Cardiotoxicity is a key reason for drug attrition from the clinic. To identify potential toxicity in drug candidates as early in drug development as possible, predictive and high throughput assays are needed. Current in vitro cardiotoxicity testing platforms have two main drawbacks. The physiologically relevant models such as primary human cardiomyocytes cultures are limited to low throughput. On the other hand, high throughput compatible models like hERG overexpressing cell lines lack the relevantophysical responses, such as sensitivity to changes in cardiac contractility. The availability of hiPSC-derived cardiomyocytes (hiPSC-CMs) provides the opportunity to develop high-throughput compatible assays allowing detection of changes in cardiac contractility with a high sensitivity, therefore, applicable for early phases of drug discovery.

Using proprietary hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) that recapitulate a human cardiomyocyte’s contractile and electrophysiological profile, we developed an assay to assess effects of cardiotoxic compounds on the Ca$^{2+}$-flux (a surrogate for contractility) of Pluricyte® Cardiomyocytes using the fast kinetic fluorescence imaging system FLIPR Tetra® and the FLIPR® Calcium-8 kit. The assay is fully automated using 384-well plate format, enabling multi-parameter analysis including beating frequency, average peak amplitude, and peak width. Data analysis was performed automated using Dotmatics Vortex® software.

To validate the assay, we tested known cardiotoxic compounds such as the β-adrenoceptor agonist isoproterenol, the hERG blocker E4031, the L-type Ca channel blocker Nifedipine and the Ca channel agonist BayK 8644. Next, we profiled a selected panel of 60 commercially available compounds representing different mechanisms ofcardiotoxicity at 30 minutes post-treatment.

Our data show that optimal cell handling and assay optimization is crucial to establish a reproducible assay with low intra-well and intra-plate coefficient variation (%CV) of peak amplitude and beat frequency.

Figure 1. Characteristics of Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium.

A: Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium exhibit a high degree of ultra-structural sarcomere organization as determined by immunofluorescence (Green: alpha-actinin; Red: myosin heavy chain 7).

B: A representative calcium transient fluorescent signal of Pluricyte® Cardiomyocytes. Calcium transient data are obtained from Pluricyte® Cardiomyocytes treated with 0.1% DMSO (negative control) using the FLIPR Tetra® platform. The FLIPR Calcium 6 Assay Kit (Molecular Devices) was used to detect calcium-transients.

Figure 2. Experimental setup of high-throughput compound screening in calcium transient assay.

A: Pluricyte® Cardiomyocytes were cultured with Pluricyte® Cardiomyocyte Medium for 8 days in 384-well plates. A selection of 60 compounds chosen from the Screen-well® Cardiotoxicity library (Enzo Life Sciences) was screened in single doses (10 μM) and in four replicates per plate. Reference controls E4031, Nifedipine, BayK 8644, and isoproterenol were used to generate concentration response curves (CRC), ranging from 3 nM up to 100 μM (right to left, each concentration in duplicate). 0.1% DMSO was used as vehicle control.

B: Representative screenshot of one of the replicate plates 30 minutes after the compound treatment. Reference compounds showed the expected effect and were used as positive controls for relevant parameters as follows: Isoproterenol increased beat rate and decreased peak spacing, BayK 8644 increased peak spacing and increased amplitude, Nifedipine decreased peak amplitude and E4031 caused arrhythmia like events (see also Figure 4B).

To develop a robust calcium transient assay suitable for high throughput compound screening the effect of cell confluency on the data quality was assessed. A: Bright Field images (IncuCyte ZOOM) obtained from cells with >95% confluency showed a significant peak amplitude, and low variation of amplitude and peak width (top row and Fig. 3B red bars). Plates containing wells with lower levels of confluency (bottom row and Fig. 3B white bars) showed a poor peak amplitude, and high variation which is not favorable for high throughput compound screening.

B: In order to set a threshold for automated QCing of plates prior to the compound screening, different experiments with various seeding densities were thoroughly analyzed. Based on the obtained data, two parameters were selected for the validation of plates prior to the compound screening: 1) Above 90% of the wells per plate should be fully confluent, 2) average peak amplitude obtained from the Ca-flux baseline measurement should be at least 1000 per plate. The QC-passed plates (red bars) exhibit low intra-well variation of the peak amplitude and peak width which is required for high throughput compound screening.

Figure 3. Full confluency (> 95%) of the monolayer is required for a robust calcium transient assay.

A: The effect of compounds on amplitude, beat frequency and peak spacing assessed 30 minutes after treatment. As mentioned in Figure 2, corresponding positive controls were used for relevant parameters. 0.1% DMSO was used as the negative control. The shaded boxes represent the assay window which is calculated based on the %CV of the negative control. The compounds present in the shaded box or further than the positive controls are considered as a significant effect.

B: List of compounds identified to have significant effect on the amplitude, beat frequency and/or peak spacing.

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