Detection of Ca++ Transients in iPS-derived Cardiomyocytes: an HTS-ready Method of Measuring Cardiomyocyte Function

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Introduction

The detection of Ca++ transients in (recombinant) cell lines is widely used in HTS drug screenig due to high specificity, robustness, throughput and sensitivity.

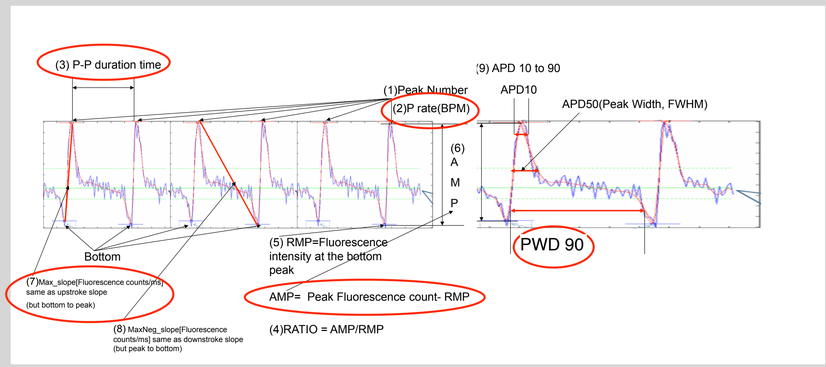
Since calcium ions are the major trigger for the initiation of contraction in cardiomyocytes, such set-up can be used for HTS screening in early cardiac safety testing.

Stemcell-derived cardiomytocyes display a primary-like phenotype and a regular beating pattern, therefore being an ideal model to detect changes in calcium handling. The Hamamatsu FDSS kinetic plate readers are equipped with high-speed cameras, integrated dispenser heads and temperature control, allowing for detection of fast calcium signals under physiological conditions.

We have used mouse stemcell-derived Cor.At® and human iPS-derived Cor.4U® cardiomyocytes in 96w and 384w 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 5 - 7 days in 384w plates, stable Ca++- transient signals could be measured over 45 min. The effect of several compounds on Ca++- transients in human iPS-derived cardiomyocytes was detected using the Calcium 5 Assay Kit in the FDSS µCell.

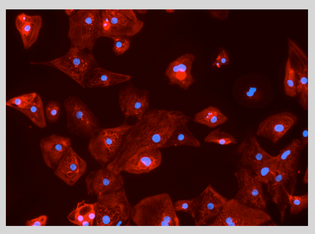
Fig. 1: Parameters measured and evaluated ( )



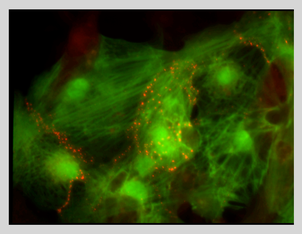
(1)	Peak number (Total, BPM)
(2)	P-P time[ms] (Ave, Std, Max, Min)
(3)	Ratio (Ave, Std) Ratio= (AMP+RMP)/RMP
(4)	AMP(Ave, Std)
(5)	RMP(Ave, Std)
(6)	Slope(Ave, Std) Rising slope : Slope from bottom to peak Falling slope : Slope from peak to bottom peak bottom setting can be selected 0%-100% 10%-90%, 20%-80%, 30%-70%
(7)	Area Undercurve(Ave, Std)
(8) - (16)	PWD(PWD10 - 90)[ms] (Ave, Std)

