iCell Cardiomyocytes^2 Application Protocol Multiwell-MEA

Measuring Cardiac Electrical Activity: Field Potential Detection on the Multiwell-MEA-System (Multi Channel Systems MCS GmbH)

Introduction

iCell® Cardiomyocytes^2 are human cardiomyocytes derived from induced pluripotent stem cells. They have been optimized for rapid recovery from cryopreservation. They fully recapitulate biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. These properties combine to make iCell Cardiomyocytes^2 an optimal in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

Multi Channel Systems MCS GmbH's microelectrode array (MEA) technology enables non-invasive, label-free measurements of local field potentials of electrically active cells including neurons, islets of Langerhans and cardiomyocytes and thus to decipher the function and dysfunction of the underlying ion channels. iCell Cardiomyocytes^2 can be cultured on MEA plates to form an electrically stable and mechanically active syncytium amenable to electrophysiological examination. Together, iCell Cardiomyocytes^2 and the Multi Channel Systems 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^2 for use on the Multi Channel Systems Multiwell system using the 96-well MEA plate.

Required Equipment, Consumables, and Software

The following equipment, consumables, and software are required in addition to the materials specified in the iCell Cardiomyocytes^2 User's Guide

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Non-profit foundation. Authority of foundation: Regional council of Tuebingen, Ref. No. 0563-16 RT, DE146484816,
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Item	Vendor	Catalog Number
Equipment		
8-channel Pipettor, 20-200 µl	Multiple Vendors	
1-channel Pipettor, 0.5-10 µl	Multiple Vendors	
Low adhesion pipette tips 10 µl	Carl Roth	PC91.1
Multiwell-System	Multi Channel Systems MCS	
Storage box	Multiple Vendors	
Optional: Crème-brulé Bunsen burner	Multiple Vendors	
Consumables		
iCell Cardiomyocytes² Kit (Cardiomyocytes)	Cellular Dynamics International (CDI)	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)	Life Technologies	14190-094
Fibronectin (human)	Roche	1105 1407001
Multiwell MEA Plates	Multi Channel Systems MCS	96W700/100F
Sterile Water	Multiple Vendors	
Software		
Multiwell-Screen v. 1.5.5.0 and above	Multi Channel Systems MCS	http://www.multichannelsystems.com/software/multiwell-screen
Optional further analysis software of your choice	Multiple Vendors	

Workflow

The cardiomyocytes are thawed and plated into 96-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.

Methods

Preparing the MEA Plate

All tasks need to be performed under sterile conditions.

1) Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20°C. This is the stock solution.
2) Prepare a 50µg/ml fibronectin solution by diluting stock fibronectin solution 1=20 in D-PBS immediately before use.
3) Optional: Using a Crème-brulé Bunsen burner and a forceps to soften a 10 µl-tip . Kink the tip to approximately 30-40°. This angle will make placing Fibronectin on the MEA plate ergonomically (Fig. 1).
4) Tilt the MEA plate at an angle so that the bottom of each well is visible. Dispense a 4 µl/droplet of fibronectin solution over the recording electrode area of the well of the MEA plate.

   Note:
   a) Even though gold electrodes are robust try to avoid touching the electrode area to prevent damaging. Ideally form a drop on the tip of the pipette and place this onto the electrode field.
   b) avoid to bewet the reference electrode which is the golden ring-shaped structure at the bottom of each well.

5) On the bottom of a storage box plainly place a paper towel and add 5-10 ml distilled water to it (Fig. 2). Place the Multiwell plate in it and loosely place put the lid on top of the box. Incubate for a minimum of 1 h in an incubator at 37°C.

