Functional Enhancement of Human iPSC-derived Cardiomyocytes Enabling Assessment of Inotropic Compounds and Improved Prediction of Compound Risk

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Toxicology and Safety Pharmacology

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Outline – Cellular Dynamics and iCell Cardiomyocytes

- CDI Company Overview

- iCell Cardiomyocytes
  - Understand your model
  - Relative to the ‘ideal’
  - Current utility and advantages
The Power of IPSC technology

Bringing relevant human biology and diversity to basic research, drug discovery, and therapy
Company Overview

• Cellular Dynamics International (CDI) is a leader in production and application of human iPS cells and iPS cell-derived cell types

• Acquired by FUJIFILM (4/2015); International presence
  ✓ Headquartered in Madison, WI (additional site in Novato, CA)
  ✓ Application / Distribution sites in Japan, South Korea, and Tilburg
  ✓ Local Sales and FAS support

• Currently employs ~175 total staff w/ >900yrs cumulative stem cell and differentiation experience

• >100 patents (owned or licensed)

• Portfolio includes off the shelf products, as well as, custom cell production and assay services.
CDI: Life Sciences and Regenerative Medicine

- **2 Business Divisions**
  - Life Sciences
  - Cellular Therapeutics

- **Life Sciences Division serves 4 major market areas**
  - Basic and Translational Sciences
  - Safety Pharmacology & Toxicity
  - Drug Discovery and Bioengineering
  - Specialty Markets

- **Cellular Therapeutics Division has 2 focal areas**
  - Internal cell therapy programs
    - Ocular, Cardiac, Neurodegenerative, and Oncology
    - Contract Development and Manufacturing partnerships

An unyielding commitment to consistent and robust iPSC-based solutions for current and future research and therapeutic applications
Life Science Research: Current Product Portfolio

**iCell Products**
- Healthy donor
- Large-scale production

**MyCell Custom Manufacturing**
- Customer defined samples
- Reprogramming, genetic engineering, differentiation

**Disease and Diversity**

**New Products in development:**
*Early access availability*

**iCell RPEs**

**Custom Assay Services**
- Customer defined end points
- Assay development, data collection, technology transfer
Human iPSC Models
Functional recapitulation

**Neurons**
- Neurite outgrowth / retraction
- Synaptogenesis / pruning
- Ion channel and synaptic activity

**Cardiomyocytes**
- Electrical activity
- Ca$^{2+}$ handling
- Contractility
iCell Cardiomyocytes; Contextual Relevance

**Functional and Structural toxicity**

**Functional Toxicity** - 1° effect is on electrical/mechanical function

- Electrical
  - Ion channels, Action Potentials, GPCRs

- Cell Signaling
  - \( \text{Ca}^{2+} \) signaling (EC coupling)
  - Biochemical

**Structural Toxicity**

1° effect is on general cellular processes

- Viability
- Lipid accumulation
- Mitochondrial function
- Oxidative stress
- Bioenergetics
- etc.....

**Mechanical Contractility**

Contextual relevance enables both functional and structural *mechanistic toxicity* testing

Processes are linked, thus downstream biology is a *phenotypic biomarker* for upstream activity

October 2, 2017
iPSC-Cardiomyocytes moving from novelty to mainstream

- Contemporary model with great interest
  - Exponential increase in publications
- Regulatory evaluation
  - CiPA, JiCSA, CSA-Hi
- Not entirely free of debate
  - Focus here will be functionality, utility, and advantages w/rspct to current models

>300 publications on toxicity
>5900 publications stem+cell+cardiomyocytes
Differences Between iPSC and Adult Cardiomyocytes

