Cardiac safety applications using Axiogenesis Cor.4U® cardiomyocytes and the Maestro™ MEA platform

Axiogenesis' Cor.4U are human induced pluripotent stem cell-derived (hiPSC) cardiomyocytes that exhibit typical biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Due to their human origin, high-purity, functional relevance, and ease of use, Cor.4U represent an optimal test system for interrogating cardiomyocyte biology in basic research, drug discovery, and safety screening.

The Maestro microelectrode array (MEA) platform from Axion Biosystems is a non-invasive, label-free assay that measures activity of electrically-active cells in throughputs up to 96 wells. Cor.4U can be cultured on Axion MEA plates to form a stable syncytium amenable to electrophysiological interrogation. With robust, physiologically-relevant responses, Cor.4U and Axion's MEA technology together form an innovative platform for *in vitro* screening of compound effects on human cardiomyocyte physiology.

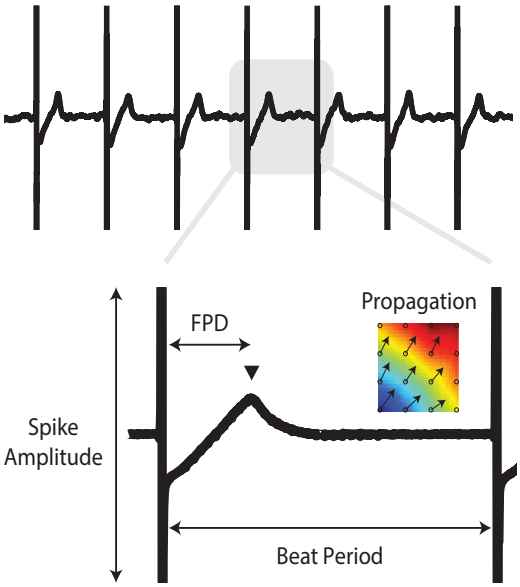


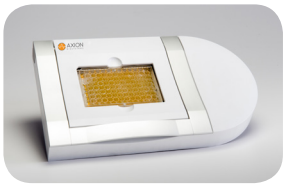
Figure 1. Top: Maestro MEA recording of cardiomyocyte activity. Bottom: The Maestro's fast data collection rate (12.5 kHz), sensitive microvolt resolution, and industry-leading electrode density enables collection of numerous cardiomyocyte activity metrics. This figure shows measures commonly used in cardiac safety studies.

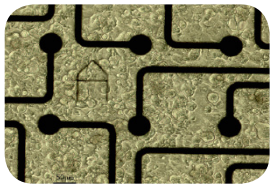
Maestro/Cor.4U advantage

Cor.4U recapitulate human cardiac electrophysiology *in vitro* enabling predictive, high throughput safety screening with the Maestro.

Both acute and chronic exposure experiments are possible as a result of Cor.4U spontaneous beating and Maestro label-free detection.

The large repolarization feature of Cor.4U facilitates accurate T-wave detection and precise Field Potential Duration (FPD) calculation.


Maestro MEA platform


Cor.4U on Axion electrodes

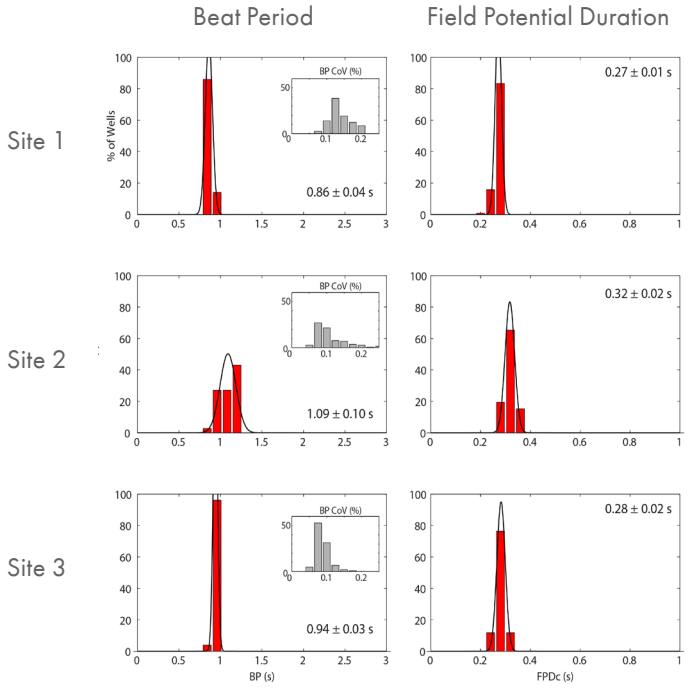


Figure 2. Cor.4U display consistent phenotypes across plates and between Maestro recording sites. Three independent laboratories measured baseline activity across a minimum of two 48-well MEA plates. Aggregate plate data is displayed.

Applications: Comprehensive *in vitro* Proarrhythmia Assay (CiPA)

Physiologically-relevant responses

The Comprehensive i*n vitro* Proarrhythmia Assay (CiPA) initiative, led by the US FDA, SPS, CSRC and HESI, aims to improve current regulatory guidance by introducing predictive technologies, including human stem cell-derived cardiomyocytes (hSC-CMs), into preclinical cardiac safety assessment. A multi-site blinded global pilot study employing microelectrode arrays (MEA) evaluated the effects of eight compounds with varying cardiac risk (low, medium, high) on hSC-CM's electrophysiological function. As shown in Figure 3, Cor.4U hiPSC-derived cardiomyocytes demonstrated accurate and reproducible results for all eight compounds on the Maestro platform.

