Predicting the Pro-arrhythmic Potential of Compounds Using Human Embryonic Stem Cell (hESC)-derived Cardiomyocytes on Multielectrode Array

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Introduction

Drug-induced delayed cardiac repolarization, a recognized risk factor for pro-arrhythmia, has become the single most common cause for the withdrawal of prescription drugs. The ability to identify detrimental off-target effects earlier has the potential to improve drug safety and reduce the cost of drug development. The vast majority of drugs known to prolong the repolarization of the cardiac membrane preferentially inhibit the delayed rectifier current (I_{Kr}) by binding to the hERG K^+ channel. Consequently, functional *in vitro* assays for predicting a drug's potential to delay cardiac repolarization typically include evaluating hERG K^+ channel block in transgenic cell lines, or action potential duration assays with primary canine or rabbit Purkinje fibres. The predictive value of these existing assays is limited, however, due to species differences and the lack of complex ion channel interactions in cell lines over-expressing hERG K^+ channel. The introduction of assays utilizing human embryonic stem cell-derived (hESC) cardiomyocytes could potentially address the shortcomings of these existing models and form the basis of more predictive assays. Here, we describe the use of hESC-derived cardiomyocytes on the multielectrode array platform (MEA) to assess the prospect of using the measured extracellular field potential as a pre-clinical cardiotoxicity screen.

Methods

Preparation of Cytiva™ Plus cardiomyocytes - To induce CM phenotype, hESCs (H7 cell line) were subjected to a controlled differentiation process. Briefly, the hESCs were adapted to alternative growth conditions, subjected to growth factor induction, followed by a period of cardiomyocyte maturation. At the end point of differentiation, cardiomyocytes were harvested and cryo-preserved at 1E06 cardiomyocytes per vial.

Seeding MEA plates - Cytiva™ Plus cardiomyocytes were seeded direct from thaw onto 48-well MEA plates (Axion Biosystems) at a density of 60,000 cells per well. On day 4 post-thaw half the seeding medium was replaced with fresh medium.

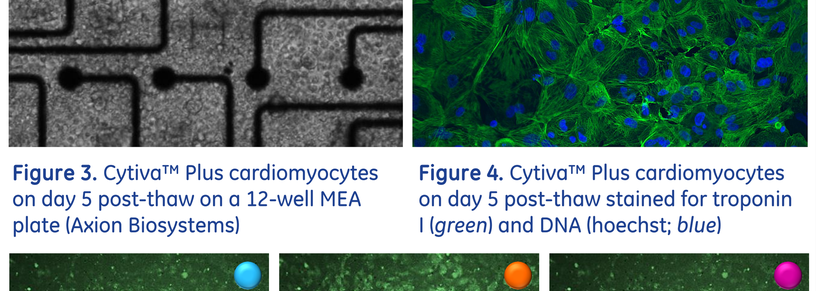


Figure 1. Time-line depicting cell seeding and maintenance for MEA experiments

MEA recordings - MEA recordings were made on day 5 post-thaw at 37°C following the compound-addition protocol outlined below.

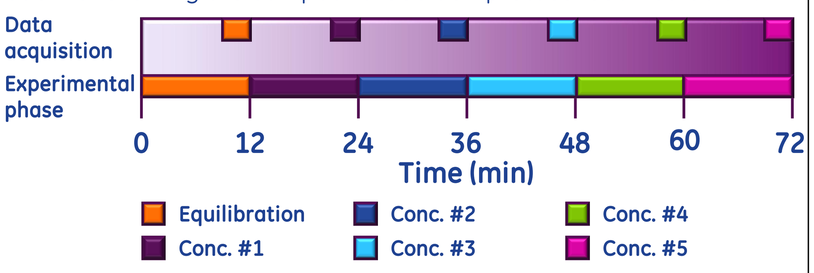


Figure 2. Time-line depicting MEA experimental protocol

Cytiva™ Plus cardiomyocyte characterization

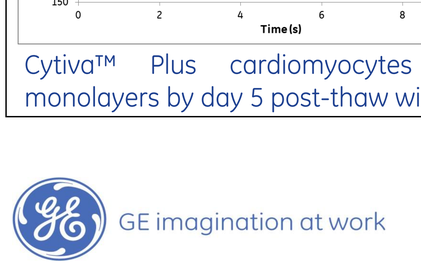


Figure 3. Cytiva™ Plus cardiomyocytes on day 5 post-thaw on a 12-well MEA plate (Axion Biosystems)

