

Submission ID #: 68907

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21016683>

Title: Simple and Effective Procedure for Hemostasis in Mouse Arteries

Authors and Affiliations:

Jinpeng Liu^{1*}, Qing Xin^{1,2*}, Mingxuan Zhang^{3*}, Haifeng Li⁴, Feng Jiang^{1,2}, Shuyang Feng⁴, Hongwei Xin³, Ying Zhang⁵, Xiaohu Han^{1,2}

¹College of Animal Science and Veterinary Medicine, Shenyang Agricultural University

²Key Laboratory of Livestock Infectious Diseases, Ministry of Education, and Key Laboratory of Ruminant Infectious Disease Prevention and Control (East), Ministry of Agriculture and Rural Affairs, College of Animal Science and Veterinary Medicine, Shenyang Agricultural University

³Manzhouli International Travel Health Care Center

⁴Shenyang Baihao Biotechnology Co., Ltd.

⁵The Affiliated Animal Hospital of Jinzhou Medical University, Collaborative Innovation Center Zoonosis Prevention and Treatment of Jinzhou Medical University

* These authors contributed equally

Corresponding Authors:

Xiaohu Han	hxx8849@syau.edu.cn
Ying Zhang	zhangy99321@163.com
Hongwei Xin	4403668@qq.com

Email Addresses for All Authors:

Jinpeng Liu	1187096511@qq.com
Qing Xin	xq05182023@163.com
Mingxuan Zhang	502549076@qq.com
Haifeng Li	hf10899@126.com
Feng Jiang	jf8849@163.com
Shuyang Feng	fengshuyang@huibaibio.com
Xiaohu Han	hxx8849@syau.edu.cn

Ying Zhang
Hongwei Xin

zhangy99321@163.com
4403668@qq.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**

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

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.

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: /əˈnesθəˈtaɪzd/

Phonetic Spelling: uh·nes·thuh·tyzd

2. Isoflurane

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

IPA: /ˌaɪsoʊˈflorem/

Phonetic Spelling: eye·soh·floor·ayn

3. Cervical

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

IPA: /ˈsɜːvɪkəl/

Phonetic Spelling: ser·vih·kuhl

4. Cranial
Pronunciation link: <https://www.merriam-webster.com/dictionary/cranial>
IPA: /'kreɪniəl/
Phonetic Spelling: kray·nee·uhl
5. Depilator
Pronunciation link: <https://www.merriam-webster.com/dictionary/depilator>
IPA: /'depəˌleɪtər/
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: /'eθəˌnɔːl/
Phonetic Spelling: eh·thuh·nawl
8. Celsius
Pronunciation link: <https://www.merriam-webster.com/dictionary/Celsius>
IPA: /'selsiəs/
Phonetic Spelling: sel·see·uhs
9. Supine
Pronunciation link: <https://www.merriam-webster.com/dictionary/supine>
IPA: /'suːˌpaɪn/
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: /'ædəˌpoʊs/
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: /ˌstɜːnoʊˈhaɪɔɪd/
Phonetic Spelling: ster·noh·hy·oyd
14. Sternocleidomastoid
Pronunciation link: <https://www.merriam-webster.com/dictionary/sternocleidomastoid>
IPA: /ˌstɜːnoʊˌklaɪdoʊˈmæstɔɪd/
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: /dɪˈseksjən/
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: /ˈlɪɡətʃər/
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: /ˌhi:məˈtə:ksɪlɪn/
Phonetic Spelling: hee·muh·tok·suh·lin
27. Eosin
Pronunciation link: <https://www.merriam-webster.com/dictionary/eosin>
IPA: /ˈi:əʊsɪn/
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: /nʊˈrɑ:nəl/
Phonetic Spelling: nyoo·rah·nuhl
32. Perfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/perfusion>
IPA: /pərˈfju:ʒən/
Phonetic Spelling: per·fyoo·zhuhn
33. Speckle
Pronunciation link: <https://www.merriam-webster.com/dictionary/speckle>
IPA: /ˈspekəl/
Phonetic Spelling: spek·uhl