Fig. 2: Characterisation of iPS-derived cardiomyocytes

A: Immunostaining of Cor.4U® human cardiomyocytes



IF for cardiac alpha-actinin at d4 post thaw



IF for Connexin 43 at d14 post thaw

B: Essential cardiac ion channels in Cor.4U human cardiomyocytes

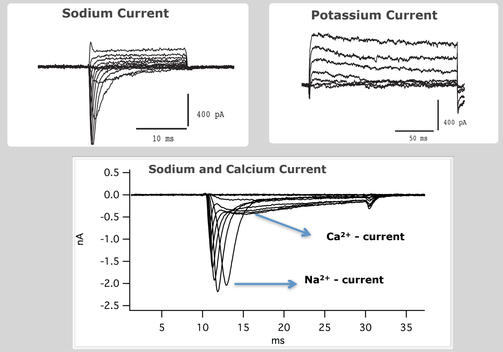
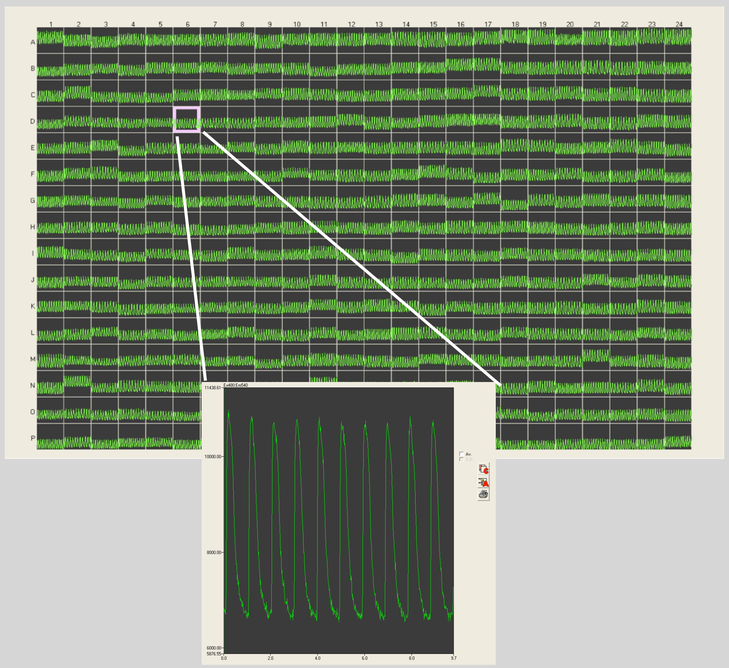
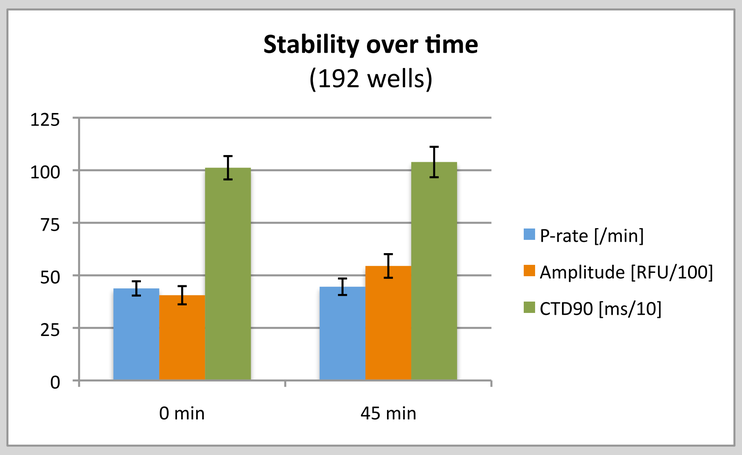


Fig. 3: Ca++ - transients in iPSc-derived human Cor.4U® cardiomyocytes

A: hiPS-Cor.4U® plating efficency in a 384w plate



B: hiPS-Cor.4U® plating efficency in a 384w plate



During a first measurement, half of a 384w plate was left untreated and served as assay control. P-rate, amplitude, and CTD90 were determined at start of measurement and after 45min. The graph shows mean and SD of the average value of the measurement interval (20s at each timepoint) of 192 wells.

