axion BioSystems

Cell Culture on Microelectrode Arrays

Cell Type: Cryopreserved Human iPSC-Derived Cardiomyocytes (Direct Plating)

Protocol

v. 1.2

Human iPSC-derived Cardiomyocytes from Cellular Dynamics

Introduction

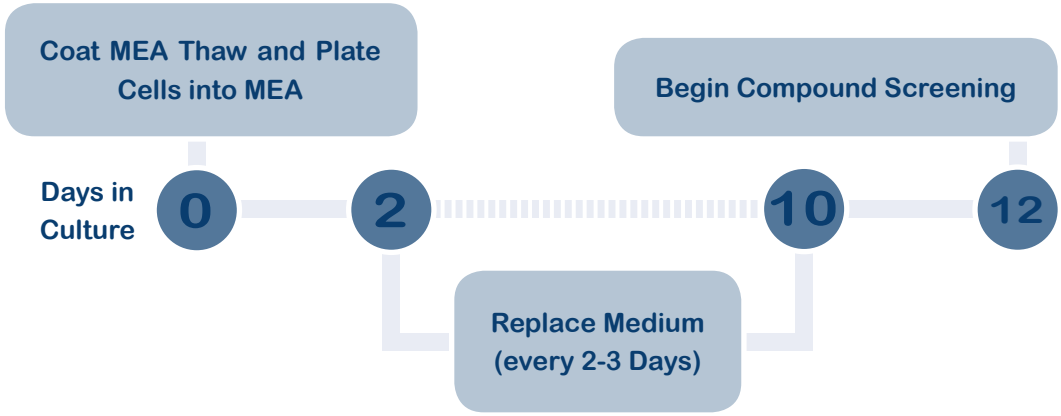
iCell Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes that exhibit typical biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Due to their human origin, high-purity, functional relevance, and ease of use, iCell Cardiomyocytes represent an optimal test system for interrogating cardiomyocyte biology in basic research and many areas of drug development.

The Maestro multielectrode array (MEA) system from Axion BioSystems is a non-invasive, label-free platform that measures local field potentials of electrically active cells, representing summed activity of underlying ion channels. iCell Cardiomyocytes can be cultured on MEAs to form an electrically and mechanically active stabile syncytium amenable to electrophysiological interrogation. Together, iCell Cardiomyocytes and Axion's MEA technology form an excellent, non-invasive platform for *in vitro* screening of compound effects on human cardiomyocyte physiology.

This Application Protocol describes how to handle iCell Cardiomyocytes for use on The Maestro MEA system and provides basic instructions for compound treatments, data acquisition, and analysis.

Workflow

iCell Cardiomyocytes are thawed into Axion 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 until day 10. From day 10-14 post-plating, baseline activity is recorded, cells can be treated with compounds, and the cardiac activity recorded on the MEA plate.



Links to Resources

Axion BioSystems
Technical Support
Applications

Notes:

Methods

Preparing the MEA Plate

1. Place a 8 µl droplet of fibronectin (1:20 in D-PBS, final concentration of 50 µg/ml) over the recording electrode area of each well in the MEA plate. See Figure 1 for appropriate drop placement.

> **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.**

2. Add 2-6 mL of sterile deionized water to the area surrounding the wells (MEA reservoirs) of the MEA plate to prevent substrate evaporation. Do not allow the water into the wells of the MEA plate.

> **Tip** **MEA reservoir water is no longer required following the media addition in Step 30.**

> **Care should be taken when using higher volumes of water in the MEA reservoirs to prevent sloshing and spilling.**

3. Incubate the fibronectin-coated MEA plate in a cell culture incubator at 37°C, for at least 60 minutes.

Culturing iCell Cardiomyocytes

4. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide.

5. Remove a sample of the cell suspension and count the cardiomyocytes using a hemocytometer to determine both the viability and total number of viable cells. Transfer the cell suspension to a 15 ml conical tube.

