Submission ID #: 61566
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18777678

Title: Dynamic Measurement and Imaging of Capillaries, Arterioles, and Pericytes in Mouse Heart

Authors and Affiliations: Guiling Zhao[1], Humberto C. Joca[1], and W. Jonathan Lederer[1]

[1]Laboratory of Molecular Cardiology, Center for Biomedical Engineering and Technology and Department of Physiology, University of Maryland School of Medicine

Corresponding Author:
Guiling Zhao
gzhao@som.umaryland.edu

Co-authors:
jlederer@som.umaryland.edu
hjoca@som.umaryland.edu

Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y**, **Dissecting microscopes: Olympus, Japan, SZX12 and Nikon**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **51**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Guiling Zhao**: This method can be used to study coronary microcirculation in living murine heart tissue and vascular tree components by ex vivo monitoring of the arterial perfusion pressure and flow **[1]**.

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

REQUIRED:

1.2. **Humberto C. Joca**: This method provides a useful platform for studying the function of pericytes and reagents on microcirculation within the heart by simultaneously measuring the vascular diameter and arterial luminal pressure **[1]**.

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

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at University of Maryland School of Medicine.

Protocol

2. Cannula Preparation

2.1. To prepare a cannula for the experiment, use a micropipette puller to produce two cannulae with long, thin tips from a single clean, borosilicate glass tube **[2]**.

2.1.1. WIDE: Talent pulling pipette **TEXT: 047" X .040" 6-8" Length**

2.2. Use a fine pair of scissors and a dissecting microscope to cut the tip of each cannula to a final tip diameter of 100-150 micrometers **[1]** and fire polish the tips to slightly round the sharp edges **[2]**.

2.2.1. SCOPE: Cannula tip being cut *Videographer: Important step*
2.2.2. Tip being polished *Videographer: Important step*

2.3. Use a platinum wire positioned on the side of the shaft approximately 2 millimeters from the tip to bend the cannula along the shaft to an about 45-degree angle **[1]** and insert the open end of the cannula into the cannula holder **[2]**.

2.3.1. Shaft being bent with wire *Videographer: Important step*
2.3.2. Cannula being inserted into cannula holder

2.4. Then tighten the fitting **[1]** and use a 5-milliliter syringe to flush the cannula and tubing with Tyrode's solution. Mount the cannula holder onto micromanipulation of a pressure myograph chamber **[2]**. NOTE: The added VO text was originally numbered 2.5 but there seem to be no shots associated with it, so I moved it to this step.

2.4.1. Fitting being tightened
2.4.2. Talent flushing cannula and tubing, with Tyrode's solution container visible in frame

3. Mouse Heart Extraction

3.1. To collect a mouse heart for the experiment, first intraperitoneally inject 500 microliters of heparin into an anesthetized, 8 to 16-week-old C57BL-6 mouse **[1-TXT]**.

3.1.1. WIDE: Talent injecting mouse *Videographer: More Talent than mouse in shot* **TEXT: Heparin**

3.2. After 10 minutes, place the mouse onto a heated bed in the supine position **[1]** and stabilize the paws with labeling tape **[2]**.

3.2.1. Talent mouse onto bed NOTE: 3.2.1 and 3.2.2 in one shot *Videographer: More Talent than mouse in shot*
3.2.2. Paw being taped

3.3. Use forceps and surgical scissors to make an incision in the abdominal skin above the diaphragm **[1]** and cut the diaphragm and the sternum **[2]** to allow dissection of the heart from the thoracic cavity **[3-TXT]**.

3.3.1. Incision being made NOTE: 3.3.1 - 3.4.1 in one shot
3.3.2. Diaphragm and/or sternum being cut
3.3.3. Heart being dissected **TEXT: Cut as close as possible to dorsal thoracic wall**

3.4. Place the heart into ice-cold Tyrode's solution containing 300-millimolar BDM (B-D-M) **[1-TXT]** and remove any connective tissues from the heart as necessary **[2]**.

