Measuring Cardiac Electrical Activity:
Manual Perforated Patch Clamp

Introduction

iCell® Cardiomyocytes recapitulate the electrophysiological characteristics of native human cardiac myocytes, expressing cardiac Na^+, Ca^{2+}, and K^+ channels in their native environment. With proper handling, cardiac action potentials can be recorded from iCell Cardiomyocytes for electrophysiological interrogation, providing an ideal cardiac model for drug discovery and detection of potential compound toxicity.

Standard ruptured patch clamp causes intracellular dialysis and ionic current rundown, altering the properties of the action potential waveform. The perforated patch clamp allows the recording of a more stable action potential by minimizing these effects. The procedure presented here has demonstrated efficacy and consistency in recording action potentials from iCell Cardiomyocytes using the perforated patch clamp.

Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell Cardiomyocytes User's Guide and in the appendices (Appendix A, Saline Solutions Preparation, Appendix B, Voltage Protocol Settings, and Appendix C, Glassware Acid Washing).

Item	Vendor	Catalog Number
Equipment		
Heating System	Multiple Vendors	
Patch Clamp Instrumentation with Voltage and Current Clamp Modes	Multiple Vendors	
Perfusion System	Multiple Vendors	
Pipette Puller	Multiple Vendors	
Consumables		
12-well Cell Culture Plates	Multiple Vendors	
15 mm Coverslips - Electrophysiology Applications	Warner Instruments	64-0703
Borosilicate Capillary Glass	Warner Instruments	GC150TF-10
Dimethyl Sulfoxide (DMSO)	Multiple Vendors	
Gramicidin	Sigma	G5002

Methods

Notes

Culturing iCell Cardiomyocytes

1. Place a 15 mm coverslip into each well of the 12-well cell culture plate(s).
2. Coat the 12-well cell culture plate(s) containing the coverslips with 1 ml/well of 0.1% gelatin for at least 1 hour according to the iCell Cardiomyocytes User's Guide.
3. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide.
4. Dilute the iCell Cardiomyocytes cell suspension in iCell Cardiomyocytes Plating Medium to 20,000 - 40,000 viable cells/ml. See the iCell Cardiomyocytes User's Guide for instructions to calculate the *Viable Cell Density.*
5. Aspirate the gelatin solution. Immediately add 2 ml/well of the cell suspension to achieve a final density of 40,000 - 80,000 viable cells/well.
6. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO_2.

   Note: *iCell Cardiomyocyte culture has been performed at both 5% and 7% CO_2 with no detected functional impact.*
7. Maintain the cardiomyocytes according to the User's Guide for at least 10 days.

   Note: *Additional time in culture may improve perforated patch clamp success rates and seal stability.*

Preparing for the Perforated Patch Clamp

See Appendix A, Saline Solutions Preparation, for intracellular and extracellular saline solution formulations.

1. Pull recording pipettes to have a resistance of ~3.5 (3 - 4.5) MΩ and a short taper on the day of experiment.

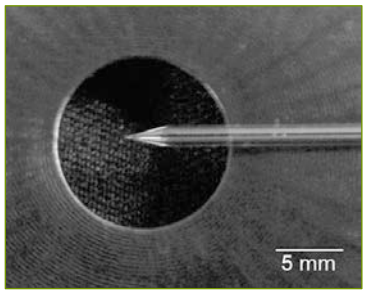

Figure 1: Recording Pipette
A short-tapered tip is important to avoid introducing air bubbles into the recording pipette when backfilling gramicidin-containing intracellular saline solution.

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2. Load extracellular saline solution into the perfusion system. Adjust the flow rate to 1.5 ± 0.04 ml/min.

   Note: *If recording in multiple solutions, such as for a drug application, adjust the flow rates to maintain a consistent speed between each solution application.*

3. Prepare a 50 mg/ml gramicidin stock solution in DMSO.

   Note: *If necessary, store gramicidin stock solution at 4°C for up to 1 week.*

4. Dilute 1 µl of 50 mg/ml gramicidin stock solution in 1 ml of intracellular saline solution to a final concentration of 50 µl/ml.