> **Ensure the cardiomyocytes are evenly suspended before removing an aliquot to count.**

6. Centrifuge the cell suspension at 180 x g for 5 minutes.

7. Aspirate the supernatant, being careful not to disturb the cell pellet.

8. Dilute the cell suspension 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.

Notes:

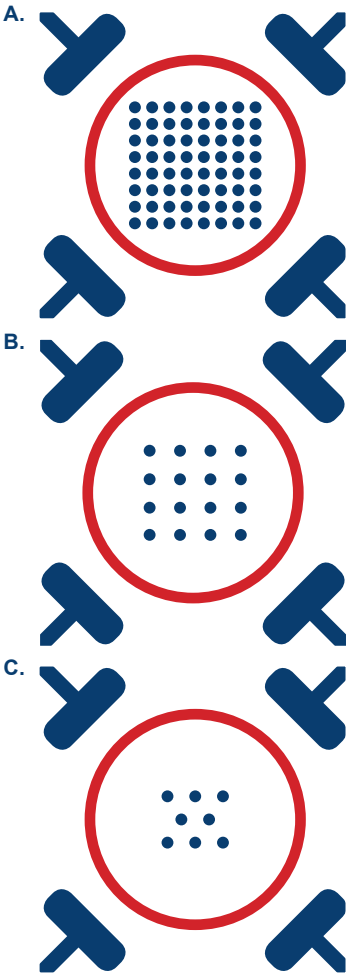


Figure 1: Drop Placement Diagram

The layouts above represent the bottom surfaces of wells in (A) a 12-well MEA, (B) a 48-well MEA, and (C) a 96-well MEA. The number of electrodes per well is different across the plate formats, however the drop placement is the same, with the drop (red circle) centered on the recording electrodes and staying within the ground electrodes.

9. Transfer the cell suspension to a sterile 1.5 ml Eppendorf tube.

<u>Plating iCell Cardiomyocytes onto the MEA</u>

10. Aspirate, from a single row or column, most of the fibronectin solution from the MEA surface.

11. Place a 8 µl droplet of iCell Cardiomyocytes suspension (approx. 16,000 cardiomyocytes) over the recording electrode area of each well. See Figure 1 for appropriate drop placement.

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 the well should then be ignored as cardiomyocyte attachment will be suboptimal.

12. Repeat Steps 10 and 11 until all rows or columns have been plated.

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

14. Gently add 150 µl of Maintenance Medium to each well of the MEA. Adding medium too quickly will dislodge the adhered cardiomyocytes.

Using a pipettor, add medium first in a semi-circle along the outer edge of the flat bottom area. Progressively add medium so it fills evenly towards the center, stopping before contact is made with the droplet in the center. Gently bridge the gap with additional medium. The goal is to prevent a rush of medium in either direction that might dislodge the cardiomyocytes.

15. Slowly add the remaining volume to reach the plate recommended media volume. Recommended well volumes for each plate type: **12-well = 500µL, 48-well = 300µL, 96 well = 200µL**.

16. Incubate in a cell culture incubator at 37°C, 5% CO_2.

17. On day 2 post-plating, replace 100% of the Plating Medium with iCell-Cardiomyocytes Maintenance Medium (Maintenance Medium).

18. Culture iCell Cardiomyocytes on the MEA plate replacing 50% of the spent medium with Maintenance Medium every 2-3 days.

19. For optimal results, perform MEA recordings 10-14 days after plating.

Notes:

Data Acquisition & Analysis

Electrical activity of iCell Cardiomyocytes in Axion MEA plates is acquired using the Maestro recording instrument and AxIS Software. Product manuals can be downloaded at www.axion-biosystems.com. The waveform recorded by each electrode on the multi-well MEA plate reflects the field potential at that electrode relative to ground electrodes. Raw voltage signals from MEAs show easily-identifiable features corresponding to the depolarization and repolarization phases of the cardiomyocyte action potential. The following figures illustrate key characteristics of the MEA waveforms and performance of iCell Cardiomyocytes.

