Rapid Intensity Modulation of a Single Light Source Allows Excitation of Voltage Sensitive Dye and Intermittent Activation of Channel Rhodopsin in hiPSC Derived Cardiomyocytes (hiPSC-CMs)

Zamora V. [1], Hortigon-Vinagre M.P. [2], Bruegmann T. [3], Costa A. [4], Craig M.A. [4], Burton F.L. [2,4], Sasse P. [3] and Smith G.L. [2,4]

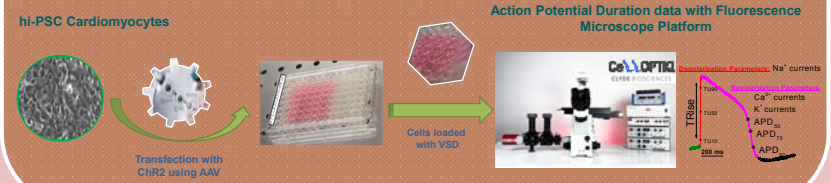
[1] Department of Biomedical Engineering, University of Glasgow, United Kingdom
[2] Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom
[3] Institute of Physiology I, University of Bonn, Germany
[4] Clyde Biosciences Ltd, University of Glasgow, United Kingdom

Abstract

One inconvenient aspect of using human induced pluripotent stem-derived cardiomyocytes (hiPSC-CM) is their spontaneous activity which can vary during the assay and affect the underlying action potential duration. It can be solved by controlling the beating rate. The wider way to carry it out is based on electrical stimulation, but it has troublesome such as low-throughput due to the well-plate format necessary and the release of electrolytes that can damage or contaminate the cells. The second and more suitable alternative to overcome these problems is the optogenetic approach. Up until the implementation of the optogenetic protocol, the cardiotoxicity assays were performed using spontaneous clusters of hiPSC-CMs, correcting the Action Potential Duration values for changes on cycle length in based of correction factors. The employment of cell transfected with channel rhodopsin (ChR2) offers the possibility of controlling stimulation rate via pulses of light, despite excitation wavelengths overlapping with those required to excite the VSD di-4-ANEPPs (λexc = 470nm). Using this approach hiPS-CMs were stimulated at a range of frequencies (0.5-2 Hz).

Methods & Materials

Commercially available hiPSC-CMs (Cellular Dynamics Inc.) were selected to carry out the studies. To test dual use of VSD and ChR2, hiPSC-CMs were transfected with ChR2 using an adeno-associated virus (AAV) vector (Penn Vector Core AAV1.CAG.hChR2(H134R)-mCherry). The multiplicity of Infection (MOI) employed were a range of concentration between 20000 and 100000 particles per cell. To analyze and record the membrane potential signal at the different pacing rates applied, the cells were transiently loaded with fluorescent voltage sensitive dye di-4-ANEPPS and the cardiac electrical activity was monitored from iPSC-cardiomyocytes using CellOPTIQ platform which is equipped with a chamber suitable for multi-well plates which controls CO_2 and temperature while the dye is illuminated via epi-fluorescence optics via an inverted microscope (40x 0.6NA objective). The sampling rate employed for this assay was 10,000 Hz. Recordings were performed over 30 seconds time windows, and were subsequently analyzed off-line using proprietary software (Clyde Biosciences).



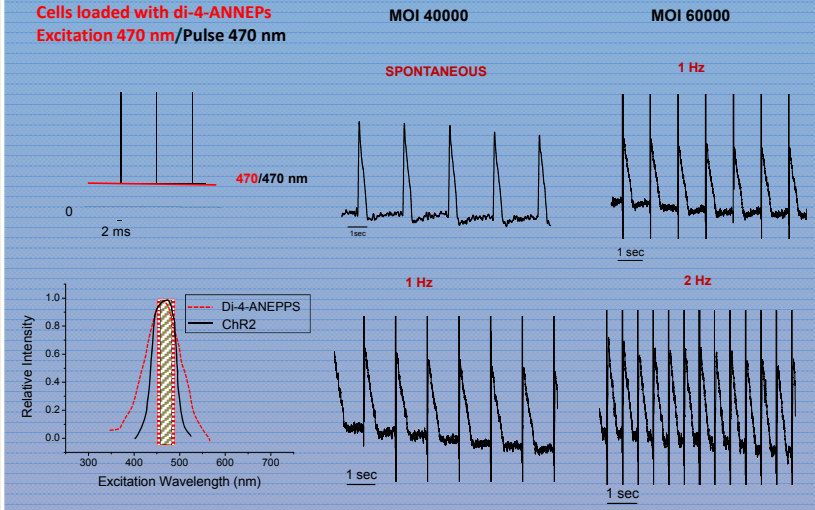
References

- Optogenetic activation of Gq signalling modulates pacemaker activity of cardiomyocytes. Beiert T. et al. Cardiovascular Research
- Optical pacing of the heart: The long way to enlightenment. Philipp Sasse. Circulation Arrhythmia and Electrophysiology

Results

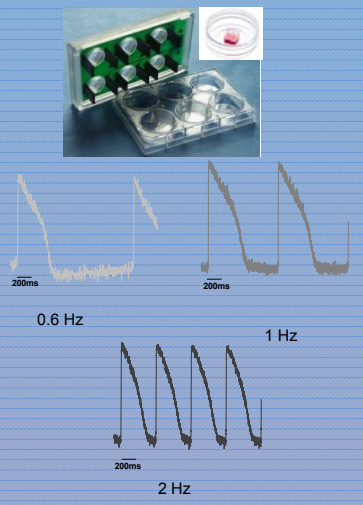
Transfection at >20,000 MOI for 48h was successful when used at early stages after plating (day 2-4). 24h after removing the virus, ChR2 expression was sufficient to allow cells to be stimulated using light pulses. To avoid interference, Di-4-ANEPPS was excited with continuous low intensity (approx. 0.05-0.1mW) illumination which is well below the threshold to excite ChR2. Pulsing illumination at ~1-2mV for ≥2ms was sufficient to initiate action potentials at rates up to 2Hz.

Optogenetic Approach



Simple optical intensity switching offers the possibility of incorporating fixed stimulation rates into a high-throughput assay for cardiac electrophysiology to increase data reproducibility.

Conventional Field Stimulation



Field stimulation normally requires solid-state electrodes that cannot easily be applied without disrupting the culture conditions and cannot be applied to 96 well plates preventing the performance of high throughput experiments.

Conclusions

- ✓ Adeno Associated Virus transfection of ChR2 is effective on commercial source of iPSC-CMs
- ✓ Optogenetic stimulation of iPSC-CMs can be integrated with optical measurements of electrical activity
- ✓ Low ChR2 expression allows single wavelength modulation to pace and measure electrical activity
- ✓ Higher ChR2 expression facilitates pacing at high frequency

Acknowledgements

This work was supported by the Biotechnology and Biological Sciences Research Council (UK), Clyde Biosciences (UK). M.P. H-V. is recipient of a postdoctoral fellowship from Fundacion Alfonso Martin Escudero (Spain).

Contact Information
Víctor Zamora Rodríguez
victor.zamorarodriguez@glasgow.ac.uk

14TH ANNUAL MEETING