Optimization of chronic pacing protocols for functional maturation of hiPSC-derived cardiomyocytes

Anthony Nicolini, Colin Arrowood, Heather Hayes, Daniel Millard
Axion BioSystems, Atlanta, GA

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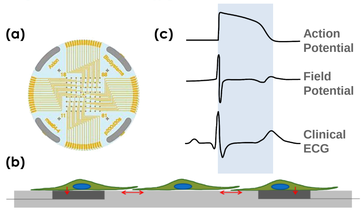
Multiwell MEA Technology

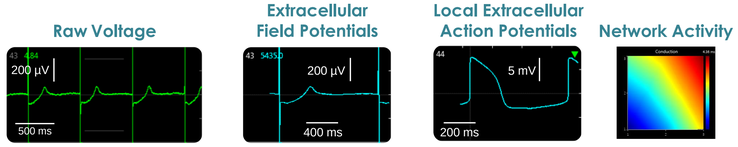
Microelectrode Array Technology

The flexibility and accessibility of neural and cardiac *in vitro* models, particularly induced pluripotent stem cell (iPSC) technology, has allowed complex human biology to be reproduced *in vitro* at unimaginable scales. Accurate characterization of neurons and cardiomyocytes requires an assay that provides a functional phenotype. Measurements of electrophysiological activity across a networked population offer a comprehensive characterization beyond standard genomic and biochemical profiling.

Axion BioSystems' Maestro™ multiwell microelectrode array (MEA) platform provides this comprehensive functional characterization. The Maestro is a non-invasive benchtop system that simply, rapidly, and accurately records functional activity from cellular networks cultured on a dense array of extracellular electrodes in each well.

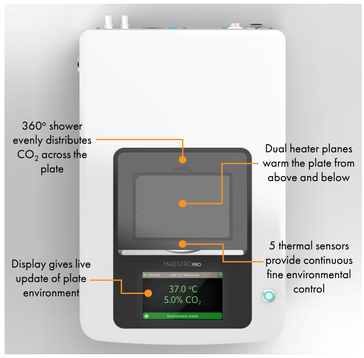


A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), to model complex, human systems. Electrodes detect changes in raw voltage (c) and record extracellular field potentials.



Raw voltage signals are processed in real-time to obtain extracellular field potentials from across the network, providing a valuable electrophysiological phenotype for applications in drug discovery, toxicological and safety screening, disease models, and stem cell characterization

Introducing the Maestro Pro™ and Maestro Edge™



- **Label-free, non-invasive recording** of extracellular voltage from cultured electro-active cells
- **Integrated environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the depolarization waveform
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data from across the entire culture
- **Scalable format (6-, 24-, 48- and 96-well plates)** meets all throughput needs on a single system
- **State-of-the-art electrode processing chip (BioCore v4)** offers stronger signals, ultra-low frequency content, and enhanced flexibility

The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

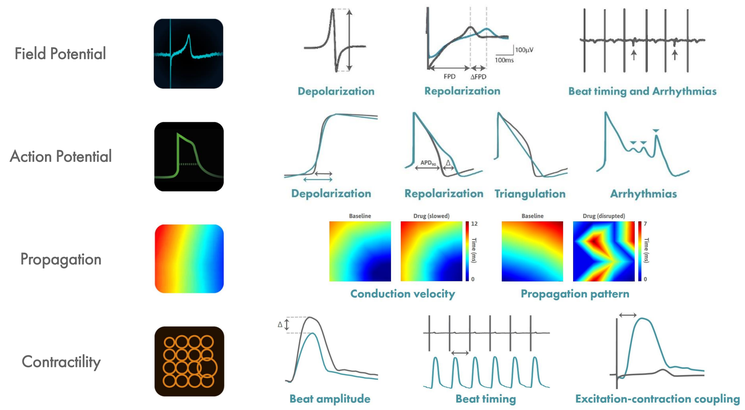
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	6-, 24-Well	6-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	Yes	Yes

MEA Assay with Cardiomyocytes

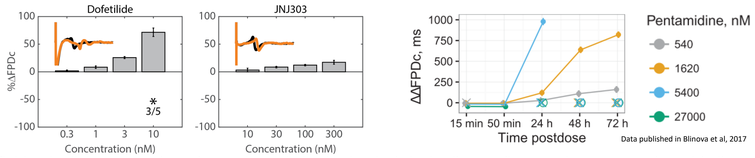
Cardiomyocyte Electrophysiology Phenotypes

The need for simple, reliable, and predictive pre-clinical assays for cardiac safety has motivated initiatives world-wide, including the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) and Japan iPS Cardiac Safety Assessment (JiCSA). The Maestro MEA platform enables assessment of functional *in vitro* cardiomyocyte activity with an easy-to-use benchtop system. The Maestro detects and records signals from cells cultured directly onto an array of planar electrodes in each well of the MEA plate, with each of the following four modes providing critical information for in vitro assessment:

- **Field Potential** – "gold standard" measurement for multiwell cardiac electrophysiology.
- **LEAP** – first truly scalable technique for acquiring action potential signals from intact cardiac monolayers.
- **Conduction** – detect speed and direction of action potential propagation.
- **Contractility** – assess the contractility of cardiac monolayers adhered to the 2D array.