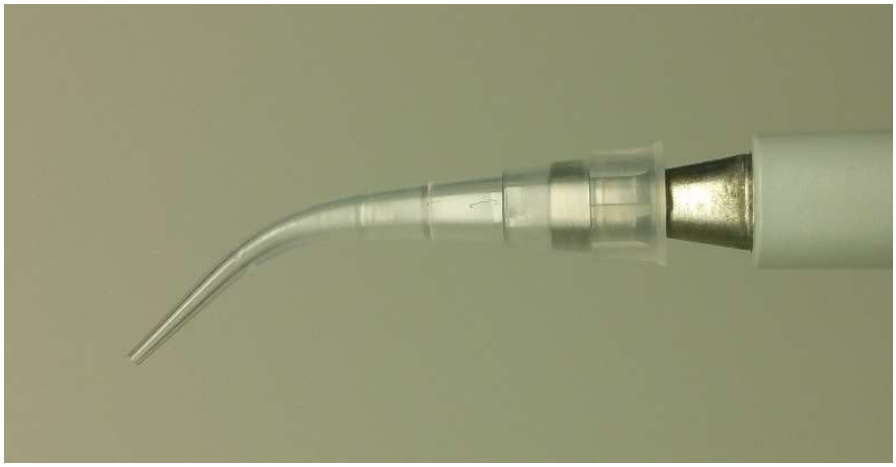

Fig. 1 Kinked 10 µl pipette. The tip was softened using a crème-brule burner and a forceps.

Fig. 2 Exemplary storage box with paper towel, holding 3 Multiwell plates

Thawing Cardiomyocytes^2

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 9,000,000 viable cardiomyocytes/ml using the number of viable cells from the certificate of testing. Alternatively use the value from the performed viability test (s. #3) as reference. Use a 1 ml pipette to carefully resuspend the cell pellet.
7) Transfer the cell suspension to a sterile 1.5 ml centrifuge tube.

Plating Cardiomyocytes^2 into the MEA Plate

The following procedure details plating the cardiomyocytes into a 96-well MEA plate.

1) Remove the fibronectin-coted MEA plate from the cell culture incubator and aspirate the fibronectin from each well (optionally using a kinked pipette tip). A rinsing step is not necessary.

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

2) Dispense a 4 µl/well droplet from the cell suspension (approx. 36,000 cells/well) over the recording electrode area of the MEA plate (optionally using a kinked pipette tip). Again try to avoid plating onto the reference electrode (Fig. 3).

   Note:
   Timing is critical in this step. It is highly recommended to plate cardiomyocytes one row at a time. Cell attachment is compromised if the fibronectin is allowed to evaporate (recognizable by the formation of crystalline structures at the bottom of the well).

3) Again put the plate in the storage container and loosely put the lid on. Store the MEA plate in a cell culture incubator at 37°C at 5% CO_2 for 1 hour.
4) During incubation, equilibrate an aliquot of Maintenance Medium in a 37°C water bath.
5) Remove the MEA plate from the incubator. Using an 8-channel pipettor gently add 200 µl of Maintenance Medium down to the side of the well of the MEA plate one row at a time. For this carefully place the tips at the edge between bottom and side of the wells and add the medium (approx. within 2 sec. per load), carefully lifting the pipettor during aspiration.

   Note:
   Timing is critical in this step. Cardiomyocyte performance is compromised if the droplets are allowed to evaporate.
   Adding the medium too quickly will dislodge the adhered cells.

6) Transfer the plate into the incubator (avoid major vibrations). Culture the cardiomyocytes at 37°C, 5% CO_2.

Maintaining Cardiomyocytes^2 in the MEA Plate

1) Immediately before use, equilibrate an aliquot of Maintenance Medium (approx. 20 ml) in a 37°C water bath.
2) Replace 190 µl/well with 37°C Maintenance Medium on day 2 post-plating.
3) Maintain the cardiomyocytes in the MEA plate, replacing 190 µl/well of the spent medium with Maintenance Medium every 48 hours
7) Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.
8) Perform MEA recordings between day 4-7 post-plating

