Measuring Cardiac Electrical Activity:

Field Potential Detection on the Maestro Multielectrode Array

Introduction

iCell® Cardiomyocytes², human cardiomyocytes derived from induced pluripotent stem cells, have been optimized for rapid recovery from cryopreservation. As an extension of the validated iCell Cardiomyocytes product line, they fully recapitulate biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. These properties combine to make iCell Cardiomyocytes² 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 Cardiomyocytes² can be cultured and maintained in MEA plates to form an electrically stable and mechanically active syncytium amenable to electrophysiological examination. Together, iCell Cardiomyocytes² 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 Cardiomyocytes² 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 Cardiomyocytes² User's Guide.

Item	Vendor	Catalog Number
Equipment		
12-channel Pipettor, 20 and 200 µl	Multiple Vendors	
Maestro Multielectrode Array (MEA) System	Axion BioSystems	
Consumables		
iCell Cardiomyocytes² Kit (Cardiomyocytes)	Cellular Dynamics International (CDI)	CMC-100-012-000.5 (0.5 unit) CMC-100-012-001 (1 unit)
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-KAP-48 (48-well)
Sterile Water	Multiple Vendors	

Item	Vendor	Catalog Number
Software		
Axion Integrated Studio (AxIS), version 2.0 or higher	Axion BioSystems	

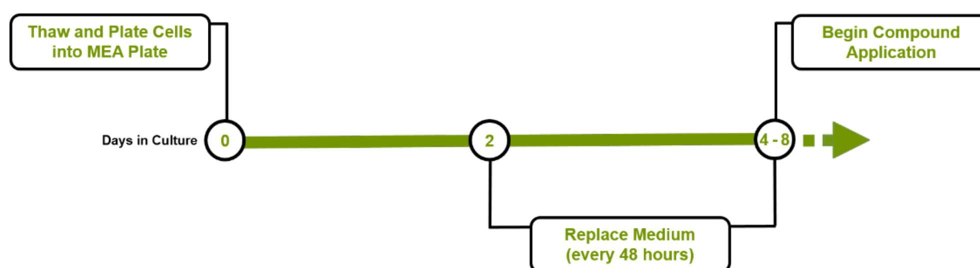
* This Application Protocol provides instructions for using 48-well MEA plates. Contact CDI's Technical Support (support@cellulardynamics.com; +1 (877) 320-6688 (US toll-free) or (608) 310-5100) for instructions for using other plate formats.

Notes

Workflow

The cardiomyocytes are thawed and plated into 48-well MEA plates previously coated with fibronectin. Replace the spent medium with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) every 48 hours. From day 4 - 8 post-plating, baseline activity is recorded, cells can be treated with compounds, and the cardiac activity recorded.

Note: *An alternative weekend-free workflow may be acceptable. Contact CDI's Technical Support for more information.*



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 a 5 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.*

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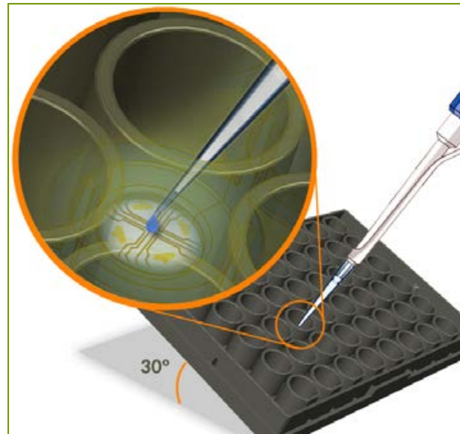

Figure 1: Droplet Placement
Tilt the MEA plate 30 degrees and dispense a 5 µl droplet over the recording electrode area of each well.

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 should be added to the area outside of the wells to prevent the droplet from evaporating.*

Thawing Cardiomyocytes

1. Thaw the cardiomyocytes according to their User's Guide.
2. Transfer the cell suspension to a 15 ml centrifuge tube.
3. Remove a sample of cells to confirm viability 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. Calculate the final volume of iCell Cardiomyocytes Plating Medium (Plating Medium) needed to resuspend the cell pellet to 10,000,000 viable cardiomyocytes/ml using the number of viable cells/vial from the Certificate of Testing. Use a 5 ml pipette to resuspend the cell pellet.
7. Transfer the cell suspension to a sterile 1.5 ml centrifuge tube.

