FINAL SCRIPT: APPROVED FOR FILMING

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Title: Multilevel Microdissection and Functional-Structural Profiling of Human Renal Arterial Branches

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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.

   LEICA S9D

   2.3.1, 2.4.1, 3.1.1, 3.2.1, 3.2.2, 3.3.1, 3.3.2, 3.3.4, 3.4.1, 3.4.2.

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes.**

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

Current Protocol Length
Number of Steps: 11
Number of Shots: 26

Introduction

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

1.1. **Xuya Kang:** Our research aims to establish standardized methods to isolate and functionally assess human renal arterial branches, uncovering mechanisms of vascular dysfunction and guiding targeted therapies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 1*

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

1.2. **Yahan Liu:** Previous research relied on animal models or indirect imaging, while our wire myography directly measures human renal artery function in vitro.

1.2.1. 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?

1.3. **Xuya Kang:** Our method enables precise, human-specific vascular analysis, overcoming species limitations and improving translational drug development.

1.3.1. 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 Urology Department of Peking University First Hospital and conducted following the Helsinki Declaration

Protocol

2. Renal Artery Isolation for Functional Assessment

Demonstrator: Xuya Kang

2.1. To begin, ensure the kidney tissue remains fully submerged in liquid during transport to maintain tissue viability and structural integrity **[1-TXT]**.

2.1.1. Establishing shot of the talent with kidney placed in a transport container filled with liquid. **TXT: Kidney tissue was obtained from renal carcinoma patients**

2.2. Visually identify the coronal plane by locating the renal hilum and aligning the cut to pass through both the renal pelvis and the lateral convex border **[1]**. Using a sterile scalpel, bisect the kidney along this coronal plane to create two symmetrical halves **[2]** and expose internal structures such as the renal pyramids and columns **[3]**.

2.2.1. Talent pointing to the renal hilum and aligning the coronal plane.

2.2.2. Talent using a sterile scalpel to cut the kidney along the identified coronal plane.

2.2.3. Talent exposes the internal structures such as the renal pyramids and columns.

2.3. Under a stereomicroscope, use micro-dissection scissors and forceps to meticulously separate the interlobar, arcuate, and interlobular arteries in a 10-centimeter black-bottomed culture dish **[1]**.

2.3.1. SCOPE: SCOPE2-3-1.mp4: 00:13-00:16, 01:53-01:57, 02:40-02:52, 03:17-03:19

2.4. Then, gently remove any surrounding tissue and fat from the dissected arteries in the same 10-centimeter black-bottomed culture dish to clean them thoroughly **[1]**.

2.4.1. SCOPE: SCOPE2-4-1.mp4: 00:29-00:42

3. Arterial Ring Mounting

3.1. Using micro-dissection scissors, section the cleaned arteries into rings approximately 2 millimeters in length for vascular function studies **[1]**.

3.1.1. SCOPE: SCOPE3-1-1 00:12-00:26

3.2. Carefully insert the first guide wire into an arterial ring in the dish **[1]**. Bend one side of the guide wire at a 90-degree angle **[2]** and transfer the arterial ring with the wire into the chamber **[3]**.

3.2.1. SCOPE: SCOPE3-2-1.mp4: 00:13-00:20

3.2.2. SCOPE: SCOPE3-2-2.mp4: 00:03-00:06.

3.2.3. SCOPE: SCOPE3-2-3.mp4: 00:39-00:4, 00:56-01:01

3.3. Before fixing the arterial ring on the sample holder, record the vessel length. Place the ring between the two holders and read the micrometer scale, where 1 scale division equals 10 micrometers **[1]**. Subtract the initial scale value measured when the holders just touch each other to calculate the arterial ring's length and width **[2]**. Then, fix the arterial ring onto the clamp-type sample holder using the instrument-provided screws, tightening them in a clockwise direction **[3]. ** NOTE: The VO adjusted for the deleted shot

~~3.3.1. SCOPE: Talent noting the arterial ring's initial placement and preparing to measure.~~ NOTE: Not filmed

3.3.2. SCOPE: SCOPE3-3-1.mp4: 00:11-00:40

3.3.3. Talent subtracting the initial value to determine vessel dimensions.

3.3.4. SCOPE: SCOPE3-3-4.mp4: 00:10-00:15, 00:20-00:24, 00:39-00:43, 01:02-01:04

3.4. Then, thread a second guide wire through the arterial ring **[1]**. Wind the wire clockwise around the fixing screws on both ends, securing it tightly to the surface of the sample holder **[2]**.

3.4.1. SCOPE: SCOPE3-4-1.mp4: 00:11-00:25

3.4.2. SCOPE: SCOPE3-4-2.mp4: 00:20-00:23, 00:53-00:56, 01:10-01:16

4. Vessel-Specific Normalization for Optimal Initial Tension Determination

4.1. Select Normalization Settings from the **DMT** *(D-M-T)* menu and set the Eyepiece calibration as 1 millimeter per division, target pressure as 13.3 kilopascal, IC1/IC100 *(I-C-One-By-I-C-Hundred)* as 0.9, online averaging time as 3 seconds, and delay time as 60 seconds **[1]**.

4.1.1. SCREEN: SCREEN4.1.1.mp4

4.2. Select the channel corresponding to the target artery and open the Normalization screen from the **DMT** menu **[1]**. In the appropriate fields, enter the tissue endpoints as **a1** equals 0 and **a2** equals the measured vessel length in millimeters **[2]**. Input the wire diameter as 40 micrometers and enter the micrometer reading from the scale **[3]**. Click **Add point** to save the data **[4]**.