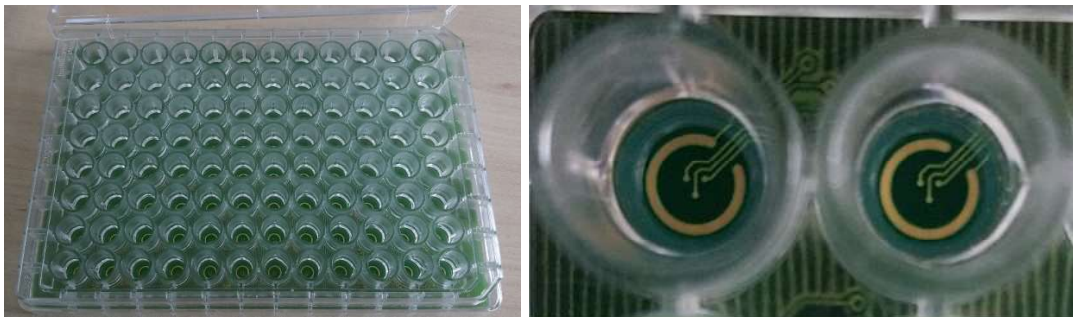

Fig. 3 96-well Multiwell plate. Left: Overview, right: Microscopic view into two wells. Note the three electodes in the center of the well and the circular reference electrode. Cells ideally are only plated in the center without covering the reference electrode.

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Data Acquisition and 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 repolarization and repolarization phases of the cardiomyocyte action potential. The following figure illustrates key characteristics of the MEA waveforms and performance of iCell Cardiomyocytes^2.

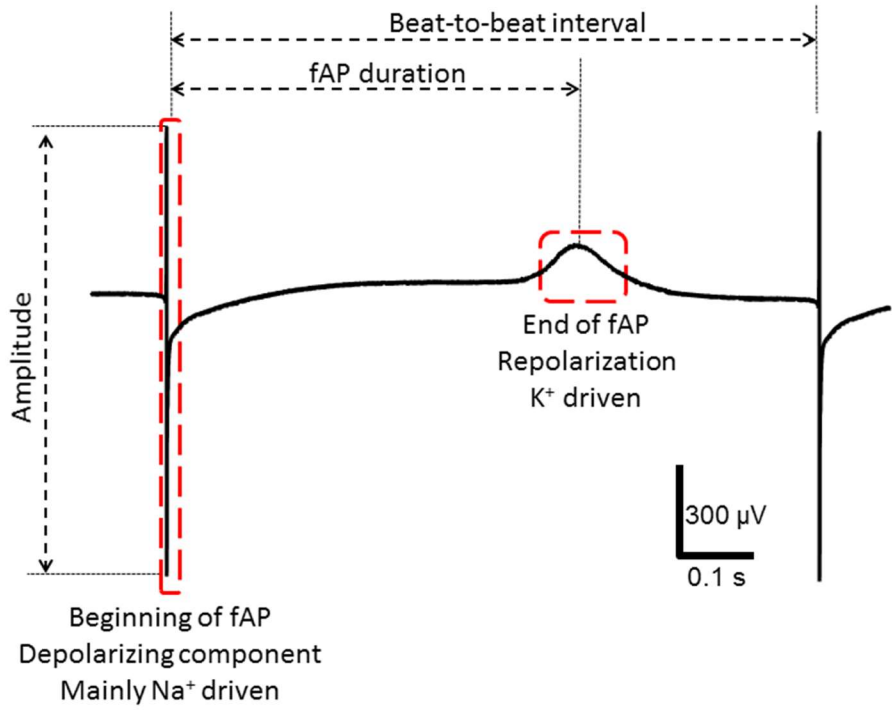

Fig. 4 Cardiac field action potential (fAP). The initial and very rapid voltage deflection represents the depolarization of the cardiomyocytes, mainly driven by activation of voltage activated Na+ channels. The small deflection after approx. 400 ms represents the repolarization of the cells at the end of the action potential, mainly generated by the flux of K+ ions across the membrane via the hERG channel. The time between the two deflections defines the fAP duration. The time interval between two action potentials defines the beat rate.

