Measuring Cardiac Activity:

Impedance Detection with xCELLigence RTCA Cardio System

Introduction

iCell® Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes that recapitulate the 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 in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

The xCELLigence RTCA Cardio System (RTCA Cardio system) is a non-invasive, label-free platform that utilizes impedance changes across the cardiac monolayer to measure indirectly cardiomyocyte viability, contractility, and electrical activity. iCell Cardiomyocytes can be cultured and maintained on an E-Plate for extended durations, thus enabling measurement of acute and sub-acute drug-induced effects. Together, iCell Cardiomyocytes and the RTCA Cardio system offer an excellent 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 RTCA Cardio system and provides basic instructions for compound treatments, data acquisition, and analysis.

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.

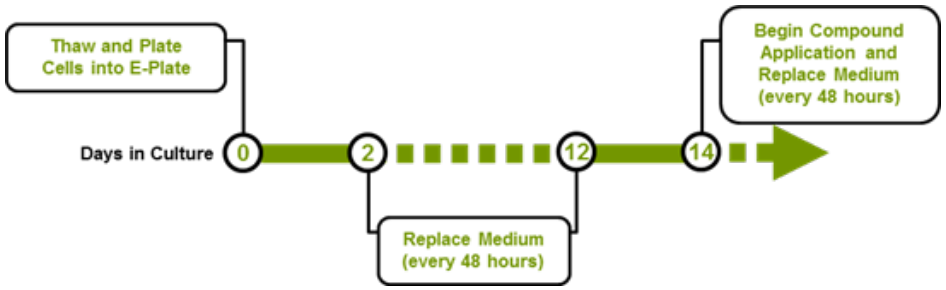
Item	Vendor	Catalog Number
Equipment		
12-channel Multichannel Pipettor (20 and 200 µl)	Multiple Vendors	
xCELLigence RTCA Cardio System	ACEA Biosciences	
Consumables		
iCell Cardiomyocytes Kit	Cellular Dynamics International (CDI)	CMC-100-010-001 CMC-100-010-005
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Invitrogen	14190
E-Plate Cardio 96 (E-Plate)	ACEA Biosciences	06417051001 06417035001
Fibronectin	Roche Applied Science	11051407001 11080938001
Sterile 50 ml Conical Tubes	Multiple Vendors	

Item	Vendor	Catalog Number
Sterile Reagent Reservoirs	Multiple Vendors	
Software		
RTCA Cardio Instrument Software	ACEA Biosciences	

Notes

Workflow

iCell Cardiomyocytes are thawed and plated into an E-Plate previously coated with fibronectin. From day 2 post-plating, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) every 48 hours. From day 14 post-plating, cells can be treated with compounds, and the cardiac activity recorded.



Methods

Preparing the E-Plate

The E-Plate is prepared the day of plating iCell Cardiomyocytes.

1. Dilute 1 mg/ml fibronectin solution in sterile D-PBS to a final concentration of 10 µg/ml 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. Add 50 µl/well of the 10 µg/ml fibronectin solution to the E-Plate to evenly coat the bottom of the well.
3. Incubate at 37°C for at least 1 hour.

Culturing iCell Cardiomyocytes

1. Aspirate the fibronectin solution from the E-Plate. Immediately add 50 µl/well of 37°C iCell Cardiomyocytes Plating Medium (Plating Medium).
2. Equilibrate the E-Plate in a cell culture incubator at 37°C, 5% CO_2 for 5 - 10 minutes.
3. Record a background measurement according to the RTCA Cardio Instrument Operator's Guide.
4. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide to a final volume of 5 ml Plating Medium, and count the cells.

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5. Dilute the cell suspension in Plating Medium to a final concentration of 400,000 plated cells/ml. See the User's Guide for instructions to calculate the *Target Plating Density* based on *Plating Efficiency*.
6. Remove the E-Plate from the RTCA Cardio Instrument and equilibrate to room temperature for 5 - 10 minutes.
7. Add 50 µl/well of the iCell Cardiomyocytes cell suspension (20,000 plated cells/well) using a multichannel pipettor.
8. Leave the E-Plate undisturbed in the biological safety cabinet at room temperature for 20 - 30 minutes to allow the cardiomyocytes to settle and ensure an even distribution.
9. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2 for 48 hours.

   Note: *Place the E-Plate in a low traffic incubator and away from the door to minimize fluctuations in temperature and air movement. Minimize opening the incubator's door during the first 24 hours.*
10. Replace the spent medium with Maintenance Medium every 48 hours. Tilt the E-Plate, remove the spent medium using a multichannel pipettor, and gently add 100 µl/well of 37°C Maintenance Medium to the side of the well to avoid disturbing the cardiomyocyte monolayer.

