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High speed (over 100Hz) HTS using human iPSCs-derived cardiomyocytes on FDSS

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Introduction

Over the last few years, many non-cardiovascular drugs representing a wide variety of structural classes have come under scrutiny or been withdrawn from the market because of reported incidences of ventricular arrhythmias, particularly torsade de pointes (TdP). 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. The stem cell (ES or iPS)-derived cardiomyocytes are especially attractive because they express ion channels and demonstrate beating and action potentials similar to primary cardiac cells. Recently, we have developed and modified a Hamamatsu FDSS platform that is capable of detecting the Ca^{2+} and membrane potential changes with high speed (over 100Hz) under environmental control. The Changes in rate of intracellular calcium transient fluxes associated with cardiomyocyte contractions after adding drug were monitored using Ca^{2+} sensitive and membrane potential-sensitive fluorophores.
Additionally we have compared CDI's cardiomyocytes with one from ReproCELL using calcium sensitive dye. We believe that a high throughput and high speed data acquisition system like this is useful in earlier drug discovery process, for ion channel targets and in prediction of cardio toxicity using human iPSCs-derived cardiomyocytes.

Material and Methods

Fluorescent based cardio toxicity assay using the FDSS

FDSS based cell based assays for measuring the impact of phpmacological compounds on the rate and magnitude of beating the lamp or sheet cardiomyocytes with the cell based assay platforms. Changes in rate of intracellular calcium transient fluxes associated with cardiomyocyte contractions after adding drug were monitored using Ca^{2+} sensitive fluorophores on the HAMAMATSU FDSS/μCELL system. We have also monitored other functional aspects underlying cardiomyocyte contractions including the action potential change by using membrane potential -sensitive fluorophores (MP-sensitive dye).
Intracellular Ca^{2+} transient fluxes underlying cell contractions were monitored by using a high throughput screening (HTS) compatible Fluo-8 AM calcium kit readout established on the FDSS/μCELL system. In 96-well format, beat rates were calculated and observations recorded regarding changes in beat rhythm and amplitude possibly associated with cardiotoxicity.
iPS cell-derived Cardiomyocyte cells were grown in the same manner as imaging in 96 well plates. Fluo-8 and MP sensitive dye were added to the plates and incubated 0.5-1 hour at 37 ˚C in 5% CO_2 . Compound plates were pre-warmed to 37 ˚C inside the FDSS/μCELL instrument. During detection, 5X compound (80 μL+20 μL) was added to the wells. Images were taken of the beating cells every 0.06 seconds to capture the changes in intracellular Ca^{2+} concentration.

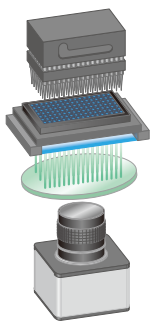
Two different human iPSCs-derived cardiomyocytes

- iCell®Cardiomyocytes from iPS academia Japan, INC, Cellular Dynamics International (CDI), Madison, WI
- ReproCardio2 from ReproCELL INC (Yokohama, Japan)

Reagents and assay plate

Fluorescent dye : final 2 μM Fluo-8AM (AATbioquest USA)
Buffer: ReproCardio assay medium (RCESD007) from ReproCELL INC (Yokohama, Japan) and HHBS buffer (AATbioquest USA).
Assay plate: Coster black bottom clear standard 96 plate or small volume 96 well plate from Coster (Japan)
Compounds: *dl*-Sotalol and others (Sigma Aldrich)

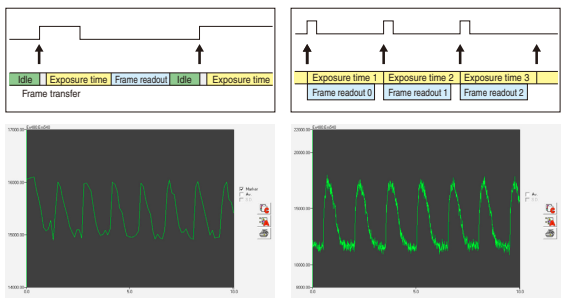
Application of high speed cell-based assay on FDSS7000EX and FDSS/μCELL



High speed mode of Hamamatsu high sensitive CCD camera

The FDSS/μCELL is a kinetic plate reader with an integrated dispensing head and imaging-based detector. Simultaneous dispensing into the entire 96/384 well plates and simultaneous detection of the kinetics of the fluorescence or luminescence intensity allow quick measurements with no time lag for the 96/384 well format. The technologies employed in the FDSS series are integrated into a compact body, enabling simple-to-use operation, suitable for assay development or in research basic cell-based kinetic assay.

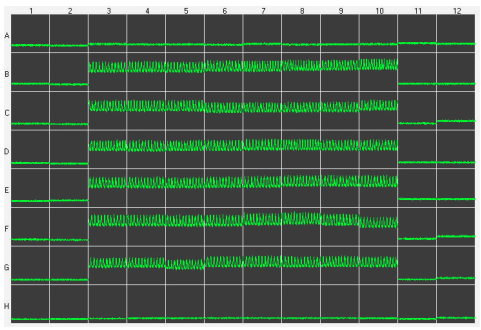
Advantage of high speed mode for image the Ca^{2+} response from human iPSCs-derived cardiomyocytes



8.2 ms (122 Hz) for 96 well
15.6 ms (64 Hz) for 384 well

	AMP_ave	Std	%CV
120 ms	1196.77	98.10	8.20
8 ms	6626.94	196.46	2.96

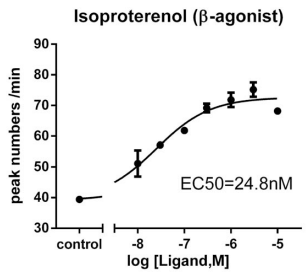
Data uniformity of Ca^{2+} response from small volume 96 well plate (10000 cells/well)



