FINAL SCRIPT: APPROVED FOR FILMING

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Title: Microvascular Embolism Mouse Model for In Vivo Two-Photon Microscopy Using Fluorescent Polystyrene Microspheres

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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**

   Authors: Please create scope videos of the shots labeled as SCOPE and upload the files to your project page as soon as possible: https://review.jove.com/account/file-uploader?src=21026443

   SCOPE: 2.2.1, 2.2.2, 2.3.1, 2.3.2, 2.4.1, 2.4.2, 2.4.3, 2.5.1, 2.5.2, 2.6.1, 2.6.2, 2.7.1, 2.7.2, 2.7.3,
   3.5.1, 3.5.2, 3.6.1, 3.6.2, 3.7.2, 3.7.3, 3.8.2, 3.9.1, 3.10.1, 3.11.1, 3.12.1, 3.12.2, 3.13.1, 3.14.2, 3.15.1, 3.15.2, 3.16.1, 3.16.2, 3.18.1, 3.18.2, 3.18.3, 3.19.1

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, 50 meters apart**

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. **Yes**

Current Protocol Length
Number of Steps: 27
Number of Shots: 55

Introduction

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

1.1. **Kevin Mol:** Using this microsphere injection model, we aim to unravel the short and long-term consequences of capillary plugging and investigate potential clearing mechanisms of the embolus from the cerebral capillary network.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.2.1*

What advantage does your protocol offer compared to other techniques?

1.2. **Kevin Mol:** It allows for real-time *in vivo* imaging and tracking of the fluorescent particles in the cortical capillary network. The microspheres and their local consequences can be followed for weeks after injection.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.10.1*

How will your findings advance research in your field?

1.3. **Kevin Mol:** Our studies help to understand local tissue damage caused by microvascular occlusion. These results may hopefully lead to new treatment strategies.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

What new scientific questions have your results paved the way for?

1.4. **Kevin Mol:** We can now study, day by day and for weeks, how capillary plugging alters vessels, activates cells, changes blood flow, and damages the blood brain barrier.

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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. **Inge Mulder, Assistant Professor at Amsterdam UMC**: (authors will present their testimonial statements live)

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Inge Mulder, Assistant Professor at Amsterdam UMC**: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Central Committee on Animal Experiments of The Netherlands (AVD11400202316817) and the local animal welfare body at the Amsterdam UMC

Protocol

2. External Carotid Artery Catheterization

Demonstrator: Kevin Mol

2.1. To begin, pinch the toe of an anesthetized mouse to verify the depth of anesthesia **[1-TXT]**. Make a midline incision of approximately 0.5 centimeter over the trachea below the mandible using surgical scissors and dissecting forceps **[2]**.

2.1.1. WIDE: Talent performing toe pinch test on anesthetized animal. **TXT: Anesthesia: 3 - 4 % isoflurane inhalation**

2.1.2. SCOPE: 2.1.2.mp4 00:08-00:30

2.2. Dissect the connective tissue superficial to the cervical fascia using micro-suture-tying forceps to expose the underlying sternohyoid muscles **[1]**. Now, separate the left and right sternohyoid muscles by gently tearing the connective tissue between them **[2]**.

2.2.1. SCOPE: SCOPE-2.2.1.mp4 00:00-00:21

2.2.2. SCOPE: SCOPE-2.2.2.mp4 00:14-00:28, 01:21-01:30

2.3. Retract the right omohyoid muscle caudo-laterally with a tissue hook **[1]**. Ensure that the right carotid triangle including the common carotid artery, internal carotid artery, and external carotid artery is clearly visible **[2]**.

