Submission ID #: 68907
Scriptwriter Name: Poornima G
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Title: Simple and Effective Procedure for Hemostasis in Mouse Arteries

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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**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes, all done
   SCOPE: 2.4.3 TO 3.9.1.

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**

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: 19
Number of Shots: 35

Introduction

Videographer: Obtain headshots for all authors available at the filming location.
Do not film the interviews

NOTE TO VO Producer:
Please generate the answers

INTRODUCTION:

1.1. This research develops a method to seal arterial punctures while preserving blood flow. It investigates whether fat wrapping prevents ischemia after arterial puncture.

1.1.1. ***B roll: 2.3.1***

1.2. Achieving hemostasis after arterial puncture is difficult without causing arterial occlusion.

1.2.1. ***B roll: 2.5.1***

CONCLUSION:

1.3. The present method aims to seal the puncture site post-procedure while maintaining normal blood flow, thereby preventing postoperative ischemia.

1.3.1. ***B roll: 3.3.1***

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 at the Shenyang Agricultural University

Protocol

2. Animal Preparation and Surgical Site Access

2.1. To begin, position the anesthetized mouse on the surgical table **[1-TXT]**.

2.1.1. WIDE: Talent placing the anesthetized mouse on the surgical table. **TXT: Anesthesia: Induction: 4% Isoflurane; Maintenance: 2% Isoflurane Videographer's NOTE:** This step has 6 shots, and I have labeled the file names. When editing, follow this order to edit

2.2. After confirming anesthesia, remove hair from the cervical and cranial regions using a depilator **[1]**. Disinfect the exposed skin with povidone-iodine followed by 75 percent ethanol to eliminate any residue **[2]**.

2.2.1. Talent applying depilatory cream to the cervical and cranial regions of the anesthetized mouse. **Videographer's NOTE:** There are 2 shots in this step, and I have labeled the file names

2.2.2. Talent wiping the area with povidone-iodine and then with 75 percent ethanol using separate sterile gauze pads.

2.3. Place the mouse on a heating pad set to 37 degrees Celsius and keep it there for the entire duration of the experimental procedures **[1]**.

2.3.1. Talent positioning the mouse on a heating pad and adjusting its body alignment.

2.4. Reposition the mouse in a supine position on the heating pad **[1]**. Make a midline cervical incision to expose subcutaneous tissue **[2]** and excise a 3 by 3 by 1 millimeter subcutaneous adipose tissue graft for future use **[3]**.

2.4.1. Talent turning the mouse onto its back on the heating pad.

2.4.2. Talent making a midline cervical incision using fine surgical scissors.

2.4.3. SCOPE: 2.4.3.

2.5. Perform blunt dissection of the submandibular gland, sternohyoid muscle, and sternocleidomastoid muscle to expose both common carotid arteries **[1]**.

2.5.1. SCOPE: 2.5.1.

2.6. Then, carefully free the common carotid artery to expose the internal and external carotid arteries **[1]**.

2.6.1. SCOPE: 2.6.1.

2.7. Place vascular clamp 1 on the distal end of the common carotid artery **[1]** and then, place vascular clamp 2 on the proximal end of the artery **[2]**.

2.7.1. SCOPE: 2.7.1.

2.7.2. SCOPE: 2.7.2.

2.8. Next, puncture the common carotid artery between the two clamps using a 23-gauge needle **[1]**.

2.8.1. SCOPE: 2.8.1.

3. Application of the Fat Pad

3.1. After removing the needle, wrap the common carotid artery at the puncture site using the cut surface, or non-serosa side, of the previously harvested fat pad **[1]**.

3.1.1. SCOPE: 3.1.1.

3.2. Place a 4-0 *(4-Oh)* suture beneath the fat-wrapped artery **[1]**. Gently tie a single throw over the fat surface to apply pressure for secure contact between the fat pad and the vessel wall **[2]**. Then, adjust the ligature gradually to balance pressure and achieve hemostasis **[3]**.

3.2.1. SCOPE: 3.2.1.

3.2.2. SCOPE: 3.2.2.

3.2.3. SCOPE: 3.2.3.

3.3. Elevate the distal common carotid artery using a moistened cotton swab **[1]**. Release vascular clamp 1 **[2]** and slowly withdraw the swab to allow a gradual return of blood flow toward the puncture site **[3]**.

3.3.1. SCOPE: 3.3.1.

3.3.2. SCOPE: 3.3.2.

3.3.3. SCOPE: 3.3.3.

3.4. Now, check for blood flow at the puncture site **[1]**. If active bleeding is observed, reapply vascular clamp 1 on the distal common carotid artery **[2]**.

3.4.1. SCOPE: 3.4.1.

3.4.2. SCOPE: 3.4.2.

3.5. After 1 minute, slowly release vascular clamp 1 **[1]**. If bleeding persists, reposition the fat pad or slightly tighten the ligature to increase pressure until full hemostasis is achieved **[2]**.