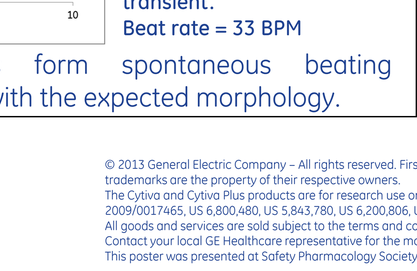
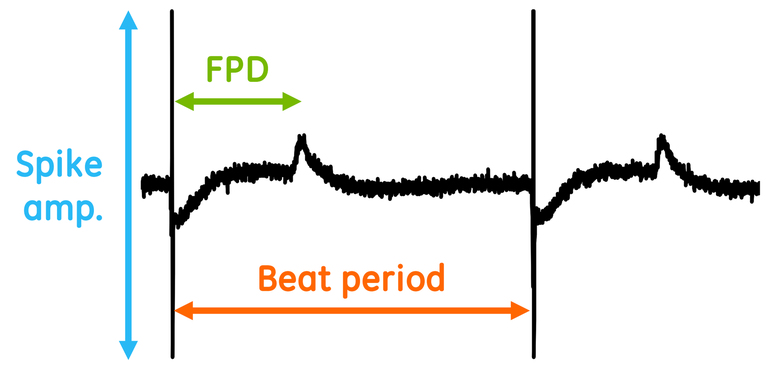


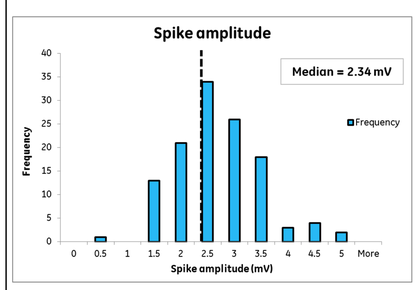
Figure 4. Cytiva™ Plus cardiomyocytes on day 5 post-thaw stained for troponin I (*green*) and DNA (hoechst; *blue*)

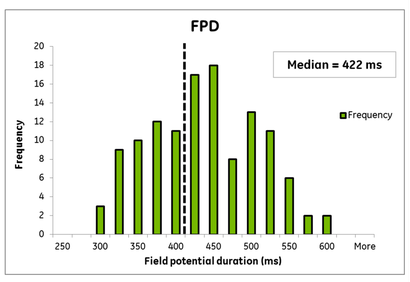
Figure 5. Spontaneous Ca^{2+} transients in Cytiva™ Plus cardiomyocytes on day 5 post-thaw imaged on IN Cell 2200 Analyzer with Fluo-4AM. Coloured circles indicate images from respective points in the Ca^{2+} transient.
Beat rate = 33 BPM

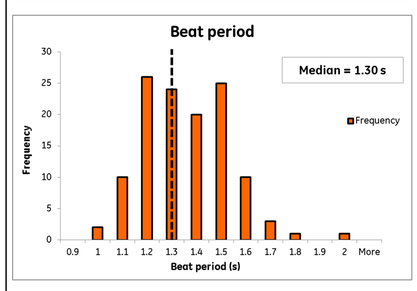
Cytiva™ Plus cardiomyocytes form spontaneous beating monolayers by day 5 post-thaw with the expected morphology.

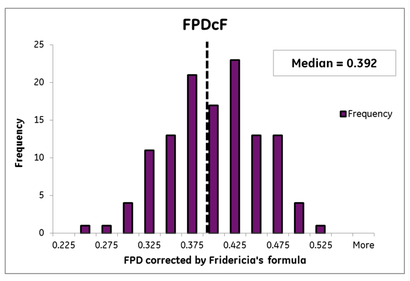
Cytiva™ Plus cardiomyocyte performance on MEA