    Note: *Do not allow the pipettor tips to touch the bottom of the well during medium removal or addition. Medium replacement may cause transient alterations to beating rhythm. Allow normal beating patterns to recover after medium replacement prior to drug application.*
11. Maintain the cardiomyocytes for 14 days, replacing medium every 48 hours.

Data Acquisition and Analysis

The RTCA Cardio Instrument Software (RTCA software) offers a wide variety of options for data acquisition and analysis. The instructions here are meant to provide a general guidance. See the RTCA Cardio Instrument Operator's Guide for specific instructions.

The beating pattern stabilizes approximately 12 - 14 days post-plating iCell Cardiomyocytes to the E-Plate. The amplitude may continue to increase with prolonged culture times. The optimal time window to perform an assay is between approximately 12 and 14 days post-plating.

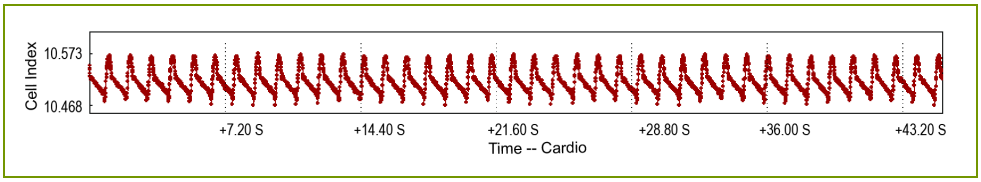

Figure 1: Stably Beating iCell Cardiomyocytes on the RTCA Cardio System
Cardiomyocytes typically beat between 30 and 50 beats per minute, with a change in amplitude greater than 0.03 cell index units and a beating rhythm irregularity less than 10% per well.

Applying Compounds

1. Replace the Maintenance Medium 2 - 4 hours before recording. Tilt the E-Plate, remove the Maintenance Medium using a multichannel pipettor, and gently add 90 µl/well of Maintenance Medium to the side of the well to avoid disturbing the cardiomyocyte monolayer.

   Note: *Evaporation rates can vary across the E-Plate. Changing the Maintenance Medium before compound treatment is required to ensure uniform medium volumes across the E-Plate.*

2. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.
3. Monitor the activity of the cardiomyocytes on the E-Plate to ensure regular beating rate and stable whole-peak amplitude values are reached.
4. Prepare test compounds in Maintenance Medium at 10X the final concentration in a regular 96-well cell culture plate.

   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. Equilibrate the 96-well cell culture plate containing the 10X compound solutions in a cell culture incubator at 37°C, 5% CO_2.
6. Quickly transfer 10 µl/well of the 10X compound solutions from the 96-well cell culture plate to the E-Plate.

   Note: *Beating rate and amplitude are temperature-dependent. The E-Plate should not be kept outside the incubator for more than 5 minutes while compounds are added.*

Data Acquisition and Analysis Using the RTCA Software

See the RTCA Cardio Instrument Software Guide for specific instructions on using the RTCA software for data acquisition and analysis.

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Example Data

Beating rate, amplitude, and beating rhythm irregularity were calculated with the RTCA software. Data were normalized to the last measurement point before compound treatment and averaged across the replicate wells. Results displayed in Figures 2 and 3 were obtained with 1-minute recordings acquired 60 minutes after drug treatment.

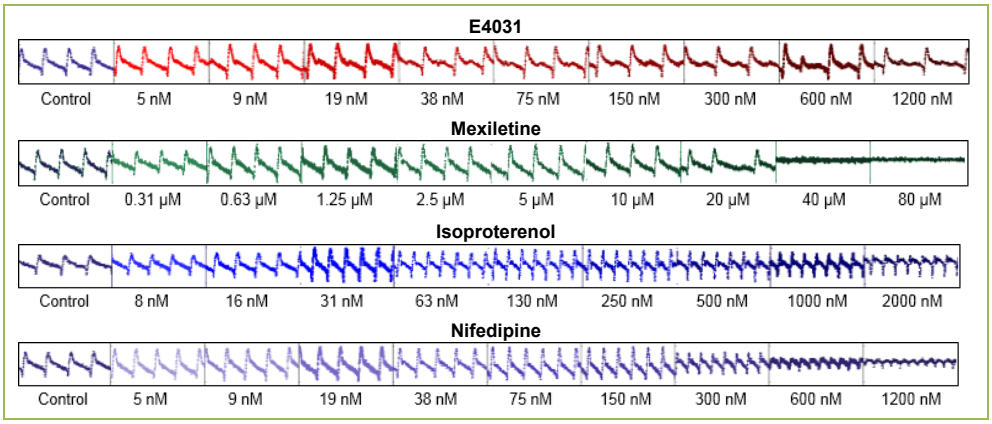

Figure 2: Representative Effects of Cardioactive Drugs on iCell Cardiomyocytes
Modulating ion channel and GPCR activity alters the spontaneous contractile activity of iCell Cardiomyocytes. Blocking I_{Kr}, $I_{Ca\text{-}L}$, and I_{Na} with E4031, nifedipine, and mexiletine and stimulating the β-adrenergic pathway with isoproterenol produced the expected effects on the beat waveforms.