3.5.1. SCOPE: 3.5.1.

3.5.2. SCOPE: 3.5.2.

3.6. Elevate the proximal common carotid artery using a moistened cotton swab **[1]**. Next, release vascular clamp 2 **[2]** and slowly withdraw the swab to permit controlled blood flow toward the puncture site **[3]**.

3.6.1. SCOPE: 3.6.1

3.6.2. SCOPE: 3.6.2

3.6.3. SCOPE: 3.6.3.

3.7. If bleeding occurs, reapply vascular clamp 2 on the distal common carotid artery **[1]**.

3.7.1. SCOPE: 3.7.1.

3.8. Now, slightly tighten the single throw of the suture to increase pressure **[1]** and wait for 1 minute **[2]**. Then, elevate the proximal common carotid artery **[3]** and release vascular clamp 2 by slowly rolling the moistened swab toward the puncture site **[4]**.

3.8.1. SCOPE: 3.8.1.

3.8.2. SCOPE: 3.8.2.

3.8.3. SCOPE: 3.8.3.

3.8.4. SCOPE: 3.8.4.

3.9. Add another throw using the same suture to form a double knot over the initial throw, improving fat pad fixation and promoting balanced hemostasis **[1]**.

3.9.1. SCOPE: 3.9.1.

3.10. Confirm that the common carotid arteries on both sides of the fat pad are filled and maintain consistent vessel diameter, indicating successful blood flow restoration **[1-TXT]**.

3.10.1. Close-up of the carotid arteries showing symmetrical diameter and restored blood flow. **TXT: Repeat the procedure on the contralateral common carotid artery**

3.11. Finally, suture the cervical incision **[1]** and apply an antiseptic to the wound site **[2]**.

3.11.1. Talent closing the surgical incision with sutures.

3.11.2. Talent dabbing the site with antiseptic solution.

Results

4. Results

4.1. Pre- and post-operative laser speckle contrast imaging and perfusion unit measurements in both sham and operated mice showed no significant changes, confirming that cerebral blood flow was not compromised by the arterial hemostasis technique **[1]**.

4.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight A and then B*

4.2. Histological examination of brain tissue by hematoxylin and eosin staining at 7, 14, and 28 days post-operation in operated mice, and in sham mice, showed intact tissue architecture in the cortex, hippocampus, and thalamus without pathological changes **[1]**.

4.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the "HE" column showing brain tissue sections for "Sham", "Postop. 7d", "Postop. 14d", and "Postop. 28d" sequentially*.

4.3. Nissl staining showed organized neuronal alignment, normal cellular structure, and preserved Nissl bodies across all groups and time points, indicating no structural damage due to arterial closure **[1]**.

4.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the "Nissl" column showing neuronal structure for "Sham", "Postop. 7d", "Postop. 14d", and "Postop. 28d" sequentially.*

1. Anesthetized
   Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetized
   IPA: /əˈnesθəˌtaɪzd/
   Phonetic Spelling: uh·nes·thuh·tyzd
2. Isoflurane
   Pronunciation link: https://www.merriam-webster.com/dictionary/isoflurane
   IPA: /ˌaɪsoʊˈflʊreɪn/
   Phonetic Spelling: eye·soh·floor·ayn
3. Cervical
   Pronunciation link: https://www.merriam-webster.com/dictionary/cervical
   IPA: /ˈsɝːvɪkəl/
   Phonetic Spelling: ser·vih·kuhl

4. Cranial
   Pronunciation link: https://www.merriam-webster.com/dictionary/cranial
   IPA: /ˈkreɪniəl/
   Phonetic Spelling: kray·nee·uhl
5. Depilator
   Pronunciation link: https://www.merriam-webster.com/dictionary/depilator
   IPA: /ˈdepəˌleɪtər/
   Phonetic Spelling: dep·uh·lay·ter
6. Povidone-iodine
   Pronunciation link: https://www.merriam-webster.com/dictionary/povidone-iodine
   IPA: /ˌpɑːvəˌdoʊnˈaɪəˌdaɪn/
   Phonetic Spelling: pah·vuh·dohn·eye·uh·dyne
7. Ethanol
   Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
   IPA: /ˈeθəˌnɔːl/
   Phonetic Spelling: eh·thuh·nawl
8. Celsius
   Pronunciation link: https://www.merriam-webster.com/dictionary/Celsius
   IPA: /ˈselsiəs/
   Phonetic Spelling: sel·see·uhs
9. Supine
   Pronunciation link: https://www.merriam-webster.com/dictionary/supine
   IPA: /ˈsuːˌpaɪn/
   Phonetic Spelling: soo·pine
10. Subcutaneous
    Pronunciation link: https://www.merriam-webster.com/dictionary/subcutaneous
    IPA: /ˌsʌbkjuːˈteɪniəs/
    Phonetic Spelling: sub·kyoo·tay·nee·uhs
11. Adipose
    Pronunciation link: https://www.merriam-webster.com/dictionary/adipose
    IPA: /ˈædəˌpoʊs/
    Phonetic Spelling: ad·uh·pohs
12. Submandibular
    Pronunciation link: https://www.merriam-webster.com/dictionary/submandibular
    IPA: /ˌsʌbˌmænˈdɪbjələr/
    Phonetic Spelling: sub·man·dib·yuh·ler
13. Sternohyoid
    Pronunciation link: https://www.merriam-webster.com/dictionary/sternohyoid
    IPA: /ˌstɝːnoʊˈhaɪɔɪd/
    Phonetic Spelling: ster·noh·hy·oyd
14. Sternocleidomastoid
    Pronunciation link: https://www.merriam-webster.com/dictionary/sternocleidomastoid
    IPA: /ˌstɝːnoʊˌklaɪdoʊˈmæstɔɪd/
    Phonetic Spelling: ster·noh·kly·doh·mas·toyd

