Cross-site reliability in a cardiac safety assay using multiwell microelectrode array (MEA) technology: preliminary results from the CiPA Pilot Study

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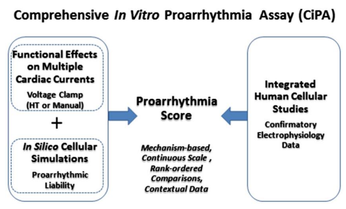
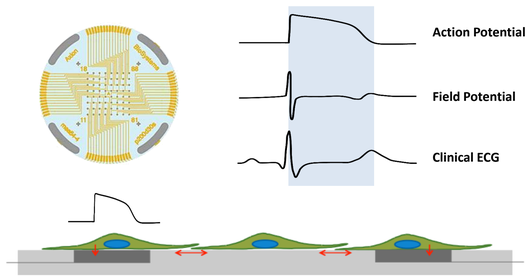
I. Introduction

- Current safety testing standards evaluate the block of the hERG potassium channel to inform cardiac liability.
- The CiPA initiative aims to facilitate the adoption of a new paradigm for assessment of clinical TdP risk, including integrated human cellular studies[1].
- Microelectrode arrays (MEA) have been used extensively with human iPSC-derived cardiomyocytes for safety testing[2,3].
- We present data from a recent CiPA pilot study supporting the predictivity and reliability of a microelectrode array based *in vitro* cell assay.

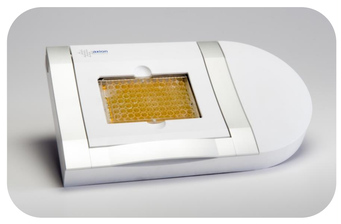


The new CiPA paradigm will combine information from *in silico* reconstructions of cellular electrophysiology and integrated human cellular studies using stem cell derived cardiomyocyte networks to provide a comprehensive pro-arrhythmia score.

II. The Maestro Multiwell MEA Platform



A grid of microelectrodes interfaces with electro-active tissue, modeling complex, human systems in a dish.



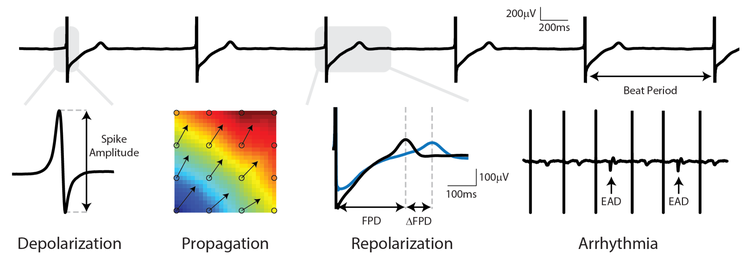
Axion's Maestro multiwell microelectrode array (MEA) platform enables high throughput evaluation of cardiac safety on the benchtop.

- **Label-free and non-invasive recording** of extracellular voltage from cultured cardiomyocytes on Axion MEA plates
- **Environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the magnitude of depolarization events
- **Sensitive voltage resolution** detects subtle repolarization features, Field Potential Duration (FPD) changes, and arrhythmic events
- **Industry-leading array density** provides high quality data through the integration of information from multiple locations in the culture
- **Scalable format (12-, 48- and 96-well plates)** meets all throughput needs on a single system

III. Cell Culture Protocol

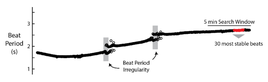
- Cells were plated according to manufacturer recommended specifications in an 8 uL drop on the electrode array, with media added to achieve a well volume of 300 uL.
- Media was changed the evening prior to the experiment and then moved straight from the incubator to the Maestro on the day of recording. The ECmini was used to supply 5% CO2 and maintain pH buffering, while the embedded heater plate kept the MEA culture plate at 37C.
- A 30 minute baseline recording was obtained, followed by a 60 minute recording after dosing. A "single dosing" scheme was utilized, with compounds prepared at 10x concentration for a 1:10 dilution upon withdrawal/addition of 30 uL to the well volume.
- Four reference compounds (E-4031, Mexiletine, Nifedipine, JNJ303) and four test compounds (Moxifloxacin, Flecainide, Quinidine, Ranolazine) were tested at 4 concentrations and 3 replicates each. Each plate contained 3 untreated and vehicle control (DMSO) wells.