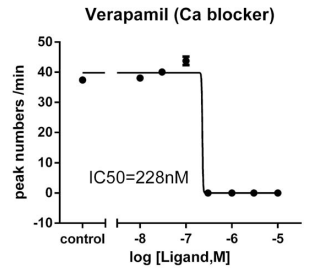
	ave	Std	%CV
Peak rate [/min]	37.71	2.37	6.28
P.P time [ms]_ ave	1597.53	91.45	5.72
AMP_ave	7933.99	380.63	4.80

N=48 well

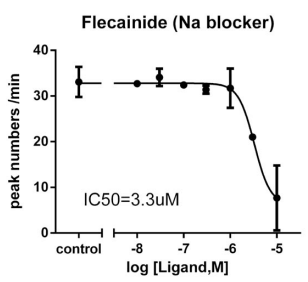
Dose response to the reference compounds of human iPSCs-derived cardiomyocytes



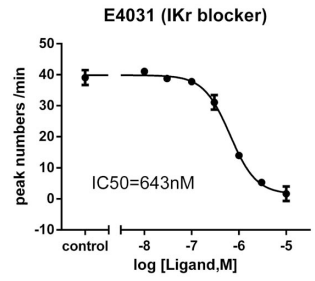
A sympathomimetic β-receptor stimulant possessing the cardiac excitatory. Cells were subsequently stimulated with a dilution series of Isoproterenol. Fluorescence emission values were obtained and plotted against the indicated concentrations of Isoproterenol.



L-type calcium channel blocker of the phenylalkylamine class and Verapamil's mechanism in all cases is to block voltage-dependent calcium channels. Cells were subsequently stimulated with a dilution series of Verapamil. Fluorescence emission values were obtained and plotted against the indicated concentrations of Verapamil.

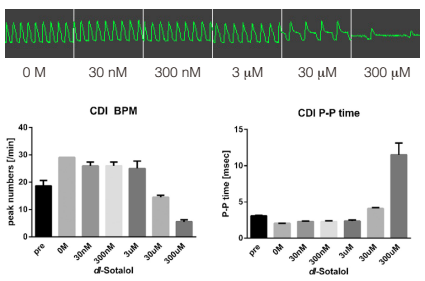


Flecainide acetate is a class Ic antiarrhythmic agent used to prevent and treat tachyarrhythmias (abnormal fast rhythms of the heart). Cells were subsequently stimulated with a dilution series of Flecainide. Fluorescence emission values were obtained and plotted against the indicated concentrations of Flecainide.

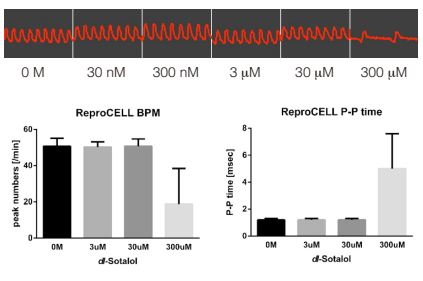


E-4031 acts on a specific class of voltage-gated potassium channels mainly found in the heart, the hERG channels. Cells were subsequently stimulated with a dilution series of E-4031. Fluorescence emission values were obtained and plotted against the indicated concentrations of E-4031.

Comparison of calcium response in two cell type of human iPSCs-derived cardiomyocytes on *dl*-Sotalol



Human iPSCs-derived cardiomyocytes (20,000 cells/well for CDI) were plated in a 96-well format. Cells were then incubated with Fluo-8 AM for 45 min. at 37 ˚C. Cells were subsequently stimulated with a dilution series of *dl*-Sotalol. Fluorescence emission values were obtained and plotted against the indicated concentrations of *dl*-Sotalol.



Dose-response to *dl*-Sotalol to ReproCardio2 semi clamp iPSCs-derived cardiomyocytes loaded with Fluo-8 dye on the FDSS System. Fluorescence emission values were obtained and plotted against the indicated concentrations of *dl*-Sotalol.

Conclusions

Intracellular Ca^{2+} transient fluxes underlying cell contractions were monitored by using a high throughput screening (HTS) compatible Fluo-8 calcium kit readout established on the FDSS/μCELL system, with high speed data acquisition. In 96-well format, beat rates were calculated and observations were recorded regarding changes in beat rhythm and amplitude possibly associated with cardiotoxicity, in comparison to two different cell formats. The unique properties of the high speed (over 100Hz) data acquisition were applied to an analysis the Ca^{2+} transient from fluorescence images of Fluo-8-loaded iPSCs-derived cardiomyocytes. The high photosensitivity of the camera, combined with the FDSS and the wave analysis software, permitted collection of fluorescence images <10 ms apart.
Using this technology, we have monitored several parameter(BPM, Peak to Peak time and AMP) from human iPSCs-derived cardiomyocytes. *dl*-Sotalol, a typical 'false negative' when using the hERG binding assay does not cause any reaction using the traditional binding assay. Using this HTS method on FDSS, we can success to image the intercellular Ca^{2+} change form the *dl*-Sotalol.
We believe that a high throughput and high speed data acquisition system like this is useful in earlier drug discovery process, for ion channel targets and in prediction of cardio toxicity using human iPSCs-derived cardiomyocytes.

Acknowledgments

We thank Dr. Asai Yasuyuki and Dr. Yoshida Shunsuke (ReproCELL Inc.) and Dr. Saotome Hideo (iPS Academia Japan, Inc.) for support in this collaboration.

Reference

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