Introduction

A large percentage of new drugs fail in clinical studies due to cardiac toxicity. Therefore development of highly predicative in vitro assays suitable for high throughput screening is of paramount importance for drug development. Human cardiomyocytes derived from stem cells may greatly accelerate the development of novel therapeutic entities and improve safety by offering more clinically relevant cell-based models than those presently available. Stem cell derived cardiomyocytes are especially attractive because they express cardiac ion channels and demonstrate spontaneous electrical and mechanical activity similar to native cardiac cells. Here we demonstrate cell based assays for measuring the impact of pharmacological compounds on the rate of beating cardiac myocytes we would like to determine a beat rate. The system allows saving data as a video, presenting intensity curves, and automatic analysis of beat rates.

Imaging & Analysis of Beating Cardiomyocytes

Cardiomyocyte contraction parameters calculated by Figure 4. Left: 1.7 0.2 9.1 2.7 0.4 15.0 3.6 0.5 14.7

• Data was acquired at ~8 fps

10000

Decay Time

Rise

30000

2+

Screenworks Peak Pro™ Software

Exp1 24 wells Exp2 200 wells Exp3 24 wells

Peaks = 19 Freq = 21.5 bpm

[Image -103160x-5752476 to -96275x-5204643]

[Image -61337x-8533235 to -60662x-8434281]

[Image 523x714 to 548x822]

Methods

Cell Preparation

- Cardiomyocytes were derived from human induced pluripotent stem cells (iPS cells) and were cultured on gelatin coated plates following published protocols.
- Cardiomyocytes were expanded in a chemically defined, serum-free medium designed for optimal cardiomyocyte growth and differentiation.
- Cardiomyocytes were plated onto 24-well plates at a density of 10^5 cells per well.
- Cardiomyocytes were maintained in a humidified incubator at 37°C and 5% CO2.
- Cardiomyocytes were differentiated for 3-5 days. The presence of spontaneous contractions indicated that the cells had differentiated properly.

High Content Imaging

- Image acquisition was done using a high-throughput imaging microscope (Tetra System®) with automated stage and camera control to capture images from multiple wells simultaneously.
- Images were acquired at high resolution using a digital camera and processed using proprietary software for data analysis.
- Cells were imaged in a time-lapse manner to capture the temporal response of the cells to different stimuli.
- The images were analyzed using ImageJ software to quantify cell contraction parameters.

Screenworks® Peak Pro™ Software

Automated Peak Analysis

Automated data analysis is required in order to make the FLIPR Tetra System practical for running assays on a large number of compounds. Manual counting of 384 traces is time consuming and subject to human error. Exporting the data to a separate program in order to analyze the traces removes subjectivity, but adds additional steps that compromises the usefulness for environments that require true automation and high throughput such as screening labs.

An automated peak detection and analysis algorithm was added to the Screenworks software to process the data and provide user selectable outputs.

First, signal peaks are detected based on a dynamic thresholding and derivative analysis. The peaks are then fit with a binomial function to provide amplitude, time, and width values. The analysis occurs immediately after acquisition and result are presented on a web-by-web basis to the user. The results can also be exported to a standard comma-separated variable (csv) file for further analysis.

High Throughput FLIPR® Tetra System Cardiac Beating assay

A method complementary to imaging uses the FLIPR Tetra System to monitor changes in intracellular Ca2+ fluxes associated with cardiomyocyte contractions using the FLIPR Calcium 5 Assay Kit. The FLIPR system allows automatic addition of reagents and compounds simultaneously with reading from 96, 384, or 1536 wells. This has been found to be 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 similar to those measured by imaging methods. Temporal response curves for analysis and visualization of beating can be acquired in ~ 2 min per plate for this assay suitable for high throughput screening of compound libraries.

Predictive Assays for High Throughput Assessment of Cardiac Toxicity and Drug Safety

Oksana Sirenko, Carole Crittenden, Jayne Cressey, Yen-Wen Chen, Carlos Funes, Debra Gallant, and Evan F. Cromwell

Molecular Devices, LLC, 1311 Orleans Drive, Sunnyvale, CA 94089

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 FLIPR Tetra CellScreen System for high content screening.
- Intracellular Ca2+ transient fluxes underlying cell contractions were monitored by using FLIPR Calcium 5 Assay Kit readout.
- Peak parameters were automatically measured on the FLIPR Tetra System using the Screenworks PRO software and proprietary analysis algorithms. The easy-to-use interface and intuitive setup provide fast and reproducible results that agree well with manual measurements.
- We demonstrate applications of these assays for toxicology screening using iPS-derived cardiomyocytes by measuring the impact of various chronicotropes and ion channel blockers on the beating rate and Ca2+ transient fluxes. Assay formats modulated the frequency of beating in line with their mode-of-action showing the functional expression of β-adrenergic and acetylcholine receptors.