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Title: Precision Ultrasound-Guided Stem Cell Delivery for Vascular Repair in Aortic Diseases

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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? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 13
Number of Shots: 32

Introduction

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

1.1. **Baoqi Yu:** Our research focuses on vascular stem cells in blood vessel diseases, investigating how they contribute to disease progression and their potential for therapeutic use in treating vascular conditions.

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

What technologies are currently used to advance research in your field?

1.2. **Qiuyue Gao:** Tissue-specific injection and intravenous infusion are common, but our ultrasound-guided epi membrane injection technique overcomes challenges in aortic wall delivery, advancing vascular stem cell research.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.10*

What advantage does your protocol offer compared to other techniques?

1.3. **Qing Xu:** This technique is minimally invasive, easy to perform, and does not require major surgeries like thoracotomy or laparotomy, reducing complications and recovery time.

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

How will your findings advance research in your field?

1.4. **Qiuyue Gao:** This technique supports localized stem cell therapy, offering a new approach for treating vascular diseases and opening possibilities for targeted regenerative treatments.

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 ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.

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

1.5. **Baoqi Yu:** Publishing with JoVE will enable our research to reach a wider audience, thereby enhancing its impact and promoting more extensive collaboration within the global scientific community.

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. **Baoqi Yu:** After publishing with JoVE, I expect to get more opportunities for collaboration, and our work might be cited more frequently.

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

AUTHORS: Please deliver the testimonials in both Chinese and in English
Videographer: Please capture the testimonials in both Chinese and in English

Ethics Title Card

This research has been approved by the Animal Research Committee at Capital Medical University

Protocol

2. Ultrasound-Guided Injection of Vascular Wall Stem Cells into the Aortic Region

Demonstrator: Qiuyue Gao, Qing Xu

2.1. To begin, add 0.05% trypsin to vascular stem cells 1 hour before injection **[1]**. Use a hemocytometer to count the cells **[2]**. Then centrifuge the cell suspension at 200 *g* for 5 minutes at room temperature before removing the supernatant **[3]**.

2.1.1. WIDE: Talent adding trypsin to a culture flask containing vascular stem cells.

2.1.2. Talent using a hemocytometer under a microscope to count the cells.

2.1.3. Talent placing the tube in a centrifuge.

2.2. Resuspend the cell pellet in Matrigel that has been prethawed at 4 degrees Celsius **[1-TXT]**. Gently mix the mixture by pipetting. **[2]** and place it on ice until injection **[3]**.

2.2.1. Talent pipetting the cold Matrigel into a tube containing the cell pellet. **TXT: Final concentration: 4 x 10^7 cells/mL**

2.2.2. Talent mixing the tube by gentle pipetting.

2.2.3. Talent placing the tube in an ice bucket.

2.3. Next, position an anesthetized mouse dorsally on a heated platform at 37 degrees Celsius to expose the abdomen **[1-TXT]**. Apply conductive gel to each of the four platform electrodes **[2]**. Then use medical tape to secure the mouse's paws to the electrodes for physiological monitoring **[3]**.

2.3.1. Talent positioning the mouse on a heated surgical platform with the abdomen facing upward. **TXT: Anesthesia: 1.5 - 2% isoflurane inhalation**

2.3.2. Talent applying conductive gel to electrodes.

2.3.3. Talent taping each of the mouse's paws onto the platform electrodes

2.4. Using a sterile cotton swab, apply depilatory cream to the intended injection area **[1]**. After a minute, gently wipe the area clean with a new swab **[2]**. Then disinfect the site by applying iodine tincture three times **[3]**.

2.4.1. Talent applying depilatory cream with a cotton swab.

2.4.2. Talent using a clean swab to wipe away the cream.

2.4.3. Talent dabbing the area with iodine tincture three times.

2.5. Drape the surgical site **[1].** Aseptically prepare a sterile syringe and maintain a sterile field throughout the procedure **[2]**.

2.5.1. Talent placing a sterile drape over the mouse.

2.5.2. Talent preparing the syringe using sterile technique.

2.6. For ultrasound-guided injection, first adjust the ultrasound platform to an angle of 30 degrees relative to the floor **[1]**. Have the operator face the animal's right side with the animal's head to the left **[2].** Then apply ultrasound gel to the cleaned and disinfected area **[3]**.

2.6.1. Talent tilting the ultrasound platform.

2.6.2. Talent positioning the animal correctly.

2.6.3. Talent applying ultrasound gel to the mouse's upper chest.

2.7. Position the ultrasound probe on the right side of the sternum **[1]** to visualize the long axis of the aortic arch **[2]**.

