Calcium Transient Assay for Cardiac Arrhythmic Potential Evaluation: using human iPS-Derived Cardio Myocytes

Commonly used acronym: *CTCM on hiPS-CMs*

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health, Other
The Method is situated in	Regulatory use - Routine production, Translational - Applied Research, Other: Safety Pharmacology
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	hiPSC-derived cardiomyocites

DESCRIPTION

Method keywords

Calcium transient
Proarrhythmia
Stem cell
Fluorescent Dye Calcium-sensitive Imaging
Cardiomyocyte
hiPS-CMs
Human induced Pluripotent Stem Cell

Scientific area keywords

Safety Pharmacology
Side effects
medium-high throughput assay
cardiovascular derisking
regulatory

Method description

Cell Culture and Reagents hiPSC-CMs can be obtained commercially either as living pre-plated cells seeded onto fibronectin-coated 96-well mClear plates (Greiner Bio-One, No. 655090) or can be plated in house at a density (~25,000 cells/well) suited to forming a confluent synchronously beating mono-layer. Most commercially offered cardiomyocyte lines represent a mix of ventricular, atrial and nodal cells and derived from one human donor. Cells were cultured with commercial culture medium (optimized for the specific cell line) in a humidified incubator at 37oC and 5% CO2, with medium being changed once a day. On the day of the experiment, the culture medium was replaced with Tyrode's solution (Sigma, No. T2397) supplemented with 10 mM HEPES together with KCl to represent isokalemic (4.2 mM K+) conditions. As a calcium-sensitive fluorescence dye Cal-520 AM (Cat. No. 36,338; AAT Bioquest) was used to capture the intracellular calcium transients. Accordingly, Cal-520 was incubated for 45 min followed by a washout and a 30-min recovery before starting the experiments. Calcium Transient Measurements Spontaneous beating activity of hiPSC-CMs was assessed through measurement of the Ca2+ fluorescence signal integrated over the whole well. Fluorescence signals were measured using the Hamamatsu FDSS/mCell platform and the records subsequently analyzed offline using NOTOCORD-hem software (version 4.3), containing EXT modules and an algorithm developed by XiTechniX to detect beat-by-beat Amp, BR, and CTD90 parameters. All wells within a plate were measured simultaneously using the following FDSS/mCell settings: sampling frequency 66.7 Hz, exposure time 14.6 ms, excitation wavelength 480 nm, emission wavelength 540 nm, temperature controlled at 37oC. First, the experimental plates were put into the FDSS/mCell to stabilize for 10 min. Next, a baseline recording was run for 3 min followed by compound addition. The effect of a compound was recorded (5-min recording time) around 15 and 30 min after compound addition. CTD90, BR, and Amp were quantified for baseline and 30-min compound effects as the median value of all beats (calcium transients) measured within a 1-min interval of the recording. The recording around 15 min was used only for observation of Early-After-Depolarisation (EADs) or fibrillation-like events. EADs were manually monitored and evaluated. Cessation of beating was defined after 30 min in case BR was <5 beats/min. Wells that temporarily stopped beating during compound addition but recovered at the 30-min time point were not defined as beat stop.

Lab equipment

Laminar flow hood ;
Cell Incubator ;
amamatsu FDSS/mCell platform ;
Analysis software.

Method status

Internally validated
Published in peer reviewed journal
Validated by an external party (e.g. OECD, EURL ECVAM,…)

PROS, CONS & FUTURE POTENTIAL

Advantages

Early (fast) evaluation of arrhythmic potential using human cells

Challenges

Maturation of cells remains debate ;
Cell-layer don't reflect 3D-complexity of a (human) heart ;

Commercial cell line represents n = 1.

Modifications

Use of more mature cells (due to improved culture conditions ?) ;
Improved phenotypic appearance (obtaining closer cellular resemblance of the human heart).

Future & Other applications

Calcium transient method can potentially be applied for measuring activity of other cell types as well

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Lu HR et al., Assessing Drug-Induced Long QT and Proarrhythmic Risk Using Human Stem-Cell-Derived Cardiomyocytes in a Ca^{2+} Imaging Assay: Evaluation of 28 CiPA Compounds at Three Test Sites. Toxicol Sci. 2019 Aug 1;170
Kopljar I. et al., Development of a Human iPSC Cardiomyocyte-Based Scoring System for Cardiac Hazard Identification in Early Drug Safety De-risking. Stem Cell Reports. 2018 Dec 11;11(6):1365-1377
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