15. Carotid
    Pronunciation link: https://www.merriam-webster.com/dictionary/carotid
    IPA: /kəˈrɑːtɪd/
    Phonetic Spelling: kuh·rah·tid
16. Dissection
    Pronunciation link: https://www.merriam-webster.com/dictionary/dissection
    IPA: /dɪˈsekʃən/
    Phonetic Spelling: dih·sek·shuhn
17. Vascular
    Pronunciation link: https://www.merriam-webster.com/dictionary/vascular
    IPA: /ˈvæskjələr/
    Phonetic Spelling: vas·kyuh·ler
18. Proximal
    Pronunciation link: https://www.merriam-webster.com/dictionary/proximal
    IPA: /ˈprɑːksɪməl/
    Phonetic Spelling: prok·sih·muhl
19. Distal
    Pronunciation link: https://www.merriam-webster.com/dictionary/distal
    IPA: /ˈdɪstəl/
    Phonetic Spelling: dis·tuhl
20. Hemostasis
    Pronunciation link: https://www.merriam-webster.com/dictionary/hemostasis
    IPA: /ˌhiːmoʊˈsteɪsɪs/
    Phonetic Spelling: hee·moh·stay·suhs
21. Ligature
    Pronunciation link: https://www.merriam-webster.com/dictionary/ligature
    IPA: /ˈlɪgətʃər/
    Phonetic Spelling: lig·uh·cher
22. Serosa
    Pronunciation link: https://www.merriam-webster.com/dictionary/serosa
    IPA: /səˈroʊsə/
    Phonetic Spelling: suh·roh·suh
23. Contralateral
    Pronunciation link: https://www.merriam-webster.com/dictionary/contralateral
    IPA: /ˌkɑːntrəˈlætərəl/
    Phonetic Spelling: kon·truh·lat·uh·ruhl
24. Antiseptic
    Pronunciation link: https://www.merriam-webster.com/dictionary/antiseptic
    IPA: /ˌæntiˈseptɪk/
    Phonetic Spelling: an·tee·sep·tik
25. Histological
    Pronunciation link: https://www.merriam-webster.com/dictionary/histological
    IPA: /ˌhɪstəˈlɑːdʒɪkəl/
    Phonetic Spelling: his·tuh·lah·ji·kuhl

26. Hematoxylin
    Pronunciation link: https://www.merriam-webster.com/dictionary/hematoxylin
    IPA: /ˌhiːməˈtɑːksɪlɪn/
    Phonetic Spelling: hee·muh·tok·suh·lin
27. Eosin
    Pronunciation link: https://www.merriam-webster.com/dictionary/eosin
    IPA: /ˈiːoʊsɪn/
    Phonetic Spelling: ee·oh·sin
28. Hippocampus
    Pronunciation link: https://www.merriam-webster.com/dictionary/hippocampus
    IPA: /ˌhɪpəˈkæmpəs/
    Phonetic Spelling: hip·uh·kam·puhs
29. Thalamus
    Pronunciation link: https://www.merriam-webster.com/dictionary/thalamus
    IPA: /ˈθæləməs/
    Phonetic Spelling: thal·uh·muhs
30. Nissl
    Pronunciation link: https://www.merriam-webster.com/dictionary/Nissl
    IPA: /ˈnɪsəl/
    Phonetic Spelling: nih·suhl
31. Neuronal
    Pronunciation link: https://www.merriam-webster.com/dictionary/neuronal
    IPA: /nʊˈrɑːnəl/
    Phonetic Spelling: nyoo·rah·nuhl
32. Perfusion
    Pronunciation link: https://www.merriam-webster.com/dictionary/perfusion
    IPA: /pərˈfjuːʒən/
    Phonetic Spelling: per·fyoo·zhuhn
33. Speckle
    Pronunciation link: https://www.merriam-webster.com/dictionary/speckle
    IPA: /ˈspɛkəl/
    Phonetic Spelling: spek·uhl