2.7.1. Talent placing the probe on the right side of the sternum.

2.7.2. SCREEN: 2.7.2.mp4 00:00-00:12

2.8. Load 25 microliters of the vascular stem cell suspension into a 29-gauge microinjection needle **[1]** and wipe the needle tip with sterile alcohol **[2]**.

2.8.1. Talent loading the suspension into the syringe.

2.8.2. Talent wiping the needle with alcohol.

2.9. Secure the syringe onto the ultrasound-guided injection apparatus **[1]**, ensuring the needle is aligned with the probe and angled 45 degrees relative to the chest wall **[2]**.

2.9.1. Talent mounting the syringe onto the apparatus.

2.9.2. Talent adjusting the angle precisely.

2.10. Now slowly move the injection needle into the ultrasound field of view **[1]**, align it to the focal plane, and match it with the guiding line on the screen **[2]**.

2.10.1. SCREEN: 2.10.1.mp4 00:00-00:16 .

2.10.2. SCREEN: 2.10.2.mp4 00:05-00:25 .

2.11. As the needle nears the skin edge, use blunt forceps to lift the skin and abdominal wall **[1]**, then rapidly insert the needle through the chest wall **[2]**.

2.11.1. Talent lifting the skin with blunt forceps.

2.11.2. SCREEN: 2.11.2.mp4 00:03-00:14 .

2.12. Under ultrasound guidance, guide the needle to the outer membrane between the innominate artery and the aortic arch **[1]**. Gently lift and lower the syringe to confirm the correct position **[2]**.

2.12.1. SCREEN: 2.12.1.mp4 00:20-00:44 .

2.12.2. Talent performing subtle up-down movements of the syringe while checking the ultrasound screen.

2.13. Press the syringe to inject the cell suspension into the designated area **[1]**. Slowly withdraw the needle **[2]** and disinfect the puncture site using standard post-injection protocol **[3]**.

2.13.1. SCREEN: 2.13.1.mp4 00:30-00:51

2.13.2. Talent withdrawing the needle.

2.13.3. Talent dabbing the site with antiseptic.

Results

3. Results

3.1. mCherry *(m-Cherry)* -labeled vascular stem cells remained in the aortic wall 4 weeks after injection **[1]** and migrated from the adventitial layer toward the media and intima of the vessel wall **[2]**.

3.1.1. LAB MEDIA: Figure 3A. *Video editor: Please emphasize the red fluorescent stem cells.*

3.1.2. LAB MEDIA: Figure 3B (right zoom-in). *Video editor: Zoom in on yellow arrows*

3.2. Ultrasound imaging revealed a visible reduction in ascending aorta diameter in stem cell-treated mice 4 weeks post-treatment compared to untreated controls **[1]**.

3.2.1. LAB MEDIA: Figure 4A (top row). *Video editor: Please highlight the images under "4 weeks post-treatment"*

3.3. Quantitative analysis confirmed that the increase in ascending aorta diameter was significantly lower in the treated group compared to untreated mice **[1]**.

3.3.1. LAB MEDIA: Figure 5A. *Video editor: Please highlight the orange bar*

Pronunciation Guide:

- **Trypsin**

Pronunciation link: https://www.merriam-webster.com/dictionary/trypsin
IPA: /ˈtrɪp.sɪn/
Phonetic Spelling: trip-sinpeople.ucas.ac.cn

- **Hemocytometer**

Pronunciation link: https://www.howtopronounce.com/hemocytometer
IPA: /ˌhiː.moʊ.saɪˈtɒm.ɪ.tər/
Phonetic Spelling: hee-moh-sigh-tom-uh-ter

- **Centrifuge**

Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛn.trəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyooj

- **Matrigel**

Pronunciation link: https://www.howtopronounce.com/matrigel
IPA: /ˈmeɪ.trɪ.dʒɛl/
Phonetic Spelling: may-trih-jelenglish.anhuinews.com

- **Isoflurane**

Pronunciation link: https://www.merriam-webster.com/dictionary/isoflurane
IPA: /ˌaɪ.səˈflʊr.eɪn/
Phonetic Spelling: eye-suh-floo-raynPhysical Review LinkAMiner+11people.ucas.ac.cn+11SpringerLink+11

- **Depilatory**

Pronunciation link: https://www.merriam-webster.com/dictionary/depilatory
IPA: /dɪˈpɪl.əˌtɔːr.i/
Phonetic Spelling: dih-pill-uh-tor-ee