<table>
<thead>
<tr>
<th>Structure</th>
<th>Adult-CM</th>
<th>iPSC-CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alignment</td>
<td>Longitudinally aligned</td>
<td>Chastically organised</td>
</tr>
<tr>
<td>Nucleation</td>
<td>30% cells bi- or poly-nuclear</td>
<td>Very limited bi-nucleation</td>
</tr>
<tr>
<td>Sarcomere organisation</td>
<td>Highly organised</td>
<td>Disorganised</td>
</tr>
<tr>
<td>Aspect ratio</td>
<td>1.5–5:1</td>
<td>2–3:1</td>
</tr>
<tr>
<td>Banding</td>
<td>Z-discs, I, H, A, and M-bands</td>
<td>Mainly Z-discs and I-bands</td>
</tr>
<tr>
<td>Sarcomere length</td>
<td>2.2 μm</td>
<td>1.6 μm</td>
</tr>
<tr>
<td>SR proteins</td>
<td>e.g. Cx43, PLN, RYR2, SERCA2/ATP2A2</td>
<td>Expression lower than adult</td>
</tr>
<tr>
<td>T-Tubules</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Exp. Gene expression</td>
<td>MYH7 (α-MHC) &gt; MYH6 (β-MHC) &gt; TNNT1 (β-TnT) &gt; TNNT2 (α-TnT)</td>
<td>MYH2/MYH7 ratio not determined</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Mainly fatty acids</td>
<td>Glucose and lactate but can use fatty acids</td>
</tr>
<tr>
<td>Energy production</td>
<td>Mainly oxidative phosphorylation</td>
<td>Mitochondria consume 20–40% of cell volume</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Throughout cell occupies 20–40% of cell volume</td>
<td>Near nuclei; numbers increase during differentiation</td>
</tr>
<tr>
<td>Beatng</td>
<td>Quiescent</td>
<td>Many cells spontaneously</td>
</tr>
<tr>
<td>Force</td>
<td>40 to 80 mV/mm² (muscle strips)</td>
<td>0.08–4 mV/mm² (3D constructs)</td>
</tr>
<tr>
<td>Capacitance</td>
<td>150 nF</td>
<td>200–400 nF (single cells)</td>
</tr>
<tr>
<td>Conductance</td>
<td>~80 to ~90 mS</td>
<td>~60 mS</td>
</tr>
<tr>
<td>Conduction velocity</td>
<td>150–350 V/s</td>
<td>90–50 V/s</td>
</tr>
<tr>
<td>Ion channel density (μA/pF)</td>
<td>2.3 to 100</td>
<td>0.19 to 0.58</td>
</tr>
<tr>
<td>Ca^{2+} kinetics</td>
<td>0.5 s</td>
<td>0.8 s</td>
</tr>
</tbody>
</table>

What is the impact of some of these differences?
- What is the limit of utility?

Example procedures to ‘mature’ cardiomyocytes past these differences

Denning et al., 2016
Cardiomyocyte Electrophysiology

Differences between native ventricular myocytes and iPSC cardiomyocyte action potentials

Differing ion channel / current stoichiometry
iPSCs primarily show:
- Decreased $I_{Na}$
- Decreased $I_{K1}$
- Increased $I_{funny}$
- Mixture of cellular subtypes

$Spontaneously beating iPSC-cardiomyocytes with depolarized MDP$
iCell Cardiomyocytes and iCell Cardiomyocytes

Overview

Human Cardiomyocytes

- >95% pure
- Normal human biology
- Predictive human reagent
- Gold Standard
  - ~100 publications
  - > 90% Top Pharma
  - Used by International Regulatory Agencies

Toxicity Testing

- Functional and Structural Toxicity
- Greater predictivity
- MOA Identification

Disease modeling /Target ID / Screening

- Hypertrophy
- Dilated Cardiomyopathy
- Diabetic Cardiomyopathy
- Ischemia/reperfusion

Regenerative Medicine

- Cardiac Patch / Catheter Delivery

Regulatory Interactions

- C. Scott Tox Sci 2014
- Guo 2011, 2013
- Potency Data & Correlation between IonOptix and Impedance

- Good correlation (r² = 0.76)

