

Measuring Cardiac Electrical Activity: *Field Potential Detection with Multielectrode Array*

Introduction

The propensity to induce cardiac arrhythmias can result in approval delays, black box warnings, and market withdrawal of new pharmaceuticals. iCell® Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes that exhibit typical biochemical, electrophysiological, and mechanical activities expected of primary human cardiomyocytes. Due to their human origin, native cell-like behavior, and ease of use, iCell Cardiomyocytes represent an optimal test system for basic cardiomyocyte biology in all areas of drug development, drug discovery, toxicology, and safety pharmacology.

Multielectrode array (MEA) technology enables non-invasive measurements of local field potentials of electrically active cells and thus the activity of the underlying ion channels. With proper handling, iCell Cardiomyocytes can be cultured on MEA chips to form a stable electrically and mechanically active syncytium amenable to electrophysiological interrogation. Together, iCell Cardiomyocytes and MEA technology form an excellent, non-invasive platform for assessing the potential pro-arrhythmic properties of compounds.

Required Equipment, Consumables, and Software

The following equipment, consumables, and software are required in addition to the materials specified in the iCell Cardiomyocytes User's Guide.

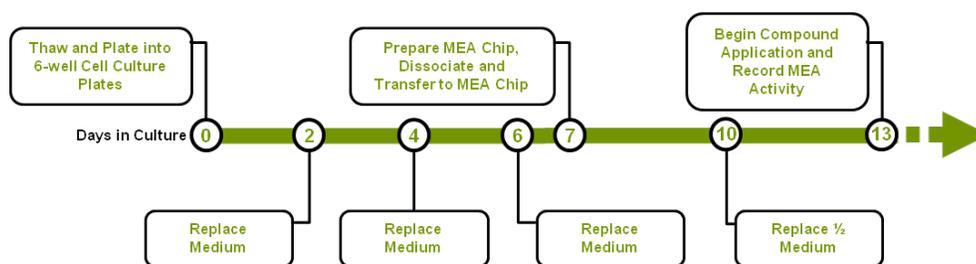
Item	Vendor	Catalog Number
Equipment		
1 ml Pipettor and Sterile Tips	Multiple Vendors	
2 µl Pipettor and Sterile Tips	Multiple Vendors	
Environmental Chamber	Multiple Vendors	
MEA System*	Multiple Vendors	
Picking/Dissecting Hood with Microscope (optional)	Multiple Vendors	
Consumables		
iCell Cardiomyocytes Kit	Cellular Dynamics International (CDI)	CMC-100-110-001 CMC-100-110-005 CMC-100-010-001 CMC-100-010-005
6-well Cell Culture Plates	Nunc	140675
6-well Multielectrode Array (MEA) Chip*	MCS	6wellMEA200/30iR-Ti-tcr
100 mm Diameter Cell Culture Plates	Becton Dickinson	353003

Item	Vendor	Catalog Number
Blood Gas Mixture (5% CO ₂ , 20% O ₂ , Balance N ₂)	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline with Ca ²⁺ and Mg ²⁺ (D-PBS)	Invitrogen	14040
Fibronectin	Roche Applied Science	11051407001
Gelatin, Type A, Cell Culture Tested	Sigma	1890
Sterile 1.5 ml Centrifuge Tubes	Multiple Vendors	
Sterile 15 and 50 ml Conical Tubes	Multiple Vendors	
Sterile Water	Multiple Vendors	
Trypsin 0.5%-EDTA (10X), no phenol red	Invitrogen	15400
Software		
Data Analysis Software*	Various Vendors	

* Several MEA systems, chips, and data analysis software are available. The cell culturing and data acquisition procedures described here were optimized for the MEA60-Inv-BC-System-E (Multi Channel Systems) using 6-well MEA chips (Multi Channel Systems). Data analysis was completed using Spike2 Software (Cambridge Electronic Devices).

Workflow

iCell Cardiomyocytes are thawed and plated into a 6-well cell culture plate previously coated with gelatin. On days 2, 4, and 6 post-plating, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium). On day 7 post-plating, cells are transferred to the MEA chip and half of the medium is replaced every 2 - 3 days thereafter. From days 5 - 7 post-transferring to the MEA chip, cells can be treated with compounds, and the cardiac activity recorded on the MEA chip.



