

Submission ID #: 70639

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Project Page Link: https://review.jove.com/files_upload.php?src=21544943

Title: Engineering High-fidelity Preclinical Mouse Model for Brain Metastasis Using Interlock Pulsatile Injection Technique and Microsurgical Vascular Repair

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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, we have another microscope with recording ability in another lab.

SCOPE Shots: All shots from 2.1 - 4.2 (Except 2.1.1)

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? **Yes**

If **Yes**, how far apart are the locations? **21km**

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: 11

Number of Shots: 40

Introduction

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

INTRODUCTION:

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

- 1.1. **Yu Bai:** This protocol establishes a high-fidelity brain metastasis model that preserves cerebral blood flow and avoids CCA ligation-induced hemodynamic damage.
 - 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. **Yu Bai:** Traditional models disrupt cerebral flow and BBB; this protocol uses IPI with microsurgical repair to restore physiology and reduce mortality.
 - 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. **Yu Bai:** The key challenge while practicing this protocol is the need for advanced microsurgical expertise and a steep learning curve overall.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Yu Bai:** Drug testing, IHC, molecular assays, and multi-modal imaging can be performed to further analyze metastasis and evaluate therapeutic responses.
 - 1.4.1. 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.~~

- 1.5. **Yu Bai:** Future studies can build on this work by exploring different cancers, molecular pathways, and developing automated injection devices for improved modeling.
 - 1.5.1. 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 the Institute of Biophysics, Chinese Academy of Sciences

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

NOTE: All SCOPE shots were recorded by the videographer

2. Surgical Exposure of the Right Carotid Artery

Demonstrator: Yu Bai

- 2.1. To begin, obtain an anesthetized C57BL/6 (*C-fifty-seven black six*) mouse after completing all preoperative procedures [1-TXT]. Disinfect the neck region by applying povidone-iodine solution and allow the skin to dry completely [2]. Drape the mouse aseptically using a sterile surgical drape, exposing only the neck region [3].
 - 2.1.1. WIDE: The anesthetized mouse is being positioned on a clean surface, fully prepared for surgery. **TXT: Anesthesia: 2.5% Tribromoethanol, 0.2 mL/10 g body weight**
 - 2.1.2. SCOPE: Talent applying povidone-iodine solution to the mouse neck and allowing it to air dry.
 - 2.1.3. SCOPE: Talent placing a sterile drape over the mouse, leaving the neck region exposed.
- 2.2. Use sterile surgical scissors to make a midline cervical incision approximately 1 centimeter in length [1]. Use fine forceps to retract the tissue [2]. Then, use fine angled forceps to perform blunt dissection of the superficial fascia and underlying muscles to expose the right carotid sheath [3].
 - 2.2.1. SCOPE: Talent making a midline cervical incision with scissors
 - 2.2.2. SCOPE: Talent retracting tissue with forceps.
 - 2.2.3. SCOPE: Talent performing blunt dissection to expose the carotid sheath.
- 2.3. Isolate the right external carotid artery from the carotid sheath and ligate it [1]. Next, isolate the right common carotid artery or CCA (*C-C-A*) within the carotid sheath while preserving the vagus nerve located laterally and beneath the vessel [2]. Place a small piece of rubber glove and a saline-moistened cotton ball beneath the isolated right CCA to elevate and stabilize the vessel [3].
 - 2.3.1. SCOPE: Talent isolating the external carotid artery and ligating it.
 - 2.3.2. SCOPE: Talent isolating the CCA while avoiding the vagus nerve.

2.3.3. SCOPE: Talent positioning a rubber piece and cotton ball under the artery.

2.4. Put two loose knots with open loops on the CCA distal and proximal to the cotton ball [1] and then tie the proximal knot [2]. Apply a microvascular clip on the CAA near the distal untightened knot [3].

2.4.1. SCOPE: Talent placing two loose knots on the artery.

2.4.2. SCOPE: Talent tightening the proximal knot.

2.4.3. SCOPE: Talent applying a microvascular clip to the artery.

3. Cancer Cell Implantation

3.1. Use a 32-gauge (*thirty two gauge*) needle with the bevel facing upward to puncture the elevated segment of the right CCA [1]. Withdraw the initial syringe while maintaining the puncture site [2]. Vortex-mix the cancer cell suspension [3]. Draw 100 microliters into the microliter syringe without any air bubbles [4]. Insert the needle tip of the microliter syringe into the puncture site and hold it steadily without initiating injection [5].