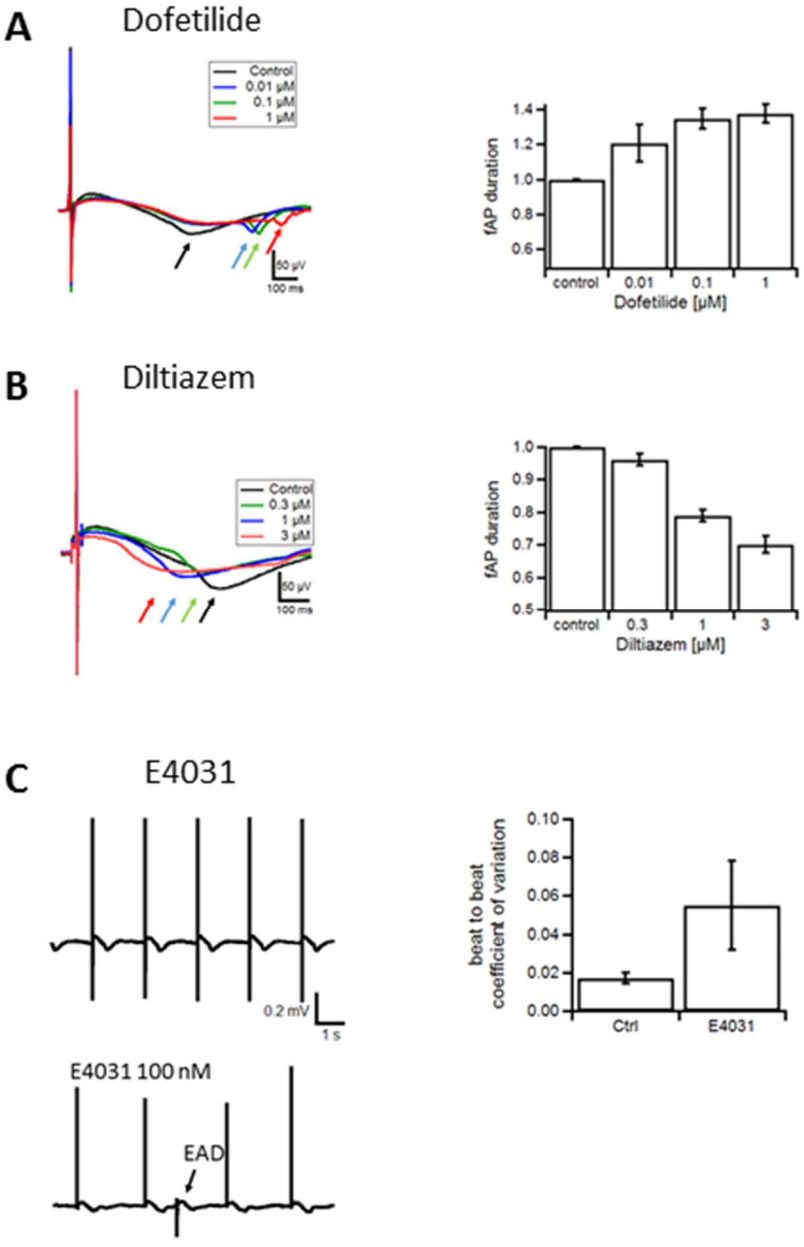

Fig. 5 Modulation of electrophysiological properties of iCell cardiomyocytes^2 by application of cardioactive compounds. A: Dofetilide, known to inhibit the hERG ion channel, concentration-dependently prolongs the duration of the fAP. B: Inhibition of the L-type Ca^{2+} channel by Diltiazem shortens the fAP duration. C: Application of the hERG inhibitor E4031 not only prolongs the fAP duration (not shown) but also expresses a proarrhythmic potential, indicated by an increase of the beat-to-beat variability and the occurrences of early afterdepolarizations (EAD).

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 Multi Channel Systems MCS Multiwell 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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We are core site of the CiPA Myocyte Team
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