Live Cell Beating Assay Using Human iPSC-derived Cardiomyocytes for Evaluation of Drug Efficacy and Toxicity

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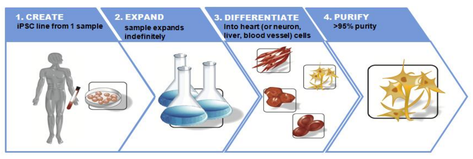
Abstract

A large percentage of new drugs fail in clinical studies due to cardiac toxicity. Therefore development of highly predictive in vitro assays suitable for screening, safety assessment, or other environments is extremely important for drug development. Human cardiomyocytes derived from stem cell sources can greatly accelerate the discovery of cardiac drugs and improve drug safety by offering more clinically relevant cell-based models than those presently available. Derived from human induced pluripotent cells and highly purified, iCell® Cardiomyocytes are especially attractive because they express ion channels and demonstrate beating and action potentials similar to primary cardiac cells. Here we demonstrate cell based assays for measuring the impact of pharmacological compounds on the rate and magnitude of beating cardiomyocytes with different instrument platforms

Introduction

Preclinical safety is an important part of drug discovery and drug development. Early assessment of cardiac toxicity would allow the industry to reduce the number of drugs failing in clinical trials because of unacceptable toxicity. An emerging application for iPSC-derived cardiomyocytes is for use as a model cell-based system for testing functional effects of ion channel blockers, GPCR antagonists, or other prospective drugs on cardiac contractility and cardiac toxicity. Cellular Dynamics International's (CDI) iCell® Cardiomyocytes are highly purified human cardiomyocytes derived from induced pluripotent cells. Using these cells in conjunction with automated imaging platforms we have shown dose-dependent atypical patterns and changes in cell beating caused by number of cardioactive and cardiotoxic compounds.



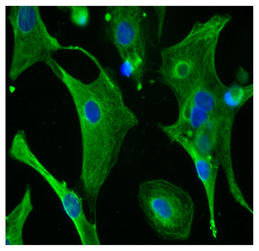


Figure 1. Left: Steps in creation of cardiomyocytes and other cell types from iPS cells. Right: Image of iCell Cardiomyocytes in culture stained with Troponin and DAPI

Spontaneous Beating of iPSC-Derived Cardiac Cells

Maturity of cardiac cells is assessed by the ability of a lawn of cells to spontaneously expand and contract (beating). Cells produced in this state in multiwell plates are very attractive for a variety of assays to monitor cardiotoxicity and cardioprotection. Cell lawn beating can be observed visually and also measured with standard electrophysiology tools. However, characterization of this activity via automated imaging remains a challenge. Here we present methods to measure beat rate in an automated fashion using time-lapsed fluorescence imaging with the ImageXpress® Micro XL and FLIPR® Tetra systems.

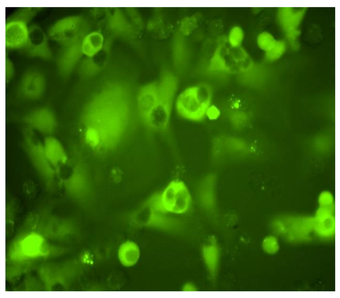
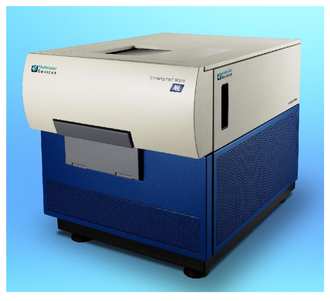


Figure 2. Single image of cardiac cells in a 96 well plate that are spontaneously beating (stained with Calcein AM)

Methods



ImageXpress® Micro XL Automated Imaging System

Cell Preparation and Imaging

- iCell Cardiomyocytes were received frozen from CDI. Cells were thawed and plated according to recommended protocol.
- Cardiomyocytes were plated 20K/96well plate or 4 K/384 well on gelatin coated plates.
- For imaging of mechanical contractions cells were stained with Calcein AM for 10min, then cells were treated with different concentrations of compounds
- Image acquisition was done on the ImageXpress Micro XL System using time-lapse images at 20x or 10x magnification, 40 images per field, at up to 100 frames per second (fps). Cells were kept under environmental control (37C, 5% CO_2).

