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Title: Modifications of the Langendorff Method for Simultaneous Isolation of Atrial and Ventricular Myocytes from Adult Mice

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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?
   No, our stereomicroscope is ZEISS stemi 305 equipped with a Axiocam 105 color camera, which can only record images but not movies.

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.
   ZEISS stemi 305

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

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group?

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

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

Current Protocol Length

Number of Steps: 17
Number of Shots: 48

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Wu Kui:** This protocol can help improve the atrial yield from a perfused mouse heart.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.1.2*

1.2. **Wu Kui:** The main advantage of this method is that the atrial and ventricular myocytes can be isolated simultaneously under the same digestion condition.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 4.1.2*

OPTIONAL:

1.3. **Wu Kui:** This method could provide insight into the mechanism of heart diseases at the cellular and subcellular level.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Animal Care and Use Committee at Capital Medical University.

Protocol

NOTE: Videographer note: The author insisted to film later some footage with her phone as the heart tissue was not perfect and has also skipped some "unimportant" steps.

2. Heart Excision and Aortic Cannulation

2.1. After euthanizing an 8 to 10-week-old male C57BL/6 (*C-fifty-seven-black-six)* mouse **[1-TXT]**, use tissue forceps to lift the skin of the xiphoid **[2]**, then using tissue scissors, make a minor lateral incision through the skin **[3]**.

2.1.1. WIDE: Talent in front of the surgical platform, euthanized mouse in view. **TEXT: See text for full mouse preparation details**

2.1.2. Talent lifting the skin of the xiphoid.

2.1.3. Talent making an incision through the skin.

2.2. Perform a blunt dissection between the skin and fascia **[1]**. Extend the skin incision toward the axillae in a V-shape on both sides **[2]** and continue the incision through the rib cage **[3]**. Then, using tissue forceps, clamp the sternum and deflect the rib cage upward to fully expose the heart and lungs **[4]**.

2.2.1. Talent performing a blunt dissection.

2.2.2. Talent extending the incision toward the axillae.

2.2.3. Talent continuing the incision through the rib cage.

2.2.4. Talent clamping the sternum and deflecting the rib cage upward.

2.3. Using curved forceps, peel off the pericardium **[1]**. If the thymus gland covers the great vessels, use two curved forceps to tear the thymus gland toward both sides **[2]**, then gently pull the base of the heart toward the tail **[3]** until the aorta and its branch arteries are visible as a “Y”-shaped blood vessel **[4]**.

2.3.1. Talent peeling off the pericardium.

2.3.2. Talent tearing the thymus gland.

2.3.3. Talent pulling the heart toward the tail.

2.3.4. ECU: Shot of “Y”-shaped blood vessel (aorta and its branch arteries).

2.4. Transect the aorta at the left common carotid artery **[1]**, then cut the brachiocephalic artery **[2]**. Excise the heart **[3]** and immediately immerse it in a Petri dish containing Tyrode’s solution to wash and pump out the residual blood **[4]**. Then, transfer the

heart to another Petri dish containing solution 1 **[5]**. *Videographer: This step is important!*

2.4.1. Talent transecting the aorta at the left common carotid artery.

2.4.2. Talent cutting the brachiocephalic artery.

2.4.3. Talent excising the heart.

2.4.4. Talent immersing the heart in a Petri dish. **TEXT: See text for all solution preparation details**

2.4.5. Talent transferring the heart to a Petri dish containing solution 1.

2.5. Under a stereomicroscope **[1]**, using fine iris scissors, trim any surplus tissue **[2]**.

2.5.1. Talent placing the Petri dish under a stereomicroscope.

2.5.2. SCOPE: Trimming of surplus tissue

2.6. Expel air bubbles from the syringe **[1]**, then with the assistance of two straight tying forceps, perform retrograde aortic cannulation, taking care that the whole cannulation process is performed under the liquid surface **[2]**.

