Automated Functional Cellular Analyses of Human iPS-derived Cardiomyocytes

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Abstract

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. iPS-derived cardiomyocytes are especially attractive because they express ion channels and demonstrate beating and action potentials similar to primary cardiac cells. This aspect, coupled with availability of such cells in large quantities, makes them useful for screening of lead compounds and potentially important for reducing animal experimentation and cost of pre-clinical development.

A critical phase in the maturation of cardiomyocytes is the formation of spontaneously beating cell lawns. Here we demonstrate live-cell assays for measuring the impact of pharmacological compounds on the rate and magnitude of beating cardiomyocytes using high content imaging, kinetic fluorescence measurement, and electrophysiology.

We developed a protocol that enables image acquisition and automatic determination of beating rate and magnitude of iPS-derived cardiomyocytes from time-lapse images of live cardiomyocytes. We have also monitored other functional aspects underlying cardiomyocyte contractions including intracellular Ca^{2+} transient fluxes by using Ca^{2+} -sensitive fluorophores and presence of functional K^+ and Ca^{2+} channels with automated patch clamp instruments.

Stem Cell Derived Cardiomyocytes

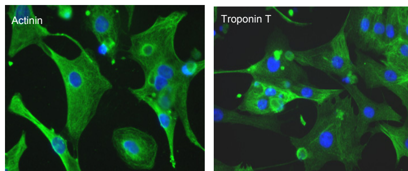
Preclinical safety is an important part of drug discovery and drug development. Early assessment of cardiac toxicity would allow us to reduce the number of drugs failing in clinical trials because of unacceptable toxicity.
One emerging application for iPS-derived cardiomyocytes is for use as a model 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.

Cell Preparation

- iPS derived cardiomyocytes were obtained from Cellular Dynamics
- Cells were plated on gelatin coated 96w plates, in the presence of maintenance media for 2-7 days
- Cells were visualized using ImageXpress® Micro Imaging System and fluorescently labeled cardiomyocyte specific markers
- Images were analyzed using standard algorithms from MetaXpress® Software

High Content Image Acquisition & Analysis

- Images were acquired with ImageXpress Micro Widefield Imaging System using a 20X Plan Fluor objective
  - Calcein AM & AF488 labeled markers: 488nm Ex, 520 nm Em
  - Hoechst dye label for nuclei: 405nm Ex, 450nm Em
- Images were analyzed using the Cell Scoring application module from MetaXpress® Software



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Spontaneous Beating of iPS-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 quite easily and also measured with standard electrophysiology tools. However, characterization of this activity via HCA remains a challenge. Here we present data on a method to measure beat rate and amplitude in an automated fashion using time-lapsed fluorescence imaging.

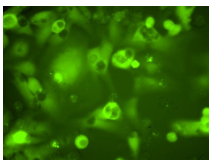

Fig. 1. Single image of cardiac cells in a 96 well plate that are spontaneously beating (stained with Calcein AM)

Use of High Content Imaging for Real Time Beat Assessment

Spontaneously beating iPS-derived cardiomyocytes were cultured in a monolayer on 96w or 384w plates resulting in synchronized contractions. Cells were stained with Calcein AM for 10min, then media was replaced and cells were treated with different concentrations of several compounds that affect heart rate: norepinephrine, epinephrine, caffeine or acetylcholine. The assay was done in duplicates for each concentration and repeats of controls for each compound group. Cells were placed into the chamber of ImageXpress® Micro System using temperature and CO_2 control. Image acquisition was done automatically using time-lapse images of live cardiomyocytes, 40 images per field. Results are shown below in Figure 2.

Automatic Algorithm for Beat Rate Analysis

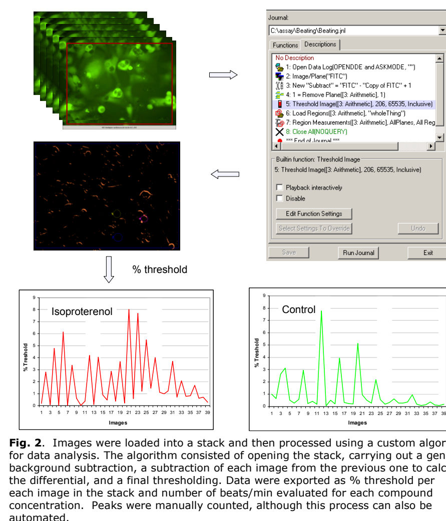

Fig. 2. Images were loaded into a stack and then processed using a custom algorithm for data analysis. The algorithm consisted of opening the stack, carrying out a general background subtraction, a subtraction of each image from the previous one to calculate the differential, and a final thresholding. Data were exported as % threshold per each image in the stack and number of beats/min evaluated for each compound concentration. Peaks were manually counted, although this process can also be automated.

