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Title: In Vitro Assessment of Cardiac Function Using Skinned Cardiomyocytes

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Author Questionnaire:

1. Microscopy: Does your protocol involve video microscopy? Y, Inverted Microscope (IX51) from Olympus.

2. Does your protocol demonstrate software usage? Y

If yes, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured files to your project page.

3. Which steps from the protocol section below are the most important for viewers to see?

3.3., 4.2.1., 4.4.2., 4.6.1

4. What is the single most difficult aspect of this procedure and what do you do to ensure success?

3.2.1. Coverslip position being rotated (we use both hands at the same we look into the microscope to rotate the coverslip).

3.2.2. Glue being placed along side of coverslip (we try to put the glue wire on top of the coverslip by looking directly to the coverslip without using the microscope).

5. Will the filming need to take place in multiple locations (greater than walking distance)? N

Section - Introduction

Videographer: Interviewee Headshots are required. Take a headshot for each interviewee.

1. **REQUIRED Interview Statements (Said by you on camera): All interview statements may be edited for length and clarity.**

    1.1. **Patrícia Gonçalves-Rodrigues**: This technique enables clarification of the pathophysiology of cardiac diseases through investigation of the correlation between in vitro and in vivo parameters in animal models and human tissue **[1]**.

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

    1.2. **Patrícia Gonçalves-Rodrigues**: The main advantage of this technique is that it allows the study of cardiac myofilament function using very small biopsies or samples that were stored frozen **[1]**.

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

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OPTIONAL Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.

1.3. **João Almeida-Coelho**: This technique allows assessing in vitro, the impact of therapeutic interventions that target myofilaments **[1]**.

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

1.4. **João Almeida-Coelho**: Before attempting an experiment, it is advisable to practice the cardiomyocyte extraction a few times to learn how to select, glue, and activate cardiomyocytes with a good size and striation **[1]**.

1.4.1. INTERVIEW: Above Talent speaking the statement above in an interview-style shot, looking slightly off-camera

Ethics title card: (for human subjects or animal work, does not count toward word length total)

1.5. Procedures involving animal subjects have been approved by Portuguese Veterinary Governmental Authorities at the Portuguese Foundation for Science and Technology.

Section - Protocol

2. Skinned Cardiomyocyte Extraction and Permeabilization

2.1. Before beginning the procedure, adjust the temperature of the testing apparatus in-chamber to 15 degrees Celsius **[1]** and turn on the force-transducer and the motor **[2]**.

2.1.1. WIDE: Talent adjusting temperature

2.1.2. Talent turning on transducer and/or motor

2.2. Thaw 3-5 micrograms of myocardial sample in a Petri dish containing 2.5 milliliters of RELAX-ISO ('relax EYE-so') solution **[1-TXT]** and use a scalpel to precisely cut the tissue into small pieces without causing unnecessary cell damage **[2]**.

2.2.1. Talent placing samples into dish, with RELAX-ISO container visible in frame
TEXT: See text for all solution preparation details

2.2.2. Tissue being cut NOTE: please use part of video take from 00:48 seconds

2.3. When all of the tissue has been cut, use a cut pipette tip to transfer the entire volume of solution and tissue fragments into a Potter-Elvehjem glass **[1]** and use a grinder to mechanically disrupt the tissue at 30-40 rotations per minute **[2]**.

2.3.1. Talent adding solution to glass
2.3.2. Tissue being homogenized NOTE: Use take 2

2.4. When a good cell suspension has been obtained, add 250 microliters of Triton to a 15-milliliter tube containing 2.25 milliliters of RELAX-ISO **[1]** and add the cell suspension to the resulting solution **[2]**.

2.4.1. Talent adding Triton to tube, with Triton and RELAX-ISO containers visible in frame
2.4.2. Cells suspension being added to the solution.

2.5. Gently invert the tube three times to mix **[1]** and let the tube at room temperature for a 1-minute **[2]** followed by a 4-minute incubation on ice **[3]**.

2.5.1. Talent inverting tube
2.5.2. Talent placing tube

2.6. At the end of the ice incubation, bring the final volume of the tube up to 15 milliliters with additional RELAX-ISO **[1]** and mix the tubes three times by inversion as demonstrated **[2]**.