- **Sternum**

Pronunciation link: https://www.merriam-webster.com/dictionary/sternum
IPA: /ˈstɜːr.nəm/
Phonetic Spelling: stur-nuhm

- **Innominate**

Pronunciation link: https://www.merriam-webster.com/dictionary/innominate
IPA: /ɪˈnɒm.ɪ.nət/
Phonetic Spelling: ih-nom-uh-nit

- **Adventitial**

Pronunciation link: https://www.howtopronounce.com/adventitial
IPA: /ˌæd.vɛnˈtɪʃ.əl/
Phonetic Spelling: ad-ven-tish-uhlJVSCIT+2SpringerLink+2SpringerLink+2

- **Intima**

Pronunciation link: https://www.merriam-webster.com/dictionary/intima
IPA: /ˈɪn.tɪ.mə/
Phonetic Spelling: in-tih-muh

- **Thoracotomy**

Pronunciation link: https://www.merriam-webster.com/dictionary/thoracotomy
IPA: /ˌθɔːr.əˈkɒt.ə.mi/
Phonetic Spelling: thor-uh-kot-uh-mee

- **Laparotomy**

Pronunciation link: https://www.merriam-webster.com/dictionary/laparotomy
IPA: /ˌlæp.əˈrɒt.ə.mi/
Phonetic Spelling: lap-uh-rot-uh-mee

- **Peristalsis**

Pronunciation link: https://www.merriam-webster.com/dictionary/peristalsis
IPA: /ˌpɛr.ɪˈstæl.sɪs/
Phonetic Spelling: per-ih-stal-sis

- **Hydrocodone**

Pronunciation link: https://www.merriam-webster.com/dictionary/hydrocodone
IPA: /ˌhaɪ.drəˈkoʊ.doʊn/
Phonetic Spelling: hy-droh-koh-doan

- **Pipette**

Pronunciation link: https://www.merriam-webster.com/dictionary/pipette
IPA: /pɪˈpɛt/
Phonetic Spelling: pih-pet

- **Cyclic**

Pronunciation link: https://www.merriam-webster.com/dictionary/cyclic
IPA: /ˈsaɪ.klɪk/
Phonetic Spelling: sigh-klik

- **Epi**

Pronunciation link: https://www.merriam-webster.com/dictionary/epi-
IPA: /ˈɛp.i/
Phonetic Spelling: eh-pee

- **Membrane**

Pronunciation link: https://www.merriam-webster.com/dictionary/membrane
IPA: /ˈmɛm.breɪn/
Phonetic Spelling: mem-brayn

- **Ultrasound**

Pronunciation link: https://www.merriam-webster.com/dictionary/ultrasound
IPA: /ˈʌl.trə.saʊnd/
Phonetic Spelling: uhl-truh-sownd

- **Stem**

Pronunciation link: https://www.merriam-webster.com/dictionary/stem
IPA: /stɛm/
Phonetic Spelling: stem

- **Cell**

Pronunciation link: https://www.merriam-webster.com/dictionary/cell

IPA: /sɛl/
Phonetic Spelling: sel

- **Aortic**

Pronunciation link: https://www.merriam-webster.com/dictionary/aortic
IPA: /eɪˈɔːr.tɪk/
Phonetic Spelling: ay-or-tik

- **Adventitia**

Pronunciation link: https://www.merriam-webster.com/dictionary/adventitia
IPA: /ˌæd.vɛnˈtɪʃ.ə/
Phonetic Spelling: ad-ven-tish-uh

- **Intima**

Pronunciation link: https://www.merriam-webster.com/dictionary/intima
IPA: /ˈɪn.tɪ.mə/
Phonetic Spelling: in-tih-muh

- **Thoracotomy**

Pronunciation link: https://www.merriam-webster.com/dictionary/thoracotomy
IPA: /ˌθɔːr.əˈkɒt.ə.mi/
Phonetic Spelling: thor-uh-kot-uh-mee

- **Laparotomy**

Pronunciation link: https://www.merriam-webster.com/dictionary/laparotomy
IPA: /ˌlæp.əˈrɒt.ə.mi/
Phonetic Spelling: lap-uh-rot-uh-mee

- **Peristalsis**

Pronunciation link: https://www.merriam-webster.com/dictionary/peristalsis
IPA: /ˌpɛr.ɪˈstæl.sɪs/
Phonetic Spelling: per-ih-stal-sis

- **Hydrocodone**

Pronunciation link: https://www.merriam-webster.com/dictionary/hydrocodone
IPA: /ˌhaɪ.drəˈkoʊ.doʊn/
Phonetic Spelling: hy-droh-koh-doan