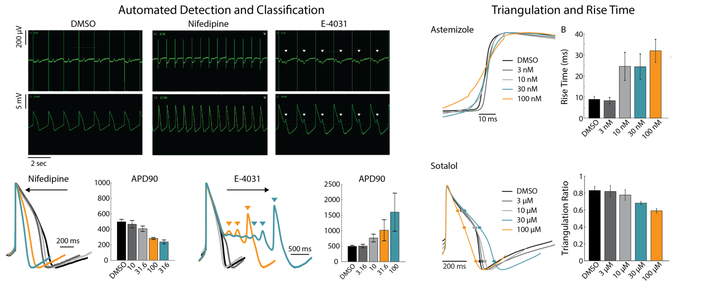


Field Potential Provides Unparalleled Sensitivity with Label-Free Measurements for Acute and Chronic Studies



Field potential measures have been characterized extensively for in vitro cardiomyocyte assays, and provide the most sensitive performance for small effects on repolarization on acute and chronic timescales. For example, dofetilide causes significant prolongation of FPDc and arrhythmia incidence, whereas JNJ303, by comparison, produces a subtle, yet detectable, prolongation of FPDc. A chronic field potential assay also detected dose-dependent trends for pentamidine, as result of effects on hERG trafficking.

LEAP Provides Measures of Action Potential Morphology



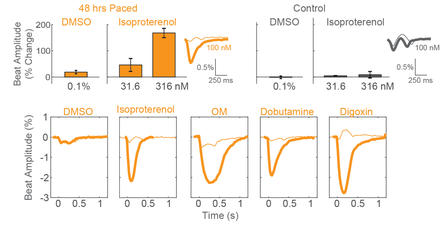
The LEAP signal provides endpoints for action potential morphology, with completely automated analysis of repolarization and EADs. Dose-dependent trends in repolarization timing were detected for nifedipine (shortening) and E-4031 (prolongation). LEAP also affords measures of rise time and triangulation, providing additional information beyond classical field potential assays.

Functional Maturation of hiPSC-CMs

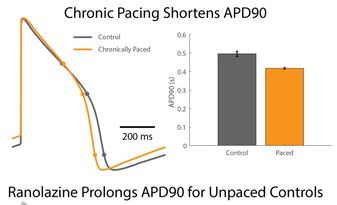
Detection of Positive Inotropes after Chronic Pacing for 48 hours

hiPSC-CMs display aspects of functional immaturity, including immature calcium handling and contractile function. Current *in vitro* protocols require 2-4 weeks of chronic pacing to improve maturity. Using array-based contractility and local electrical stimulation, we detected functionally mature phenotypes in hiPSC-CMs after only 48 hours of chronic pacing.

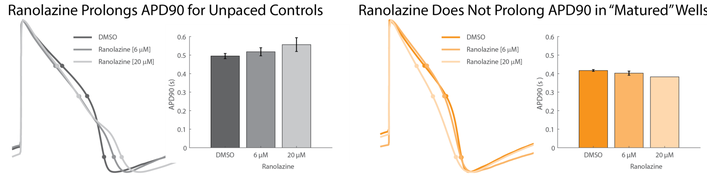
After chronic pacing at 2 Hz for 48 hours, CDI iCell CM² cardiomyocytes were dosed with positive inotropes, such as isoproterenol. Chronically paced wells (orange) showed a dose-dependent increase in beat amplitude in response to isoproterenol, while unpaced control wells (gray) showed no response. Similarly, chronically paced wells also successfully detected a variety of other positive inotropes.



Maturation of hiPSC-CM Functional Responses with Chronic Pacing

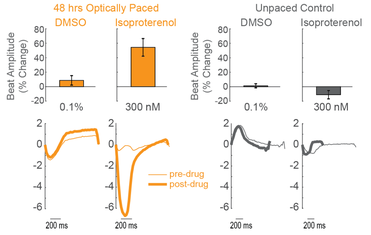


After chronic pacing at 2 Hz for 48 hours, CDI iCell CM² cardiomyocytes exhibited a shorter action potential duration (APD), as compared to unpaced control wells, when measured using LEAP technology. The APD90 was reduced by ~100 ms in "matured" wells that underwent chronic pacing, comparable to the reduction in FPD observed in this and other experiments.



After chronic pacing at 2 Hz for 48 hours, CDI iCell CM² cardiomyocytes were dosed with ranolazine to evaluate the effects of "maturation" on the functional electrophysiological response. Unpaced control wells exhibited prolongation of APD with increasing doses of ranolazine, relative to the vehicle control. By comparison, "matured" wells did not prolong APD90 following addition of ranolazine. Also, at 20uM ranolazine, three of the four "matured" replicates became quiescent.

Light-based Chronic Pacing with Optogenetics for Maturation Studies



CDI iCell CM2 cardiomyocytes were transduced with AAV6-CAG-ChR2 upon cell seeding. After 10 days in culture, the plate was paced with 5 ms blue light pulses at 2 Hz for 48 hours. Untransduced control wells were exposed to the same blue light, but did not pace in response. The cardiomyocytes were then dosed with isoproterenol, a positive inotrope, or vehicle control. Chronically paced wells (orange) showed an increase in beat amplitude in response to isoproterenol, while unpaced control wells (gray) showed no change in beat amplitude.

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural and cardiac cell culture activity with a flexible, easy-to-use benchtop system.
- AxIS Navigator software makes analysis and reporting of functional data simple and hassle-free with an array of automatically generated metrics and advanced analysis tools.
- hiPSC-derived cardiomyocytes exhibit maturation of electrophysiological and contractile function following only 48 hours of chronic pacing, as measured using turn-key assays for action potential and contractility measurements.