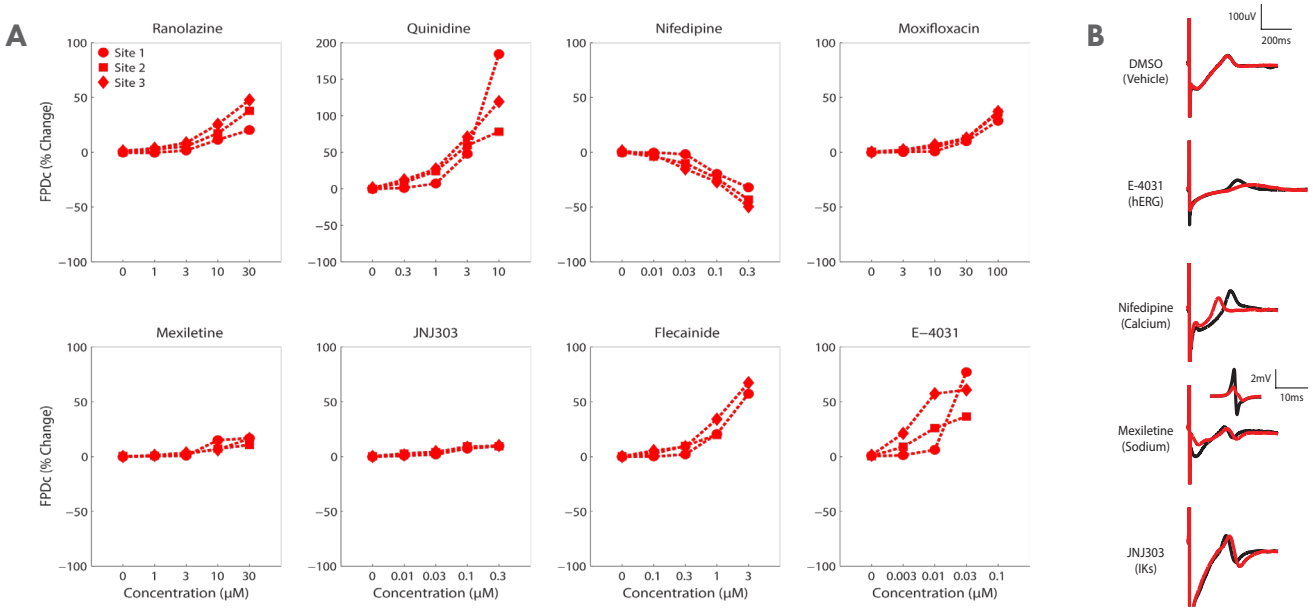


Figure 3. (A) Eight compounds with known electrophysiological effects on cardiomyocyte ion channel activity were tested using Cor.4U on the Maestro MEA platform. Data is consistent in both phenotype and magnitude across three independent testing sites. (B) Maestro voltage recordings pre- (black) and post-treatment (red) are overlayed to demonstrate the effect of specific channel inhibitors on Cor.4U. As expected, compounds targeting channels associated with repolarization (calcium and potassium) influenced Field Potential Duration (FPD). Sodium channel blockers known to affect depolarization (spike amplitude) showed no change in FPD duration, but did consistently decrease spike amplitude in the MEA recording (data not shown).

CiPA - beyond hERG channel blockers

Current FDA regulations immediately reject any drug candidate shown to block hERG channel function. However, not all hERG channel blockers are clinically unsafe (Figure 4). New CiPA guidelines which include *in vitro* cardiomyocyte-based functional activity assays will help make those distinctions clearer allowing promising compounds to remain in the pipeline.

Conclusions

Cor.4U cardiomyocytes together with the Maestro MEA platform have demonstrated the ability to provide a robust, high-throughput, physiolgically-relevant assay for cardiac safety studies. Results here strongly support use of this experimental system to achieve CiPA's iniative of incorporating functional, *in vitro* assays into pre-clinical paradigms.

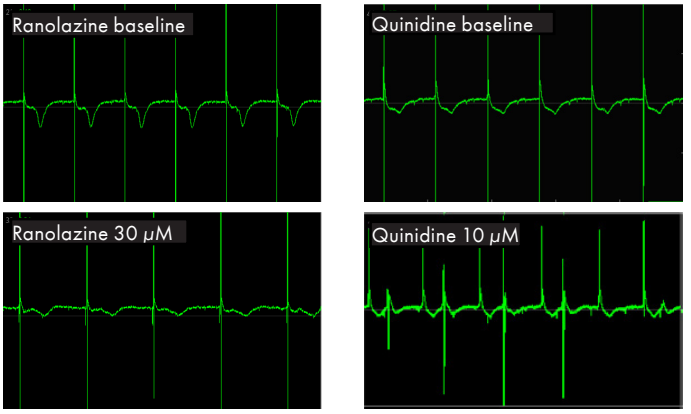


Figure 4. Raw Maestro data of Cor.4U with two compounds, Ranolazine and Quinidine. Top panels show baseline measurements on a representative electrode before compound addition. Bottom panels show response on the same electrode 30 minutes post-treatment. Though both are known hERG blockers, only Quinidine (10μM) showed arrhythmic activity in this functional assay.