2.6.1. Talent expelling air from the syringe.

2.6.2. SCOPE: Retrograde aortic cannulation being performed.

2.7. Adjust the cannulation depth such that the cannula tip is in the ascending aorta, taking care not to penetrate the aortic valves **[1]**, then with a pre-knot 3-0 *(three-oh)* suture, ligate the aorta to the cannula notch **[2]**. *Videographer: This step is important!*

2.7.1. SCOPE: Adjusting the cannulation depth.

2.7.2. SCOPE**:** Ligation of the aorta to the cannula notch.

2.8. Gently inject solution 1 from the syringe **[1]** to flush out the residual blood **[2]**. Then, connect the cannulated heart to the Langendorff apparatus, taking care not to introduce any air bubbles into the heart **[3]**.

2.8.1. Talent injecting solution 1 from the syringe.

2.8.2. SCOPE: Shot of residual blood flushing out/ heart and the atrial appendages expanding and becoming pale.

2.8.3. Talent connecting the cannulated heart to the Langendorff apparatus.

3. Heart Perfusion

3.1. After connecting the cannulated heart to the Langendorff apparatus, perfuse the heart with solution 1 **[1]** for approximately two minutes **[2]**.

3.1.1. WIDE: Talent switching on the peristaltic pump to perfuse the heart with solution 1.

3.1.2. ECU: Shot of heart being perfused.

3.2. Using a single-use sterile polyethylene pipet, draw up about 2.5 milliliters of solution 3 **[1]** and prewarm it in the water bath for later use **[2]**. Then, to digest the tissues, perfuse the heart with the remaining solution 3 **[3]** for approximately 11 to 12 minutes **[4]**.

3.2.1. Talent drawing up 2.5 mL of solution 3 in a polyethylene pipet.

3.2.2. Talent warming the solution 3 in a water bath.

3.2.3. Talent switching to solution 3 for perfusion.

3.2.4. ECU: Shot of heart being perfused.

3.3. After the first 2 minutes of perfusion with solution 3, recycle the perfused solution to the perfusate reservoirs by the peristaltic pump for reuse until digestion is completed **[1]**.

3.3.1. Talent replacing the perfusate collector with the solution 3 container to recycle solution 3.

3.4. When the heart becomes swollen, turning slightly pale and flaccid **[1]**, using toothed forceps, gently pinch the myocardium **[2]**. If an imprint is visible **[3]**, terminate the digestion **[4]** and detach the heart from the apparatus **[5]**.

3.4.1. ECU: Shot of heart becoming swollen and turning pale and flaccid.

3.4.2. ECU: Myocardium being pinched.

3.4.3. ECU: Shot of imprint of the forceps on the heart.

3.4.4. Talent turning off the peristaltic pump to terminate digestion.

3.4.5. Talent detaching the heart.

4. Cell Isolation and Calcium Reintroduction

4.1. To isolate the atrial and ventricular myocytes, use forceps **[1]** to remove the ventricles and the atria **[2]** and place them in different Petri dishes **[3]**. Then, add the prewarmed solution 3 to both dishes **[4].** *Videographer: This step is important!*

4.1.1. WIDE: Talent picking up forceps, heart in view.

4.1.2. ECU: Talent removing the ventricles and atria.

4.1.3. Talent placing the ventricles and atria in separate Petri dishes.

4.1.4. Talent adding solution 3 to the Petri dishes.

4.2. Using blunt forceps, triturate the tissues into a turbid texture **[1-TXT]**, then gently pipette the tissue for even digestion without introducing air bubbles **[2]**.

4.2.1. ECU: Talent triturating the tissue. **TEXT: Process the ventricles and the atria separately, one after the other at each step**

4.2.2. Talent pipetting the tissue.

4.3. To arrest the remaining enzyme activity, using a pipette, transfer the turbid digested tissue into solution 4 **[1]**, then centrifuge for 20 seconds at 192 × *g* **[2]**. After removing the supernatant, resuspend the cell sediment in solution 5 **[3]**.

