

Measuring Cardiac Electrical Activity: *Field Potential Detection on the Maestro Multielectrode Array*

Introduction

iCell® Cardiomyocytes are human cardiomyocytes that recapitulate the electrophysiological, biochemical, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Their derivation from human induced pluripotent stem cells, high purity, functional relevance, and ease of use, make iCell Cardiomyocytes an optimal in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

Axion BioSystems' Maestro multielectrode array (MEA) technology enables non-invasive, label-free measurements of local field potentials of electrically active cells and thus the activity of the underlying ion channels. iCell Cardiomyocytes can be cultured on MEAs to form an electrically stable and mechanically active syncytium amenable to electrophysiological examination. Together, iCell Cardiomyocytes and the Maestro MEA technology form an excellent, non-invasive platform for in vitro screening of compound efficacy and toxicity in human cardiac myocytes.

This Application Protocol describes how to handle iCell Cardiomyocytes for use on the Maestro MEA system.

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		
Maestro Multielectrode Array (MEA) System	Axion BioSystems	
12-channel Pipettor (20 and 200 µl)		
Consumables		
iCell Cardiomyocytes Kit	Cellular Dynamics International (CDI)	CMC-100-010-001 CMC-100-010-005
1.5 ml and 15 ml Centrifuge Tubes	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS)	Invitrogen	14190
Fibronectin	Roche Applied Science	11051407001
Multielectrode Array (MEA) Plates*	Axion BioSystems	M768-GL1-30Au200 (12-well) M768-KAP-48 (48-well)
Sterile Water	Multiple Vendors	

Item	Vendor	Catalog Number
Software		
Axion Integrated Studio (AxIS) (version 1.8 or newer)	Axion BioSystems	

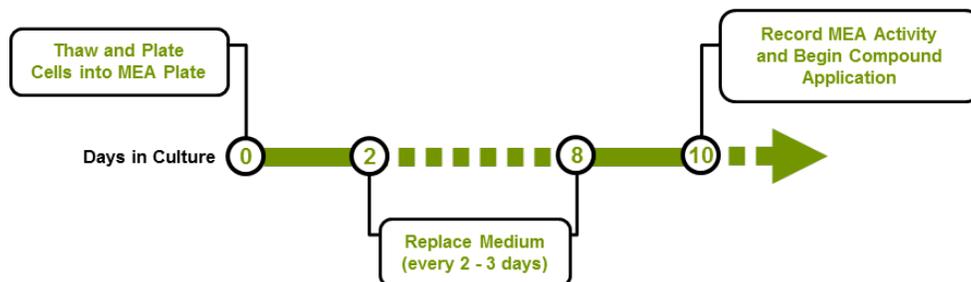
Notes

* This Application Protocol provides instructions for using 12- and 48-well MEA plates. Contact CDI's Technical Support (support@cellulardynamics.com; +1 (877) 320-6688 (US toll-free) or (608) 310-5100) for instructions for using other plate formats.

Workflow

iCell Cardiomyocytes are thawed and plated into 12- or 48-well MEA plates previously coated with fibronectin. On day 2 post-plating, replace the spent medium with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium), and replace medium every 2 - 3 days thereafter. From day 10 - 14 post-plating, baseline activity is recorded, cells can be treated with compounds, and the cardiac activity recorded.

Note: This Application Protocol is optimized for plating cells directly into an MEA plate to better fit end-use workflows. iCell Cardiomyocytes can be plated into a 6-well cell culture plate and then transferred into the MEA plate. Contact CDI's Technical Support (support@cellulardynamics.com; (877) 320-6688 (US toll-free) or (608) 310-5100) for instructions.



Methods

Preparing the MEA Plate

1. 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.

2. Tilt the MEA plate at an angle so that the bottom of each well is visible. Dispense an 8 µl/well droplet of fibronectin over the recording electrode area of the well of the MEA plate (Figure 1).

Note: Do not touch the surface of the MEA plate with the pipette tip to avoid damaging the recording electrodes.

