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Electrophysiological Characterization of iCell² hiPSC derived Cardiomyocytes: The New Generation of CDI hiPSC-CMs

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Abstract

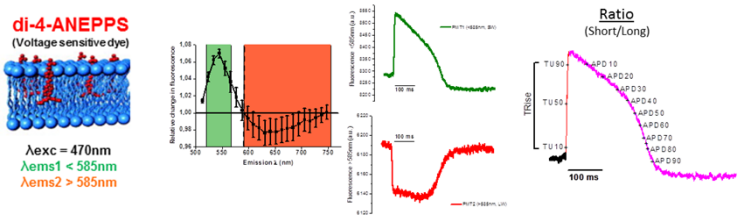
Most of the efforts in cardiac safety pharmacology are orientated towards the improvement of early stage drug screening. In this regard, the creation of in vitro human ventricular electrophysiology has been accelerated by the commercial availability hiPSC-CMs. Many studies have validated iCell cardiomycytes (Cellular Dynamics Inc.) as a robust model to carry out predictive cardiotoxicity, but the requirement of at least 10 days in vitro (DIV), increases the handling and associated risks. The electrophysiological characterization of 2nd generation of this cell type (iCell² cardiomyocytes) with accelerated maturation processes that allows their use after 4-7 DIV is presented. Cellular electrophysiology was examined using CellOPTIQ™ (Clyde Biosicences Ltd.). The cells are cultured in 96-well glass bottom plates. The electrical activity is recorded on cell monolayers loaded with di-4-ANEPPS (transient incubation in serum-free media). Baseline electrical recordings are registered after 7 DIV using the CellOPTIQ™ system (10kHz, 15s). A dose-dependent APD shortening was evident in iCell² following treatment with the L-type Ca^{2+} channel blocker nifedipine. A dose-dependent prolongation of APD is shown upon E4031 hERG blockade, with EADs evident following treatment with the highest concentration of E4031 (0.1µM). In summary, the electrophysiological characteristics of iCell² cell line are easily recorded using the CellOPTIQ™ system and are suitable for use in cardiotoxicology screening.

Results

Baseline parameters obtained for iCell² hiPSC-CMs were: cycle length 2±0.5s, APD20, APD50 and APD90 were 181.2±42.5ms, 374.2±55.6ms and 460.6±63ms respectively. Baseline rise time and diastolic interval values were 6.2±3.4ms and 1567±484ms (N=190). iCell² hiPSC-CMs were treated with a variety of ion channel blockers: Nifedipine (L-type Ca^{2+} channel blocker), E4031 (hERG channel blocker) and Flecainide (Na^{+} channel blocker). Nifedipine caused a significant dose dependent decrease of APD90 from 78.64±4.5% of baseline following 0.03µM treatment to 40.1±1.1% of baseline following 0.3µM treatment (**p<0.01, ***p<0.001; n=4). E4031 caused a significant dose dependent increase in APD90: 223.63±22, 230.02±18.9 and 665.97±229.9% of baseline respectively following 0.01, 0.03 and 0.1µM treatment (*p<0.05; **p<0.01; ***p<0.001; n=4). Flecainide caused a dose dependent increase in APD90 which was significant following 1µM treatment, 268.4±28.8% of baseline (***p<0.001; n=4). Following 3µM flecainide treatment iCell² hiPSC-CMs became quiescent.

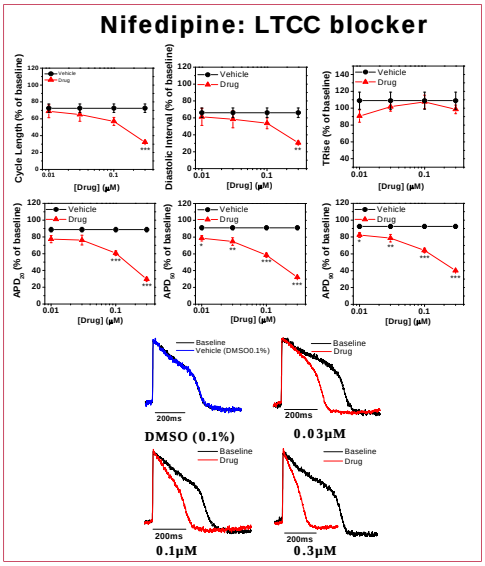
Methodology

iCell Cardiomyocytes were purchased from Cellular Dynamics International (USA) and were seeded in a fibronectin coated 96 well plate (50,000 cells/well) and cultured for 7 days. Following maturation, the cells were transferred to serum free media and transiently exposed to voltage sensitive dye (Di-4-ANEPPS). Measurements were obtained before and after drug treatment by exciting the cells with a 470nm LED. The emitted fluorescence was recorded at 10KHz from regions of iCell² cardiomyocytes for periods up to 15s on the CellOPTIQ™ electrophysiology platform (Clyde Biosciences Ltd). The records were subsequently analysed off-line using proprietary software (Clyde Biosciences Ltd). A Dunnett's test was completed to test for statistical significance of all data (*p<0.05; **p<0.01; ***p<0.001).

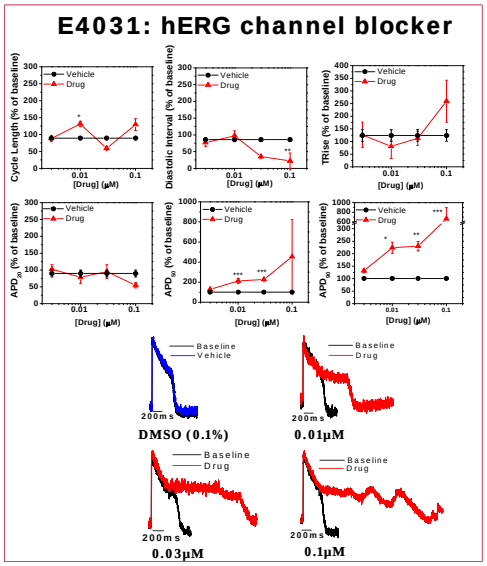


	Cycle Length (s)	Diastolic Interval (ms)	Trise (ms)	APD20 (ms)	APD50 (ms)	APD90 (ms)	N
iCell² hiPSC-CMs (mean±SD)	2±0.5	1567±484	6.2±3.4	181.2±42.5	374.2±55.6	460.6±63	190

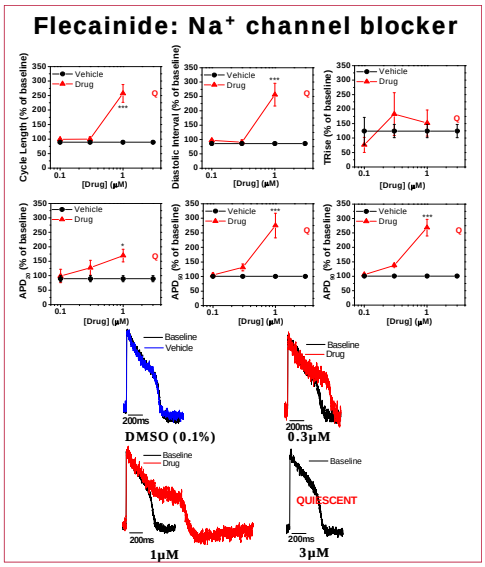
Nifedipine: LTCC blocker



E4031: hERG channel blocker



Flecainide: Na^{+} channel blocker



Conclusion

- ✓ iCell² hiPSC-CMs have a suitable electrophysiological profile for cardiotoxicology screening. In conjunction with the optical CellOPTIQ™ platform and VSD employment, the electrical properties of these cells before and after drug treatment can be studied.

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