4.3.1. Talent transferring the tissue into solution 4.

4.3.2. Talent placing the tube containing the tissue in a centrifuge.

4.3.3. Talent resuspending the cells.

4.4. To avoid calcium paradox and calcium overload, reintroduce the calcium in a stepwise manner by gradually adding a total of 50 microliters of 100 millimolar per liter calcium chloride to the cell suspension **[1-TXT]**.

4.4.1. Talent adding $CaCl_2$ to the cell suspension. **TEXT: Add 5, 10, 15, and 20 µL at 5 min intervals**

4.5. Once all the calcium chloride has been added, store the cells in Tyrode's solution for the patch clamp study **[1-TXT]**.

4.5.1. Talent adding Tyrode's solution to the cells. **TEXT: For other cellular studies, use solution 6; Complete functional studies within the next 6h**

Results

5. Results: Survival Rates and Quality of the Isolated Cardiac Myocytes

5.1. Cannulation depth is associated with the perfusion of the atria and its appendages **[1]**. When the cannula tip is at the ascending aorta, both atrial appendages are inflated, indicating sufficient atria perfusion **[2]**. However, when the cannula tip is at the aortic root, both atrial appendages are wizened, indicating insufficient perfusion **[3]**.

5.1.1. LAB MEDIA: Figure 4.

5.1.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize the two swollen structures at the top of the heart in Figure 4A*

5.1.3. LAB MEDIA: Figure 4. *Video Editor: Emphasize the shriveled structures at the top of the heart (compared to figure 4A) in Figure 4B*

5.2. Atrial myocytes isolated by cannulation at the ascending aorta **[1]** had higher survival rates than those isolated by cannulation at the aortic root **[2]** both before **[3]** and after calcium reintroduction **[4]**.

5.2.1. LAB MEDIA: Figure 8. *Video Editor: Emphasize both AMAA bars*

5.2.2. LAB MEDIA: Figure 8. *Video Editor: Emphasize both AMARbars*

5.2.3. LAB MEDIA: Figure 8. *Video Editor: Emphasize black bars for both AMAA and AMAR*

5.2.4. LAB MEDIA: Figure 8. *Video Editor: Emphasize white bars for both AMAA and AMAR*

5.3. In contrast, there was no difference in the survival rates of ventricular myocytes **[1]** isolated by cannulation at either the ascending aorta **[2]** or the aortic root **[3]**, both before **[4]** and after calcium reintroduction **[5]**.

5.3.1. LAB MEDIA: Figure 8.

5.3.2. LAB MEDIA: Figure 8. *Video Editor: Emphasize both VMAA bars*

5.3.3. LAB MEDIA: Figure 8. *Video Editor: Emphasize both VMAR bars*

5.3.4. LAB MEDIA: Figure 8. *Video Editor: Emphasize black bars for both VMAA and VMAR*

5.3.5. LAB MEDIA: Figure 8. *Video Editor: Emphasize white bars for both VMAA and VMAR*

5.4. The whole-cell patch clamp recording of the sodium current **[1]** in the isolated atrial **[2]** and ventricular myocytes **[3]**, as well as the current densities, confirm that the quality of the isolated cells meets the requirements for electrophysiological experiments **[4]**.

5.4.1. LAB MEDIA: Figure 9A, 9B.

5.4.2. LAB MEDIA: Figure 9A, 9B. *Video Editor: Emphasize Figure 9A.*

5.4.3. LAB MEDIA: Figure 9A, 9B. *Video Editor: Emphasize Figure 9B.*

5.4.4. LAB MEDIA: Figure 9C.

Conclusion

6. Conclusion Interview Statements

6.1. **Wu Kui:** When transecting the aorta, make sure to retain a sufficient length for ligation. Otherwise, the aorta may penetrate the aortic valves after ligation.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 2.4.1*