Human Stem Cell-Derived Cardiomyocyte– Bringing Cardiovascular Toxicity Screening to the Front Line

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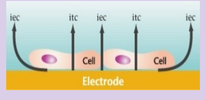
Abstract #1111Poster Board -141

Abstract

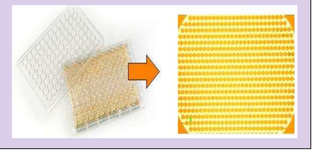
Cardiovascular (CV) Cardiovascular (CV) toxicity is a prominent cause of drug attrition and withdrawal. Introducing new *in vitro* assays with higher throughput should permit earlier hazard identification and enable medicinal chemists to design-out CV liabilities. A central hurdle to developing *in vitro* CV assays has been the dual challenge of replicating integrated cardiovascular physiology while achieving a format with the throughput and consistency required for screening. The emergence of human stem-cell derived cardiomyocytes (CM) which beat spontaneously appears to address the first of these technical challenges and provides a foundation for developing screens. One screening platform, cellular impedance assays, has been validated for CM screening showing excellent predictivity for arrhythmia (Guo et al Tox Sci 2013) and contractility (Scott et al, in preparation). To complement validation data, in this poster we provide data to illustrate 3 points. (1) Changes in beat rate and amplitude are typically related. Across a wide range of stimuli the relationship is consistent with the inverse force-frequency relationship observed in force-based assays. (2) Either beat rate or amplitude can be selectively altered by compounds indicating they can be independent variables. (3) Understanding that the amplitude-frequency relationship is inverted relative to *in vivo* can be valuable for translating cardiomyocyte data to animal studies. In addition, we compare and contrast cellular impedance with calcium flux and MEA, two other technologies for detecting CM beating. These platforms detect different endpoints within the excitation-contraction cascade. We posit a new CV toxicity screening cascade that integrates the strengths of each platform. Key criteria include driving primary screening to achieve broad, inclusive detection followed by a choice of secondary assays to enable mechanism-appropriate follow-up.

Impedance Technology

How it works
- Confluent monolayer plated on interdigitated electrodes
- Weak alternating current impeded by cell layer
- Changes in morphology alter current flow.

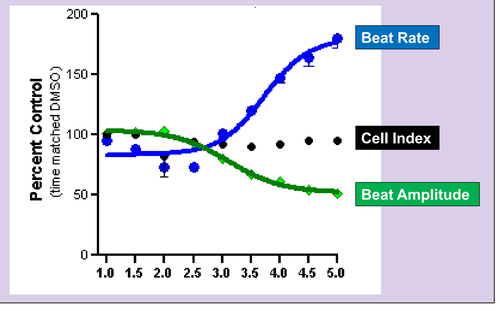


Novel Features
- Exquisite sensitivity- 1 nanometer cell diameter
- Real-time, continuous monitoring- kinetic responses
- xCELLigence, data collection speed (12 msec)
- Robust data in whole-cell

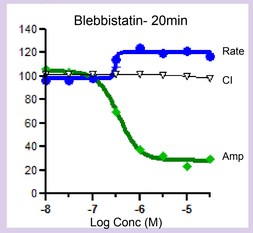


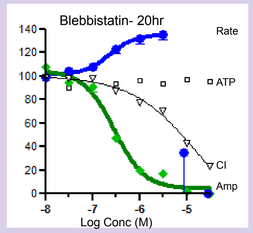
Representative Data Format

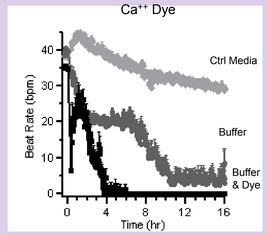
- ½ log dilutions (arbitrary units relative to top conc)
- 20 min default response
- 3 wells/point (separate plates)
- All points do have SEM error bar



Impedance Detection Attributes



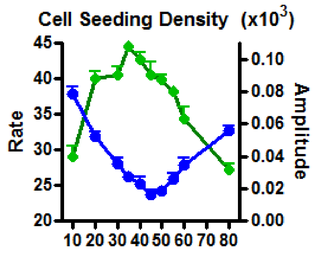


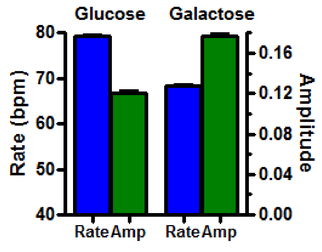


- An example of a cmpd with preferential amplitude effect is seen with acute Blebbistatin
- Impedance assays are highly sensitive to changes in contraction
- Label-free, real-time monitoring allow continuous reading in media without dye