Material and Methods

For assay optimisation, mouse stemcell-derived Cor.At® cardiomyocytes were thawed and seeded at 12k per well in fibronectin-coated 96w Plates (Greiner µClear) and precultured in standard Cor.At® Culture Medium for up to 10 days. Several Fluo dyes were tested in different buffer systems at either 2 µM or 2.5 µM with incubation at 37°C for 30 - 60 min. Measurements were performed at 37°C in the FDSS7000EX equipped with a high sensitive CCD camera, and the high speed (8 msec) camera mode was compared to the 100 msec mode.

Human iPS-derived Cor.4U® cardiomyocytes were seeded at 10k per well in fibronectin-coated 384w Plates (Greiner µClear) and precultured in standard Cor.4U® Culture Medium for 5 - 7 days prior measurement. Cells were loaded using the FLIPR® Calcium 5 Assay Kit Component A (Molecular Devices, Sunnyvale, CA) dissolved in IMDM medium w/o FBS for 30 - 60 min at 37°C. Ca++-transients were measured in the FDSS µCell at 37°C. After a background measurement was performed, compounds were added at various concentrations using the 384w injector head of the FDSS µCell, and compound effects were measured at various timepoints.

Results

During assay optimisation, the dyes Fluo-4, Fluo-4FF, Fluo-8, and Fluo-8AM were tested (data not shown). In this assay, Fluo-8AM worked best; for further experiments the Molecular Devices FLIPR Calcium 5 Assay Kit was used. Dissolving the dye in cell culture medium w/o FBS gave better results than using a HBSS buffer. Since the FDSS instruments are temperature-controlled, loading of the cells was directly monitored within the instrument. Usually, a 30 min incubation is sufficient for good results. For compound analysis, a time-matched vehicle control has to be measured on the same plate for normalization of the values.

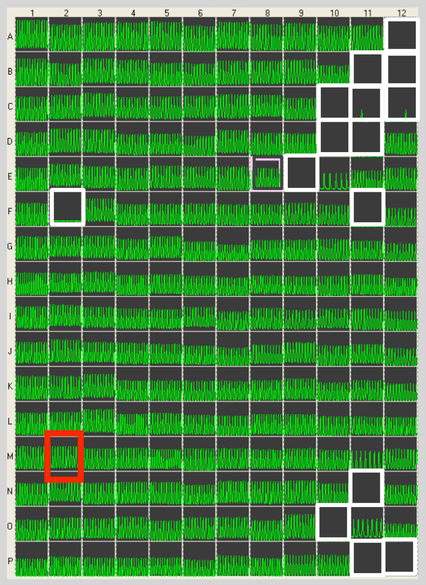
Using human Cor.4U cardiomyocytes after 5 - 7 days of culture, stable Ca++ - transients could be measured for at least 45 min (Fig. 3). In most cases, recordings from all wells of a 384w plate were possible, non-responding wells were mainly due to handling issues (Fig. 4, white boxes).

A total of 20 compounds was measured during this study, 6 control compounds and 14 inhouse compounds. Data of 4 control compounds are presented in Fig. 4.

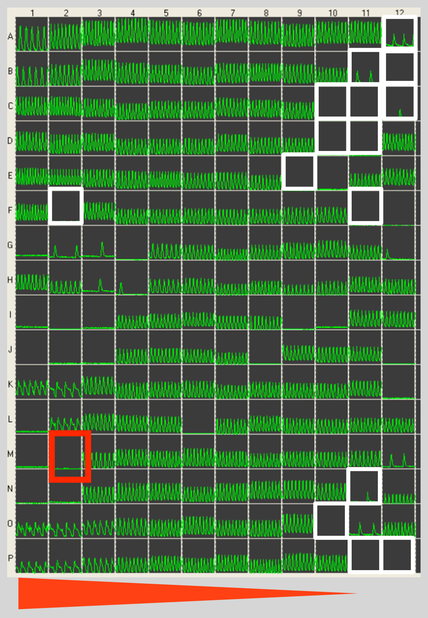
Our results suggest that the combination of the FDSS instruments with Axiogenesis' stemcell-derived cardiomyocytes is suitable as an HTS-ready assay for early cardiotoxicity screening.