IV. Analysis Methods

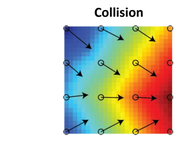


The CiPA pilot study utilized four primary endpoints derived from the MEA field potential:

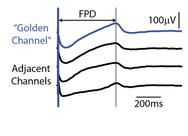
- **Spike Amplitude** – the peak to peak amplitude of the depolarization spike.
- **Beat Period** – the time interval between consecutive depolarization spikes.
- **Field Potential Duration (FPD)** – the time interval from the depolarization spike to the peak of the repolarization feature, or "T-wave".
- **Arrhythmia** – indicated by the occurrence of early afterdepolarization (EAD) events



Label free, non-invasive recording enabled:
- Continuous recording without confounding effects on cardiac function
- Identification of maximally stable signals
- Detection of rare arrhythmic events



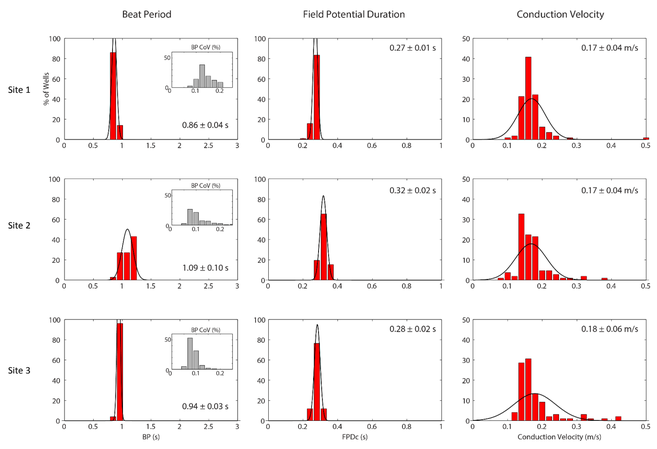
Array-based processing identified functional irregularities in beat propagation for use in quality control assessment



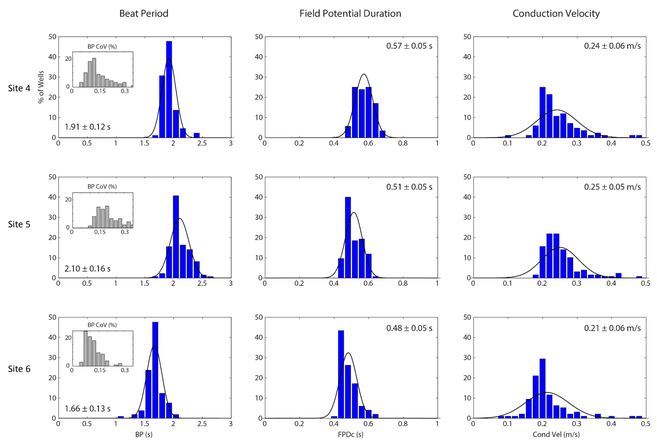
Redundancy in repolarization timing was utilized to maximize the accuracy of the measured cellular behavior

V. Baseline Reliability

- Consistency in the baseline electrophysiology across wells, plates, and sites is critical for establishing reliability in a cell-based assay.
- Cell culture and experimental protocols were optimized and rigidly defined across sites to minimize biological variability.
- Similarly, analysis methods were designed to minimize algorithmic error and compensate for remaining biological variability. Software tools were distributed and applied consistently across sites

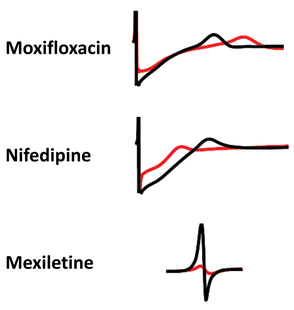


Cell type 1 exhibited consistent beat period, field potential duration (Fridericia correction), and conduction velocity across wells, plates, and sites. The beat period was also highly stable across wells (see inset, ~0.1% coefficient of variation) due to the identification of the most stable beats from the continuous recording.