Methods

Culturing iCell Cardiomyocytes

1. Coat 6-well cell culture plates with 1 ml/well of 0.1% gelatin for at least 1 hour according to the iCell Cardiomyocytes User's Guide.
2. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide.

3. Aspirate the gelatin solution. Immediately add 2 - 5 ml/well of the iCell Cardiomyocytes cell suspension to achieve a final cell density of 750,000 plated cells/well. See the iCell Cardiomyocytes User's Guide for instructions to calculate the *Target Plating Density* based on *Plating Efficiency*.

Note: Each well of the 6-well cell culture plate must contain at least 750,000 plated cardiomyocytes. Any remaining cardiomyocytes can be distributed evenly between the wells to a maximum density of 1,000,000 plated cells/well. Cardiomyocytes remaining after this step should be discarded or plated for other purposes in accordance with the specific end-use protocol.

4. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO₂.

Note: iCell Cardiomyocytes culture has been performed at both 5% and 7% CO₂ with no detectable functional impact.

5. Maintain the cardiomyocytes according to the User's Guide for 7 days.

Preparing the 6-well MEA Chip

The following procedure details preparing a 6-well MEA chip. Scale volumes appropriately for other chip formats with a different area of recording electrodes.

1. Place a dry 6-well MEA chip into a sterile 100 mm diameter cell culture plate.
2. Add ~3 ml of sterile water to the bottom of the plate to prevent substrate evaporation. Do not allow water into the wells of the MEA chip.

Note: The exact volume is not essential as long as the vessel containing the MEA chip maintains a moist environment. To keep the MEA chip stationary and maintain a moist environment, insert a laboratory tissue soaked with sterile water in the vessel.

3. Prepare a 50 µg/ml fibronectin solution by diluting stock fibronectin solution 1:20 in D-PBS immediately before use.

Note: Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20°C, and dilute to 50 µg/ml the day of use.

4. Dispense a 2 µl droplet of fibronectin directly over the recording electrode area of each well of the MEA chip.
5. Incubate the fibronectin-coated MEA chip in a cell culture incubator at 37°C, 7% CO₂ for at least 1 hour.

Note: Longer incubation times are acceptable; however, the droplet of fibronectin should not be allowed to evaporate to avoid impacting proper cell attachment.

Collecting iCell Cardiomyocytes from the 6-well Cell Culture Plate

Appropriately collecting iCell Cardiomyocytes from the 6-well cell culture plate(s) as described here is a critical step to achieve robust functionality upon transfer to the MEA chip. iCell Cardiomyocytes are sensitive to over-digestion by the dissociating enzyme and to excessive mechanical trituration. The following recommendations detail proper handling procedures for the dissociating enzyme and cardiomyocytes and ensure optimal cardiomyocyte performance on the MEA chip.

1. Before use, equilibrate an aliquot of Maintenance Medium and D-PBS in a 37°C water bath.
 2. Dilute 0.5% trypsin solution in D-PBS to a final concentration of 0.1%. Before use, equilibrate the 0.1% trypsin solution in a 37°C water bath.
- Note:** To ensure a consistent dissociation, dispense a stock of 0.5% trypsin solution into single-use aliquots and store frozen until use. Thaw 0.5% trypsin aliquot(s) at 4°C overnight. Always use freshly prepared 0.1% trypsin and equilibrate trypsin in a 37°C water bath for 10 minutes. Avoid prolonged equilibration periods of time.
3. Aspirate the Maintenance Medium from the 6-well cell culture plate containing iCell Cardiomyocytes.
 4. Wash cardiomyocytes twice with 2 ml/well of 37°C D-PBS.
 5. Add 1 ml/well of 37°C 0.1% trypsin solution. Incubate in a cell culture incubator for 2 minutes.

Note: iCell Cardiomyocytes may not appear to be lifting from the bottom of the 6-well cell culture plate at the end of the 2-minute trypsin incubation (Figure 1B). Proceed with step 6 to wash the cardiomyocytes from the plate to avoid over-digestion with trypsin.

