Measuring Cardiac Activity:

Impedance and Extracellular Field Potential Detection with CardioExcyte 96 System

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.

The CardioExcyte 96 system is a non-invasive, label-free platform that combines field potential and impedance recording for simultaneous measurement of electrical and contractile activity, respectively. This platform allows comprehensive evaluation of viability, contractility, and electrical activity involved in excitation-contraction (EC) coupling across the cardiomyocyte monolayer. iCell Cardiomyocytes² can be cultured and maintained in a CardioExcyte 96 sensor plate for extended durations, thus enabling measurement of acute and sub-acute drug-induced effects. Together, iCell Cardiomyocytes² and the CardioExcyte 96 system offer an excellent platform for in vitro screening of compound effects on human cardiomyocyte physiology.

This Application Protocol describes how to handle iCell Cardiomyocytes² for use on the CardioExcyte 96 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 Cardiomyocytes² User's Guide.

Item	Vendor	Catalog Number
Equipment		
8-channel Multichannel Pipettor, 20 and 200 µl	Multiple Vendors	
CardioExcyte 96 System	Nanion	
Consumables		
iCell Cardiomyocytes² Kit (Cardiomyocytes)	Cellular Dynamics International (CDI)	CMC-100-012-000.5 (0.5 unit) CMC-100-012-001 (1 unit)
Dulbecco's Phosphate Buffered Saline with Ca^{2+} and Mg^{2+} (D-PBS)	Invitrogen	14040
CardioExcyte 96 Sensor Plate (CE96 Plate)	Nanion	

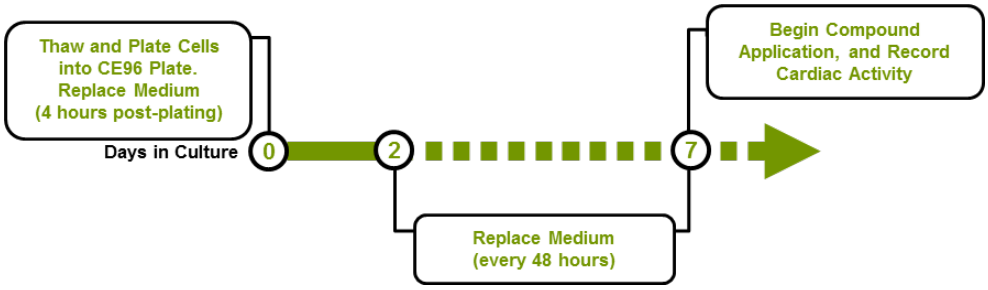
Item	Vendor	Catalog Number
Fibronectin	Roche Applied Science	11051407001 11080938001
Sterile Reagent Reservoirs	Multiple Vendors	
Software		
CardioExcyte Control Software	Nanion	
DataControl96 Software	Nanion	

Notes

Workflow

The cardiomyocytes are thawed and plated into a CE96 plate previously coated with fibronectin. 4 hours post-plating and every 48 hours thereafter, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium). From day 7 post-plating, 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 (support@cellulardynamics.com; +1 (877) 320-6688 (US toll-free) or (608) 310-5100) for more information.*



Methods

Preparing the CE96 Plate

The CE96 plate is prepared the day of plating 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 center of the wells of a CE96 plate to evenly coat the bottom of the well.
3. Incubate at 37°C for at least 1 hour.

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Thawing Cardiomyocytes

1. Aspirate the fibronectin solution from the CE96 plate. Immediately add 50 µl/well of 37°C iCell Cardiomyocytes Plating Medium (Plating Medium) to the center of the wells.

   Note: *Do not allow the fibronectin-coated surface to dry.*

2. Equilibrate the CE96 plate in a cell culture incubator at 37°C, 5% CO_2 for 5 - 10 minutes.
3. Thaw the cardiomyocytes according to their User's Guide to a final volume of 5 ml Plating Medium by diluting the 1 ml cell suspension from the cryovial in 1 ml of Plating Medium rinse and 3 ml of additional Plating Medium.
4. Remove a sample of cells to confirm viability using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
5. Calculate the final volume of Plating Medium needed to obtain a final cell plating density of 1 x 10^6 viable cardiomyocytes/ml using the number of viable cells/vial from the Certificate of Testing.
6. Remove the CE96 plate from the incubator and equilibrate to room temperature for 5 - 10 minutes.
7. Add 50 µl/well of the cell suspension (50,000 cells/well) to the center of the wells using a multichannel pipettor.
8. Leave the CE96 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 the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2 for 4 hours.

   Note: *Place the CE96 plate in a low traffic incubator and away from the door to minimize fluctuations in temperature and air movement.*

Maintaining Cardiomyocytes in the CE96 Plate

1. Immediately before use, equilibrate an aliquot of Maintenance Medium in a 37°C water bath.
2. Replace the Plating Medium with Maintenance Medium 4 hours post-plating. Tilt the CE96 plate, remove the spent medium using a multichannel pipettor, and gently add 200 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.*

3. Maintain the cardiomyocytes on the CE96 plate replacing 100% of the spent medium with 200 µl/well of 37°C Maintenance Medium every 48 hours.
4. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.
5. Perform recordings from day 7 post plating.

