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Title: Single-Cell Optical Action Potential Measurement in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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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 similar? **N**

2. **Software:** Does the part of your protocol being filmed 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 video files to your project page as soon as reasonably possible.

   Videographer: Please film screen shots for reference

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? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, 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 (greater than walking distance)? **N**

Protocol Length
Number of Shots: **45**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Fitzwilliam Seibertz**: This protocol can provide high throughput measurements of cellular electrophysiology in a manner that befits the rapidly developing technology of induced pluripotent stem cell derived cardiomyocytes **[1]**.

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

REQUIRED:

1.2. **Fitzwilliam Seibertz**: The main advantage of this technique is that it is less complex and less labor intensive than the traditional patch clamp technique, yet it is able produce equivalent results **[1]**.

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

OPTIONAL:

1.3. **Niels Voigt**: This protocol can be expanded for large scale toxicity screening programs using human induced pluripotent stem cell derived cardiomyocytes, such as the comprehensive in vitro proarrythmia assay **[1]**.

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

OPTIONAL:

1.4. **Martyn Reynolds**: Optimization of the illumination and detection hardware requires careful matching to the sample. Sufficient illumination is needed to reach the sample and to allow for signal detection. Too much illumination, however, will bleach the sample. Detector amplification must be set such that the signal of interest can be reliably monitored without saturating the detector **[1][2]**.

1.4.1. B-roll: Shot of major components of the setup *Videographer: Please capture; Video Editor: please use with interview statement*

1.4.2. INTERVIEW: To be provided by authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: Authors will upload the interview in January

Protocol

2. Cell Preparation

2.1. To prepare induced pluripotent stem cell-derived cardiomyocyte cultures for the experiment, first coat individual 10-millimeter, round glass number-zero coverslips with 150 microliters of 1:60 basement membrane matrix within each well of a 24-well plate **[1-TXT]** and incubate the coverslips at 4 degrees Celsius for 4 hours **[2]**.

2.1.1. WIDE: Talent adding matrix to coverslip(s) **TEXT: See text for all solution and medium preparation details**

2.1.2. Talent placing coverslips at 4 °C

2.2. At the end of the incubation, remove the excess matrix from the coverslips **[1]** and apply the appropriate volume of cells to each coverslip **[2-TXT]**.

2.2.1. Matrix being removed

2.2.2. Talent adding cells to coverslip(s) **TEXT: See text for full cellular preparation details**

2.3. After 1 hour at 37 degrees Celsius, carefully fill each well with plating medium **[1]** and return the plate to the incubator **[2-TXT]**

2.3.1. Talent adding medium to well(s)

2.3.2. Talent placing the plate back in the incubator **TEXT: Cells can be maintained on coverslips for up to one month**

3. Microscope Setup

3.1. To prepare for voltage sensitive dye imaging, equip an inverted epifluorescence microscope with a 40x magnification, high numerical aperture lens **[1]** and couple a fast switching warm white LED to the transmitted illumination port **[2]**.

3.1.1. WIDE: Talent attaching objective to microscope

3.1.2. Talent coupling LED to port

3.2. Insert a simple red 660-nanometer filter into the transmitted light path **[1]** and mount a fast switching 470-nanometer LED head for photometry recording **[2]**.

3.2.1. Talent inserting filter into light path

3.2.2. Talent mounting LED head

3.3. Insert a 470-40 excitation filter at the epifluorescent port to clean-up the light generated by the LED **[1]** and insert a microscope cube containing a 495-nanometer long pass beam splitter in the mirror unit carousel within the microscope **[2]**.

3.3.1. Talent inserting filter

3.3.2. Talent inserting cube

3.4. Fit a detection arm containing an adjustable field diaphragm to the C-mount port to allow region of interest selection **[1]** and couple a PMT (P-M-T) detector **[2-TXT]** and a USB camera to the microscope **[3]**.

3.4.1. Talent fitting detection arm to c-mount port *Videographer: Important step*

3.4.2. Talent attaching PMT detector to microscope *Videographer: Important step*
TEXT: PMT: photomultiplier

3.4.3. Talent attaching camera *Videographer: Important step*

3.5. Insert a filter cube containing a 565-nanometer long pass beam splitter and a 535-50 emission filter into the PMT port to split the emission light between the two detectors **[1]**.