5. Transfer the coverslip containing iCell Cardiomyocytes to the recording chamber.

   Note: *Minimize the time that the cardiomyocytes are exposed to air.*

6. Begin extracellular saline solution perfusion.

7. Allow the system to heat to 35 - 37°C.

   Note: *Maintaining a stable temperature is crucial. Fluctuations in temperature may cause physical movement of the coverslip and interfere with seal integrity.*

Identifying Cardiomyocytes for Patch Clamp

Identify a cardiomyocyte suitable for patch clamp as follows:

- Target a cardiomyocyte not in contact with surrounding cardiomyocytes. Isolated cardiomyocytes ensure optimal voltage control.
- Target a stably beating cardiomyocyte. For evoked or paced experiments, target cardiomyocytes beating slower than the experimental pacing rate.

  Note: *iCell Cardiomyocytes are a nearly pure population of cardiomyocytes. Almost any cell, beating or not, may be patched under the assumption that it is a cardiomyocyte. Beating cardiomyocytes are preferable targets because beating and beat stability are easily identifiable markers. For evoked or paced experiments, non-beating cardiomyocytes may be suitable but difficult to assess for general cell health before performing the perforated patch clamp.*

- Target a cardiomyocyte with a defined edge. Ensure the edge of the cell where the recording pipette will make contact is well-defined and free of large vesicles.

Note: *When plated at a low density, many iCell Cardiomyocytes become broad and flat. Avoid performing the perforated patch clamp on flat cardiomyocytes as it may prove challenging. Suitable cardiomyocytes often have a flat outer region with a raised center (Figure 2).*

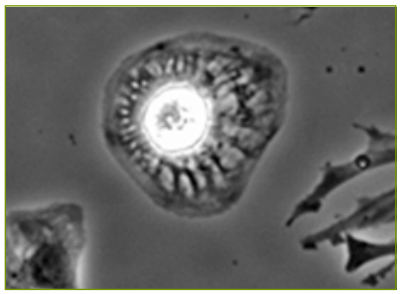

Figure 2: Cardiomyocyte Suitable for Patch Clamp
The cell is isolated from neighboring cells, and its center region is raised with a well-defined edge and an absence of vesicles.

Filling the Recording Pipette

Filling the recording pipette with intracellular saline solution is an important step in perforated patch clamp. The tip of the recording pipette is filled with gramicidin-free intracellular saline solution to facilitate sealing onto the cardiomyocyte, followed by backfilling with gramicidin-containing intracellular saline solution to facilitate pore formation. Too much gramicidin-free intracellular saline solution in the tip creates a diffusion barrier and prolongs the time to achieve a usable access resistance. Too little causes the target cell to be exposed to the gramicidin before sealing, deteriorating the membrane quality.

1. Dip the tip of a recording pipette into gramicidin-free intracellular saline solution for 5 seconds.
2. Insert the micropipette filler into the recording pipette, ensuring the micropipette filler is free of air bubbles.

   Note: *Avoid air bubbles when adding gramicidin-containing intracellular saline solution to the recording pipette. Tapping the recording pipette to remove bubbles is not recommended as it will mix the gramicidin-containing and gramicidin-free intracellular saline solutions.*
3. Touch the inside of the recording pipette, just above the taper, with the micropipette filler. Allow a small amount of gramicidin-containing intracellular saline solution to leak from the micropipette filler and slowly fill the remaining volume of the recording pipette tip.
4. Add gramicidin-containing intracellular saline solution such that the intracellular saline solution comes into contact with the end of the recording electrode. Do not completely fill the recording pipette.

Forming the GΩ Seal

The time it takes to form a GΩ seal is important when using perforated patch clamp because gramicidin begins to diffuse across the gramicidin-free barrier as soon as the recording pipette is filled. Thus, to maintain a gramicidin-free barrier, never fill a recording pipette before identifying a cell to record.

