Safety toxicity with cardiomyocytes derived from induced human pluripotent stem cells on FDSS platform

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1. Overview

Purpose:
- Introduction of selected, pure human cardiomyocytes derived from induced pluripotent stem cell (hiPSCM) into a calcium transient imaging high throughput screening (HTS) assay to assess cardiotoxicity and drug efficacies

Methode:
- Monitoring of intracellular free calcium $[Ca^{2+}]_i$ with a Ca^{2+} sensitive fluorescent dye under serum-free conditions
- Recording of $[Ca^{2+}]_i$ with the high speed fluorescence imaging camera using the Hamamatsu FDSS/µCell (Hamamatsu Photonics K.K., see fig. 1)
- Monitoring of $[Ca^{2+}]_i$ modulation in the presence of reference compounds
- Offline raw data analysis with the Hamamatsu CalDio software

Results:
- 39 compounds were successfully tested in triplicate and 10 concentrations each
- hiPSCM responded as expected to the reference compounds under serum-free condition in BMCC Medium

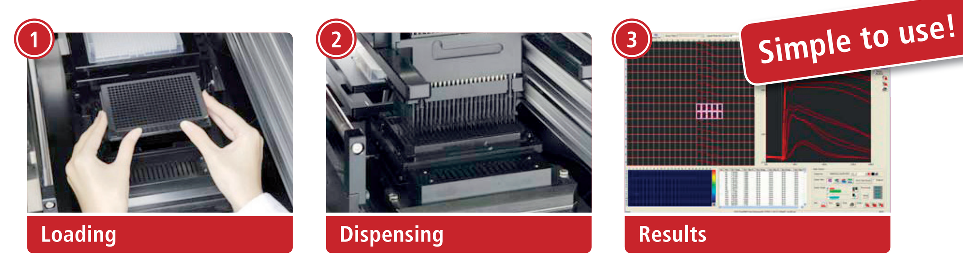
2. Introduction

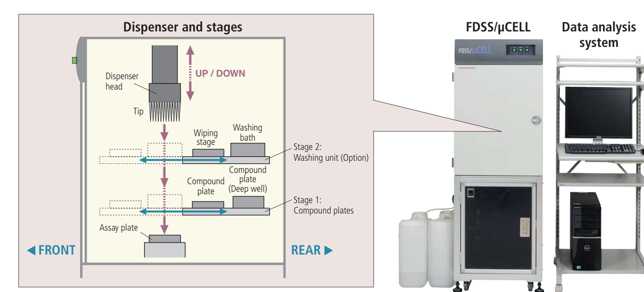
Toxicity is the main cause of attrition in the Drug Discovery (DD) process today and can be seen on two specific cell types: cardiomyocytes and hepatocytes. Over the years, one challenge for pharmaceutical companies has been to be able to determine compound toxicity in the early stages of DD in order to avoid additional cost in compound development and improvement. Since their first discovery in late 2007, iPS derived cells represent one of the few cases where DD has taken to novelty so fast. These cells allow researchers to obtain pluripotent stem cells without using embryos, thus avoiding critical ethical issues. It comes as no surprise, considering the massive demand, that the first commercially available iPS derived cells were cardiomyocytes. With cells easily available in high quantity and independently from donors, cardiotoxicity can be assessed much more early in the DD process and in some companies is now part of the secondary screening phase. Furthermore, researchers do not have to limit themselves to the very low throughput of functional assays or electrophysiology but now have access to high throughput fluorescence based methods as a first broad-range screening.

We present here a study made by Axiogenesis with Hamamatsu FDSS kinetic plate readers equipped with a high-speed camera, an integrated dispenser head and temperature control, allowing for detection of fast calcium signals under physiological temperatures. We have used cryopreserved hiPS-derived Cor.4U® cardiomyocytes in 384 well plates to optimise assay conditions and to detect changes in calcium transients induced by cardiac ion channel modulators. Using hiPS-derived Cor.4U® cardiomyocytes precultured for 3 - 5 days in 384 well plates, stable $[Ca^{2+}]_i$ signals were measured over 35 min and the effect of more than 30 compounds on $[Ca^{2+}]_i$ transients in human iPS-derived cardiomyocytes were detected and analyzed.