2.3.1. SCOPE: SCOPE-2.3.1.mp4. 00:20-00:31

2.3.2. SCOPE: SCOPE-2.3.2.mp4 00:21-00:42

2.4. Next, remove any remaining fascia and adipose tissue surrounding the common carotid artery **[1]**. Carefully separate the common carotid artery from the vagus nerve **[2]**. Place two 4/0 *(four-by-oh)* 1.5 threads around the common carotid artery and tie them loosely ensuring the knots do not impede blood flow **[3]**.
AUTHOR'S NOTE: Scope shots filmed together for 2.4.1-2.4.2

2.4.1. SCOPE: SCOPE-2.4.1-&-2.4.2.mp4. 00:07-00:21

2.4.2. SCOPE: SCOPE-2.4.1-&-2.4.2.mp4. 00:25-00:35

2.4.3. SCOPE: SCOPE-2.4.3.mp4. 00:18-00:24, 00:39-00:40, 01:25-01:45, 02:02-02:06

2.5. Remove fascia and adipose tissue from the internal carotid artery and posterior parietal artery **[1]**. Then temporarily ligate the posterior parietal artery and all internal carotid

side branches including the occipital artery by tying a knot with a 4/0 1.5 thread **[2]**.

2.5.1. SCOPE: SCOPE-2.5.1.mp4. 00:14-00:20, 01:00-01:10,02:37-02:44

2.5.2. SCOPE: SCOPE-2.5.2.mp4. 00:30-00:35, 03:07-03:17,03:37-03:50

2.6. Remove fascia and adipose tissue from the external carotid artery and the temporal artery **[1]**. Place two loose knots around the external carotid artery and temporal artery using four-slash-zero size one-point-five threads **[2]**.

2.6.1. SCOPE: SCOPE-2.6.1.mp4 00:31-00:50

2.6.2. SCOPE: SCOPE-2.6.2.mp4. 01:34-01:54, 03:20-03:28

2.7. Position the most distal thread as far away from the Y-shaped bifurcation as possible and use it to permanently ligate the external carotid artery and temporal artery. Leave the proximal knot untied **[1]**.
AUTHOR'S NOTE: All Scope shots filmed together for 2.7

2.7.1. SCOPE: SCOPE-2.7.mp4 00:11-00:46

~~2.7.2. SCOPE: Shot of the distal thread being used to ligate the ECA and temporal artery.~~

~~2.7.3. SCOPE: Shot of the untied proximal knot.~~

3. Controlled Injection of FITC-Dextran 10-Micrometer Microspheres into the Mouse Internal Carotid Artery

3.1. Homogenize and sonicate the ten-micrometer microspheres to obtain a uniform suspension of individual particles **[1]**.

3.1.1. Talent sonicating the microsphere solution.

3.2. Add 20 microliters of homogenized microspheres to 140 microliters of FITC *(Fit-C)*- Dextran to obtain 160 microliters of mixture containing 1.44 × **[1-TXT]**.

3.2.1. Talent adding 20 µL microspheres solution to the FITC-Dextran mixture. **TXT: Final volume: 160 µL, 1.44 x 10^5 microspheres**

3.3. Briefly retract the syringe connected to the catheter to create an airlock **[1]**. Then immerse the catheter tip into the prepared FITC-Dextran/Tween-20/microsphere *(Fit-C-Dextran-Tween-Twenty-Microsphere)* mixture **[2]** and slowly pull back the syringe until the mixture is in the catheter **[3]**. Ensure no air bubbles are present in the catheter **[4-TXT]**.

3.3.1. Talent retracting syringe to create airlock.

3.3.2. Talent Immersing catheter tip into mixture.

3.3.3. Shot of the mixture being drawn into the catheter.

3.3.4. Talent checking the catheter for air bubbles. **TXT: If bubbles are present, empty mixture into 0.5 mL container and re-perform retraction and immersion**

3.4. Place the syringe with the loaded catheter in the syringe pump **[1]**. Set the pump to ten microliters per minute **[2]** and run until a small drop of microsphere mixture appears at the catheter tip **[3]**.

3.4.1. Talent mounting catheter in the syringe pump.

3.4.2. Talent setting the flow rate on pump.

3.4.3. Shot of a small drop forming at catheter tip.

3.5. Now, place a vessel clip on the internal carotid artery **[1]** and tighten one of the previously prepared proximal knots around the common carotid artery **[2]**.