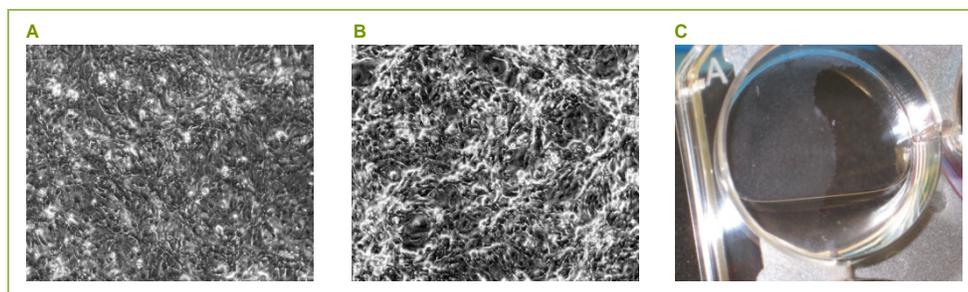


Figure 1: iCell Cardiomyocytes before and after Enzyme Treatment

iCell Cardiomyocytes were cultured for 7 days on a 6-well cell culture plate: (A) confluent monolayer of cardiomyocytes before enzyme treatment, (B) after enzyme treatment, and (C) washed from half of the well as described in step 6.

6. Quickly wash the cardiomyocytes from the plate surface using a 1 ml pipettor by tilting the plate and repeatedly aspirating and dispensing the trypsin solution over the plate surface 2 - 3 times (Figure 1C).
 7. Rotate the plate 180° and repeat the previous step.
 8. Add 3 ml/well of 37°C Maintenance Medium to quench the trypsin.
 9. Tilt the plate and triturate the cardiomyocytes 3 - 4 times using a 5 ml serological pipette. Avoid excessive trituration and introduction of air bubbles.
 10. Transfer the cell suspension to a 15 ml tube.
- Note:** Use a 1 ml pipettor to collect any remaining cell suspension from the wells after the cell transfer.
11. Centrifuge the cell suspension at 200 x g for 4 minutes.
 12. Aspirate the supernatant, being careful not to disturb the cell pellet.

13. Add 3 ml of 37°C Maintenance Medium and mix gently to resuspend the cardiomyocyte pellet.
14. Remove an aliquot to count the fraction of viable cardiomyocytes using a hemocytometer.
Note: *Ensure the cardiomyocytes are evenly suspended before removing an aliquot to count.*
15. Centrifuge the cell suspension at 200 x g for 4 minutes.
16. Aspirate the supernatant, being careful not to disturb the cardiomyocyte pellet.
17. Add 37°C Maintenance Medium to resuspend the cardiomyocyte pellet at a final concentration of 8,000 viable cardiomyocytes/ μ l.
Note: *Be mindful of cardiomyocyte viability after dissociation. Low viability (<70%) after dissociation could indicate a rough dissociation and lead to excess debris and poor performance of the cardiomyocyte monolayer on the MEA chip.*
Note: *Plating efficiency values are not used when transferring pre-plated cardiomyocytes to the MEA chip.*

Plating iCell Cardiomyocytes into the MEA Chip

The following procedure details plating iCell Cardiomyocytes into a 6-well MEA chip. Scale volumes appropriately for other chip formats with a different area of recording electrodes.

1. Remove the fibronectin-coated MEA chip from the cell culture incubator and aspirate the fibronectin from each well. Additional rinsing is not necessary.
2. Dispense a 2 μ l droplet of iCell Cardiomyocytes cell suspension (approximately 16,000 viable cardiomyocytes) directly over the recording electrode area of each well of the MEA chip. Use of microscope in a picking/dissecting hood is highly recommended for this step.
Note: *Timing is critical in this step. Cardiomyocyte attachment is compromised if the fibronectin is allowed to dry. Under typical conditions, the well will begin to dry within a few minutes after aspiration of the excess fibronectin solution. At this point the residual fibronectin in the well will begin to crystallize, turn white, and should be discarded as cardiomyocyte attachment will be suboptimal.*
3. Incubate the MEA chip containing iCell Cardiomyocytes in a cell culture incubator at 37°C for 1 hour.
4. Gently add 200 μ l/well of Maintenance Medium under sterile conditions to the MEA chip. Adding the medium too quickly will dislodge the adhered cardiomyocytes.
Note: *CDI recommends adding the medium to the outside wall (along the circumference) of the well first by evenly distributing the medium along an edge using a pipettor. Then slowly add additional medium so that it fills the interior of the well in an even manner from the outside toward the center. Stop prior to reaching the cardiomyocyte droplet and very slowly add additional medium until the droplet is reached. Adding the medium too forcefully during this step can easily dislodge cardiomyocytes from the plating substrate.*
5. Gently add an additional 300 μ l/well of Maintenance Medium for a final volume of 500 μ l/well.

6. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO₂.
7. For optimal conditions, perform MEA recordings 5 - 7 days post-plating.

Note: Wait 3 days after initial plating before replacing medium to allow for proper attachment. Then, replace the medium every 2 - 3 days. To avoid potential monolayer detachment, CDI recommends replacing half of the medium during regular maintenance.

Cleaning the MEA Chip

After data acquisition, carefully clean and appropriately store the MEA chip according to the manufacturer's instructions.

Data Acquisition and Analysis

Data Acquisition

Beating activity typically stabilizes 5 - 7 days after incubating iCell Cardiomyocytes on an MEA chip, at which point the monolayers are suited for data acquisition. Follow the manufacturer's instructions for the MEA system to perform data acquisition.

Data Analysis

The waveform recorded by each electrode on the MEA chip reflects differences in the field potential at that electrode relative to the ground electrode. Similar to an electrocardiogram (ECG), the exact shape of the waveform depends on the local ion channel activity and the direction of electrical propagation. Thus, it is expected for different electrodes to show different waveform phenotypes. In general, waveforms showing clear depolarization and repolarization characteristics are chosen for analysis. Typical dose-response relations are generated through a cumulative compound addition. In such experiments, it is imperative that the same electrode(s) be used for the entire analysis across all control and compound additions.

The following figures illustrate key characteristics of the MEA waveform and performance of iCell Cardiomyocytes. Data are shown that exemplify the effects of modulating crucial ion channel and G protein-coupled receptors (GPCRs) activities through cumulative compound addition.

Raw MEA data were acquired using MC-Rack Software (Multi Channel Systems) and then exported to Spike2 Software (Cambridge Electronic Devices) to analyze the following parameters:

- Beat frequency
- Field potential duration
- Initial spike amplitude

Representative tracings were averaged over 60 seconds, unless otherwise noted. Data in the bar graphs show the mean \pm sem (n = 2 - 4 wells, each condition) of the respective parameter averaged over the final 5 minutes of each control and dose application.

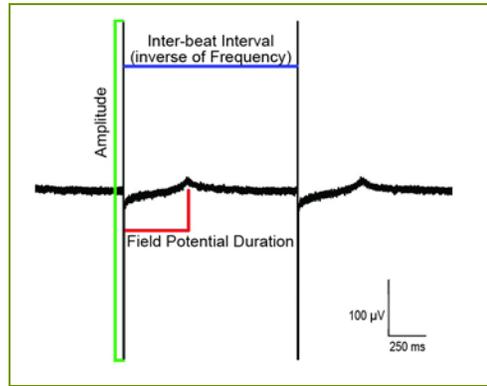


Figure 2: MEA Waveforms Suitable for Analysis

The components of the field potential waveform represent distinct electrical behavior. The initial peaks indicate depolarization while the secondary deflection indicates repolarization. The field potential duration (FPD) is taken as the duration between depolarization and repolarization. Beat-to-beat interval (the inverse of frequency) is measured as the duration between the initial peaks of sequential beats.