Beat Rate Modulation

The impact of four pharmacological agents on the beat rate was tested. Three of the agents, isoproterenol, epinephrine, and caffeine are known stimulants. The fourth, acetylcholine, is a known inhibitor of cardiac contraction. The tested reagents modulated the frequency of beating in line with their mode-of-action showing the functional expression of ß-adrenergic and acetylcholine receptors.

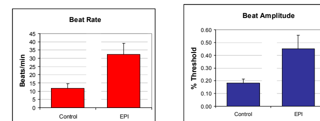

Fig 3. Average amplitude and number of beats of cardiomyocytes after dosing with EPI for 10 minutes. Contractions were measured over a 15 second period with images taken every 300msec. Average change in the cell lawn was determined by a differential threshold algorithm. Beat Amplitude represents the average amplitude of all beats.

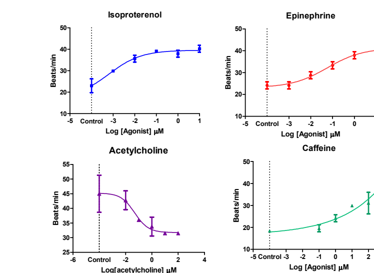

Fig. 4: Beat rate modulation of cardiac cells by four agents. Beat rates were measured in iPS derived cardiomyocytes cells (15000c/w). Initial beat rate varied from one cell lot to another (~20-40 beats/min). Beat frequency was typically determined 10min after compound addition, however the beat rates remained stable between 5-60min after compound addition (not shown).

Beat Rates Determined by Ca^{2+} Fluxes

Intracellular transient Ca^{2+} fluxes underlying cardiomyocyte contractions were studied using Calcium 5 Kit Assay on the FLIPR® Tetra system. Cells were incubated with dye for 1 hour at 37C in 5% CO_2. The frequency of Ca^{2+} transients was found to be sensitive to isoproterenol, epinephrine, and caffeine. The absolute beat rates were found to be very similar to that measured by HCA.

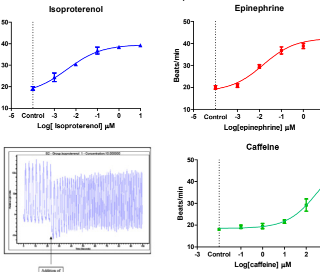

Fig. 5: Frequency modulation of Ca^{2+} transients in iCells. Ca^{2+} transients were measured by the FLIPR Tetra system in Calcium 5 loaded iPS derived cardiomyocytes (15000c/w). Frequency was determined immediately after compound addition. Values were similar to those seen using high content imaging.

iPS-Derived Cardiomyocytes for Electrophysiology Studies

The presence of functional ion channels was tested with the automated patch clamp instrument IonWorks Barracuda™. The system has 384 parallel patch clamp amplifiers which measure both ligand and voltage-gated ion channels simultaneously in 384 recording sites. Both K^+ and Ca^{2+} channels were detected in the iPS derived cardiac cells.

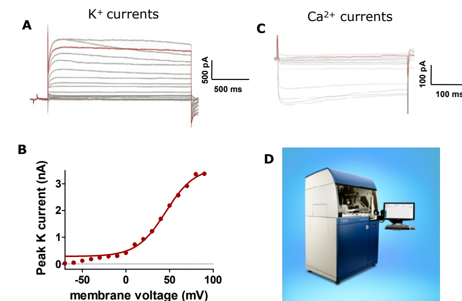

Fig 6: Patch Clamp recordings of endogenous ion channel currents with IonWorks Barracuda™ system. **A)** Sample recording of total outward potassium currents (Vh = -70mV, sampling rate 1k Hz, single hole mode); **B)** Current-voltage relationship of potassium currents recorded in panel A; **C)** Sample recording of total calcium currents (Vh = -80mV, sampling rate 10k Hz, single hole mode); **D)** Side view of the IonWorks® Barracuda™ automated electrophysiology system.

Summary

- We demonstrate live-cell assays for measuring the impact of pharmacological compounds on the rate and magnitude of beating cardiomyocytes using ImageXpress Micro Automated Microscope for high content imaging and MetaXpress software for image analysis.
- We demonstrate applications of these assays for prospective toxicity screening using iPS-derived cardiomyocytes by measuring the impact of isoproterenol, epinephrine, caffeine or acetylcholine on the beating rate and Ca^{2+} transient fluxes. Tested reagents modulated the frequency of beating in line with their mode-of-action showing the functional expression of ß-adrenergic and acetylcholine receptors.
- Intracellular Ca^{2+} transient fluxes underlying cell contractions were monitored by using a high throughput screening (HTS) compatible FLIPR Calcium 5 Kit readout established on the FLIPR Tetra system.
- The presence of functional K^+ and Ca^{2+} channels in iPS-derived cardiomyocytes was determined with the IonWorks Barracuda automated patch clamp instrument.

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