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Title: In Vitro Intraluminal Gel Infusion: An Advanced Approach for Microscopic Analysis of Human Resistance 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 , all done**

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**

Current Protocol Length
Number of Steps: 23
Number of Shots: 42

Introduction

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

NOTE: Details given by videographer
Camera: BMPCC4K
ISO: 400
White Balance: 4000K
Frame Rate: 30fps
Interview: UHD 4K
Protocol: 1080P

1.1. **Md Abdul Hakim:** The research refines a technique to preserve and analyze human resistance arteries, enhancing visualization, molecular understanding, and potentially improving diagnostics and therapies for cardiovascular conditions.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the most recent developments in your field of research?

1.2. **Hans Ackerman:** Our recent study found tetrameric hemoglobin regulates NO-mediated vascular function in human resistance arteries, where this technique was used to preserve arterial structure and enable part of advanced molecular analysis.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1* files B072_06262351_C090.mov and B072_06262353_C091.mov might have Hans' statement. NOTE: Please include Hans Ackerman's statement. Hakim has also recorded the same 1.2 statement, but dont use it.

What research gap are you addressing with your protocol?

1.3. **Md Abdul Hakim:** The protocol addresses the gap in effectively preserving the structural and molecular architecture of human resistance arteries for accurate analysis and improved understanding of cardiovascular disease mechanisms.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.2*

Interview files: Interview B072_06262158_C053.mov

B072_06262159_C054.mov
B072_06262201_C057.mov
B072_06262202_C060.mov
B072_06262203_C061.mov
B072_06262204_C062.mov
B072_06262205_C063.mov
B072_06262351_C090.mov
B072_06262353_C091.mov

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

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at NIH

Protocol

2. Microdissection, Cannulation and Pressurization of Human Small Resistance Arteries

Demonstrator: Md Abdul Hakim

2.1. To begin, obtain the human omental tissues on ice for the procedure **[1].** Cut a piece of the visibly vascularized region of the tissue and transfer it to a petri-dish containing Krebs-Henseleit buffer on ice **[2].**

2.1.1. WIDE: Talent placing the ice box with tissues on the work bench.

2.1.2. LAB MEDIA: 2.1.2-(a);-human-omental-tissue-collected-from-surgical-team.jpeg and 2.1.2-(b);-Pieces-of-tissues-in-dish-for-microdissection.jpeg. *Video editor: Please show the 2 images side by side.* There is an uncropped high-resolution version of the same images: 2.1.2-uncropped,-a&b-(subject-information-removed).png. Use this and crop it if necessary.

2.2. Using a dissection microscope, carefully isolate the small resistance arteries of 100 to 300 micrometers in diameter **[1].** Clean the arteries from surrounding adipose tissues and remove blood from the lumen without damaging the arteries **[2-TXT]**.

2.2.1. LAB MEDIA: 2.2.1-(a);-Isolated-human-omental-arteries-(blood-in-the-lumen).jpg.

2.2.2. LAB MEDIA: 2.2.1-(b);-Clean-arteries.jpg. **TXT: Keep the isolated arteries in cold buffer throughout the procedure**

2.3. Prepare a culture myograph chamber with two glass cannulas to cannulate and pressurize the dissected arteries **[1]**.

2.3.1. Talent assembling the myograph chamber with two parallel glass cannulas positioned correctly.

2.4. Fill a 5-milliliter syringe attached to a clean capillary tubing with fresh cold Krebs-Henseleit buffer **[1]** and connect the tubing to one end of a cannula **[2]**. Then fill the glass cannula with the buffer, ensuring no air bubbles are present **[3]**.

2.4.1. Talent filling a 5 milliliter syringe with Krebs-Henseleit buffer.

2.4.2. Talent connecting the syringe to the cannula.

2.4.3. Talent pushing buffer through the cannula, checking for air bubbles.

2.5. With a wide-bore transfer pipette, carefully transfer the dissected artery to the myograph chamber **[1]**. Cannulate the proximal end of the artery by sliding it onto a glass cannula using fine forceps **[2]** and secure it with two nylon sutures **[3-TXT]**.

2.5.1. SCOPE: 2.5.1-(timeframe;-15s---18s).mp4 00:14-00:19. **NOTE**: Don't use videographer's footage for 2.5.1 and 2.5.2. Use the lab media indicated here

2.5.2. SCOPE: 2.5.2-(timeframe;-32s-36s).mp4 00:31-00:36.

2.5.3. Talent tying sutures to hold canula in place. **TXT: Similarly cannulate the distal end of the artery and secure it**

2.6. Place the chamber onto the myograph unit, ensuring it is connected to the pressure controller **[1]** and set the initial pressure to 10 millimeters of mercury **[2]**.