4.2.1. SCREEN: SCREEN4.2.1&4.2.2.mp4: 00:00-00:05

4.2.2. SCREEN: SCREEN4.2.1&4.2.2.mp4: 00:07-00:17

4.2.3. SCREEN: SCREEN4.2.3-2&4.2.4.mp4: 00:01-00:06,

4.2.4. SCREEN: SCREEN4.2.3-2&4.2.4.mp4: 00:09-00:11, 01:10-01:13

4.3. Now apply passive stretch and wait for 3 minutes **[1]**. Enter the new micrometer reading as the next point and click **Add point [2]**. Add 5 milliliters of 60 potassium ion solution to the chamber to induce a potassium-mediated contraction of the vessel **[3]**. To wash out the 60-potassium ion solution, add 5 milliliters of Krebs solution three times **[4]**. Finally, add 5 microliters of phenylephrine stock solution to the chamber containing 5 milliliters of Krebs buffer **[5]**.

4.3.1. Talent initiating passive stretch and setting a timer for 3 minutes.

4.3.2. SCREEN: SCREEN4.3.2.mkv

4.3.3. Talent pipetting 5 milliliters of 60K+ solution into the chamber.

4.3.4. Talent washing out the chamber by pipetting and discarding 5 milliliters of Krebs solution three times.

4.3.5. Talent adding 5 microliters of phenylephrine stock into the Krebs buffer-filled chamber.

Results

5. Results

5.1. The interlobar artery was successfully dissected from the renal medulla, showing a thick vascular wall and surrounding adipose tissue that required careful removal to preserve structural integrity **[1]**. The arcuate artery was isolated at the corticomedullary junction, displaying a thinner vascular wall and an arched trajectory **[2]**.

5.1.1. LAB MEDIA: Figure 1C. *Video editor: Highlight the thick white artery running vertically, labelled as the interlobar artery*

5.1.2. LAB MEDIA: Figure 1C**.** *Video editor: Highlight the arch-shaped vessel branching horizontally, labelled as the arcuate artery*

5.2. The interlobular artery was identified running linearly through the renal cortex with a very thin wall and tightly integrated with cortical tissue **[1]**.

5.2.1. LAB MEDIA: Figure 1C**.** *Video editor: Highlight the thin vertical artery labelled as the interlobular artery*

5.3. Histological analysis revealed that the interlobar artery had the thickest vascular wall with a distinct adventitia **[1],** while arcuate and interlobular arteries exhibited progressively thinner walls and fewer smooth muscle layers **[2]**.

5.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the largest pink-stained circular structure labeled "Interlobar artery" at the left.*

5.3.2. LAB MEDIA: Figure 2. *Video editor: Highlight the images labelled as "Arcuate artery" and "Interlobular artery".*

5.4. Arterial normalization involved repeated mechanical stretching and potassium-induced stimulation, establishing stable baseline tension conditions across samples **[1]**.

5.4.1. LAB MEDIA: Figure 5.

5.5. Cumulative addition of phenylephrine from 10^{-9} to 10^{-4} molar concentrations induced progressively stronger contractions in arterial rings in a dose-dependent manner **[1]**. In phenylephrine-precontracted arteries, increasing concentrations of acetylcholine from 10^{-8} to 3×10^{-5} molar produced concentration-dependent vasodilation **[2]**.

5.5.1. LAB MEDIA: Figure 6. *Video editor: Trace the rising trend of the line graph as each concentration (from left to right) is added.*

5.5.2. LAB MEDIA: Figure 7. *Video editor: Highlight the declining slope of the graph as concentration of acetylcholine increases, beginning at the leftmost arrow*.

1. Identified Terms & Pronunciation Links

Hilum

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/hilum
- **IPA (American):** /ˈhɪləm/
- **Phonetic Spelling:** HIL-əm

Coronal

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/coronal
- **IPA (American):** /kəˈroʊnəl/
- **Phonetic Spelling:** kuh-ROH-nəl

Renal

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/renal
- **IPA (American):** /ˈriːnəl/
- **Phonetic Spelling:** REE-nəl

Pyramids (in the context of "renal pyramids")

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/pyramid
- **IPA (American):** /ˈpɪrəmɪd/
- **Phonetic Spelling:** PEER-ə-mid

Interlobar

- **Pronunciation link:** No confirmed link found
- **IPA (American):** /ˌɪntərˈloʊbɑːr/
- **Phonetic Spelling:** in-tuhr-LOH-bar

Arcuate (arteries)

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/arcuate
- **IPA (American):** /ˈɑːrkjuˌeɪt/

- **Phonetic Spelling:** AR-kyoo-ayt

Interlobular

- **Pronunciation link:**
  No confirmed link found
- **IPA (American):** /ˌɪntərloʊˈbjuːlər/
- **Phonetic Spelling:** in-tur-loh-BYOO-lər

Stereomicroscope

- **Pronunciation link:**
  https://www.merriam-webster.com/dictionary/stereomicroscope
- **IPA (American):** /ˌstɛri.oʊˈmaɪkrəˌskoʊp/
- **Phonetic Spelling:** STEER-ee-oh-MY-kruh-skohp

Phenylephrine

- **Pronunciation link:**
  https://www.merriam-webster.com/dictionary/phenylephrine
- **IPA (American):** /ˌfiːniˈlɛfrɪn/
- **Phonetic Spelling:** fee-nee-LEF-rin