Data Acquisition and Analysis

The CardioExcyte Control Software and DataControl96 Software offer a variety of options for data acquisition and analysis. The instructions here are meant to provide a general guidance. See the CardioExcyte 96 system's manual for specific instructions.

The beating pattern stabilizes on day 4 post-plating cardiomyocytes into the CE96 plate. Example baseline activity and compound treatment at day 7 post-plating can be seen in Figure 1.

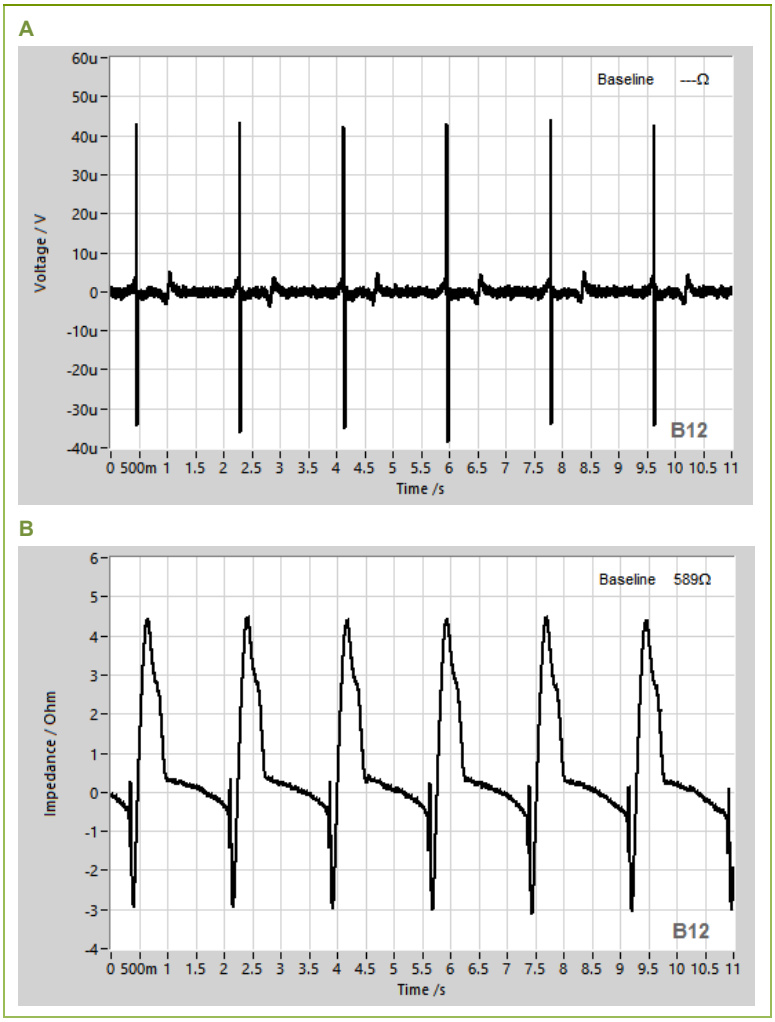

Figure 1: iCell Cardiomyocytes² and the CardioExcyte 96 System Enable Multiplexed Endpoints for Easy and Robust Interrogation of Cardiomyocyte Function
Electrical and contractile activities define the EC process. The example traces show 11 second recordings where (A) electrical activity is recorded as the extracellular field potential and (B) contractile activity is captured as changes in impedance readings from iCell Cardiomyocytes². The combined detection of these recordings illustrates the ease with which compounds can be assessed for effects on electrical, contractile, or both activities.

Notes

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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 recording. Tilt the CE96 plate, remove the Maintenance Medium using a multichannel pipettor, and gently add 180 µl/well of Maintenance Medium to the side of the well to avoid disturbing the cardiomyocyte monolayer.

   Note: *Evaporation rates can vary across the CE96 plate. Changing the Maintenance Medium before compound treatment is required to ensure uniform medium volumes across the plate.*
3. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.
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 20 µl/well of the 10X compound solutions from the 96-well cell culture plate to the CE96 plate. Gently mix by pipetting 3 - 5 times.

   Note: *Beating rate and amplitude are temperature-dependent. The CE96 plate should not be kept outside the incubator for more than 5 minutes while compounds are added.*

Data Acquisition and Analysis

The CardioExcyte Control Software enables automatic detection and analysis of different parameters of the beat traces. See the CardioExcyte Control Software manual for specific instructions on using the CardioExcyte Control Software for data acquisition and analysis. Further analysis, such as IC_{50} calculations, can be performed using the DataControl96 Software provided with the system.

Example Data

Beating rate, amplitude, pulse width, and beat rate regularity were calculated with the CardioExcyte Control Software. Data were normalized to pre-compound addition values. Results displayed in Figure 2 were generated with recordings acquired 60 minutes (dofetilide) or 30 minutes (nifedipine) after drug treatment compared to untreated wells.