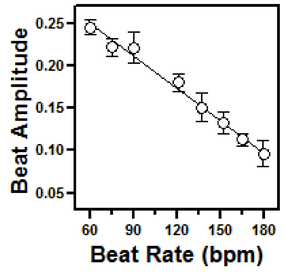
Negative Force-Frequency Relationship

Examples of rate/amplitude inverse variation

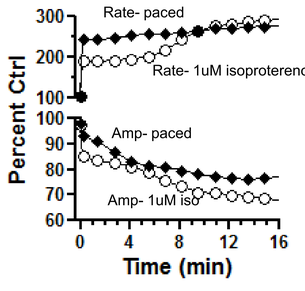




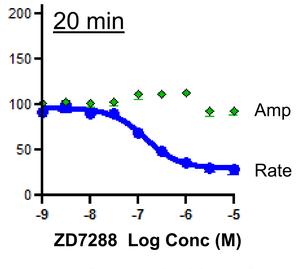
Confirmation: paced beating

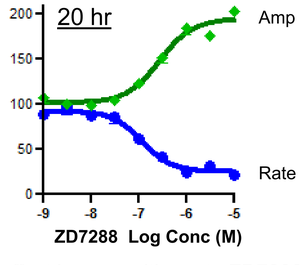


Time Dependence



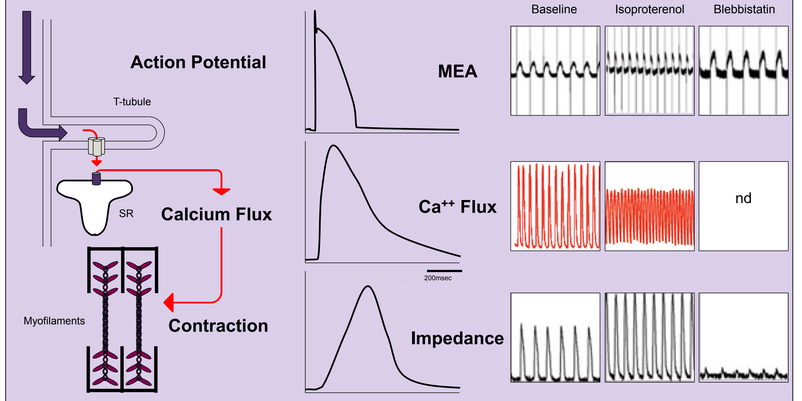
Detecting inverse, time-dependent pharmacology





- An example of a cmpd with preferential Rate effect is seen with acute ZD7288
- ZD7288- inhibits the I_f pacemaker current and has selective rate effects *in vivo*

Combining Assays to Span Excitation-Contraction Coupling Cascade



Emerging Cascade of Complementary Assays

Cardiovascular Toxicity Risk				
Arrhythmia	Contractility	Heart Rate	Blood Pressure	CV Structural Tox
Non-hERG Arrhythmia	Kinase Inhib. CV Tox		Idiosyncratic Function Tox	

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Primary Screens						
hERG	Cellular Impedance					

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Secondary Screens, SAR Studies, Mechanistic Investigation						
Patch clamp	MEA	Ca++ Flux	Impedance	Cantilever	IonOptix	Imaging

↓

CV assessment *in vivo*
GP MAP, Rat & Dog Telemetry

- Complementary but independent assays are critical to a cascade and multiple diverse CM assays have emerged
- Impedance assays offer capacity for both primary and secondary screening
- The introduction of CMs enable fundamental shift by allowing early functional cardiotox testing

Conclusions

- **The amplitude-rate relationship mirrors the force-frequency relationship and is important for understanding and translating spontaneous beat function.**
- **Cellular impedance assays with stem cell derived cardiomyocytes are robust measure of pharmacology with the versatility of real-time and label-free detection.**
- **The down-stream endpoint (contraction) allows inclusive detection of diverse pathways/mechanisms and is well suited to a front-line screen.**
- **Pairing impedance-based detection with additional secondary assays enables mechanism-appropriate follow-up.**
- **Combining diverse CM functional assays has the potential to allow earlier identification of CV hazards and an opportunity to develop SAR to mitigate liability.**