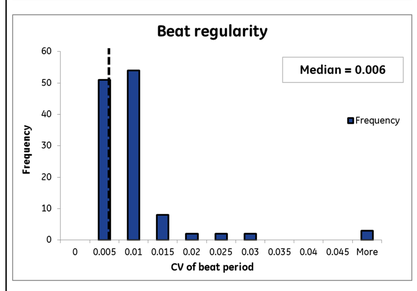


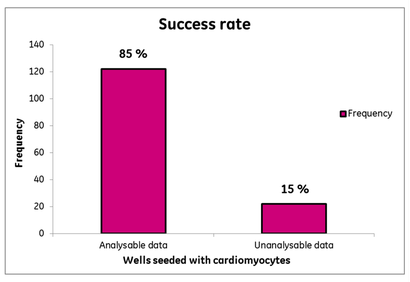






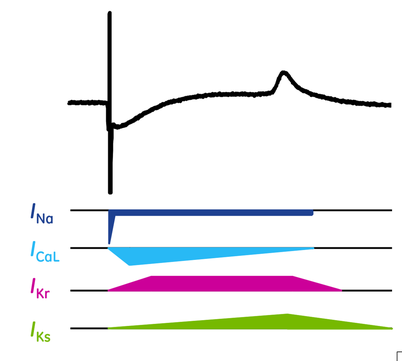




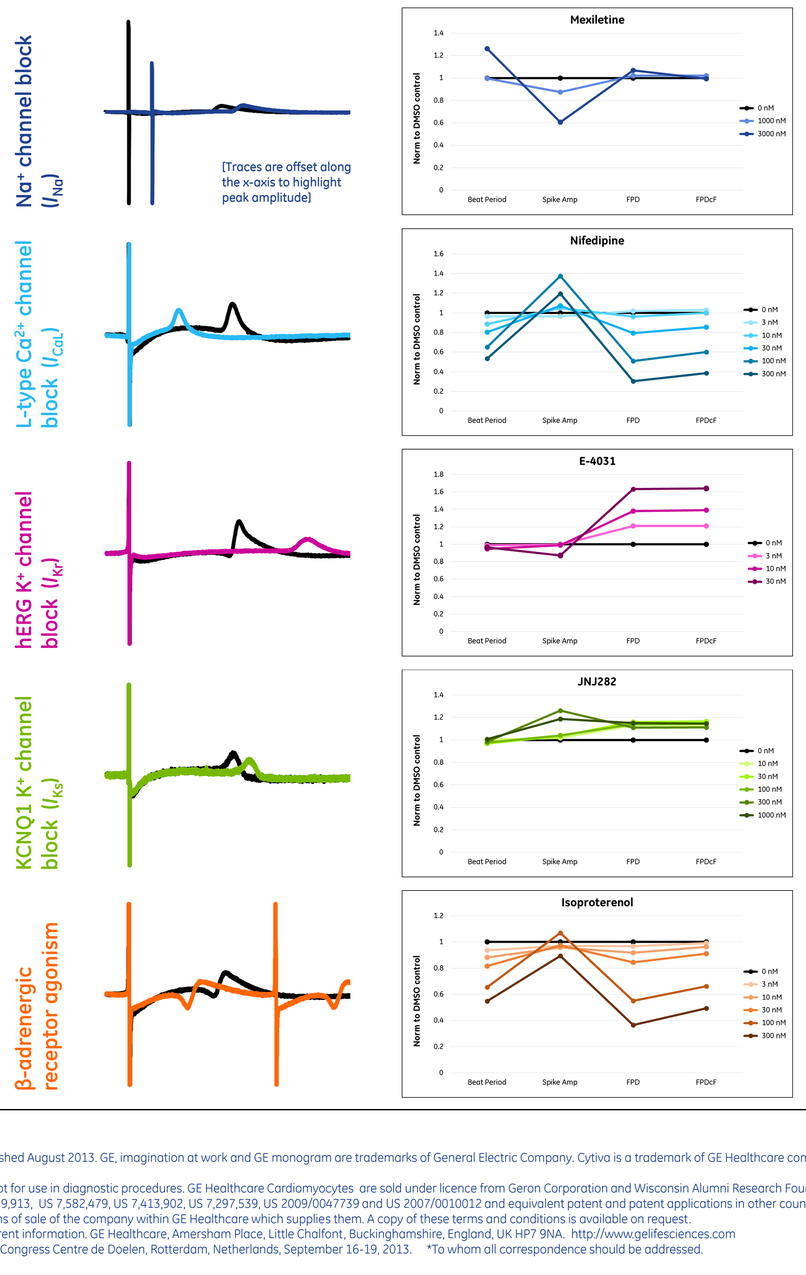


Spontaneous beating characteristics of the Cytiva™ Plus cardiomyocytes at 37°C. $FDPcF = FPD/\sqrt[3]{\text{Beat period}}$

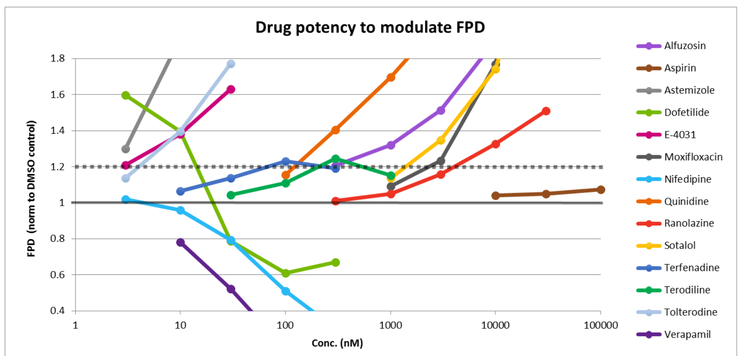
Pharmacological modulation of the Cytiva™ Plus cardiomyocyte waveform



Many different ion channels contribute to the measured extracellular field potential. Blockade of a specific ion channel results in the characteristic modulation of the Cytiva™ Plus cardiomyocyte waveform as illustrated below.