3.5.1. SCOPE: SCOPE-3.5.1.mp4. 00:05-00:20

3.5.2. SCOPE: SCOPE-3.5.2.mp4 00:08-00:22

3.6. Using micro-scissors make a small diagonal incision in the external carotid artery approximately 0.5 millimeters distal to the ligation thread ensuring the incision size is slightly smaller than the catheter diameter **[1]**. Absorb any blood with a sterile cloth **[2]**.
AUTHOR'S NOTE: All Scope shots filmed together for 3.6

3.6.1. SCOPE: SCOPE-3.6.mp4 00:00-00:30

3.6.2. SCOPE: SCOPE-3.6.mp4. 00:40-00:50

3.7. Briefly start the syringe pump to form a drop of microsphere mixture at the catheter tip confirming that no air remains in the catheter tip **[1]**. Open the external carotid artery incision and insert the catheter using fine micro-forceps **[2-TXT]**. Then secure the catheter in the external carotid artery by tightening the proximal suture around the external carotid artery **[3]**.

3.7.1. Shot of the pump being started.

3.7.2. SCOPE: SCOPE-3.7.2.mp4. 00:16-00:31
TXT: Avoid touching any other surfaces to prevent microsphere loss

3.7.3. SCOPE: SCOPE-3.7.3.mp4. 00:04-00:25

~~3.8. Start the syringe pump at ten microliters per minute to ensure no leakage or bubbles **[1]**. If either is present detach the catheter and return to catheter insertion **[2]**.~~

~~3.8.1. Shot of pump running at ten microliters per minute.~~

~~3.8.2. SCOPE: Talent detaching catheter.~~
AUTHOR'S NOTE: Step not filmed

3.9. Gradually pump at ten microliters per minute until the artery visibly enlarges and the green FITC dye fills the external carotid artery, confirming successful injection **[1-TXT]**.
AUTHOR'S NOTE: SCOPE steps 3.9.1 and 3.10.1 are the same. Should be merged together. Recording is therefore the same, named '3.9.1 & 3.10.1'

3.9.1. SCOPE: SCOPE-3.9.1-&-3.10.1.mp4. 00:14-00:40 **TXT: Maintain adequate**

pressure in catheter and vasculature to match the animal's arterial pressure

~~3.10. Visually confirm the injection of the microsphere mixture into the external carotid artery and that the buildup of pressure is successful by observing the green FITC dye filling the arteries **[1].**~~

NOTE: VO of 3.10 is merged with 3.9

~~3.10.1. SCOPE: SCOPE-3.9.1 & 3.10.1.mp4.~~

3.11. Next, remove the vessel clip from the internal carotid artery ~~and the ligation from the common carotid artery~~ **[1],** then increase the syringe pump flow rate to twenty microliters per minute **[2]**.

3.11.1. SCOPE: SCOPE-3.11.1.mp4. 00:00-00:07

3.11.2. Talent adjusting pump flow rate to twenty microliters per minute.

3.12. Confirm successful microsphere injection by verifying that the internal carotid artery distal to the posterior parietal artery bifurcation is filled with FITC dye **[1]**. ~~If confirmation is not seen, reposition the posterior parietal artery ligation further distally from the bifurcation then tighten the ligation **[2].**~~

3.12.1. SCOPE: SCOPE-3.12.1.mp4 00:00-00:10

3.12.2. ~~SCOPE: Talent repositioning and tightening the PPA ligation~~.
AUTHOR'S NOTE: Shot not filmed

3.13. Once injection is successful, remove the thread that ligated the common carotid artery to enable the microsphere mixture to flow into the internal carotid artery **[1]**.During the injection, the FITC dye can still be observed in the external carotid artery **[2].**