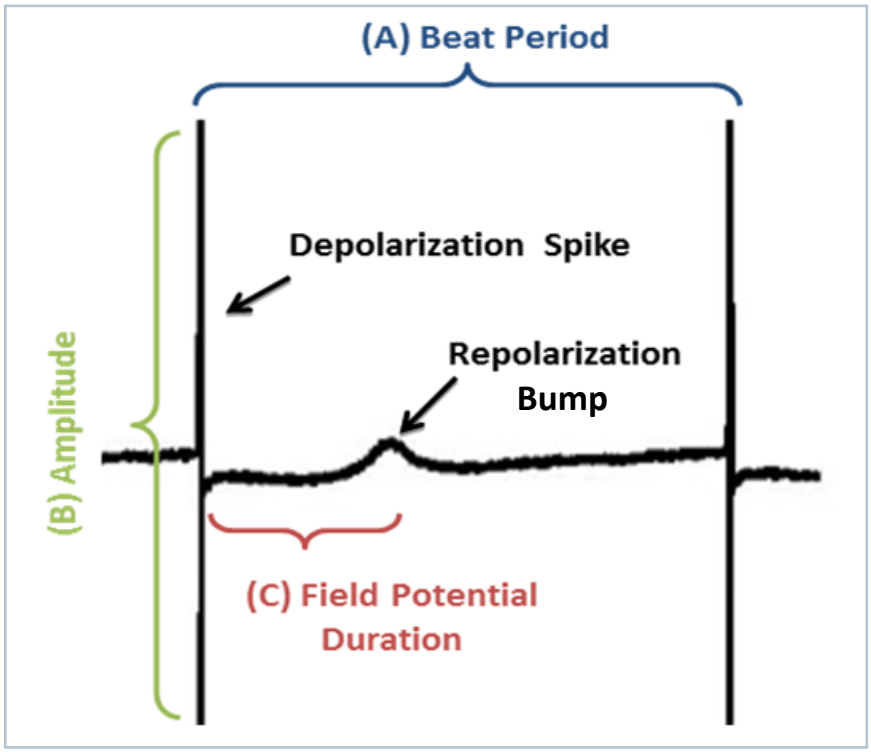


Figure 3. Example Cardiac Field Potential and Analysis Parameters

The depolarization and repolarization phases are labeled on the example cardiac spike complex in Fig. 3, along with corresponding regions for beat period (A), amplitude (B) and field potential duration (C). For the following figures MEA data were acquired and analyzed using AxIS Software and exported to Microsoft Excel or MATLAB for presentation. A 1 min baseline recording was made before the addition of compounds. Compounds were then added to the wells and the plate was placed in an incubator for 30 minutes before recording. For Figures 3, 4 and 5, a 1 minute period following the first 15 seconds of the recording after dosing was used for analysis in AxIS.