3. Material and Methods

Cryopreserved Cor.4U hiPSCM (Axiogenesis AG) were thawed and seeded in 384-well microplates (Greiner Bio-one) coated with a 1:100 diluted fibronectin solution (Sigma). Three days after thawing and seeding the hiPSCM revealed stable synchronous beating and were ready to be introduced into the assay. Two hours before the experiment the medium was changed to the serum-free BMCC Medium (Axiogenesis) to a total volume of 25 µl. A 0.5x dilution of the FLIPR Calcium 5 Assay dye (Molecular Devices) was prepared according to the manufacturers instructions and 25 µl were loaded onto the cells for a 20 min incubation at 37 °C and 5 % CO_2. The assay chamber of the FDSS/µCELL was heated to 37 °C before the hiPSCM were transferred into the device. After an additional 20 min equilibration time, the baseline $[Ca^{2+}]_i$ was recorded of all wells. Subsequently, 25 µl of 3x concentrated compound dilutions or DMSO vehicle control were added to each well at the same time with the 384 dispenser head of the FDSS/µCell and recordings were taken after 5 min each for a total time of 35 min. Fluorescence images of all wells of the microplate were recorded every 0.016 s to capture changes of $[Ca^{2+}]_i$. The raw data were analyzed offline for a total of 16 parameters of the $[Ca^{2+}]_i$ waveform with the CalDio software (see fig. 2 and 3).





4. Results

The hiPSC-derived Cor.U cardiomyocytes have been successfully introduced into an HTS assay to monitor intracellular free calcium with a calcium sensitive fluorescent dye under serum-free condition with the Hamamatsu FDSS/µCell. Within a period of 10 days 39 compounds were tested in 10 concentration dose responses and at least triplicates for each concentration and vehicle controls. Using the CalDio software it was possible to analyze the action of the compounds on the calcium transients and it was possible to cluster the compounds due to their action on the analyze parameter.

5. Conclusions

- Human iPS-derived Cor.4U cardiomyocytes can be easily applied to fast HTS screening assay to monitor druginduced intracellular free calcium transients.
- The fast camera of the FDSS/µCell allows for a reliable and stable recording of Ca^{2+} transients in a 384 well format.
- Thus this assay is an ideal and cost effective, fast medium to high throughput screening assay to assess cardiotoxicity and drug efficacy at an early time point of the drug development process.

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Fig. 1: Hamamatsu FDSS/µCell

