑːtɪd/
Phonetic Spelling: kuh·rah·tid
24. Arterial
Pronunciation link: <https://www.merriam-webster.com/dictionary/arterial>
IPA: /ɑːrˈtɪriəl/
Phonetic Spelling: ar·teer·ee·uhl
25. Subcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌbkjuːˈteɪniəs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs
26. Perfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/perfusion>
IPA: /pərˈfjuːʒən/
Phonetic Spelling: pur·fyoo·zhuhn
27. Postoperative
Pronunciation link: <https://www.merriam-webster.com/dictionary/postoperative>
IPA: /ˌpoʊstˈɑːpərətɪv/
Phonetic Spelling: pohst·op·uh·ruh·tiv
28. Erythema
Pronunciation link: <https://www.merriam-webster.com/dictionary/erythema>
IPA: /ˌerəˈθiːmə/
Phonetic Spelling: eh·ruh·thee·muh
29. Viable
Pronunciation link: <https://www.merriam-webster.com/dictionary/viable>
IPA: /ˈvaɪəbəl/
Phonetic Spelling: vye·uh·buhl
30. Necrosis
Pronunciation link: <https://www.merriam-webster.com/dictionary/necrosis>
IPA: /nəˈkroʊsɪs/
Phonetic Spelling: nuh·kroh·suhs
31. Debridement
Pronunciation link: <https://www.merriam-webster.com/dictionary/debridement>
IPA: /diˈbriːdmənt/
Phonetic Spelling: dih·breed·muhnt
32. Dehiscence
Pronunciation link: <https://www.merriam-webster.com/dictionary/dehiscence>
IPA: /diˈhɪsəns/
Phonetic Spelling: dih·hiss·uhns

33. Rejection

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

IPA: /rɪˈdʒekʃən/

Phonetic Spelling: ree·jek·shuhn