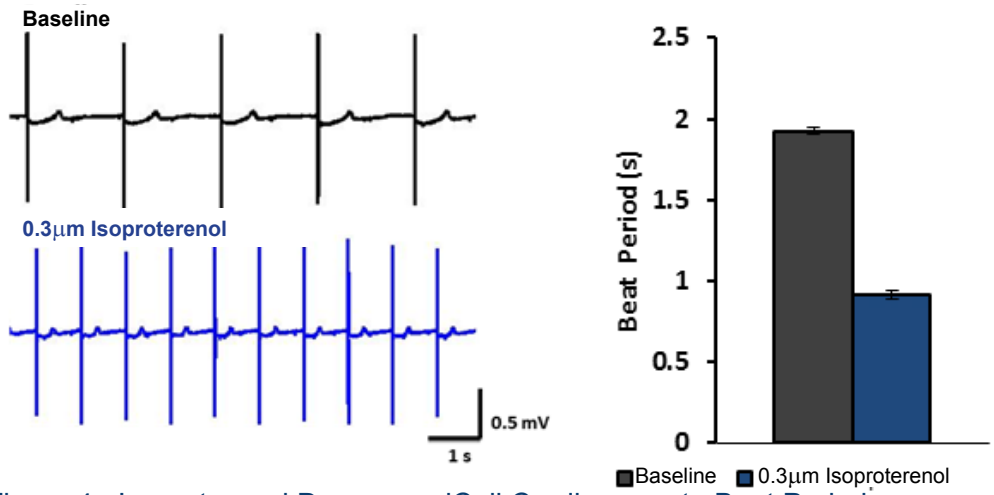


Figure 4. Isoproterenol Decreases iCell Cardiomyocyte Beat Period

On the left are example traces from iCell Cardiomyocytes for a baseline period (black) and a period of increased beat rate 30 minutes after addition of 0.3 µM isoproterenol. At right are the mean beat period ± SD for the two conditions.

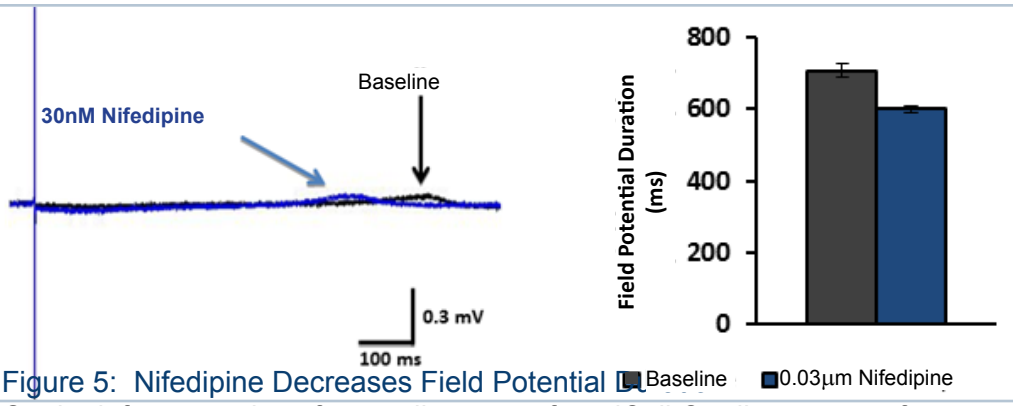


Figure 5: Nifedipine Decreases Field Potential D

On the left, an overlay of two spike traces from iCell Cardiomyocytes from a pre-dose baseline period (black) and a period 30 minutes after addition of 30 nM nifedipine. On the right, field potential duration (mean ± SD) from two wells before (gray bar) and after (blue bar) addition of 30 nM nifedipine.

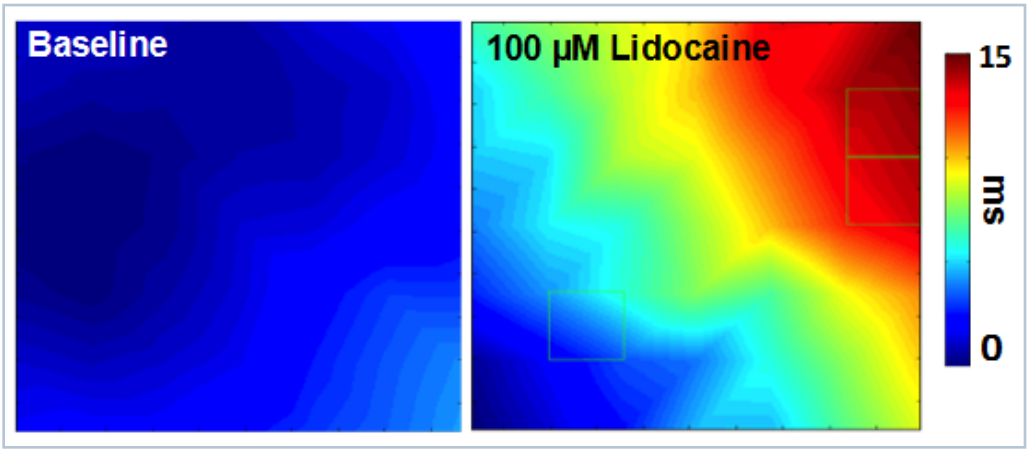


Figure 6: MEA Propagation Maps for Spontaneous Action Potentials in Cultured iCell Cardiomyocytes