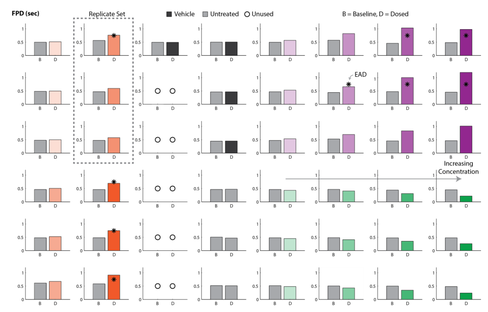


Cell type 2 also exhibited consistent beat period, field potential duration (Fridericia correction), and conduction velocity across wells, plates, and sites. The beat period was longer for cell type 2, but demonstrated the same stability across wells (see inset, ~0.1% coefficient of variation).

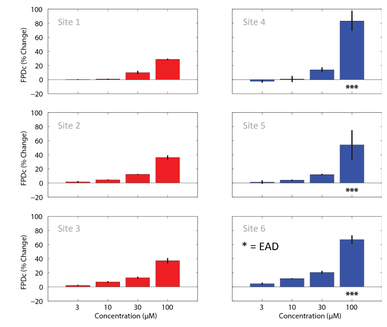
VI. Signal Analysis Workflow



Raw field potential signals show distinct phenotypic changes for compounds that act via different mechanisms.

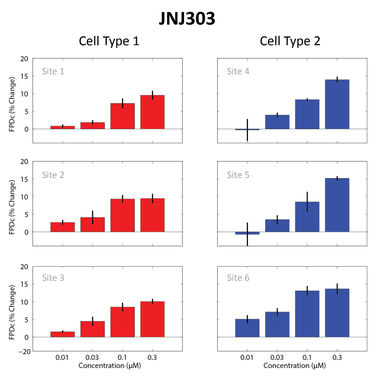


Raw measures of repolarization agree across well replicates and display dose-dependent changes in response to compound introduction. By comparison, no change in repolarization timing was observed for untreated and vehicle control wells.

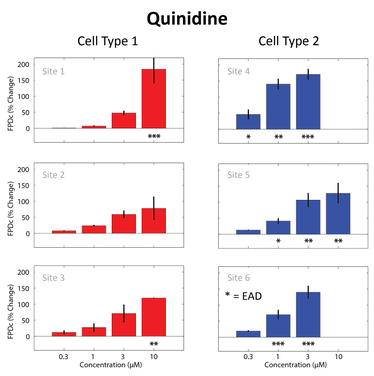


Moxifloxacin prolonged repolarization consistently across replicates, sites, and cell types. The magnitude of prolongation was consistent across sites for a given cell type, and the trend was consistent across cell types.

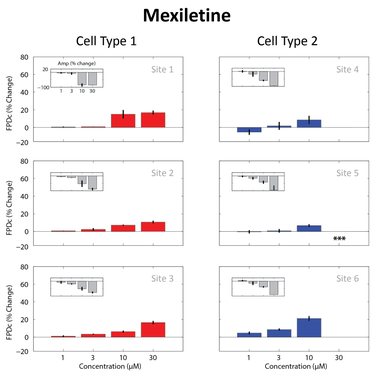
VII. Compound Case Studies



JNJ303, an I_{KS} blocker, produces modest, yet reliable, prolongation of repolarization. The CM-MEA assay can resolve these sensitive changes.