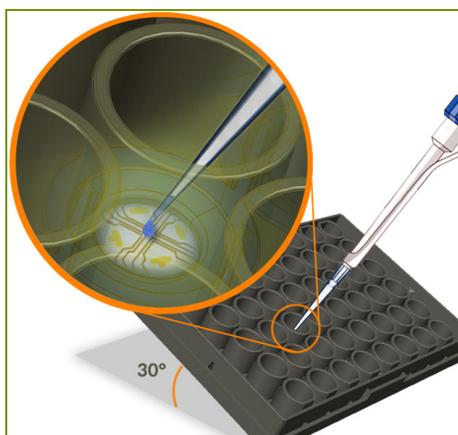


Figure 1: Droplet Placement

Tilt the MEA plate 30 degrees and dispense an 8 μ l droplet over the recording electrode area of each well.

3. Incubate the fibronectin-coated MEA plate in a cell culture incubator at 37°C, 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. Sterile water may be added to the area outside of the wells to prevent the droplet from evaporating.

Thawing iCell Cardiomyocytes

1. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide.
2. Transfer the cell suspension to a 15 ml centrifuge tube.
3. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
4. Centrifuge the cell suspension at 180 x g for 5 minutes.
5. Aspirate the supernatant, being careful not to disturb the cell pellet.
6. Gently resuspend the cell pellet in iCell Cardiomyocytes Plating Medium (Plating Medium) to 2,000,000 plated cardiomyocytes/ml. See the iCell Cardiomyocytes User's Guide for instructions to calculate the *Target Plating Density* based on *Plating Efficiency*.
7. Transfer the cell suspension to a sterile 1.5 ml centrifuge tube.

Plating iCell Cardiomyocytes into the MEA Plate

The following procedure details plating iCell Cardiomyocytes into a 48-well MEA plate. Instructions for 12-well MEA plates are provided in the notes.

1. Remove the fibronectin-coated MEA plate from the cell culture incubator and aspirate the fibronectin from each well. Additional rinsing is not necessary.

Note: It is recommended to aspirate fibronectin one row at a time to avoid evaporation or crystallization of the fibronectin following aspiration.

- Dispense an 8 μl /well droplet of iCell Cardiomyocytes cell suspension (approximately 16,000 plated cardiomyocytes) over the recording electrode area of the MEA plate (Figure 1).

Note: For 12-well MEA plates dispense the same volume.

Note: Timing is *critical* in this step. It is recommended to plate cardiomyocytes one row at a time. Cardiomyocyte attachment is compromised if the fibronectin is allowed to evaporate.

- Incubate the MEA plate containing iCell Cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂ for 1 hour.

Note: Sterile water may be added to the area surrounding the wells to prevent droplet evaporation.

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

- Before adding medium, load a 12-channel pipettor with sterile tips and remove tips from the positions identified in Figure 2.

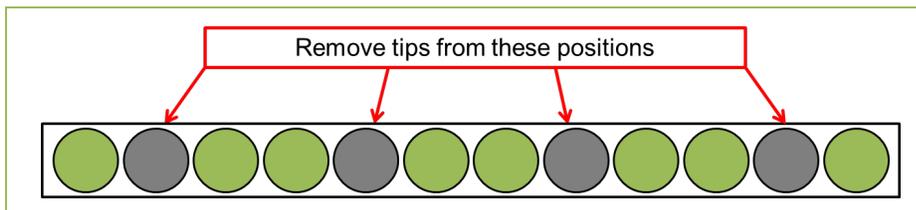


Figure 2: Tip Loading Strategy for a 12-channel Pipettor

A 12-channel pipettor loaded with sterile tips arranged in the highlighted positions (green) is suitable for medium addition to a 48-well MEA plate.

- Tilt the MEA plate at 30 degrees (Figure 1). Gently add 150 μl /well of Plating Medium down the side of the well of the MEA plate one row at a time using the 12-channel pipettor. Adding the medium too quickly will dislodge the adhered cardiomyocytes.

Note: For 12-well MEA plates dispense the same volume.

Note: Timing is *critical* in this step. Cardiomyocyte performance is compromised if the droplets are allowed to evaporate.