Plating Cardiomyocytes into the MEA Plate

The following procedure details plating the cardiomyocytes into a 48-well MEA plate.

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.*

2. Dispense a 5 µl/well droplet of the cell suspension (approximately 50,000 cells) over the recording electrode area of the MEA plate (Figure 1).

   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.*

3. Incubate the MEA plate containing the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.

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

4. During the incubation, equilibrate an aliquot of Maintenance Medium in a 37°C water bath.

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

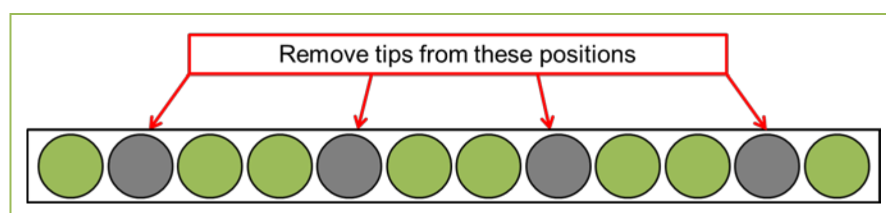

Figure 2: Tip Loading Strategy for a 12-channel Pipettor
A 12-channel pipettor loaded with sterile tips arranged in the highlighted positions (green) is suitable for medium addition to a 48-well MEA plate.

6. Tilt the MEA plate at 30 degrees (Figure 1). Gently add 150 µl/well of 37°C Maintenance 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: *Timing is critical in this step. Cardiomyocyte performance is compromised if the droplets are allowed to evaporate.*

7. 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.

8. Turn the MEA plate 180 degrees and slowly add an additional 150 µl/well of 37°C Maintenance Medium down the side of the well to reach a final volume of 300 µl/well.

9. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.

Maintaining Cardiomyocytes in the MEA Plate

1. Immediately before use, equilibrate an aliquot of Maintenance Medium in a 37°C water bath.

2. Replace 100% of the medium with 37°C Maintenance Medium on day 2 post-plating.

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3. Maintain the cardiomyocytes in the MEA plate, replacing 100% of the spent medium with Maintenance Medium every 48 hours.
4. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.
5. Perform MEA recordings from day 4 - 8 post-plating.

Data Acquisition and Analysis

Data Acquisition

Beating activity typically stabilizes on day 4 post-plating cardiomyocytes in the 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. Immediately before use, equilibrate an aliquot of Maintenance Medium in a 37°C water bath.
2. Replace the Maintenance Medium 4 - 24 hours before compound application. Tilt the MEA plate, remove the Maintenance Medium one row at a time using the 12-channel pipettor, and gently add 135 µl/well of 37°C Maintenance Medium down the side of the well twice to reach a final volume of 270 µl/well.
3. Prepare test compounds in Maintenance Medium at 10X the final concentration in a cell culture plate.
4. Equilibrate the cell culture plate containing the 10X compound solutions in a cell culture incubator at 37°C, 5% CO_2.

   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.*
5. Quickly transfer 30 µl/well of the 10X compound solutions from the cell culture plate to the MEA plate. Gently mix by pipetting 3 - 5 times.

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[2].

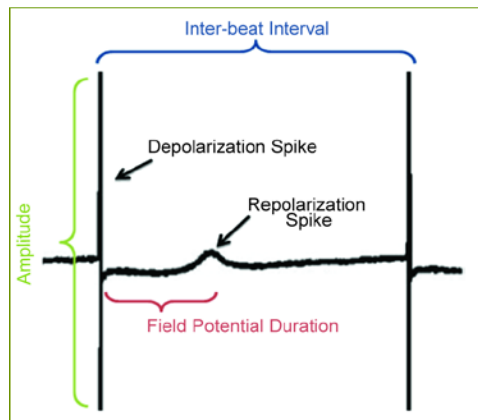

Figure 3: MEA Cardiac Field Potential and Analysis Parameters
The components of the field potential waveform represent distinct electrical behavior. The initial peaks indicate depolarization while the secondary deflection indicates repolarization. The field potential duration is taken as the duration 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.