3.13.1. SCOPE: SCOPE-3.13.1.mp4 00:04-00:30

Added shot: 3.13.2: SCOPE: SCOPE-3.13.2.mp4 00:00-00:10

~~3.14. If retrograde flow into the common carotid artery occurs, decrease the syringe pump flow to ten microliters per minute **[1].** If retrograde flow continues, gently squeeze the external carotid artery, internal carotid artery and common carotid artery using micro-forceps to ensure no obstruction is present due to a thrombus **[2].**~~
AUTHOR'S NOTE: Steps 3.14-3.16 not filmed

~~3.14.1. Talent reducing syringe pump flow.~~

~~3.14.2. SCOPE: Shot of ECA, ICA and CCA being squeezed with micro-forceps.~~

~~3.15. Visually confirm that no air embolus is present inside the vasculature **[1 TXT].** If present, stop the syringe pump **[1].** Move the air embolus to the external carotid artery by gently squeezing the artery using micro-forceps **[2].**~~

~~3.15.1. SCOPE: Shot of vasculature with air embolus. **TXT: If present, stop syringe pump**~~

~~3.15.2. SCOPE: Shot of the artery being squeezed with micro-forceps to move air~~

~~embolus.~~

~~3.16. Then ligate the common carotid artery and place a vessel clip on the external carotid artery **[1].** Remove the catheter and air embolus and return to catheter insertion **[2 TXT].**~~

~~3.16.1. SCOPE: Shot of the CCA being ligated and a clip being placed on the ECA.~~

3.16.2. ~~SCOPE: Talent removing the catheter and air embolus.~~ ~~**TXT: If obstructed but retrograde flow continues, close CCA with vessel clip**~~

3.17. Switch off the syringe pump once the mixture in the catheter reaches the 80-microliter mark to stop injection **[1]**

3.17.1. Talent switching off pump.

3.18. Then ligate the common carotid artery, clip the internal carotid, and gently remove the catheter while keeping the securing thread in place. **[1]**. Permanently ligate the external carotid artery by tightening the thread that had secured the catheter **[3]**.

3.18.1. SCOPE: SCOPE-3.18.1-&-3.18.2.mp4. 00:00-00:10

AUTHOR'S NOTE: SCOPE 3.18.1 and 3.18.2 are in the same recording named; '3.18.1 & 3.18.2'

3.18.2. SCOPE: SCOPE-3.18.3.mp4. 00:15-00:36

3.19. Remove the vessel clip and threads around the common carotid artery, internal carotid artery and posterior parietal artery to restore blood flow **[1]**. Then moisten the surgical area with a few drops of saline **[2]**.

3.19.1. SCOPE: SCOPE-3.19.1.mp4 00:27-00:50

3.19.2. Talent applying saline drops to surgical field.

3.20. Suture the skin with a reverse cutting 12-millimeter long, 3/8 *(Three-by-Eight)* curved needle and a non-absorbable three-zero silk suture **[1]**.

3.20.1. Talent suturing skin with specified needle and suture.

Results

4. Results

4.1. The success of the microvascular embolism surgery was confirmed by full-body and full-brain post-mortem in situ imaging 4 days after surgery **[1]**. A sagittal brain slice showed lodging of microspheres in the brain **[2]**.

4.1.1. LAB MEDIA: Figure 3

4.1.2. LAB MEDIA: Figure 3 *Video Editor: Please highlight A*

4.2. Extracted mouse brains imaged 1 day post-surgery showed that microspheres were predominantly lodged in the ipsilateral hemisphere, within the flow territory of the middle and anterior cerebral arteries **[1]**. Microsphere counts in brain slices were significantly increased when Tween20 was added to the microsphere mixture **[2]**.

4.2.1. LAB MEDIA: Figure 3 *Video Editor: Please highlight B,C*

4.2.2. LAB MEDIA: Figure 3E. *Video editor: Highlight the bar labeled with Tween20*

4.3. Mice lost less weight and recovered faster after surgery when injected with a microsphere mixture that contained Tween20 **[1]**.