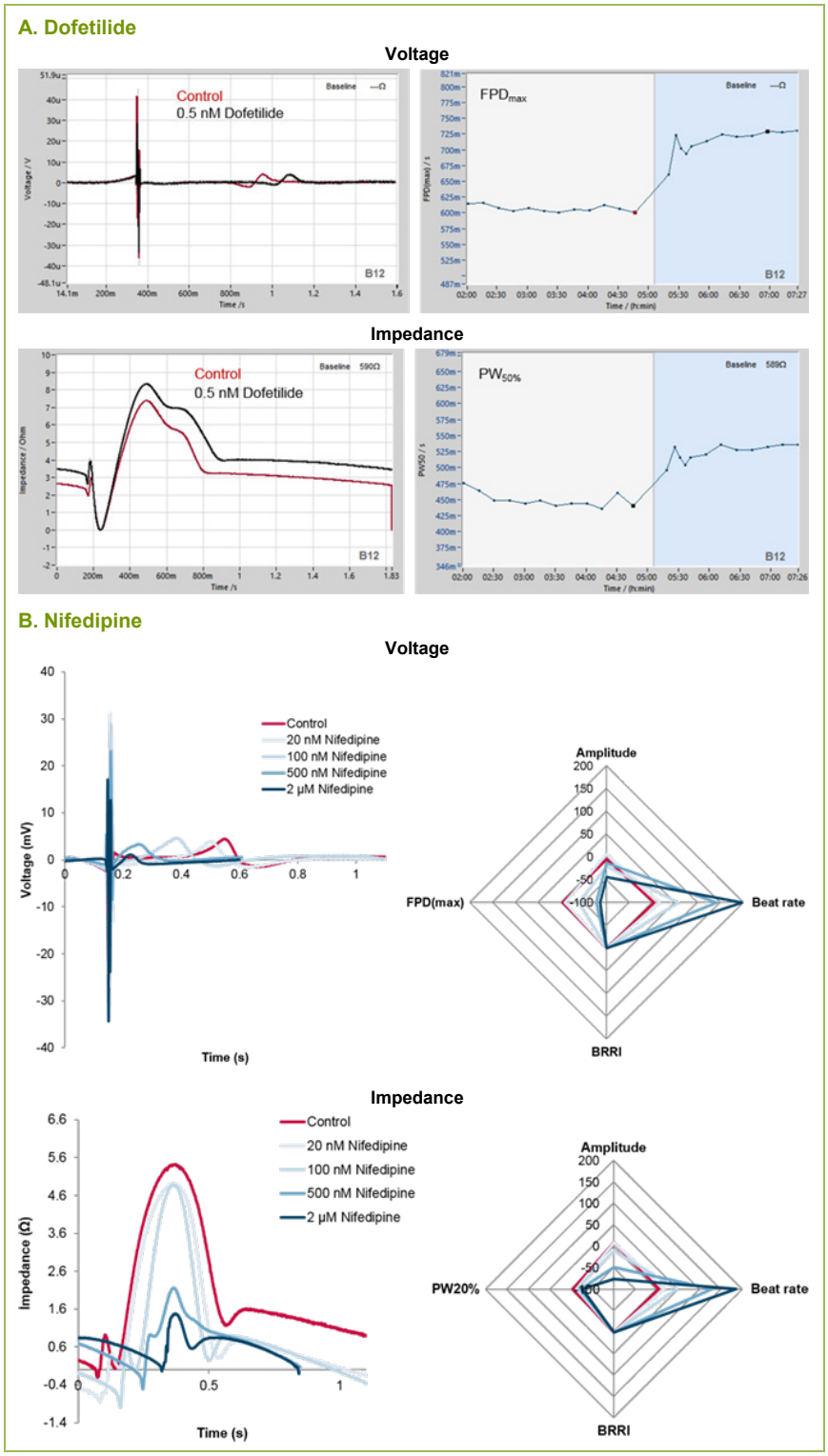

Figure 2: iCell Cardiomyocytes[2] and the CardioExcyte 96 System Enable Simultaneous Detection of Different EC Endpoints

Modulating ion channel and mechanical cardiac activities alters the spontaneous EC process of iCell Cardiomyocytes[2]. Blocking I_{Kr} and $I_{Ca\text{-}L}$ with dofetilide and nifedipine, respectively, produced the expected phenotypic effects on both field potential and impedance waveforms. The CardioExcyte Control Software and DataControl96 Software offer export formats to provide (A) single or (B) multiple parameter analysis. FPD = field potential duration; PW = pulse width; BRRI = beat rate regularity index.

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Summary

iCell Cardiomyocytes² provide an in vitro test system that equilibrates rapidly upon reanimation from cryopreservation to recapitulate native human cardiac myocyte physiology and function while the CardioExcyte 96 system provides a label-free technology for non-invasive monitoring of electrical and mechanical cellular functions. 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. The stable signals and the adaptive peak algorithm of the system enable automated peak detection and discrimination of compound effects on different components of the EC process. 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.

Revision History
Version: February 2017
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