3.4.1. Talent placing heart into solution, with BDM container visible in frame **TEXT: BDM: 2,3-butanedione monoxime**
3.4.2. Tissue being removed

3.5. Then place the heart into a pre-chilled dissecting chamber filled with fresh Tyrode's solution supplemented with 30-millimolar BDM **[2]**.

3.5.1. Talent placing heart into chamber, with solution container visible in frame

4. Septal Artery Preparation and Cannulation

4.1. To prepare the septal artery for cannulation, turn on the servo pump **[1]** and set the servo controller pressure to "flow" mode **[2]**.

4.1.1. WIDE: Talent turning on pump NOTE: 4.1.1 and 4.1.2 in one shot
4.1.2. Talent setting pressure to flow

4.2. Pin the heart onto the PDMS, taking care to avoid damage to the middle area of the tissue **[1-TXT]**, and place the tissue under a dissecting microscope **[2]**.

4.2.1. Heart being pinned **TEXT: PDMS: polydimethylsiloxane** NOTE: 4.2.1 and 4.2.2 *maybe* in one shot
4.2.2. Microscope arm being placed above dish

4.3. Remove both the right and left atria **[1]** and cut open the right ventricle **[2]**.

4.3.1. SCOPE: At least one atrium being removed NOTE: Scope shots are inverted
4.3.2. SCOPE: Ventricle being opened

4.4. Remove the right ventricular free wall **[1]** and expose the septal artery **[2]**.

4.4.1. SCOPE: Wall being removed *Videographer: Important step*
4.4.2. SCOPE: Artery being exposed *Videographer: Important step*

4.5. Use a 30-micrometer-diameter nylon thread to tie a loose knot around the septal artery **[1]**.

4.5.1. SCOPE: Thread being placed/knot being tied *Videographer: Important step*

4.6. Use fine scissors to remove the left ventricular free wall **[1]** and transfer the papillary muscle preparation to the experimental chamber **[2]**.

4.6.1. SCOPE: Wall being removed *Videographer: Important step*
4.6.2. Talent placing tissue into experimental chamber under microscope

4.7. Use a micromanipulator to insert a cannula into the septal artery **[1]** and secure the cannula with the suture **[2]**.

4.7.1. SCOPE: Artery being cannulated *Videographer: Important step*
4.7.2. SCOPE: Suture being tightened *Videographer: Important/difficult step*

4.8. Then use pins to secure the papillary muscle to the chamber floor with the tip of the cannula parallel to the arterial wall so that a clear view of the vasculature can be obtained **[1]** and use the syringe to gradually flush the solution through the cannula **[2]**.

4.8.1. SCOPE: Muscle being pinned to chamber *Videographer: Important/difficult step*
4.8.2. SCOPE: Solution being expelled *Videographer: Important step*

5. Preparation Stabilization

5.1. To stabilize the tissue preparation, turn on a peristaltic pump **[1]** and continuously superfuse the preparation with pre-gassed physiological saline solution at a 3-4 milliliters/minute flow rate **[2]**.

5.1.1. WIDE: Talent turning on pump
5.1.2. Solution being perfused

5.2. Turn on the temperature controller for the superfusion solution **[1]** and adjust the

temperature of the bath superfusate to 35-37 degrees Celsius **[2]**.

5.2.1. Talent turning on controller
5.2.2. Talent measuring bath temperature

5.3. Connect the cannula to the servo pump **[1]** and adjust the flow to set the pressure to approximately 10 millimeters of mercury **[2-TXT]**.

5.3.1. Talent connecting cannula to pump
5.3.2. Talent adjusting flow **TEXT: Monitor flow and pressure throughout experiment**

5.4. Let the superfusion run for about 10 minutes **[1]**.

5.4.1. Talent setting timer

5.5. Then increase the flow of the luminal solution to set the artery pressure to approximately 60 millimeters of mercury **[1]** and allow the sample to stabilize for 30-60 minutes with monitoring **[2]**.

5.5.1. Talent increasing flow
5.5.2. Talent setting timer, with setup visible in frame

6. Fluorescence-Tagged Wheat-Germ Agglutinin (WGA) Loading and Confocal Arteriole and Capillary Imaging

6.1. To load the sample with wheat germ agglutinin, perfuse the preparation with 5 milliliters of Tyrode's solution supplemented with 100 micrograms of Alexa Fluor 488 (four-eighty-eight)-conjugated wheat germ agglutinin **[1]**.

