CLYDE BIOSCIENCES

axio GENESIS
Use our discoveries to advance yours

www.clydebio.com

Optical Detection of Excitation-Contraction Coupling in iPS-Derived Cardiomyocytes and its Application to Medium/High Throughput Cardiac Safety Assessment

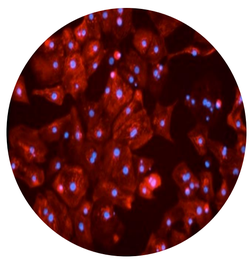
Zamora, V[1]; Hortigon-Vinagre, M.P[1]; Craig,M.A[1]; Clark, J[1]; Luerman, G[2];Smith, G[1]

1 Clyde Biosciences Ltd, Glasgow, UK
2 Axiogenesis AG, Cologne, Germany

Abstract

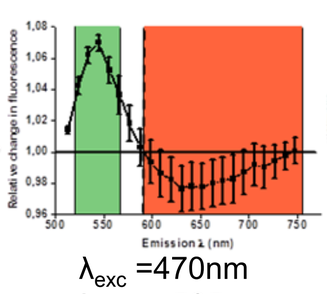
The process of excitation-contraction coupling is the key link between electrical and mechanical events in cardiac muscle and represents a range of targets for cardiotoxic effects. The characteristics of the electrical event (action potential) are important in determining the efficiency of the E-C coupling process and the relative electrical stability of the heart (arrhythmias). The amplitude and time course of the intracellular Ca^{2+} transient is a critical determinant of the inotropic status of the heart and has the potential to influence the membrane potential and the pro-arrhythmic status of the myocardium. The relationship between intracellular Ca^{2+} and the subsequent contractile event provides information on the inotropic status of the myocardium. All these functional aspects of cardiotoxicity can addressed using Axiogenesis Cor4U iPS-derived cardiomyocytes in conjunction with Clyde Biosciences Ltd CellOPTIQ™ platform

Methodology



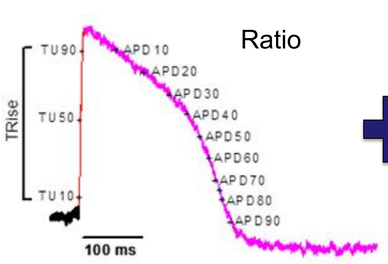
axio GENESIS

iPSC-CMs



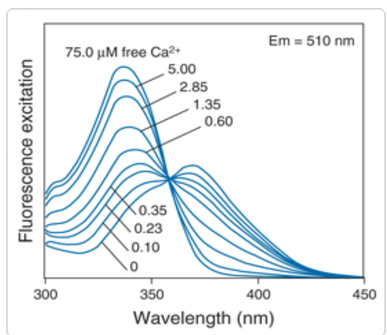
λ_{exc} =470nm
λ_{ems1} <585nm
λ_{ems2} >585nm

Electrical Activity (Di-4-Anepps)



Action Potential

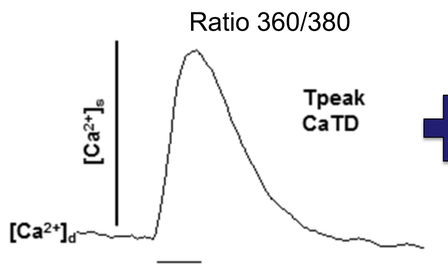
Intracellular Ca^{2+} (Fura4F-AM)

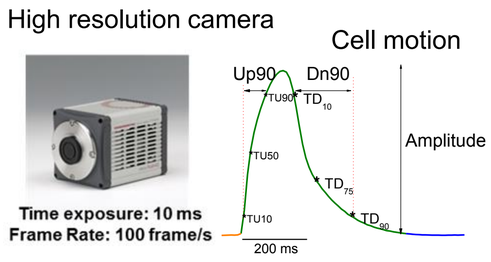


λ_{exc}=360/380nm
λ_{ems}<510nm

Calcium Transient

Contractility (Image Analysis)