2.6.1. Talent adding RELAX-ISO to tube, with RELAX-ISO container visible in frame
2.6.2. Tube being mixed

2.7. Then centrifuge the cells **[1-TXT]** and carefully remove almost all liquid, except the last 3 milliliters of the supernatant **[2-TXT]**.

2.7.1. Talent placing tubes into centrifuge **TEXT: 1 min, 348 x g, RT**
2.7.2. Supernatant being removed **TEXT: Repeat wash and centrifuge until no bubbles observed**

2.8. After the last washout, remove the supernatant up to a volume of 5-10 milliliters of cell suspension **[1]**.

2.8.1. Talent removing supernatant

3. Skinned Cardiomyocyte Selection and Gluing

3.1. For skinned cardiomyocyte selection, add a drop of cell suspension onto a coverslip placed on top of a glass slide in a confocal microscope slide holder **[1]** and use the 20X objective to select a single rod-shaped cardiomyocyte with a good striation pattern and size **[2]**.

3.1.1. WIDE: Talent adding drop to coverslip
3.1.2. LAB MEDIA: Figure 20x image

3.2. Use two hands to rotate the coverslip until the selected cardiomyocyte is positioned horizontally with its ends aligned with the needle of the force transducer and the motor **[1]** and use a cotton swab tip to place a thin line of glue along the side of the coverslip **[2]**.

3.2.1. Coverslip position being rotated *Videographer: Difficult step*
3.2.2. Glue being placed along side of coverslip *Videographer: Difficult step*

3.3. To glue the cell into place, immerse the needle tips of the force transducer and the motor into the glue line to create a glue halo around both tips **[1]** and quickly move the needle tip of the force transducer down so that it glues to one edge of the cardiomyocyte **[2]**.

3.3.1. SCOPE: Tips being immersed/halo being created NOTE: use take 3 *Videographer: Important step*
3.3.2. SCOPE: Tip being moved to glue edge of cardiomyocyte NOTE: 3.3.2 and 3.4.1 shot together *Videographer: Important step*

3.4. Repeat this procedure with the tip of the motor and the other extremity of the cell **[1]**.

3.4.1. SCOPE: Other edge being glued

3.5. After 5-8 minutes, move both micromanipulators simultaneously to lift the needles

about 15 micrometers to avoid gluing the cell to the coverslip **[1]**.

3.5.1. Needle and cells being lifted. NOTE: Missing from original script but added by author during the shoot.

4. Active, Passive and Calcium (Ca^{2+}) Sensitivity Force Measurements

4.1. To measure the active and passive forces and the calcium sensitivity, fill the first experimental well with 55-100 microliters of relaxing solution **[1]** and fill the second experimental well with 55-100 microliters of activating solution **[2]**.

4.1.1. WIDE: Talent filling well with relaxing solution, with solution container visible in frame as possible
4.1.2. Talent filling well with activating solution, with solution container visible in frame as possible

4.2. Using the camera software, set the region of interest to an area of the cardiomyocyte with a clear pattern of striation **[1]** and set the sarcomere length to 2.2 micrometers **[2]**.

4.2.1. Talent at computer, setting ROI, with monitor visible in frame *Videographer: Important step*
4.2.2. SCREEN: screenshot_1: JoVE Video Editor please emphasize SLo – Sarcomere Length 2.200

4.3. Measure the distance between the two extremes of the cardiomyocyte and record the value as the myocyte length within the software **[1]**.

4.3.1. SCREEN: screenshot_1: JoVE Video Editor please outline/emphasize rectangular box over image in top right of frame

4.4. Next, move the needles slightly higher **[1]** and gently move the microscope stage so that the cell moves from the coverslip to the well containing relaxing solution on the back of the stage **[2]**.

4.4.1. Needle height being adjusted *Videographer: Important step*
4.4.2. Stage being moved *Videographer: Important step*

4.5. Select the protocol that contains two-cell shortening to occur when the cell is sequentially emerged in the calcium and relaxing solutions **[1]**.

4.5.1. SCREEN: screenshot_2: JoVE NOTE to Video Editor: please emphasize pro/protocol_2 text in bottom left of frame

4.6. To elicit an isometric contraction, move the microscope stage so that the cardiomyocyte moves from the relaxing to the activating solution **[1-TXT]**.