Calcium Flux Assay

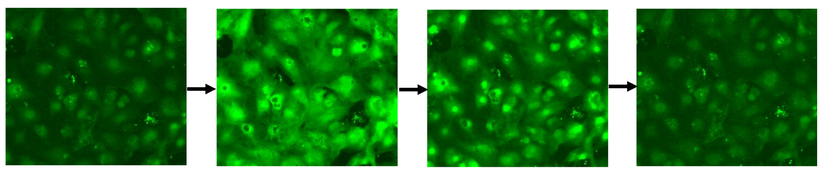
- Calcium 5 kit dye was added to the plates and incubated 1 hour at 37C in 5% CO_2.
- Compound plates were pre-warmed to 37C inside the FLIPR Tetra instrument and compound addition was done simultaneously to all wells
- Experimental plates were loaded and a pre-drug read was acquired of changes in intracellular Ca^{2+} concentration.
  - Acquisition was setup for Calcium 5 (Ex 485nm, Em 530nm)
  - Data was acquired at 8 fps
- FLIPR system reads were acquired during compound addition and at prescribed times after addition (read time ~ 2 min)
- Experimental plates were transferred to the ImageXpress Micro XL system and data was acquired as described above on a well-by-well basis.

FLIPR® Tetra Cellular Screening System

Imaging & Analysis of Beating Cardiomyocytes

Cardiac toxicity is a serious drug safety concern because it can cause arrhythmias or heart failure. We have developed methods for the ImageXpress Micro and FLIPR Tetra systems that enable image acquisition and automatic determination of beating rate of live cardiomyocytes from a series of time-lapse images. One protocol captures mechanical movement of cells; a second monitors changing in intensity of Ca^{2+} fluxes synchronous with beating. Both methods allow visual monitoring of drug impact on the beat rate, rhythm and amplitude in 96 or 384 well formats. The system allows saving data as a video, presenting intensity curves, and automatic analysis of beat rates.

Visualizing beating cardiomyocytes by Ca^{2+} fluxes



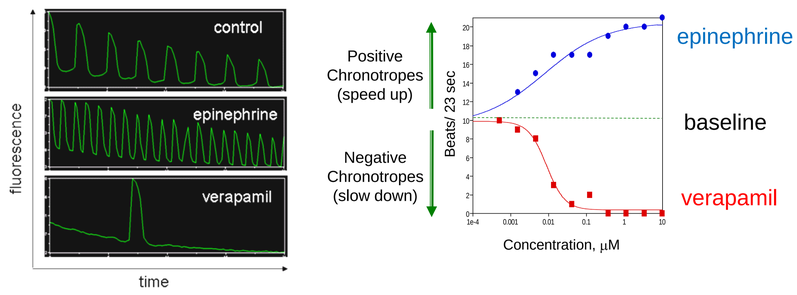


Figure 4. Top: Images of cardiomyocytes showing low and high Calcium 5 signal through beat cycles. Lower Left: Visualizing beating of cardiomyocytes by fluorescence intensity (Calcium 5 signal). Lower Right: Temporal response of signals from cells dosed with positive and negative chronotropes.