6.1.1. WIDE: Talent switching perfusate, with WGA container visible in frame

6.2. After 30 minutes, change the perfusate back to normal Tyrode's solution **[1]** and turn on the confocal microscope system **[2]**.

6.2.1. Talent changing perfusate to Tyrode's solution, with solution container visible in frame
6.2.2. Talent turning on system

6.3. Use the microscope at a low power of magnification to locate the septal artery in transmitted light mode **[1-TXT]** and begin imaging with the spinning disk confocal **[2]**.

6.3.1. Talent at microscope, locating artery **TEXT: Follow cannula position to locate septal artery** NOTE: Scope shot also available

6.3.2. Talent opening software imaging, with monitor visible in frame

6.4. Select a 40x objective magnification and the 488-nanometer excitation laser **[1]** and adjust the laser intensity and the sampling rate **[3]**.

6.4.1. Talent selecting objective
6.4.2. SCREEN: video 2: 00:00-00:08

6.5. To define the imaging range, set the top and bottom imaging positions for the microscope and set the step size and z-stack imaging parameters **[1]**.

6.5.1. SCREEN: video 3: 00:04-00:24 *Video Editor: please speed up*

6.6. Then select a folder for storing the image and click **Run** to begin recording the images **[1]**.

6.6.1. SCREEN: video 3: 00:25-00:44 *Video Editor: please speed up*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

2.2., 2.3., 4.4.-4.8.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
4.7.2., 4.8.1

Results

7. Results: Representative Capillary and Arteriole Imaging and Luminal Pressure and Atrial Diameter Assessment

7.1. When a fluorescence vascular marker is perfused into the vascular lumen **[1]**, it is possible to visualize whole vascular trees using high-speed confocal microscopy **[2]**.

7.1.1. LAB MEDIA: Figure 5 left image
7.1.2. LAB MEDIA: Figure 5 left image *Video Editor: please emphasize vessels in image*

7.2. Further magnification enables imaging of the capillary in detail **[1]**.

7.2.1. LAB MEDIA: Figure 5 left image *Video Editor: please emphasize/zoom into dashed box and show Figure 5 right image as magnification of image in dashed box*

7.3. When pinacidil, an ATP-sensitive potassium channel agonist, is served from the lumen, the diameter of the arterioles increases **[1]**.

7.3.1. LAB MEDIA: Video 1

7.4. When the vasoconstrictor endothelin-1 is applied from the lumen **[1]**, the diameter of the arteriole decreases **[2]**. When the flow set is constant, the luminal pressure increases **[3]**.

7.4.1. LAB MEDIA: Figure 6 *Video Editor: please emphasize ET-1 image in Figure 6A*
7.4.2. LAB MEDIA: Figure 6 *Video Editor: please emphasize orange data line in Figure 6B*
7.4.3. LAB MEDIA: Figure 6 *Video Editor: please emphasize black data line in Figure 6C*

7.5. In these images of papillary tissue from an NG2-DsRed (N-G-two-D-S-red)-expressing transgenic mouse **[1]**, both the capillary **[2]** and pericytes can be visualized within the papillary muscle tissue **[3]** under conditions that better mimic the physiology of live animals **[4]**.

7.5.1. LAB MEDIA: Figure 7
7.5.2. LAB MEDIA: Figure 7 *Video Editor: please emphasize green signal in right Merge image*

7.5.3. LAB MEDIA: Figure 7 *Video Editor: please emphasize red signal in right Merge image*

7.5.4. LAB MEDIA: Figure 7

Conclusion

8. Conclusion Interview Statements

8.1. **Humberto C. Joca**: It is important that no bubbles are present within the tubing at any point during the procedure **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B-roll: 2.5*

8.2. **Guiling Zhao**: Combining this method with optic imaging techniques, the preparation can be used to study microcirculation in the heart and intercellular communications between different cell types using fluorescence-tagged transgenic mice **[1]**.

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