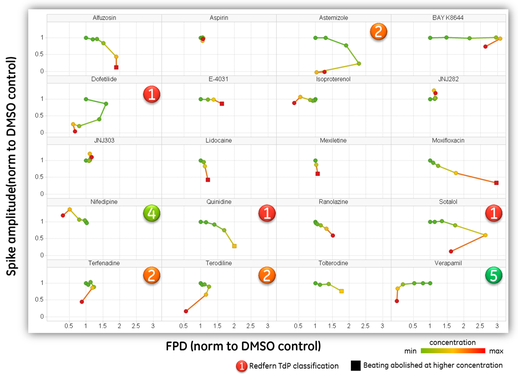
Effect of drugs known to prolong QT interval on Cytiva™ Plus cardiomyocyte FPD



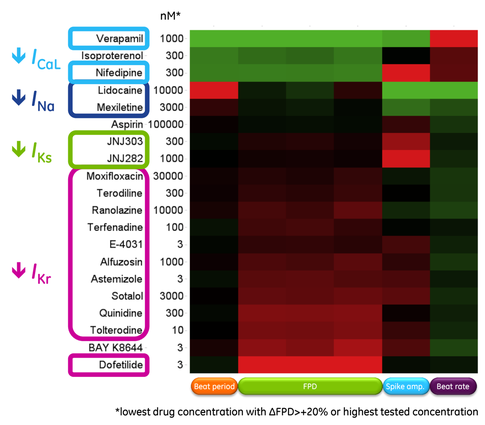
Rank (#)	Drug	Conc where FPD = +20% (nM)	hERG IC_{50} (nM)	Drug class
1	Dofetilide	< 3	10	Class III antiarrhythmic
2	Astemizole	< 3	13	Antihistamine
3	E-4031	< 3	32	Class III antiarrhythmic (experimental)
4	Tolterodine	5	10	For bladder incontinence
5	Terfenadine	90	40	Antihistamine
6	Quinidine	150	750	Class Ia antiarrhythmic
7	Terodiline	230	380	For bladder incontinence
8	Alfuzosin	300	14,000	For benign prostatic hyperplasia
9	Sotalol	1500	75,000	Class III antiarrhythmic
10	Moxifloxacin	2600	40,000	Antibacterial
11	Ranolazine	5000	12,000	Antianginal
12	Aspirin	> 1,000,000	No block	Cyclooxygenase inhibitor
13	Nifedipine	-	275	Antihypertensive/antianginal
14	Verapamil	-	540	Class IV antiarrhythmic

To validate Cytiva™ Plus cardiomyocytes as a potentially useful new *in vitro* test system for drug-induced delayed cardiac repolarization, dose escalation studies were performed for a number of drugs known to prolong the QT interval as a therapeutic or side effect. For selective hERG K^+ channel blockers, a similar rank-ordering of compounds was found for both the propensity of the drug to induce FPD prolongation and the drug's hERG IC_{50} value.

Multi-parameter analysis of MEA drug data



Correlation plots of FPD against spike amplitude for range of drug concentrations tested. Drugs with similar mechanisms of action show similar correlation graphs. Also, drugs with similar Redfern TdP classification scores [1] have similar profiles.



*lowest drug concentration with ΔFPD>+20% or highest tested concentration

Multi-parameter profiling of MEA data. Compounds with similar mechanisms of action cluster together in the hierarchical plot. Consequently, it could be possible to predict the mechanism of action of an unknown compound by where it clusters in the plot.

Summary

Cytiva™ Plus cardiomyocytes are hESC-derived cardiomyocytes that exhibit the appropriate morphology and electrophysiological responses. Cytiva™ Plus cardiomyocytes:

- Form spontaneously beating monolayers with the following extracellular field potential characteristics:
  - Spike amplitude – 2.3 mV
  - Beat period – 1.3 s
  - FPD – 420 ms
  - CV of beat period – 0.006
- Can distinguish between specific ion channel blockers. Blockade of I_{Na}, I_{CaL}, I_{Kr} or I_{Ks} results in a characteristic modulation of the Cytiva™ Plus waveform as illustrated.
- Can predict the potency of drugs that prolong the QT interval.

We propose that an MEA assay based on hESC-derived cardiomyocytes could complement or potentially replace some of the pre-clinical cardiac toxicity screening tests currently used for lead optimization and further development of new drugs.

References

[1] Redfern W.S et al. (2003) Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc Res 1;58(1):32-45.

Acknowledgements

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GE imagination at work

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