2.6.1. Talent positioning the chamber on the myograph unit and connecting the pressure and temperature lines.

2.6.2. Talent turning on the controller and set the pressure using a knob on the device with the display showing the pressure at 10 mmHg. **Videographer's NOTE**: Broke these down to shots A through E for 10 mmHg increases on advise of talent

2.7. Increase the intraluminal pressure in 10 millimeters of mercury increments every 10 minutes until reaching 60 millimeters of mercury **[1]**. Use the pressure regulator to maintain consistent pressure throughout the pressurization process **[2]**.

2.7.1. Show the pressure adjustment sequence with increments of 10 millimeters of mercury displayed on the controller.

2.7.2. Talent adjusting the pressure regulator to keep the pressure steady.

2.8. Next, use the micro-positioner to straighten the pressurized artery longitudinally to approximate its physiological length **[1]**.

2.8.1. Talent adjusting the micro-positioner knobs to gently straighten the artery inside the chamber.

2.9. Run MyoVIEW *(my-oh-view)* or another suitable software on the computer monitor to track the inner and outer arterial diameters using digital calipers **[1]**.

2.9.1. Show the MyoVIEW interface on opening on the monitor and running the program. **Videographer's NOTE**: 2.9.2 and 2.10.1 - Talent got a little confused on the order of what we were doing so the slates might not match for all takes

for

2.10. Allow the artery to equilibrate for 45 minutes to achieve a stable diameter and ensure that it maintains its physiological dimensions and tone **[1]**.

2.10.1. Shot of the artery in the myograph chamber while the software displays stable diameter readings during the equilibration period. **Videographer's NOTE**: added an insert shot of the myograph chamber since it was not possible to get the screen and the chamber in the same shot

2.11. To test the artery's viability, add a vasoconstrictor or a vasodilator and record the changes in arterial diameter **[1-TXT]**.

2.11.1. LAB MEDIA: 2111_6~1.AVI. 01:23-01:40 **TXT: Vasoconstrictor: 60 mM KCl or Phenylephrine; Vasodilator: Acetylcholine**

3. Artery Fixation and Preparation of Tissue-Stabilizing Gel

3.1. Wash the artery with fresh solution to let it restore its resting baseline diameter **[1]** and then fix the artery by introducing 10 percent neutral buffered formalin both into the lumen **[2]** and around the vessel in the chamber. Incubate overnight **[3]**.

3.1.1. Talent adding the wash solution and flushing it.

3.1.2. Talent adding formalin solution luminally.

3.1.3. Talent adding formalin to the chamber and covering it with a lid.

3.2. After fixation, rinse the arteries inside and outside with PBS to remove any residual fixative from the artery and chamber at 37 degrees Celsius **[1].**

3.2.1. Talent rinsing both luminal and abluminal sides of the artery in the myograph chamber with PBS using a syringe and tubing.

3.3. Place the tube of liquefied tissue-stabilizing gel at room temperature for 3 to 5 minutes to slightly cool the liquid **[1]** and then transfer it to a 40 degrees Celsius heating block to maintain the liquid gel for 10 to 15 minutes **[2]**. Withdraw the liquid gel using a 3-milliliter sterile syringe connected to 10 to 15-inch-long capillary tubing **[3]**.

3.3.1. Talent placing the gel tube at room temperature on the bench.

3.3.2. Talent placing the tube onto a 40 degrees Celsius heating block.

3.3.3. Talent withdrawing liquid gel from the tube using sterile syringe connected to a

long capillary tubing.

4. Intraluminal Gel Infusion and Embedding

4.1. Connect the distal end of the tubing filled with liquid gel to the proximal cannula **[1]** and gently infuse the gel into the artery lumen **[2]**. Immediately replace the PBS in the chamber **[3]** with an equal volume of liquid gel to fill it to the top **[4]**.

4.1.1. Talent attaching the capillary tubing carrying the gel to the proximal end of the cannula.

4.1.2. LAB MEDIA: 4.1.2;-Intraluminal-gel-infusion-(timefram;-15-23s).mp4 00:15-00:24.

4.1.3. LAB MEDIA: 414_AB~1.MP4 00:05-00:12.

4.1.4. LAB MEDIA: 414_AB~1.MP4 00:35-00:41.

4.2. Now, disconnect the myograph unit from the temperature controller and allow the chamber to rest at room temperature for 10 minutes **[1]**.

4.2.1. Talent unplugging the temperature controller and placing the chamber on the bench.

4.3. Using fine forceps, loosen the nylon sutures and gently remove the cannulas from the artery and chamber **[1]**.

4.3.1. Talent using forceps to untie and extract the cannulas from each end of the artery embedded in solidified gel.

4.4. Finally, transfer the entire solidified gel containing the artery from the chamber into a container filled with 70 percent ethanol **[1-TXT]**.