3.5.1. Talent inserting cube and filter into port *Videographer: Important step*

3.6. Couple the PMT to a power supply and a PMT amplifier **[1]** and connect the PMT amplifier output to an analogue input pin of a data acquisition system **[2]**.

3.6.1. Talent attaching PMT to power supply and/or amplifier

3.6.2. Talent connecting output to input pin

3.7. To fulfill Nyquist criteria and to prevent aliasing, filter the analogue data from the PMT at 1-kilohertz or higher **[1]** and digitize the data at a frequency that is at least double that of the highest frequency present in the analogue signal **[2]**.

3.7.1. Talent filtering data at 1 kHz or higher *Videographer: Important step*

3.7.2. SCREEN: 61890_3.7.2.mp4: Digitization properties being selected

3.8. **Martyn Reynolds**: The data filtering and digitizing are key features of this protocol and require minimal adjustments for optical investigation into a variety of two- and three-dimensional excitable cellular constructs **[1]**.

3.8.1. INTERVIEW STATEMENT: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: Authors will upload the interview in January

4. Voltage Sensitive Dye Cell Loading

4.1. To load the cells with voltage sensitive dye, mix 5 microliters of 1000x FluoVolt and 50 microliters of Powerload solution **[1-TXT]** and add 3 microliters of the resulting loading solution to 3 milliliters of warmed Tyrode's solution in a Petri dish **[2]**.

4.1.1. WIDE: Talent adding dye to solution, with dye and solution containers visible in frame *Videographer: Important step* **TEXT: Perform all dye-related steps in low light**

4.1.2. Talent adding solution to dish *Videographer: Important step*

4.2. Add a single induced pluripotent stem cell-derived cardiomyocyte-coated cover slip to the dish **[1]** and place the dish at 37 degrees Celsius for 20 minutes **[2]**.

4.2.1. Talent adding slip to dish

4.2.2. Talent placing dish at 37 °C

4.3. Mount a heated live cell imaging chamber onto the microscope stage **[1]** and fill the chamber with 500 microliters of fresh Tyrode's solution **[2]**.

4.3.1. Talent placing chamber onto stage

4.3.2. Talent adding solution to chamber

4.4. Then wash the coverslip with fresh Tyrode's solution at 37 degrees Celsius **[1]** and use fine point forceps to carefully transfer the coverslip to the pre-warmed bath chamber **[2]**.

4.4.1. Talent washing coverslip *Videographer: Difficult step*

4.4.2. Talent placing coverslip into chamber

5. Electrical Field Stimulation

5.1. To standardize the cellular dynamics and experimental parameters, insert two platinum electrodes into the chamber spaced 5 millimeters apart **[1]** and connect the electrodes to an external stimulator **[2]**.

5.1.1. WIDE: Talent attaching insert to chamber

5.1.2. Talent connecting stimulator to insert

5.2. Set the stimulator to 5-millisecond bipolar field pulses at 0.5 hertz **[1]** and increase the stimulus from 1 volt until the cells begin to contract **[2]**.

5.2.1. Talent setting stimulator

5.2.2. ECU: Cells contracting

5.3. Then set the voltage to roughly 25% above this threshold **[1-TXT]**.

5.3.1. Talent setting voltage **TEXT: Normal range: 1-30 V**

6. Optical Action Potential (AP) Acquisition

6.1. For optical action potential acquisition, use the transmitted light path and the USB camera to visualize the myocytes under brightfield conditions **[1]**.

6.1.1. WIDE: Talent visualizing cells, with monitor visible in frame

6.2. Select an isolated cell and use the field diaphragm to tightly crop its optical path, ensuring that only light from the cell of interest is monitored **[1]**.

6.2.1. SCREEN: 61890_6.2.1.mp4: optical path being cropped around cell.

6.3. Activate the PMT amplifier and set the PMT supply to 750 volts **[1]**.

6.3.1. Talent activating and setting PMT supply *Videographer: Important step*

6.4. Run the stimulation protocol along with the acquisition software **[1]** while simultaneously activating the 470-nanometer excitation light **[2-added]**.