- Slowly return the MEA plate to a flat position on the surface of the biological safety cabinet to allow the medium to gently cover the droplet.
- Turn the MEA plate 180 degrees and slowly add an additional 150 μl /well of Plating Medium down the side of the well to reach a final volume of 300 μl /well.

Note: For 12-well MEA plates, add 850 μl /well to reach a final volume of 1 ml/well.

- Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂.

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

Maintaining iCell Cardiomyocytes on the MEA Plate

1. On day 2 post-plating, replace 100% of the Plating Medium with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium).
2. Culture iCell Cardiomyocytes on the MEA plate replacing 50% of the spent medium with Maintenance Medium every 2 - 3 days.

Note: Replace only 50% of the spent medium to avoid potential monolayer detachment.

3. Perform MEA recordings 10 - 14 days post-plating.

Data Acquisition and Analysis

Data Acquisition

Beating activity typically stabilizes 10 - 14 days after plating iCell Cardiomyocytes on an MEA plate, at which point the monolayers are suited for data acquisition. Electrical activity on the Maestro MEA system is acquired using AxIS Software according to the manufacturer's instructions.

Applying Compounds

1. Replace 50% of spent medium with the Maintenance Medium at least 2 - 4 hours before compound application.
Note: Avoid full medium replacement during compound application as it may affect beating stability.
2. Prepare test compounds in Maintenance Medium at 10X the final concentration in a cell culture plate.
3. Equilibrate the cell culture plate containing the 10X compound solutions in a cell culture incubator at 37°C, 5% CO₂.

Note: Final DMSO concentrations above 0.1% should be used with caution. Therefore, if test compounds are dissolved in DMSO, the 10X compound solutions should not exceed 1% DMSO.

4. Quickly transfer the 10X compound solutions from the cell culture plate to the MEA plate.

Data Analysis

The waveform recorded by each electrode on the MEA plate reflects the field potential at that electrode relative to ground electrodes. Raw voltage signals from the MEA plate show easily identifiable features corresponding to the depolarization and repolarization phases of the cardiomyocyte action potential. The following figure illustrates key characteristics of the MEA waveforms and performance of iCell Cardiomyocytes.

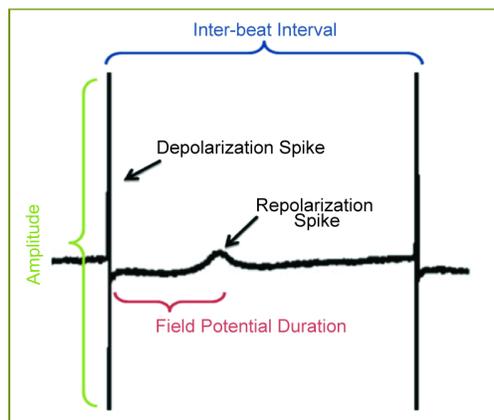


Figure 3: MEA Cardiac Field Potential and Analysis Parameters

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 is taken as the duration between depolarization and repolarization. Inter-beat interval (the inverse of frequency) is measured as the duration between the initial peaks of sequential beats.

Raw MEA data were acquired and analyzed using AxIS Software and exported to Microsoft Excel or MATLAB software for presentation. A baseline period was recorded before the addition of compounds. Compounds were then added to the wells, and the plate was equilibrated at 37°C in a cell culture incubator for 30 minutes before recording. In Figure 4, a 1-minute period following the first 3 minutes of the recording was used for analysis using AxIS Software.