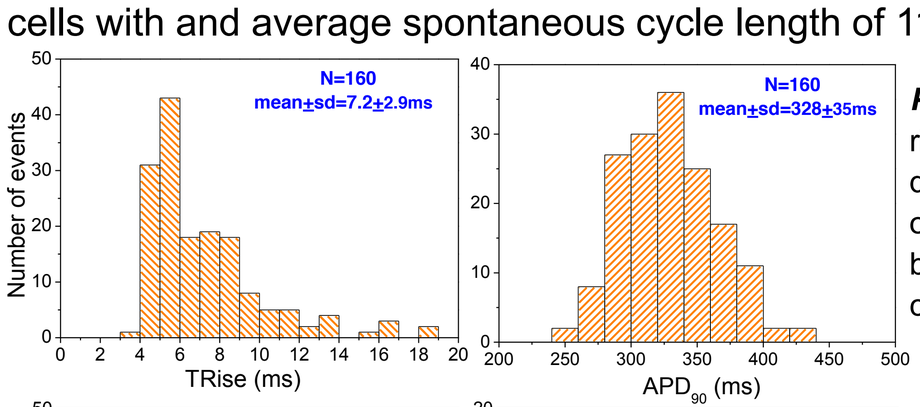


Contractile Kinetic

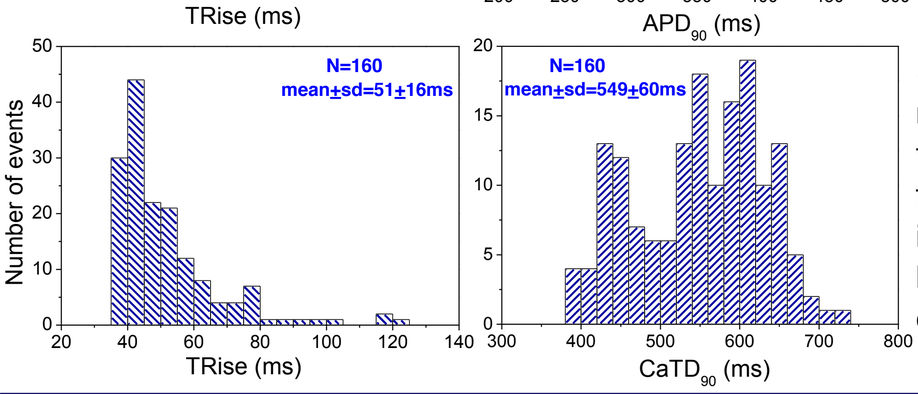
To carry out the studies, iPSC-CMs (Axiogenesis) were plated in 96 well glass bottom plate (25,000 cells/well) and were transiently loaded with di-4-ANEPPS and Fura 4F-AM. The dye Di-4-ANNEPPS is used to ratiometrically measure transmembrane voltage (10 KHz acquisition rate), Fura-4F to measure ratiometrically assess Ca^{2+} (1 KHz acquisition rate), while contractility was measured via cell motion algorithm (100 Hz frame rate) over 15 seconds time windows employing the platform CellOPTIQ™, and the records were subsequently analysed off-line using proprietary software (Clyde Biosciences).

Results

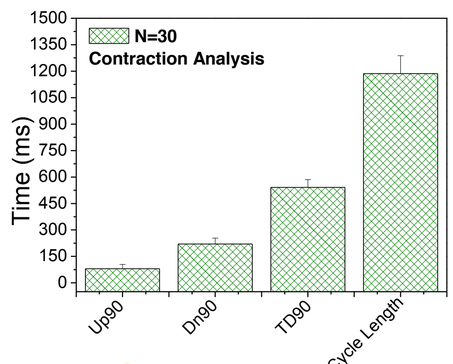
Baseline functional characteristics of the cells vary minimally across wells and plates, on average (Mean/SD) the rise time (10%-90%) was 7.2ms/2.9, the action potential duration at 90% repolarisation was 328.2ms/35.4. Ca^{2+} transients had a rise time of 51.8ms/16.3 and a duration at 90% of 549.1ms. The contractile event duration 80.5ms/23.7 and a duration at 90% of 541.6ms. These were measured from spontaneously active cells with and average spontaneous cycle length of 1186.1ms/101.9



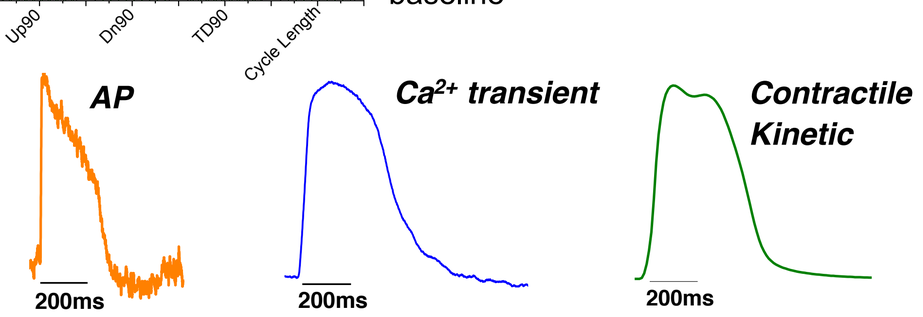
Panel A. Histogram graphs for the rise time duration and action potential duration at 90% of the repolarization on the transmembrane voltage baseline behaviour of the Cor.4U cardiomyocytes



Panel B. Histogram graphs for the rise time duration and calcium transient duration at 90% using fluorescence assay to measure the intracellular Ca^{2+} events on the baseline behaviour of the Cor.4U cardiomyocytes



Panel C. Mean and SD values for the contractile duration at 90% (UP90), gradient of the relaxation period (Dn90). TD90 is the contraction decay duration at 90% and finally the cycle length duration on the spontaneous activity of the baseline



Panel D. Representative traces of Action potential, Intracellular Ca^{2+} events and Contractile kinetic of the baseline behaviour on Cor.4U cells

Conclusions

- Axiogenesis Cor.4U® cells represent a human iPS-derived cardiomyocyte system with stable electrical and mechanical suitable for cardiotoxicity and novel lead investigation
- The small variance of these parameters means that small differences (~10%) in any of these functional parameters could be detected with low repeat numbers (n=5) providing a rapid and sensitive assay of human cardiac muscle function

For further information please contact:

Clyde Biosciences
BioCity Scotland, Bo'Ness Road, Newhouse, Lanarkshire, Scotland, UK, ML1 5UH.

T: +44 (0)1698 539920
E: info@clydebio.com

Acknowledgments

This work was supported by Axiogenesis AG (Germany), Clyde Biosciences (UK).V. Zamora is recipient of a postdoctoral fellowship from Fundacion Alfonso Martin Escudero (Spain).