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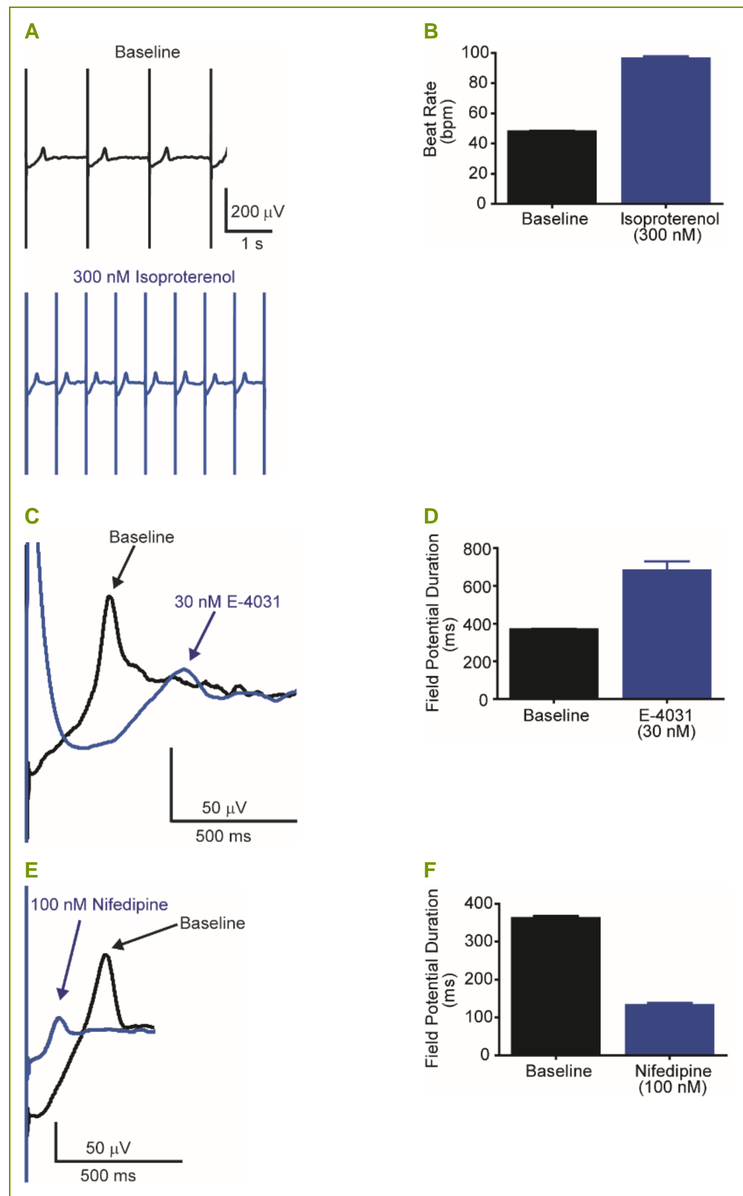

Figure 4: iCell Cardiomyocytes[2] Activity Can Be Pharmacologically Modulated and Quantified

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[2] were exposed to the indicated compounds at the concentrations listed and the effects quantified ± SEM.

Summary

iCell Cardiomyocytes[2] can be reanimated from cryopreservation directly into MEA plates where they rapidly recover to exhibit the expected electrical activity and spontaneous beating. The methods presented here highlight the ease of using iCell Cardiomyocytes[2] 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.

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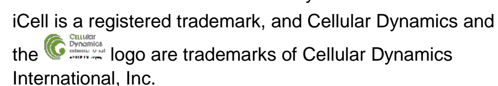
iCell is a registered trademark, and Cellular Dynamics and the  logo are trademarks of Cellular Dynamics International, Inc.

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Version: February 2016
AP-CMC2AXD160203