4.3.1. LAB MEDIA: Figure 3F. *Video editor: Highlight the orange line.*

4.4. Fluorescence microscopy images of the brain cortex taken 0.5 hours after surgery showed individual white dots representing microspheres occluding capillaries **[1]**. Two-photon z-stack projection images showed microspheres causing cerebral microvascular embolisms with impaired perfusion **[2]**.

4.4.1. LAB MEDIA: Figure 4A,B. *Video Editor: Please emphasize the red dotted square*

4.4.2. LAB MEDIA: Figure 4C,D. *Video Editor: Please emphasize the white dotted square*

4.5. Embolisms also caused disruption of the blood-brain barrier with visible dye leakage from the vessels **[1]**.

4.5.1. LAB MEDIA: Figure 4F,G. *Video Editor: Please emphasize the white dotted square*

Pronunciation Guide:

 microvascular
Pronunciation link: no confirmed link found
IPA: /ˌmaɪkroʊˈvæskjələr/
Phonetic Spelling: MY-kroh-VAS-kyuh-lər
 embolism
Pronunciation link: https://www.merriam-webster.com/dictionary/embolism
IPA: /ˈɛmbəlɪzəm/
Phonetic Spelling: EM-buh-liz-um
 in vivo
Pronunciation link: https://www.merriam-webster.com/dictionary/in%20vivo
IPA: /ɪn ˈviːvoʊ/
Phonetic Spelling: in VEE-voh
 two-photon
- **photon**
  Pronunciation link: https://www.merriam-webster.com/dictionary/photon
  IPA: /ˈfoʊtɑn/
  Phonetic Spelling: FOH-ton
  So **two-photon** would be /tuː ˈfoʊtɑn/ → “too-FOH-ton”

 microscopy
Pronunciation link: https://www.merriam-webster.com/dictionary/microscopy
IPA: /maɪˈkrɑːskəpi/
Phonetic Spelling: my-KRAS-kuh-pee
 fluorescent
Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescent
IPA: /flʊˈrɛsənt/
Phonetic Spelling: floo-RESS-ənt
 polystyrene
Pronunciation link: https://www.merriam-webster.com/dictionary/polystyrene
IPA: /ˌpɒliˈstaɪriːn/ (British) or /ˌpoʊliˈstaɪrin/ (American)
Phonetic Spelling (American): pah-lee-STAIR-een
 microspheres
Pronunciation link: no confirmed link found
IPA: /ˈmaɪkroʊˌsfɪərz/
Phonetic Spelling: MY-kroh-SFEERS
 catheterization
Pronunciation link: https://www.merriam-webster.com/dictionary/catheterization
IPA: /ˌkæθətərəˈzeɪʃən/
Phonetic Spelling: kath-uh-TER-uh-ZAY-shun
 sternohyoid
Pronunciation link: no confirmed link found

IPA: /stərˌnoʊˈhaɪɔɪd/
Phonetic Spelling: ster-noh-HY-oid

omohyoid
Pronunciation link: no confirmed link found
IPA: /ˌoʊmoʊˈhaɪɔɪd/
Phonetic Spelling: oh-moh-HY-oid

ligate / ligation

- **ligate**
  Pronunciation link: https://www.merriam-webster.com/dictionary/ligate
  IPA: /ˈlaɪgeɪt/
  Phonetic Spelling: LY-gayt
- **ligation**
  Pronunciation link: https://www.merriam-webster.com/dictionary/ligation
  IPA: /laɪˈgeɪʃən/
  Phonetic Spelling: ly-GAY-shun

perfusion
Pronunciation link: https://www.merriam-webster.com/dictionary/perfusion
IPA: /pərˈfjuːʒən/
Phonetic Spelling: per-FYOO-zhun

blood-brain barrier

- **barrier**
  Pronunciation link: https://www.merriam-webster.com/dictionary/barrier
  IPA: /ˈbæriər/
  Phonetic Spelling: BAR-ee-ər