Notes

Notes

1. Place the recording pipette over the recording electrode.
2. Lower the recording pipette into the bath.

   Note: *Maintain a neutral pressure in the recording pipette to avoid diffusion into or out of the recording pipette's tip. Diffusion of extracellular saline solution into the recording pipette may increase the time needed for membrane permeabilization and potentially depolarize the cell.*
3. Run the Membrane Test Protocol and compensate for recording pipette offset. See Appendix B, Voltage Protocol Settings, for settings for the Membrane Test Protocol.
4. Quickly bring the recording pipette tip in close proximity to the cardiomyocyte surface.
5. Gently touch the surface of the cell with the recording pipette.

   Note: *Do not puncture the cell membrane by pressing too hard. The cell surface should slightly dimple. When the recording pipette makes contact with the cell surface, the resistance increases by 0.1 - 0.2 MΩ.*
6. Apply a slow and steadily increasing negative pressure to the recording pipette using a 1 ml syringe while monitoring the resistance using the Membrane Test Protocol.

   Note: *Applying negative pressure too quickly may rupture the cell membrane. Applying too much negative pressure may suck the cell into the recording pipette. GΩ seals can often be achieved using 0.1 ml of suction from the 1 ml syringe. Gradual addition of a holding voltage may also facilitate seal formation.*
7. Release the negative pressure when the resistance is ≥1 GΩ.
8. Switch from voltage-clamp mode to current-clamp mode.

   Note: *If a holding voltage was applied to achieve a GΩ seal, system settings may convert it to a holding current when amplifier modes are switched. Switch to I=0 mode from voltage clamp mode and remove any holding voltages or currents before continuing to current clamp mode.*
9. Wait 15 - 20 minutes for gramicidin diffusion and perforation.

Recording Action Potentials

Spontaneous activity can be monitored in current-clamp mode at any time after formation of the GΩ seal. Access resistance decreases over time as gramicidin perforation occurs. During this time, maximum diastolic potential (MDP) becomes more negative and *dV/dt* of phase 1 depolarization increases. Begin your experimental procedures after MDP, *dV/dt, and APD* values stabilize. Refer to Appendix B, Voltage Protocol Settings, for performing an evoked action potential protocol.

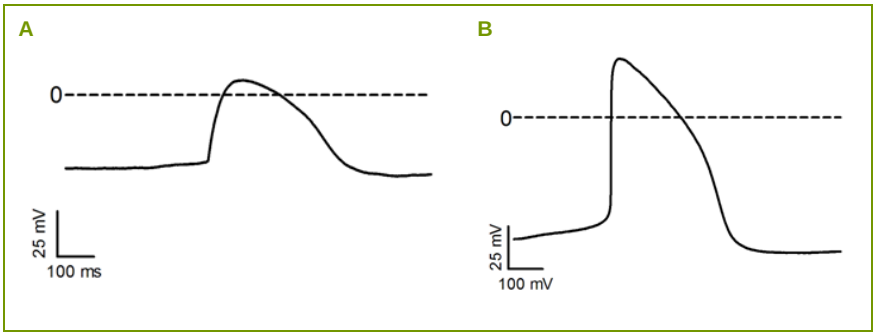

Figure 3: Alterations in Action Potential Waveform during Gramicidin Perforation
Panels A and B show example tracing of a spontaneously beating iCell Cardiomyocyte at 5 and 15 minutes after GΩ seal formation, respectively. MDP became more negative, from -45 to -79 mV, and dV/dt *increased from 2 to 39 mV/ms.*

Notes

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Appendices

Appendix A. Saline Solutions Preparation

Prepare saline solutions in ASTM type 1 grade water using clean acid-washed glassware according to Appendix C, Glassware Acid Washing. Do not use glassware that was cleaned with detergent or tap water.

Item	Vendor
Consumables	
0.2 µm Filters	Multiple Vendors
Water, ASTM Type 1 Grade	Multiple Vendors

The following table of reagents provides the final concentrations to use in the preparation of the extracellular and intracellular saline solutions.