Cell-based model for testing cardiac drugs

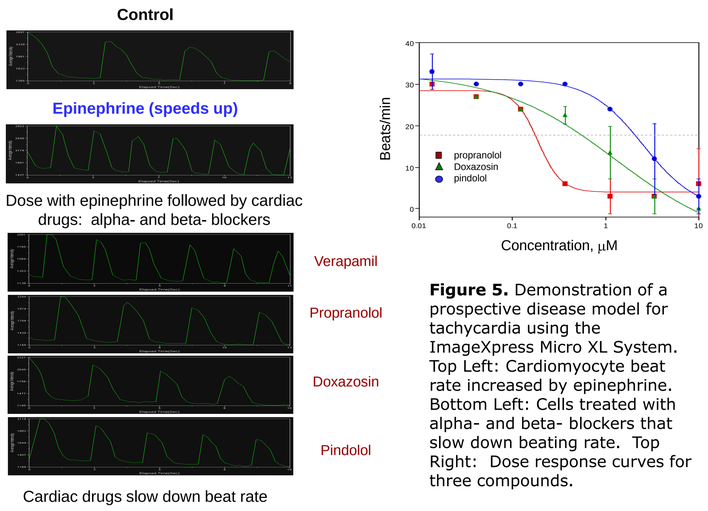
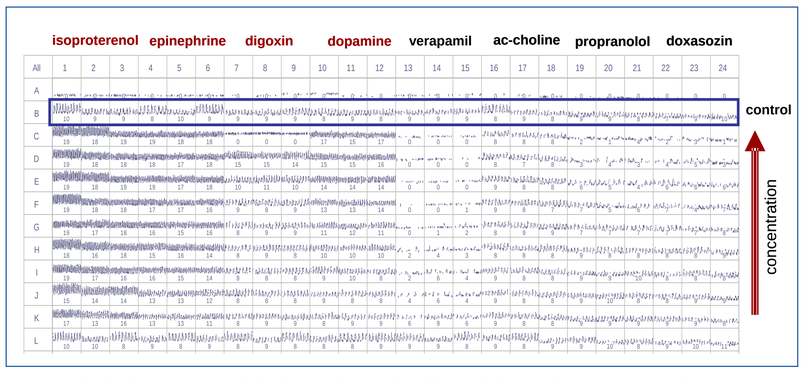


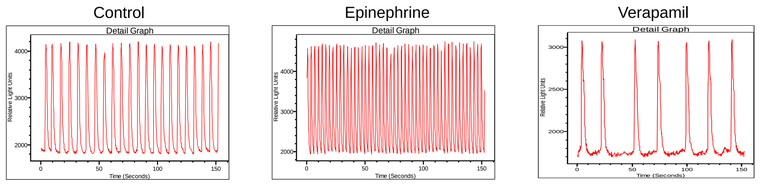
Figure 5. Demonstration of a prospective disease model for tachycardia using the ImageXpress Micro XL System. Top Left: Cardiomyocyte beat rate increased by epinephrine. Bottom Left: Cells treated with alpha- and beta- blockers that slow down beating rate. Top Right: Dose response curves for three compounds.

High Throughput FLIPR Tetra System Cardiac Beating Assay

A complementary method uses the FLIPR Tetra system to monitor changes in intracellular Ca^{2+} fluxes associated with cardiomyocyte contractions using the FLIPR® Calcium 5 Kit Assay. The FLIPR system allows automatic addition of reagents and compounds, simultaneous with reading from 96 or 384 wells. This has been found to be very advantageous for the cardiac beating assays because it reduces well-to-well variability caused by reading at different time points. The absolute beat rates were found to be very similar to that measured by imaging methods. Temporal response curves for analysis and visualization of beating can be acquired in ~ 2 min per plate making this assay suitable for high throughput screening of compound libraries.



FLIPR ScreenWorks® Software Output for 384 Well Plate 10 Minutes After Dose



Graphs of Calcium 5 Intensity vs. Time for Single Wells from FLIPR Tetra

Development of Predictive Cell-Based Assays

Development of new, more potent and safer drugs requires an in vitro system where efficacy and safety can be tested. Positive and negative inotropes are used in clinics to treat heart failure, tachycardia, arrhythmia or other cardiac diseases. We have demonstrated effects of several positive (isoproterenol, dopamine, etc.) and negative (α– and β– blockers) chronotropes on cardiac rates and determined EC50s at the expected ranges. Image based assays using calcium flux and iPSC derived cardiomyocytes are suitable for that task and could be used to estimate efficacy and approximate dosing prior to clinical studies. Results from assays run on the FLIPR Tetra system are shown below.