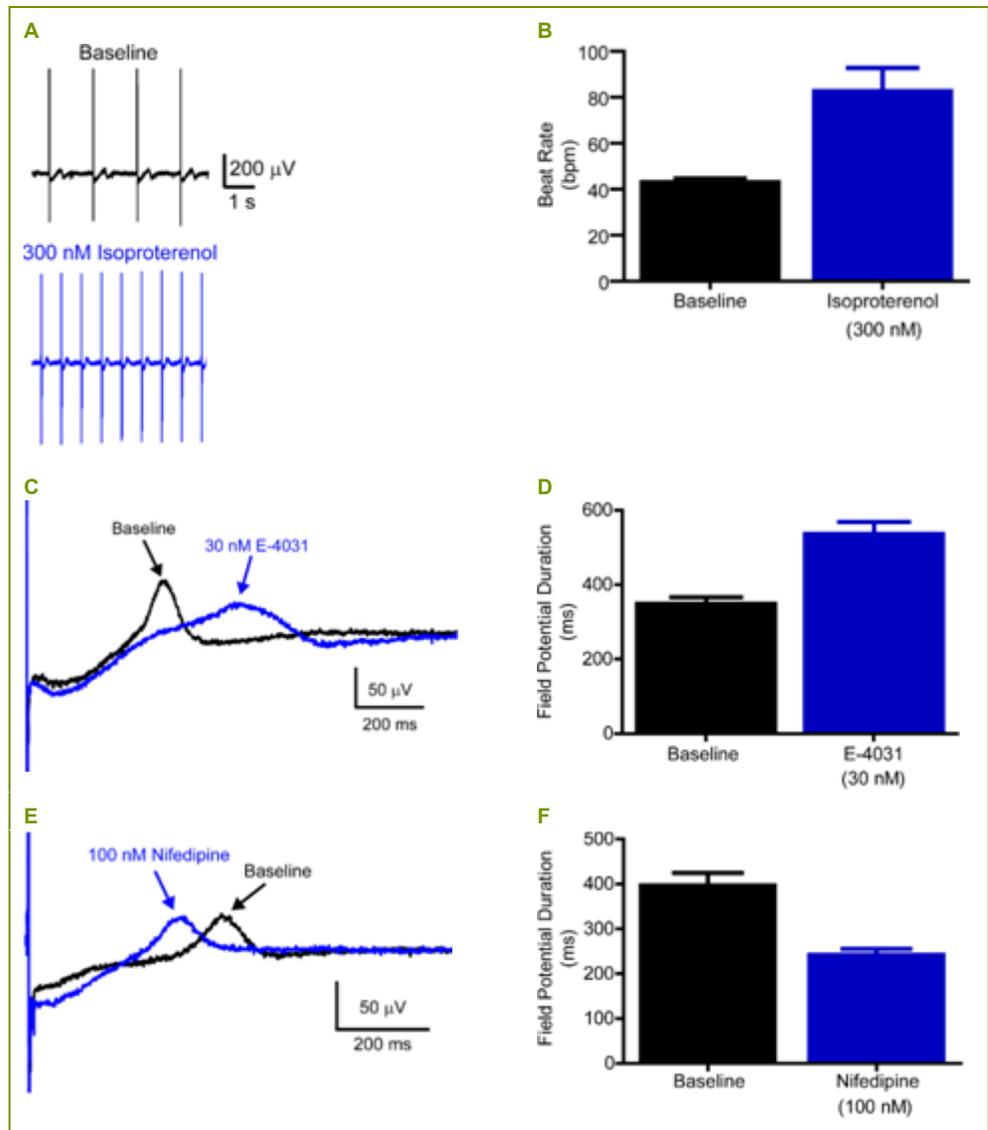


Figure 4: iCell Cardiomyocytes Activity can be Pharmacologically Modulated and Quantified

Panels A and B show the expected increase in beat rate stimulating the β -adrenergic pathway with isoproterenol. Panels C and D show the expected increase in the field potential duration blocking I_{Kr} with E-4031. Panels E and F show the expected decrease in the field potential duration blocking the L-type calcium channels with nifedipine. iCell Cardiomyocytes were exposed to the indicated compounds at the concentrations listed and the effects quantified \pm SD.

Summary

iCell Cardiomyocytes can be cultured on MEA plates where electrical activity corresponding to spontaneous beating can be monitored. The methods presented here highlight the ease of using iCell Cardiomyocytes on the Maestro MEA system. Together, these products offer a high throughput in vitro system for gathering physiologically relevant data on the electrophysiological activity of human cardiac cells.

Notes

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Version: August 2014
AP-CMCAXD140814