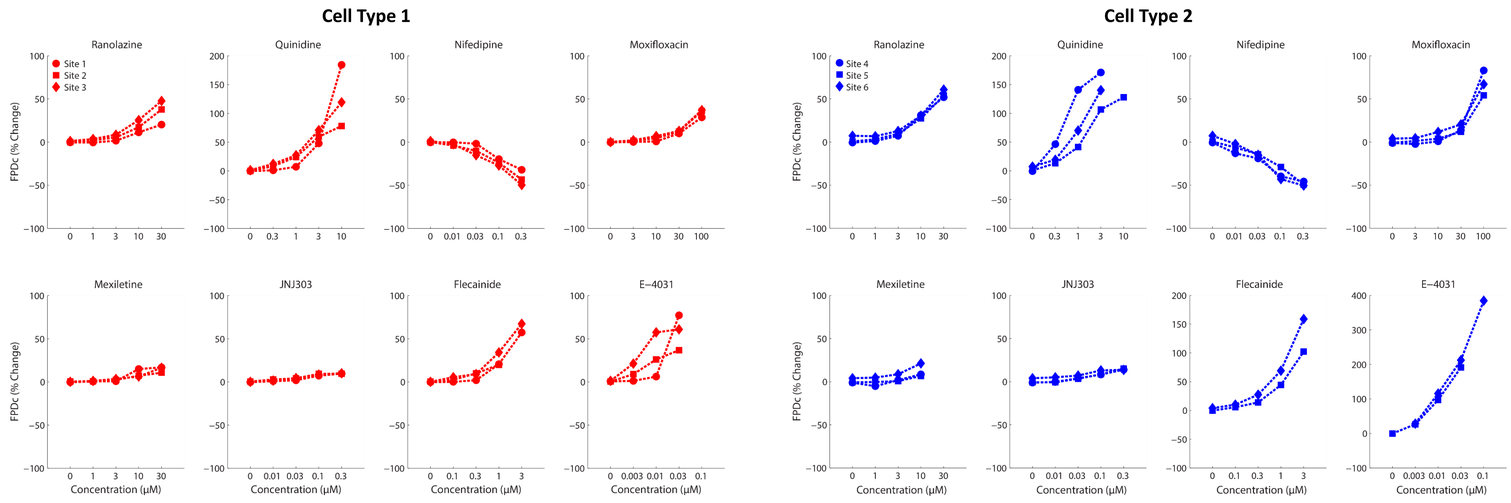


Quinidine produced EADs at intermediate to high concentrations. Across sites, wells experiencing EADs exhibited greater prolongation of repolarization.



Mexiletine, a sodium channel blocker, produces subtle changes in repolarization, but significant reduction in depolarization spike amplitude (see inset).

VIII. Maestro Results Compilation and Conclusions



The CiPA pilot study results from the Maestro sites demonstrated high reliability across replicates and sites for each cell type, while accurately detecting phenotypic changes in depolarization, repolarization, and arrhythmia occurrence for a set of 8 compounds with known mechanism of action.

IX. Conclusions

Predictivity of the myocyte-MEA assay

- Expected responses were detected for eight compounds with known mechanism.
- Prolongation and shortening of repolarization, and effects on depolarization, were detected for compounds blocking hERG, calcium, and sodium ion channels.

Reliability of the myocyte-MEA assay

- The myocyte baseline activity was highly consistent across wells, plates, and sites for each cell type.
- Consistent response trends were observed across sites and cell types for each compound.

References

1. Sager, Philip T., et al. "Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium." American heart journal 167.3 (2014): 292-300.
2. Clements, Mike, and Nick Thomas. "High-throughput multi-parameter profiling of electrophysiological drug effects in human embryonic stem cell derived cardiomyocytes using multi-electrode arrays." Toxicological Sciences 140.2 (2014): 445-461.
3. Harris, Kate, et al. "Comparison of Electrophysiological Data From Human-Induced Pluripotent Stem Cell–Derived Cardiomyocytes to Functional Preclinical Safety Assays." toxicological sciences (2013): kft113.

Acknowledgements

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