3.1.1. SCOPE: Talent puncturing the artery with a 32-gauge needle.

3.1.2. SCOPE: Talent removing the syringe while keeping the puncture stable.

3.1.3. SCOPE: Talent vortexing the suspension.

3.1.4. SCOPE: Talent loading the cancer cell suspension into a microliter syringe.

3.1.5. SCOPE: Talent inserting and stabilizing the microliter syringe in the artery.

3.2. Remove the microvascular clip applied previously [1]. Gently advance the syringe plunger in small pulses to inject the tumor cell suspension into the internal carotid artery [2]. While injecting, maintain a rate of approximately 1 microliter per second and ensure that it does not exceed 60 microliter per minute [3].

3.2.1. SCOPE: Talent removing the microvascular clip.

3.2.2. SCOPE: Talent gently advancing the syringe plunger intermittently to inject the tumor cell suspension in small pulses.

3.2.3. SCOPE: Shot of the talent checking to confirm a rate of around 1 $\mu\text{L/s}$ and monitoring the flow. *Videographer: Please record this shot as a backup.*

3.3. Monitor the vessel to identify successful delivery when the red vessel becomes temporarily transparent [1]. Confirm that no visible extravasation occurs around the puncture site [2]. After completing the injection, withdraw the microliter syringe carefully [3]. Immediately tighten the distal knot in a controlled manner [4].

3.3.1. SCOPE: Shot of the vessel turning temporarily transparent as the suspension flows through the lumen.

- 3.3.2. SCOPE: Shot showing no leakage around the puncture site.
- 3.3.3. SCOPE: Talent carefully withdrawing the microliter syringe from the vessel.
- 3.3.4. SCOPE: Talent tightening the distal knot on the vessel to seal it.

3.4. Use a 10-0 (*ten-oh*) nylon microsurgical suture to close the puncture site in a watertight manner adjacent to the defect margin [1]. Pass the microsurgical suture needle through the puncture hole and leave the suture untied temporarily [2]. Flush the surgical field with heparinized saline [3]. Secure the suture with two to three square knots [4]. Then, trim the free ends of the suture to approximately 1 to 2 millimeters [5].

- 3.4.1. SCOPE: Talent suturing the puncture site.
- 3.4.2. SCOPE: Talent passing the suture needle through the vessel wall.
- 3.4.3. SCOPE: Talent flushing the surgical field with heparinized saline.
- 3.4.4. SCOPE: Talent tying square knots on the suture.
- 3.4.5. SCOPE: Talent trimming the suture ends.

4. Restoration of the Blood Flow and Postoperative Assessment

4.1. Gradually loosen the proximal knot [1]. Use heparinized saline to flush the lumen [2]. Place a dry cotton ball in the surgical field to monitor for excessive bleeding [3]. Once active effusion stops, loosen the distal knot [4].

- 4.1.1. SCOPE: Talent loosening the proximal knot.
- 4.1.2. SCOPE: Talent flushing the lumen with heparinized saline.
- 4.1.3. SCOPE: Talent placing a cotton ball in the surgical field to check for excessive bleeding.
- 4.1.4. SCOPE: Talent loosening the distal knot.

4.2. Observe the CCA for robust pulsations [1]. Check the repair site to ensure there is no narrowing and the vessel remains open and continuous [2]. Irrigate the surgical field thoroughly with non-heparinized normal saline [3]. Close the skin incision using a continuous suture [4].

- 4.2.1. SCOPE: Shot of the artery showing pulsation.
- 4.2.2. SCOPE: Talent examining the repaired vessel to ensure there is no narrowing and that the vessel remains open and continuous.
- 4.2.3. SCOPE: Talent irrigating the surgical field.
- 4.2.4. SCOPE: Talent closing the skin incision using a continuous suture.