Fig. 4: Detection of compound effects on Ca++-transients in iPSc-derived human Cor.4U® cardiomyocytes

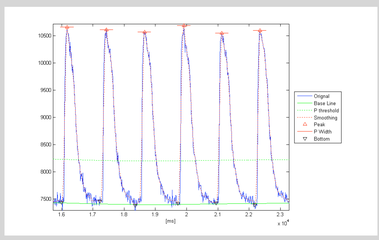
Before Drug Application



After Drug Application



Baseline recording



Addition of 50 nM E-4031; 5 min

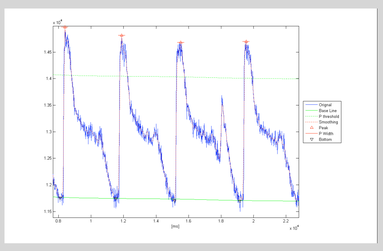
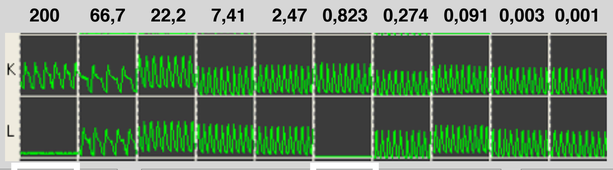
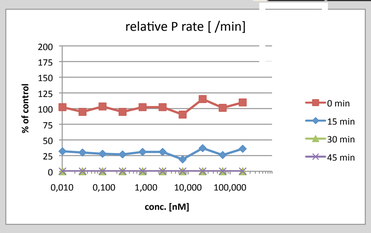


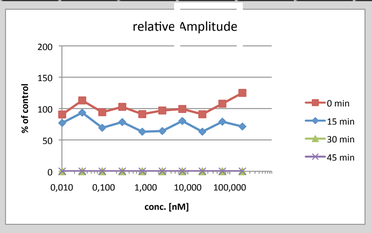
Fig. 4 ctd: Detection of compound effects on Ca++-transients in iPSc-derived human Cor.4U® cardiomyocytes