4.6.1. Stage being moved NOTE: Use take 2 *Videographer: Important step* **TEXT: Functional cell will immediately contract**

4.7. Upon reaching the force plateau, begin recording the force data **[1]**.

4.7.1. SCREEN: screenshot_3: JoVE Video Editor please emphasize red data line in top left of frame Author NOTE: Please add a line tracing the real recording of the cell activation. This would clarify the shape of the tracing recorded and removing the confounding effect of the recording noise

4.8. After 10 seconds, switch the cell to the relaxing solution **[1]** and record the data until the test stops **[2]**.

4.8.1. Stage being moved
4.8.2. SCREEN: screenshot_4: JoVE Video Editor please emphasize

4.9. To determine the calcium sensitivity, replace the activating solution with 55-100 microliters of each calcium solution **[1]** and record the data as just demonstrated **[2-TXT]**.

4.9.1. Calcium solution being added to well
4.9.2. Talent at computer, recording data, with monitor visible in frame **TEXT: Data will automatically be saved**

4.10. At the end of the measurement, stretch the tips of the force transducer and motor to remove the needles from the cell **[1]** and use a cotton swab soaked in acetone to carefully remove the glue halo from the needle tips **[2]**.

4.10.1. SCOPE: Tips being stretched
4.10.2. Tips being wiped

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Section – Results

5. Results: Representative Skinned Cardiomyocyte Assessment

5.1. Although a certain degree of deterioration and force decrease is expected after prolonged experiments, the values of active tension within the functional permeabilized cardiomyocytes should be relatively stable **[1]**.

5.1.1. LAB MEDIA: Table 6: JoVE Video Editor please emphasize Active tension data row

5.2. Here representative force traces of 3 out of 8 force recordings needed to carry out a protocol of myofilaments calcium-sensitivity are shown **[1]**.

5.2.1. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure

5.3. By transferring the cell to a well containing the activating solution **[1]**, the cardiomyocyte starts to develop force until it reaches a plateau **[2]**.

5.3.1. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure: JoVE Video Editor please add/emphasize Activating solution text in all three graphs

5.3.2. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure: JoVE Video Editor please emphasize Activating solution data line plateau in all three graphs

5.4. After a quick slack test, the baseline values of zero force can be obtained **[1]**.

5.4.1. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure: JoVE Video Editor please emphasize spike between T1 and Ktr in all three graphs

5.5. The slope of the last part of this curve can be used to determine the value of the rate of force redevelopment **[1]**, which is a measure of the apparent rate of cross-bridge attachment and detachment **[2]**.

5.5.1. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure: JoVE Video Editor please emphasize Ktr text and area of Activating solution curve in all three graphs

5.5.2. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure in all three graphs

5.6. After transferring the cell back to a well containing the relaxing solution **[1]**, the cell relaxes and its force drops **[1]**.

5.6.1. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure: JoVE Video Editor please add/emphasize blue Relaxing solution text in all

three graphs

5.6.2. LAB MEDIA: Figure 5 traces on left only without black instructive text at bottom of figure: JoVE Video Editor please emphasize blue data line after Ktr text in all three graphs

5.7. In addition to myofilament calcium-sensitivity and the length-dependent activation measurements **[1]**, the sarcomere length dependencies of the calcium-activated force per cross-sectional area **[2]** and the calcium-independent tension can be calculated **[3]**.

5.7.1. LAB MEDIA: Figure 7

5.7.2. LAB MEDIA: Figure 7: JoVE Video Editor please emphasize Tactive data line

5.7.3. LAB MEDIA: Figure 7: JoVE Video Editor please emphasize Tpassive data line

5.8. Finally, it is important to note that the way that the cardiomyocytes are isolated significantly impacts the results **[1]**.

5.8.1. LAB MEDIA: Figure 8: JoVE Video Editor please emphasize Fresh, Frozen, and Collag+Triton texts on x-axis in Figure 8A OR no animation

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Section - Conclusion

6. Conclusion Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.

6.1. **Inês Falcao-Pires**: Skinned cardiomyocytes for assessing cardiac function in vitro represent an important technique for clarifying cellular changes and myofilamentary mechanisms in a physiological and pathological context **[1]**.

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

6.2. **Inês Falcao-Pires**: This methodology has the advantages of requiring a minimal amount of myocardial sample and allowing the use of cardiomyocytes from a wide range of species, pathologies and cardiac locations **[1]**.

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

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