4.3. After recovery from the surgery, tumor progression and model establishment were evaluated through daily body weight and clinical assessments [1], bioluminescence imaging with quantitative ROI analysis [2], and histological confirmation of metastatic

lesions [3].

- 4.3.1. Shot of the talent checking the weight of the mouse post-surgery
- 4.3.2. Representative shot of the talent Place the anesthetized mouse in the imaging chamber.
- 4.3.3. Representative shot of the talent checking the hematoxylin and eosin-stained slide under a microscope.

Results

5. Results

5.1. Continuous in vivo bioluminescent imaging [1] and quantitative analysis over 4 weeks after intracarotid inoculation showed that metastatic tumor cells proliferated within the brain microenvironment [2].

5.1.1. LAB MEDIA: Figure 3A. *Video editor: Show the full row of mouse images from left to right and highlight how the bright glowing areas increase over time.*

5.1.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the upward trend of the plotted line across the weeks on the graph.*

5.2. Postoperative body weight monitoring revealed progressive weight loss in tumor-bearing mice compared with PBS controls [1].

5.2.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the red line it slopes downward and compare it to the blue control line that stays higher.*

5.3. Kaplan–Meier survival analysis further demonstrated reduced survival in the tumor suspension-injected group [1].

5.3.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the red line curve dropping fast.*

5.4. Hematoxylin and eosin staining revealed tumor cells infiltrating the brain parenchyma and effacing its normal architecture [1].

5.4.1. LAB MEDIA: Figure 3F. *Video editor: Emphasize the dense, dark-stained clustered areas that clearly look different from the lighter normal background. Also emphasize the darker part of the zoomed-in image.*

1. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈnes.θəˌtaɪzd/
Phonetic Spelling: uh·ness·thuh·tyzed
2. C57BL/6
Pronunciation link: No confirmed link found
IPA: /siː ˈfɪf.ti ˈsev.ən blæk sɪks/
Phonetic Spelling: see fifty seven black six
3. Preoperative
Pronunciation link: <https://www.merriam-webster.com/dictionary/preoperative>
IPA: /ˌpriːˈɑː.pə.ə.tɪv/
Phonetic Spelling: pree·op·uh·ruh·tiv
4. Povidone-iodine
Pronunciation link: <https://www.merriam-webster.com/dictionary/povidone>
IPA: /ˈpɑː.vəˌdoʊn ˈaɪ.əˌdaɪn/
Phonetic Spelling: pah·vuh·dohn eye·uh·dyne
5. Aseptically
Pronunciation link: <https://www.merriam-webster.com/dictionary/aseptic>
IPA: /eɪˈsep.tɪ.kli/
Phonetic Spelling: ay·sep·tihk·lee
6. Cervical
Pronunciation link: <https://www.merriam-webster.com/dictionary/cervical>
IPA: /ˈsɜː.vɪ.kəl/
Phonetic Spelling: sur·vih·kuhl
7. Forceps
Pronunciation link: <https://www.merriam-webster.com/dictionary/forceps>
IPA: /ˈfɔːr.seps/
Phonetic Spelling: for·seps
8. Retract
Pronunciation link: <https://www.merriam-webster.com/dictionary/retract>
IPA: /rɪˈtrækt/
Phonetic Spelling: ree·trakt
9. Dissection
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissection>
IPA: /daɪˈsek.ʃən/
Phonetic Spelling: dye·sek·shuhn
10. Fascia
Pronunciation link: <https://www.merriam-webster.com/dictionary/fascia>
IPA: /ˈfæʃ.i.ə/, /ˈfeɪ.ʃə/
Phonetic Spelling: fash·ee·uh, fay·shuh
11. Carotid
Pronunciation link: <https://www.merriam-webster.com/dictionary/carotid>
IPA: /kəˈrɑː.tɪd/
Phonetic Spelling: kuh·rah·tid