- Cardiac agonists/antagonists

- European Union

- Japanese National Institute of Health

- International Society for Heart and Lung Transplantation

- JCSA

- FDA

- HESI

- CIPA
iCell Cardiomyocytes possess the appropriate ion channels, action potentials, and GPCR pathways expected of a relevant human cardiomyocyte model.
Value Proposition
Physiologically Appropriate Arrhythmia Triggers

Adult Canine Purkinje Fiber APs


iCell Cardiomyocyte APs


iCell Cardiomyocytes show physiologically relevant proarrhythmic triggers
Toxicity Testing
Predictivity Screens

Proarrhythmia screening in 96 wells

Cardiomyocyte activity generates rhythmic deflections of the impedance baseline

Easily implemented higher throughput proarrhythmia screening

Larger screens with quantitative analytics provides greater predictivity

- >120 compounds
- ~equal positives and negatives
- beat rate, atypical beats, irregularity

> 90% -- QT prolongation
> 80% -- Proarrhythmia

iCell Cardiomyocytes provide a more predictive tool for detecting proarrhythmia
iPSC Cardiomyocytes

Contractility

iPSC versus adult cardiomyocytes
• Isotropic myofilament arrangement
• Isotropic cell alignment
• Negative force frequency relationship
• Limited effects of pre-load (Frank-Starling)
• Difficult to directly translate positive inotropy
Comparisons between IonOptix-based measurements of dog cardiomyocytes (gold standard) to Ca$^{2+}$ and impedance-based measurements of iCell Cardiomyocytes (higher throughput)

C. Scott Tox Sci 2014
Comparisons between IonOptix-based measurements of dog cardiomyocytes (gold standard) to Ca^{2+} and impedance-based measurements of iCell Cardiomyocytes (higher throughput)

### Potency Data & Correlation between IonOptix and Impedance

Good correlation (r^2 = 0.76)

C. Scott Tox Sci 2014

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dog cardiomyocytes</th>
<th>iCell Cardiomyocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IonOptix^1</td>
<td>FLIPR^2</td>
</tr>
<tr>
<td>sensitivity</td>
<td>83%</td>
<td>77%</td>
</tr>
<tr>
<td>specificity</td>
<td>84%</td>
<td>70%</td>
</tr>
<tr>
<td>accuracy</td>
<td>82%</td>
<td>74%</td>
</tr>
<tr>
<td>pos predict</td>
<td>90%</td>
<td>79%</td>
</tr>
<tr>
<td>neg predict</td>
<td>76%</td>
<td>67%</td>
</tr>
</tbody>
</table>


### iCell Cardiomyocytes

- Show potency correlation with gold standard model
- Demonstrate good to excellent assay validation parameters
- Provide a predictive surrogate model for measuring contractility
Small molecule KI-induced cardiotoxicity

Phenotypic Assays

FDA approved SMKI show cardiac liabilities
- Preclinical assays were insufficient
- Toxicities arose in late development / clinic
- Difficult to ascribe mechanism

Prediction hindered by:
- Highly conserved site of action-ATP-binding pocket (on vs off target effects)
- Multiple effects on overlapping endpoints

Cellular impedance assays with iCell CMs can predict KI toxicity

Model can:
- Determine on-target vs off-target KI toxicity (*MARK vs Chk KI*)
- Identify KI-related toxicity with p<0.05 (>160 cmpds via Ambit and AZ-proprietary datasets)

S. Lamore SOT 2014

iCell Cardiomyocytes provide a predictive tool for detecting KI toxicity

Implementing iCell Cardiomyocytes in toxicity testing cascade


- Structural Toxicity
  - Cell injury
  - Cell death
- Functional Toxicity
  - Proarrhythmia
  - Ca^{2+} handling
  - Contractility

Primary screen identifies a problem (or lack thereof)
Secondary investigations identify mechanism
Subsequent primary screens can be performed on med chem series
iCell Cardiomyocytes / Cardiomyocytes

Recapitulate native behavior

Predictive for proarrhythmia, altered contractility, and structural toxicity

Useful model for predicting adverse effects of small molecule and biologics-based therapies

www.cellulardynamics.com