For each beat, latency from the time at which an action potential is first detected is given a color-scale, illustrating signal conduction patterns. Boxes represent electrodes where action potential amplitude was sub-threshold, and latency is thus interpolated. In this example, application of 100 µM lidocaine decreases the conduction velocity across the network compared to the predose condition.

Summary

iCell Cardiomyocytes can be cultured on MEAs where electrical activity corresponding to spontaneous beating can be monitored. The waveform characteristics representing cardiomyocyte depolarization, repolarization, automaticity, and propagation can be readily measured, and their modulation by cardioactive compounds can be robustly quantified. The methods and data presented here highlight the ease of using iCell Cardiomyocytes on The Maestro MEA platform from Axion BioSystems. Together, these products offer a high throughput in vitro system for gathering physiologically relevant data on the electrophysiological activity of human cardiac cells.

Required Materials

Consumables

Item	Vendor	Catalog Number
iCell Cardiomyocytes Kit	Cellular Dynamics International	CMC-100-010-001
Dulbecco's PBS without Ca^{2+} and Mg^{2+}	Life Technologies	14040
Fibronectin	Roche	11051407001
KimWipes	Various	
Pipettes and Pipettors	Various	
1.5 mL Eppendorf Tubes	Various	
15 mL and 50 mL Centrifuge Tubes	Various	
Pipette Aid and Sterile Pipettes	Various	
Sterile 70% Ethanol	Various	

Equipment

Item	Vendor	Catalog Number
Maestro MEA System	Axion BioSystems	
12-Well MEA	Axion BioSystems	M768-GLx
48-Well MEA	Axion BioSystems	M768-KAP
96-Well MEA	Axion BioSystems	M768-KAP-96
Axion Integrated Studio (AxIS)	Axion BioSystems	
37°C Water Bath	Various	
Cell Culture Incubator	Various	
Hemocytometer or Automated Cell Counter	Various	
Biological Safety Cabinet	Various	
Tabletop Centrifuge	Various	
Phase Contrast Microscope	Various	
Liquid Nitrogen Storage	Various	

Trademarks

Axion BioSystems, Inc. and the  logo are trademarks of Axion BioSystems, and may not be used without the express written permission of Axion BioSystems, Inc.

All other brands, product names, company names, trademarks and service marks are the properties of their respective owners.

Restrictions and Liabilities

This document is provided “as is” and Axion BioSystems will assume no responsibility for any typographical, technical or other inaccuracies in this document. Axion BioSystems does not make any commitment to provide any changes, updates, enhancements, or other additions to this document to you within any time frame or at all.

This document might contain references to third-party sources of information, hardware or software, products or services and/or third-party websites (collectively the “Third-Party Information”). Axion BioSystems has no control over and is not responsible for any Third-Party Information, including, without limitation the content, accuracy, copyright compliance, compatibility, performance, trustworthiness, legality, decency, links or any other aspect of Third-Party Information. The inclusion of Third-Party Information in this document does not imply endorsement with your use of Axion BioSystems technology.

Conditions of Use

You are responsible for understanding and performing the protocols that are described within. Axion BioSystems makes no guarantee for any results you may achieve. These protocols are provided as a recommendation by Axion BioSystems based on its use and experience.

Origin

Axion BioSystems Microelectrode Arrays are manufactured in the United States of America.

Copyright Notice

Acknowledgement

Axion BioSystems would like to thank Cellular Dynamics International, Inc. for providing their experience and resources toward the creation of this application note.