12. Ligate
Pronunciation link: <https://www.merriam-webster.com/dictionary/ligate>
IPA: /'laɪ.geɪt/
Phonetic Spelling: lie·gayt
13. Vagus
Pronunciation link: <https://www.merriam-webster.com/dictionary/vagus>
IPA: /'veɪ.gəs/
Phonetic Spelling: vay·guhs
14. Saline
Pronunciation link: <https://www.merriam-webster.com/dictionary/saline>
IPA: /'seɪ.laɪn/, /sə'liːn/
Phonetic Spelling: say·line, suh·leen
15. Microvascular
Pronunciation link: <https://www.merriam-webster.com/dictionary/microvascular>
IPA: /,maɪ.kroʊ'væs.kjə.lə/
Phonetic Spelling: my·kroh·vas·kyuh·ler
16. Gauge
Pronunciation link: <https://www.merriam-webster.com/dictionary/gauge>
IPA: /geɪdʒ/
Phonetic Spelling: gayj
17. Bevel
Pronunciation link: <https://www.merriam-webster.com/dictionary/bevel>
IPA: /'bev.əl/
Phonetic Spelling: bev·uhl
18. Vortex-mix
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /'vɔːr.teks/
Phonetic Spelling: vor·teks
19. Microliter
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /,maɪ.kroʊ.liː.tər/
Phonetic Spelling: my·kroh·lee·ter
20. Plunger
Pronunciation link: <https://www.merriam-webster.com/dictionary/plunger>
IPA: /'plʌŋ.dʒər/
Phonetic Spelling: plun·jer
21. Tumor
Pronunciation link: <https://www.merriam-webster.com/dictionary/tumor>
IPA: /'tuː.mər/
Phonetic Spelling: too·mer
22. Extravasation
Pronunciation link: <https://www.merriam-webster.com/dictionary/extravasation>
IPA: /ɪk'stræv.ə'seɪ.ʃən/
Phonetic Spelling: ik·strav·uh·say·shuhn

23. Microsurgical
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microsurgery>
 IPA: /ˌmaɪ.kroʊˈsɜː.dʒɪ.kəl/
 Phonetic Spelling: my·kroh·sur·jih·kuhl
24. Heparinized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/heparin>
 IPA: /ˈhep.ə.əˌnaɪzd/
 Phonetic Spelling: hep·uh·ruh·nyzed
25. Lumen
 Pronunciation link: <https://www.merriam-webster.com/dictionary/lumen>
 IPA: /ˈluː.mən/
 Phonetic Spelling: loo·muhn
26. Effusion
 Pronunciation link: <https://www.merriam-webster.com/dictionary/effusion>
 IPA: /ɪˈfjuː.ʒən/
 Phonetic Spelling: ih·fyoo·zhuhn
27. Irrigate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/irrigate>
 IPA: /ˈɪr.əˌgeɪt/
 Phonetic Spelling: ir·uh·gayt
28. Bioluminescence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/bioluminescence>
 IPA: /ˌbaɪ.ooˌluː.məˈnes.əns/
 Phonetic Spelling: bye·oh·loo·muh·ness·uhns
29. Quantitative
 Pronunciation link: <https://www.merriam-webster.com/dictionary/quantitative>
 IPA: /ˈkwɑːn.təˌteɪ.tɪv/
 Phonetic Spelling: kwahn·tuh·tay·tiv
30. Histological
 Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>
 IPA: /ˌhɪs.təˈlɑː.dʒɪ.kəl/
 Phonetic Spelling: his·tuh·loh·jih·kuhl
31. Metastatic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/metastatic>
 IPA: /ˌmet.əˈstæt.ɪk/
 Phonetic Spelling: met·uh·stat·ik
32. Inoculation
 Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculation>
 IPA: /ɪˌnɑː.kjəˈleɪ.ʃən/
 Phonetic Spelling: ih·nok·yuh·lay·shuhn
33. Kaplan–Meier
 Pronunciation link: <https://www.merriam-webster.com/medical/Kaplan-Meier%20method>
 IPA: /ˈkæp.lən ˈmaɪ.ə/
 Phonetic Spelling: kap·luhn my·er

34. Hematoxylin

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

IPA: /ˌhiː.məˈtɑːk.sə.lɪn/

Phonetic Spelling: hee·muh·tok·suh·lin

35. Eosin

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

IPA: /ˈiː.ə.sɪn/

Phonetic Spelling: ee·uh·sin

36. Parenchyma

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

IPA: /pəˈrɛŋ.kə.mə/

Phonetic Spelling: puh·reng·kuh·muh