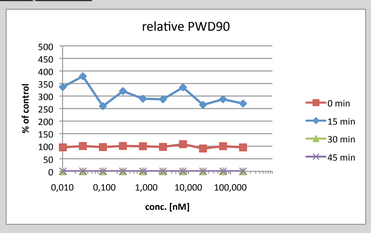
A: E-4031

200 66,7 22,2 7,41 2,47 0,823 0,274 0,091 0,003 0,001 nM



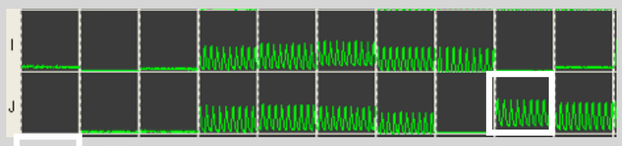


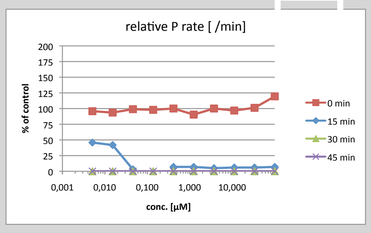


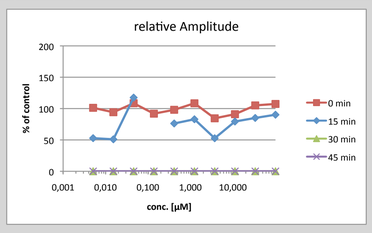


B: Isradipine

100 33,33 11,11 3,704 1,235 0,412 0,137 0,046 0,015 0,005 µM







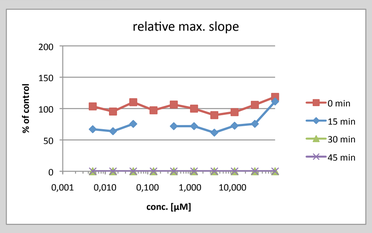
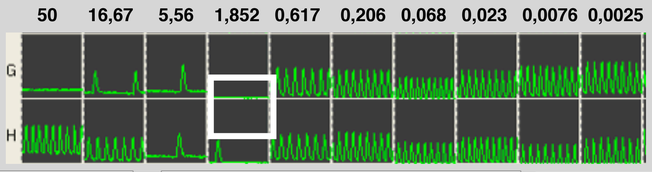
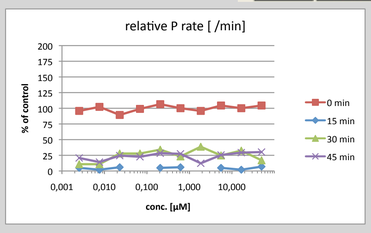


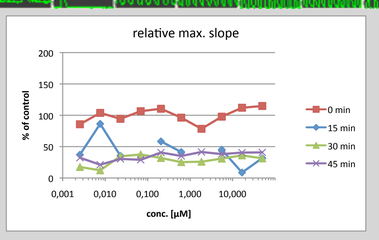
Fig. 4 ctd: Detection of compound effects on Ca++-transients in iPSc-derived human Cor.4U® cardiomyocytes

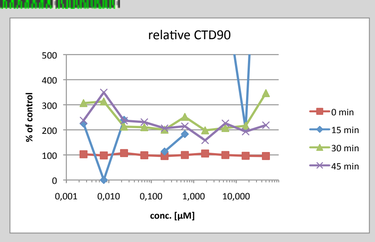
C: TTX

50 16,67 5,56 1,852 0,617 0,206 0,068 0,023 0,0076 0,0025 µM



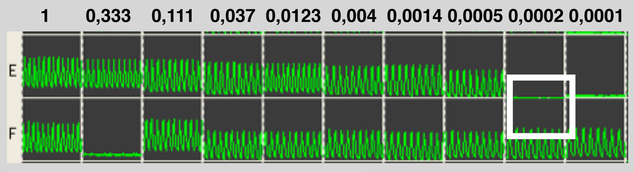




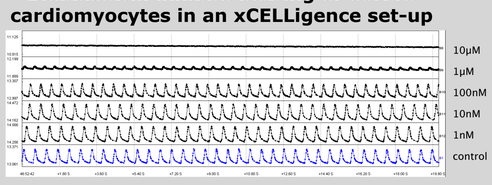


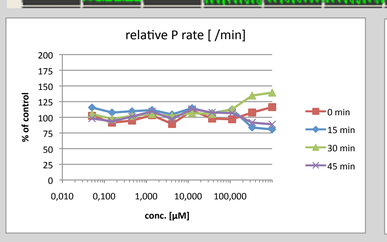
D: Blebbistatin

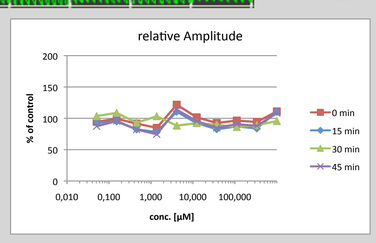
1 0,333 0,111 0,037 0,0123 0,004 0,0014 0,0005 0,0002 0,0001 µM

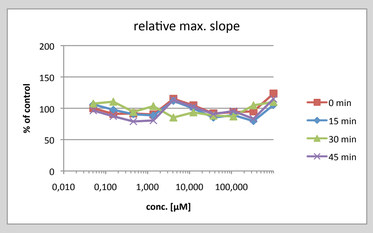


Blebbistatin inhibits beating of Cor.4U cardiomyocytes in an xCELLigence set-up









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