6.4.1. SCREEN: 61890_6.4.1.mp4: Protocol being started

6.4.2. ADDED SHOT: Blue excitation light turning on.

6.5. Record 10 sweeps, ensuring that stable action potentials are detected **[1-added]**. After the last sweep, immediately move the microscope stage while still recording to briefly acquire background signal from a region devoid of cells and turn off the excitation light **[2-TXT]**.

6.5.1. ADDED SHOT: WIDE-sweeps being recorded- monitor visible.

6.5.2. SCREEN: 61890_6.5.2.mp4: stage being moved, background signal being recorded, light being turned off **TEXT: Repeat for multiple cells**

7. Data Analysis

7.1. To analyze the action potential data, open a saved recording in the appropriate analysis software **[1]** and average 10 sweeps containing stimulated action potentials from a single cell **[2]**.

7.1.1. WIDE: Talent opening recording, with monitor visible in frame

7.1.2. SCREEN: 61890_7.1.2.mp4: Sweeps being averaged

7.2. Subtract the mean of the baseline signal, representing fluorescence offset, from the averaged trace **[1]**. Then, use the formula to calculate the change in fluorescence using diastolic fluorescence as F0 *('ef-zero')* **[2-TXT]**.

7.2.1. SCREEN: 61890_7.2.1.mp4: Mean being calculated and then subtracted from baseline signal

7.2.2. BLACK TEXT WHITE BACKGROUND: $\frac{\Delta F}{F_0} = \frac{F - F_0}{F_0}$

7.3. Identify the diastolic and action potential areas of interest and measure the desired cardiac action potential parameters including, but not limited to, the duration at 50% and 90% repolarization **[1]**.

7.3.1. SCREEN: 61890_7.3.1.mp4: AP areas of interest being identified, then AP parameters being measured

7.4. Then export the data from this single cell to a spreadsheet program **[1]**.

7.4.1. SCREEN: 61890_7.4.1.mp4: Data being exported

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.4., 3.5., 3.7., 4.1., 6.3.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
Keeping cells alive and responsive in the bath. Ensure constant heating (step 4.4) and minimal excitation light exposure (step 6.4).

Results

8. Results: Representative Optical AP Profiles of Isolated Native Murine and Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

8.1. This method allows for rapid and precise quantification of repolarization mechanics, which can provide valuable insights into cellular ionic abnormalities **[1]**.

8.1.1. SCREEN: 61890_8.1.1.mp4: Traces of action potentials

8.2. Pronounced phase 1 features can be observed in optical signals from murine cardiomyocytes **[1]**, which are morphologically distinct from those of human induced pluripotent stem cell-derived cardiomyocytes **[2]**.

8.2.1. LAB MEDIA: Figures 3A and 3B graphs Video Editor: please emphasize peak in first figure 3A graph

8.2.2. LAB MEDIA: Figures 3A and 3B graphs Video Editor: please emphasize both graphs in Figure 3B

8.3. In addition, human induced pluripotent stem cell derived cardiomyocytes are responsive to pharmacological manipulation with nifedipine, a known L-type calcium channel antagonist **[1]**.

8.3.1. LAB MEDIA: Figure 3C graphs Video Editor: please emphasize red data line in left graph

8.4. During continuous drug application, a 41.5% decrease in action potential duration at 90% repolarization was observed **[1]**, suggesting the functionality of fluorescent voltage indicator-based imaging as a platform for prospective high throughput cardiac drug screening studies **[2]**.

8.4.1. LAB MEDIA: Figure 3C graphs Video Editor: please emphasize red data points in right graph

8.4.2. LAB MEDIA: All Figure 3C

Conclusion

9. Conclusion Interview Statements

9.1. **Fitzwilliam Seibertz**: It is important to keep in mind that although the voltage-sensitive-dye in this protocol is less cytotoxic than others, the excitation light should be used sparingly to conserve cellular viability **[1]**.

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (6.4.)

9.2. **Niels Voigt**: The modular photometry equipment used in this protocol is incredibly versatile and can be used for simultaneous measurements of other electrophysiological properties, such as intracellular calcium measurements **[1]**.

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