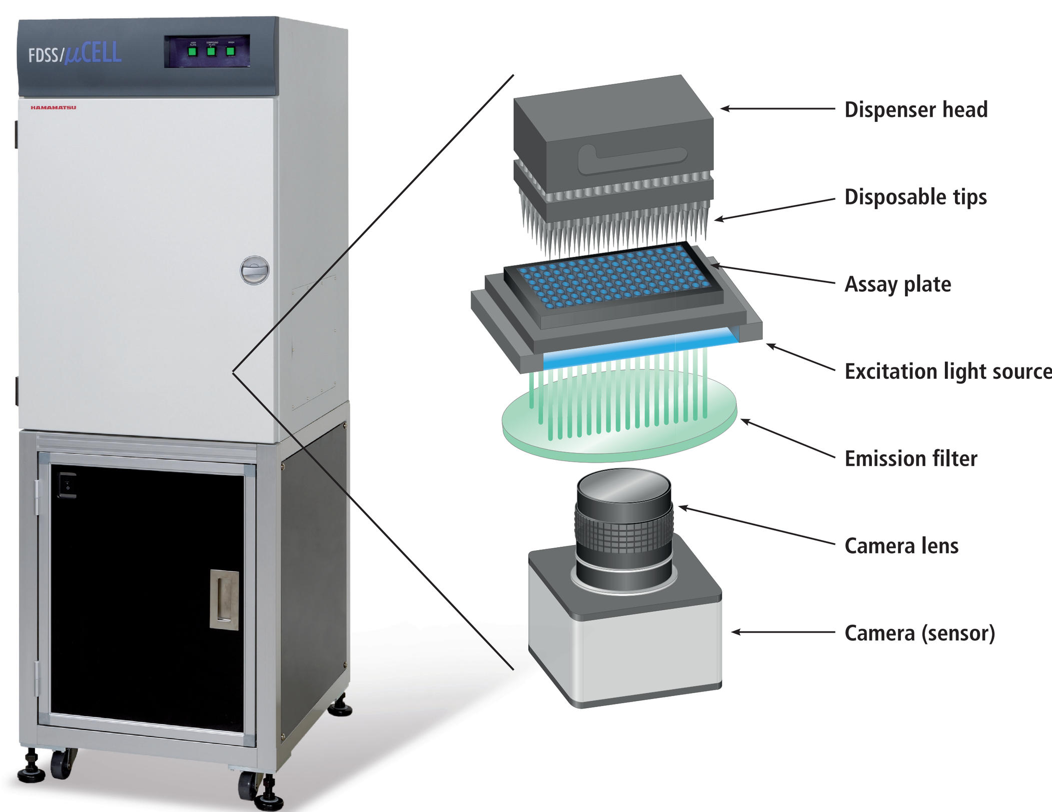
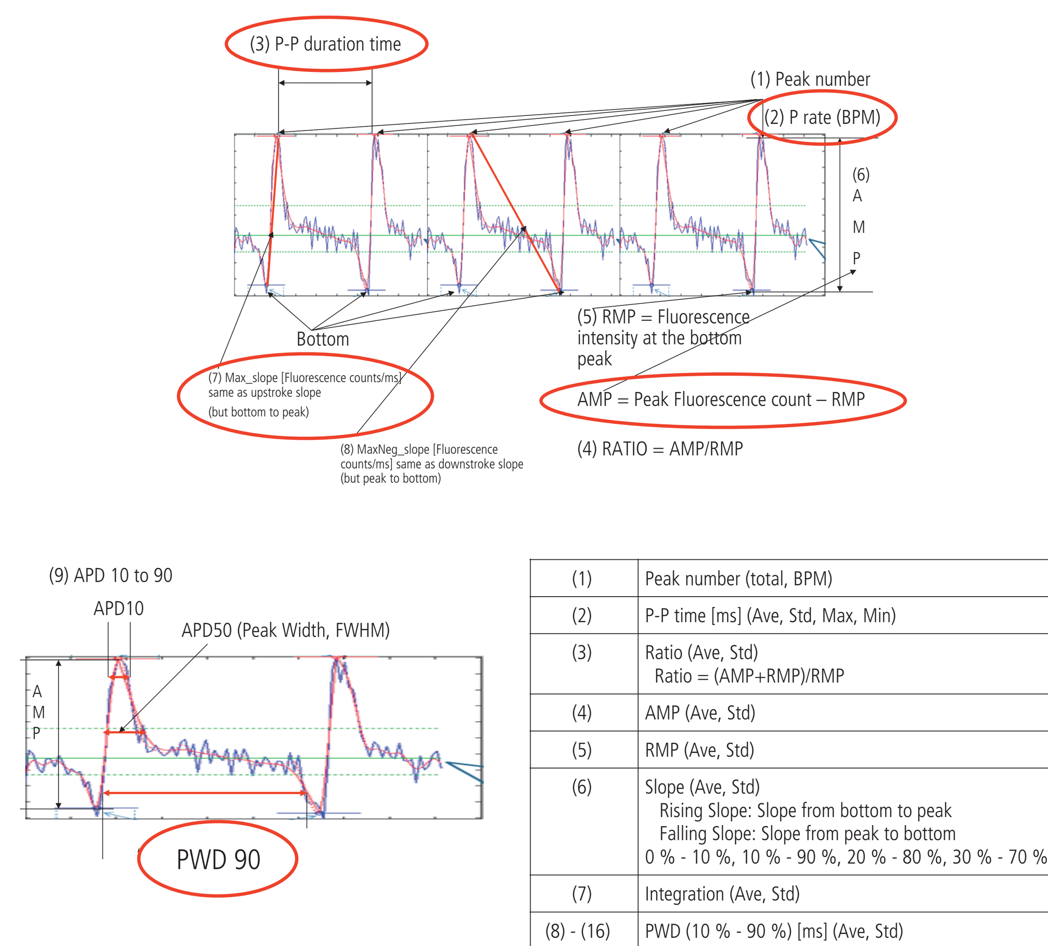


Fig. 2: Parameters calculated from raw data of Ca^{2+} transients



(1)	Peak number (total, BPM)
(2)	P-P time [ms] (Ave, Std, Max, Min)
(3)	Ratio (Ave, Std) Ratio = (AMP+RMP)/RMP
(4)	AMP (Ave, Std)
(5)	RMP (Ave, Std)
(6)	Slope (Ave, Std) Rising Slope: Slope from bottom to peak Falling Slope: Slope from peak to bottom 0 % - 10 %, 10 % - 90 %, 20 % - 80 %, 30 % - 70 %
(7)	Integration (Ave, Std)
(8) - (16)	PWD (10 % - 90 %) [ms] (Ave, Std)

Fig. 3: Clustering of compounds due to their action on parameters of the calcium transient waveforms

Fig. 4 Detection of Astemizole effects on Ca^{2+} transients in iPSc-derived human Cor.4U® cardiomyocytes

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