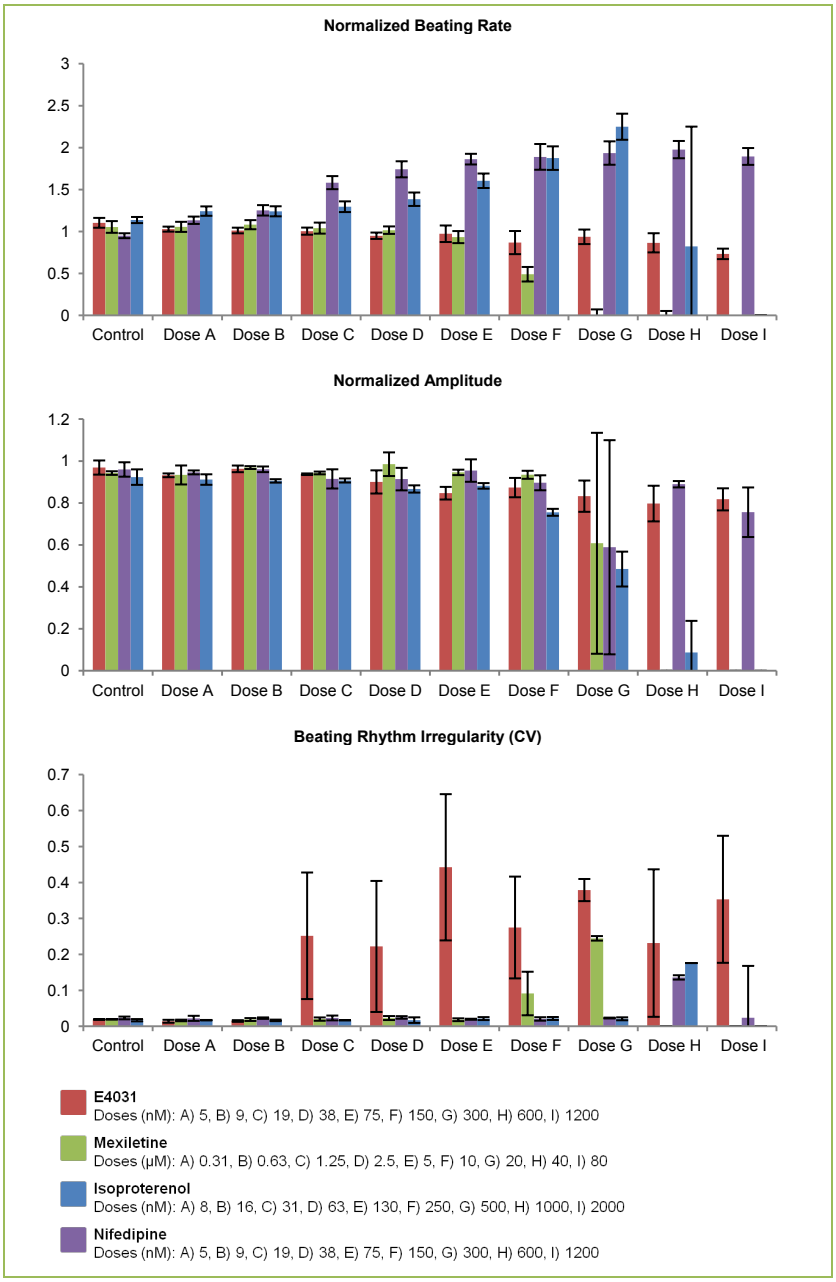

Figure 3: Quantitative Effects of Cardioactive Drugs on iCell Cardiomyocytes
Blocking I_{Kr}, $I_{Ca\text{-}L}$, and I_{Na} with E4031 (red), mexiletine (green), and nifedipine (purple) and stimulating the β-adrenergic pathway with isoproterenol (blue) produced the expected effects on beating rate, amplitude, and rhythmicity (mean ± SD; n = 3 wells for each condition).

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Summary

iCell Cardiomyocytes provide an in vitro test system that recapitulates native human cardiac myocyte physiology and function while the xCELLigence RTCA Cardio System provides a label-free technology for non-invasive monitoring of cardiomyocyte behavior and viability. The methods and results presented here highlight the ease of use with which robust and relevant data can be gathered on human cardiomyocyte viability, electrical activity, and contractility. Together these tools bring 96-well based, real-time, predictive assessments of compound efficacy, potency, and toxicity on human cardiomyocytes to the drug development process.

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