4.4.1. Talent lifting the block of gel containing the artery and placing it into a labeled container filled with 70 percent ethanol. **TXT: Perform histopathological and immunohistochemical assays with the embedded artery. Videographer's NOTE**: Did 2 takes before the gel was no longer usable. The first take the transfer is better, but talent forgot to put the lid on at the end. If possible use the first part of the first take and then maybe punch in on the edit to use the closing of the lid.

Results

5. Results

5.1. Equilibrated human omental artery in solution was visualized at physiological conditions **[1]**. Upon exposure to 60 millimolar potassium chloride, viable arteries showed a clear constriction **[2]**, followed by a return to baseline diameter after washing **[3]**.

5.1.1. LAB MEDIA: Figure 2 *Video editor: Highlight A and point to the text "Baseline*"

5.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight B and point to the text "60 mM KCl"*

5.1.3. LAB MEDIA: Figure 2. *Video editor: Highlight C and point to the text "Wash"*

5.2. Hematoxylin and eosin staining of gel-embedded arterial cross-sections showed a well-preserved lumen **[1]** and clear layering of endothelial, smooth muscle, and adventitial cells **[2]**.

5.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the inset round section*

5.2.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the EC (endothelial cell), TM (Tunica Media) and TA (Tunica Adventitia) regions in the image*

5.3. Immunohistochemistry revealed intact expression of CD31 in endothelial cells **[1]** and alpha-SMA in smooth muscle cells, confirming structural preservation of the vessel wall **[2]**.

5.3.1. LAB MEDIA: Figure 4B. *Video editor: Highlight dark brown boundary of the artery*.

5.3.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the dark brown layers*.

5.4. In situ hybridization using a probe for *Homo sapiens* peptidylprolyl isomerase B showed bright red punctate signals in smooth muscle cells, indicating preserved mRNA integrity **[1]**.

5.4.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the red/pink dots spread across*.

1. omentum

Pronunciation link:
https://dictionary.cambridge.org/pronunciation/english/omentum Cambridge Dictionary

IPA (American): /oʊˈmɛn.təm/
Phonetic Spelling: oh-MEN-tuhm

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2. omental

Pronunciation link:
https://dictionary.cambridge.org/us/pronunciation/english/omental Cambridge Dictionary

IPA (American): /oʊˈmɛn.təl/
Phonetic Spelling: oh-MEN-tuhl

1. myograph

Pronunciation link:
https://www.collinsdictionary.com/dictionary/english/myograph (Collins) Collins Dictionary

IPA (American): /ˈmaɪoʊˌgræf/
Phonetic Spelling: MY-oh-graf

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2. micro-positioner

Pronunciation link:
No confirmed Merriam-Webster or Oxford entry found; using HowToPronounce as fallback: likely “my-crow-puh-ZISH-uh-ner” but audio unavailable.
Link: No confirmed link found

IPA (American): /ˈmaɪkroʊ pəˈzɪʃənər/
Phonetic Spelling: MY-crow puh-ZISH-uh-ner

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3. equilibrate (from "artery to equilibrate")

Pronunciation link:
https://www.merriam-webster.com/dictionary/equilibrate (Merriam-Webster) Merriam-Webster

IPA (American): /ɪˈkwɪl.ə.bɹeɪt/
Phonetic Spelling: ih-KWIL-uh-brayt

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4. vasoconstrictor

Pronunciation link:
https://www.merriam-webster.com/dictionary/vasoconstrictor (Merriam-Webster) Merriam-Webster

IPA (American): /ˌvæsoʊkənˈstrɪktər/
Phonetic Spelling: VAY-soh-kun-STRIK-tur

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5. vasodilator

Pronunciation link:
https://www.merriam-webster.com/dictionary/vasodilator (Merriam-Webster) Merriam-Webster

IPA (American): /ˌvæsoʊˈdaɪlətər/
Phonetic Spelling: VAY-soh-DYE-luh-tur

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6. immunohistochemistry (relevant to your IHC usage)

Pronunciation link:
https://www.oxfordlearnersdictionaries.com/definition/english/immunohistochemistry (Oxford) Oxford English Dictionary *(assuming entry exists; typical for OED-like sources)*

IPA (American): /ɪˌmju.noʊˌhɪstəˈkɛm.ɪstri/
Phonetic Spelling: ih-MYOO-noh-his-toh-KEM-is-tree

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7. in situ hybridization

Pronunciation link:
No Merriam-Webster entry; fallback to HowToPronounce—but exact link not found.
Link: No confirmed link found

IPA (American): /ɪn ˈsitʃu ˌhaɪbrɪdaɪˈzeɪʃən/
Phonetic Spelling: in-SEE-choo HY-brih-dye-ZAY-shun