Reagent	Final Concentration
Extracellular Saline Solution	
$CaCl_2$	1.8 mM
Glucose	15 mM
HEPES	15 mM
KCl	5.4 mM
$MgCl_2$	1 mM
NaCl	150 mM
NaOH	pH 7.4
Na-Pyruvate	1 mM
Intracellular Saline Solution	
$CaCl_2$	2 mM
EGTA	5 mM
HEPES	10 mM
KCl	150 mM
KOH	pH 7.2
MgATP	5 mM
NaCl	5 mM

1. Prepare extracellular saline solution by diluting reagents in water at the final concentrations specified in the above table. Adjust pH to 7.4 with NaOH. Filter using a 0.2 µm filter.

   Note: *Extracellular saline solution may be prepared at 20X concentration and stored in 50 ml aliquots at -20°C for up to 1 year. Adjust pH to 7.4 and filter after diluting to the final concentration.*

2. Prepare intracellular saline solution by diluting reagents in water at the final concentrations specified in the above table. Adjust the pH to 7.2 with KOH. Filter using a 0.2 µm filter.

   Note: *Store intracellular saline solution in 1 ml aliquots at -20°C for up to 1 year.*

Appendix B. Voltage Protocol Settings

Notes

Membrane Test Protocol

Run the Membrane Test Protocol, setting the continuous square wave voltage at 1 mV and the holding level at 0 mV.

Spontaneous Action Potential Protocol

Record spontaneous action potentials in gap-free mode while in current clamp mode.

Evoked Action Potential Protocol

Evoke action potentials by injecting a 150 - 550 pA depolarizing current for 5 ms. Adjust the current step amplitude as necessary to consistently evoke an action potential while minimizing the step artifact.

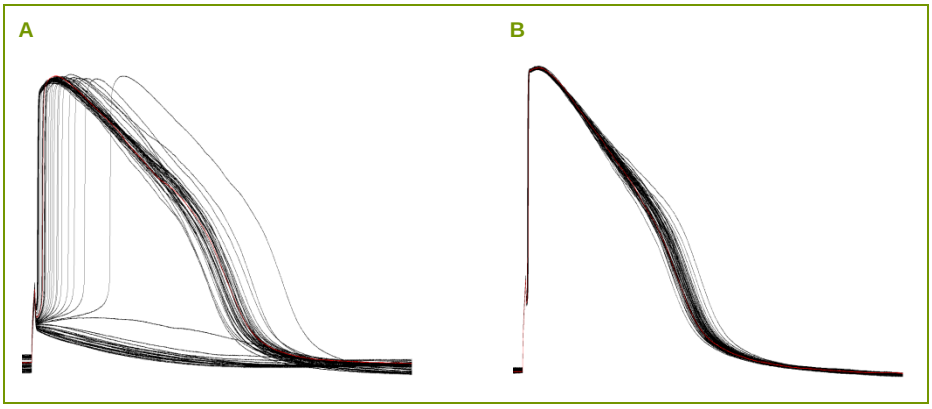

Figure 1: Evoked Action Potentials at Multiple Current Injection Amplitudes
Panel A and B show action potentials evoked from a cardiomyocyte with a current injection step of 250 pA and 350 pA, respectively. A 250 pA current injection did not always elicit an evoked action potential.

Notes

Appendix C. Glassware Acid Washing

Item	Vendor
Consumables	
Concentrated 12M HCl	Multiple Vendors
Water, ASTM Type 1 grade	Multiple Vendors

1. Prepare HCl solution by diluting 3 ml of 12M HCl in 100 ml of water.

   Note: *Always add acid to water and never the reverse.*

2. Soak new glassware in HCl solution for at least 10 minutes.
3. Rinse glassware 5 to 6 times in water and allow to dry upside-down.

   Note: *Glassware may also be baked at 180°C for 2 hours to dry.*

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