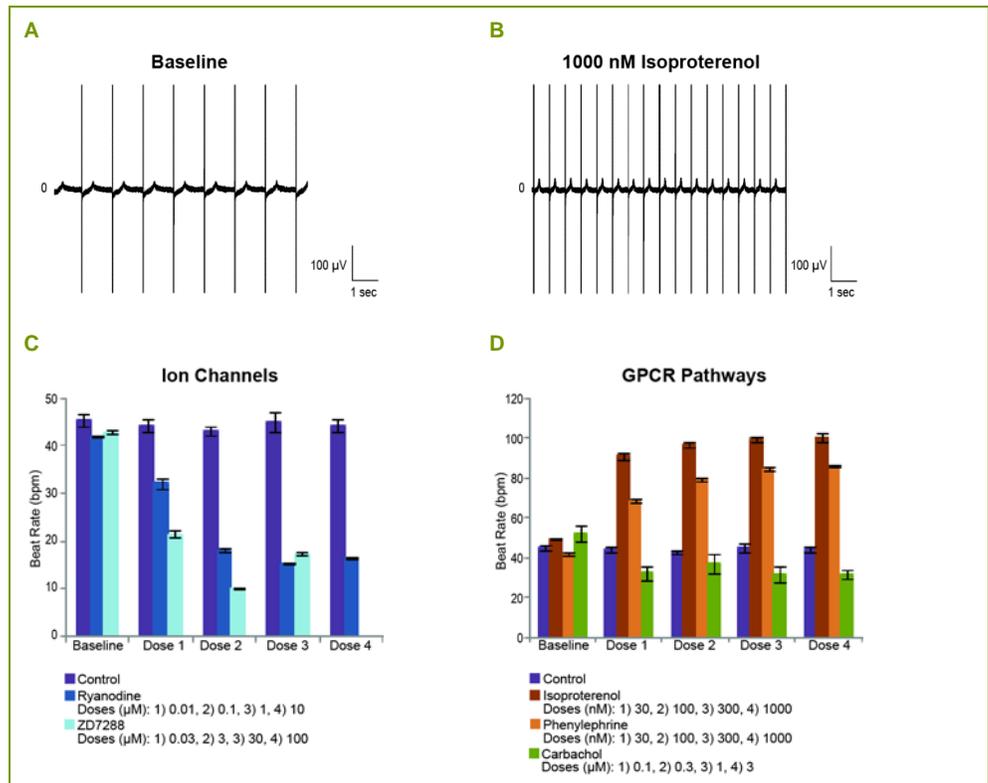


Figure 3: Effects of Ion Channel and GPCR Activity on Beat Rate

Modulating ion channel and GPCR activity can alter the spontaneous beat rate. Panels A and B show the MEA waveform under control conditions or in the presence of isoproterenol. Panels C and D quantify the effects on the beat rate of iCell Cardiomyocytes during exposure to the indicated ion channel antagonists or GPCR-agonists at the concentrations listed. The asterisk (*) indicates the concentration where beating activity ceased. Carbachol and its respective controls were averaged over the first minute of each dose recording due to desensitization.