Positive and negative chronotropic effects

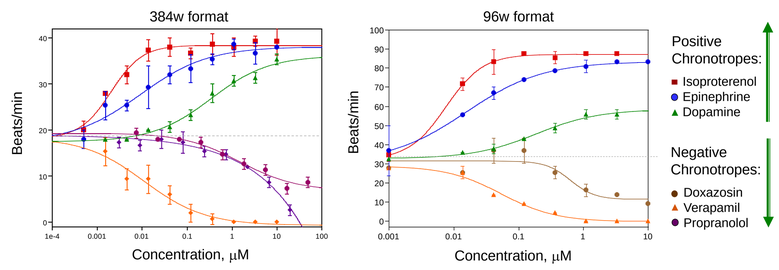
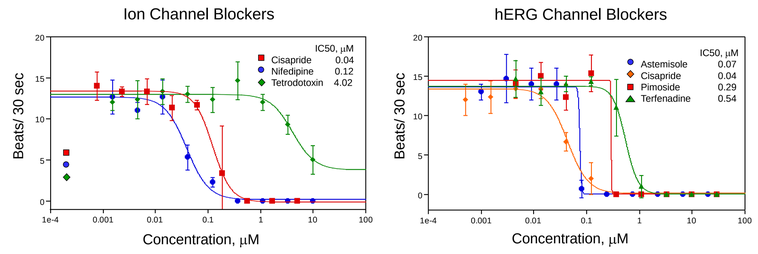


Figure 6. Frequency modulation of Ca^{2+} transients in iPSC derived cardiomyocytes. Increase in fluorescence by Ca^{2+} transients were measured by the FLIPR Tetra system in Calcium 5 loaded iPSC derived cardiomyocytes. Frequency was determined 10-15 min after compound addition.

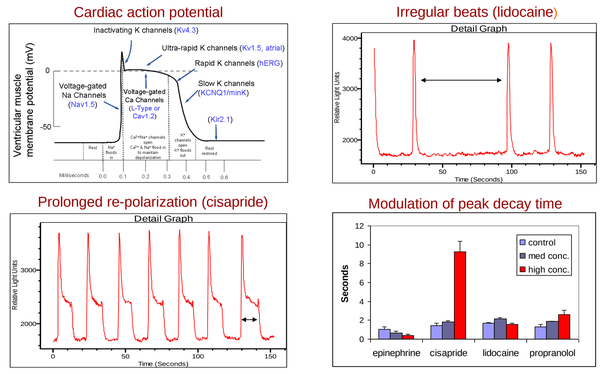
FAIL EARLY: Identification of compounds that affect beat rate and rhythm of iPS cardiomyocytes

We have shown dose-dependent atypical patterns and changes in cell beating rate caused by several known cardiotoxic compounds including hERG, Ca^{2+} and Na^{+} channel blockers. Potentially toxic compounds can be easily detected in the assay by their effect on the beat rate pattern.



Surrogate markers for cardiac toxicity assay

The assay system allows detection of atypical patterns caused by compounds known to be associated with long QT syndrome (e.g., cisapride and doxazosin) and Na^{+} channel blockers (e.g., lidocaine). Measuring peak width, peak spacing and other parameter would allow prediction of drugs inducing long QT syndrome, arrhythmia and other potentially dangerous features.



Summary

We demonstrate live-cell assays for measuring the impact of pharmacological compounds on the rate and magnitude of beating cardiomyocytes using FLIPR Tetra and ImageXpress Micro Systems

We demonstrate applications of these assays for prospective cardiac drug efficacy screening using iPSC-derived cardiomyocytes by measuring the impact of number known cardiac drugs on the beating rate and Ca^{2+} transient fluxes. Tested reagents modulated the frequency of beating in line with their mode-of-action and expected EC50

The FLIPR Tetra instrument can become a tool for identification of cardiotoxic compounds earlier in the drug discovery process. We have shown dose-dependent atypical patterns and changes in cell beating caused by several known cardiotoxic compounds including blockers of ion channels and drugs causing long QT syndrome. Potentially toxic compounds can be easily detected in the assay by their affect on the beat rate pattern.

Molecular Devices