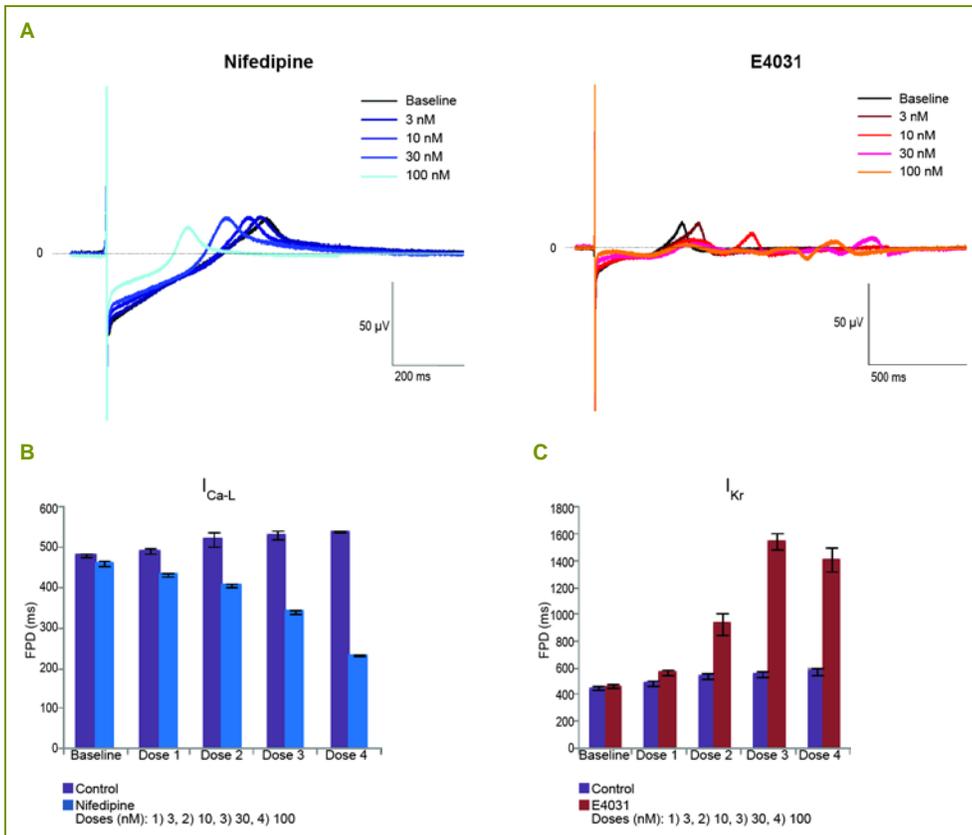


Figure 4: Effects of Ion Channel Activity on Field Potential Duration

Modulating ion channel activity can alter the field potential duration (FPD). *iCell* Cardiomyocytes were exposed to the indicated compounds at the concentrations listed. Panel A shows an overlay of averaged single-beat MEA waveforms under control conditions (baseline) and increasing concentrations of nifedipine (blue) or E4031 (red) to block I_{Ca-L} or I_{Kr} channels, respectively. Panels B and C quantify the FPD shortening and prolongation by nifedipine and E4031, respectively.

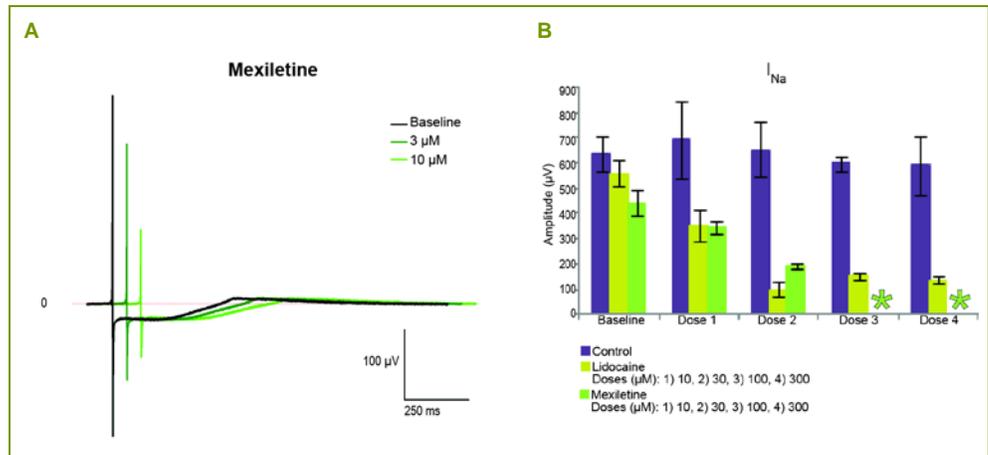


Figure 5: Ion Channel Activity Effects on Peak Amplitude

Modulating ion channel activity can affect the amplitude of the initial peaks. iCell Cardiomyocytes were exposed to lidocaine or mexiletine at the concentrations listed to block the I_{Na} channel. (A) Representative tracings illustrate the decrease in amplitude where the traces from each drug application have been offset along the X-axis to highlight the initial peaks. (B) Cumulative additions of compound show a clear dose relation. Asterisks (*) indicate concentrations where beating activity ceased.

Summary

iCell Cardiomyocytes can be cultured on MEA chips where the spontaneous electrical activity can be monitored. The waveform characteristics representing cardiomyocyte depolarization, repolarization, automaticity, and propagation (data not shown) can be readily measured and their modulation by cardioactive compounds can be robustly quantified. iCell Cardiomyocytes are highly pure, human cardiomyocytes, and the methods and data presented here highlight the ease with which human-based, mechanistic data regarding cardiomyocyte electrophysiology can be generated.

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