Rising to the Challenges of Human iPSC-derived Cells for Tox & Drug Screening

Paul Bello, PhD
Director of Operations
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“A long, risky road”

Need for early toxicity testing and improved prediction

Slide taken from the Pivotal Point Group, LLC

Source: Pharmaceutical Research and Manufacturers of America
Overview of Presentation

**iPSC-Derived Neural Stem Cells**

*Neurotoxicity in drug safety testing*

- **Functional Integrity**
  - Gene Expression, Electrophysiology, Multi-Electrode Array,
  - Effects of developmental neurotoxin

**iPSC-Derived Cardiomyocytes**

*Cardiotoxicity in drug safety testing*

- **Functional Integrity**
  - Express major cardiac-selective markers
  - Beat spontaneously in culture, Ca$^{2+}$ imaging

- **Electrophysiology, Pharmacology**

**Hepatocytes**

*Hepatotoxicity in drug safety testing*

- Metabolism studies, Hepatotoxicity studies, Genotoxicity micronucleus studies
iPSC-Derived Neural Cells

The way forward for more predictive toxicity testing
Neurotoxicity in Drug Safety Testing

Functional Integrity
- Gene Expression
- Protein Expression
- Electrophysiology
- Multi-Electrode Array
- Whole Cell Patch

Disease Modeling
- Responsive to drug treatments
- Expression disease-relevant phenotypes

- Patch clamp
- Gene expression
- Biochemical analysis
- Multi-Electrode Array
- Neurite outgrowth
General Characterization of NSCs

Axol confirms expression of neural stem cell markers like SOX2, PAX6, Ki67 and ZO1
Characterization of Cortical Neurons

We looked at cerebral cortical neuronal markers like VGluT1, Tbr1, Cux1, Tuj1 by ICC. Our transcriptomic data confirmed down regulation of iPSCs markers and up regulation of cortical neuronal markers like MAP2, NMDA, VGlut1, Cux1, etc.
Electrophysiological Characterization

Spontaneous activity

Data from our collaborators
Neurotoxin Effects on iPSC-Derived Neural Stem Cells

Data provided from Dr. Kisby’s lab by Michael Czulinski and Morgan Florek: MAM, Methylazoxymethanol
Induction of Epileptiform Activity & Effects of Anti-Epilepsy Drugs

- Induced epilepsy by adding PTZ (pentylentetrazole) (>1 mM)

- Anti-epilepsy drugs, phenytoin & sodium valproate (VPA) were able to reverse the high frequency synchronized bursts evoked with PTZ

These results suggested that long-term electrophysiological measurements in iPSC-derived neurons using a MEA system may be beneficial for drug screening applications (ePoster 107)
Neurotoxicity Summary

• iPSC-derived NSC
  • Express neural markers at gene & protein level
  • Excellent neurite outgrowth & branching
  • Electrophysiologically functional
  • Capable of synaptic plasticity

• iPSC-derived NSCs are more sensitive to the developmental neurotoxin MAM & can replace cell lines for neurotoxin screening
• Responsive to drug treatment
• Can be cultured long-term
• Physiologically relevant tool for toxicity & drug discovery studies
iPSC-Derived Cardiomyocytes

A way forward for more predictive toxicity testing
Why iPSC-Derived Cardiomyocytes?

- Benefits of a synchronously beating monolayer
  - React as a unit syncytium of cells, electrically coupled
- Robust & Reproducible
- Large quantities available
- High purity
- Functional on xCelligence, for calcium imaging & for electrophysiology
iPSC-Derived Cardiomyocytes Showing Synchronized Beating

Benefits of a synchronously beating monolayer
- Electrically coupled,
- Physiologically relevant to human heart
Functional iPSC-Derived Cardiomyocytes

Protein Expression

Signaling & Stress-Response

Telethonin (green) suggested signalling & stress-response functions is present iPSC-CMs with a pattern of sarcomeric striation observed in patches inside some cells. (All actin, red)

Ankyrin repeat domain 1 (ANKRD1) (green) could be used a marker of toxic stress, showed similar expression to telethonin. (All Actin, red)

Human iPSC-CMs (hiPSC-CMs) express more cardiac troponin-T (cTnT) & α-Actinin than human skin fibroblasts (hSFs)

Data from Abigail Robertson from University of Manchester
Near Pure Population Ventricular Cardiomyocytes

- Negligible effect on AP parameters (n=8)
  - Positive effect of carbachol observed with atrial-derived HL-1 cells
  - Suggests majority of cells do not display an atrial phenotype

Ventricular myosin light chain (87%) and atrial myosin light chain (13%) (Does not include nodal population)
Dofetilide ($I_{Kr}$)

- Significant prolongation to APD90
- Negligible effect on other AP parameters
Effect of Dofetilide on Calcium Imaging

Without treatment
Using Fluo-4 calcium dye to measure calcium transients

Dofetilide treatment prolongs the calcium transient

Control
Dofetilide 5nM 0.3Hz
prolonged

Average Dofetilide treatment

Delayed after depolarization (DAD) apparent in some cells

Data provided by Dr Frances Brook at Oxford University
**In-Vitro Models for Cardiotoxicity Studies**

Isoproterenol (β-adrenoceptor antagonist) & Carvedilol (β-adrenoceptor agonist) were added at 265h.

Data shared by Dr Jason Gill, Durham University

iPSC-derived cardiomyocytes responded to both compounds in a dose-dependent fashion & strongly indicate the clinical relevance of these cells & their utility for drug screening applications.
Cardiotoxicity Summary

iPSC-derived cardiomyocytes (CMs) could be used in cardiotoxicity & pharmacology studies

- iPSC-derived CMs express definitive cardiac markers & form organized sarcomeres
- iPSC-derived CMs show synchronized beating as a monolayer culture at high confluency
- Functional on xCelligence & for calcium imaging
- Pharmacology consistent electrophysiological measurement of Aps
- iPSC-derived CMs can form ‘cardiospheroids’ (ePoster 107)
Hepatocytes

A way forward for more predictive toxicity testing
Hepatotoxicity in Drug Safety Testing

We need:

• Reliable genotoxicity testing, predictive hepatotoxicity screens
• Cells expressing adult hepatocyte markers & no fetal phenotype
• Large batch sizes from the same donor for consistency for toxicity and high-throughput screening

TO DATE, human primary hepatocytes have much greater functionality than iPSC-derived hepatocytes
Assay-Ready Expanded (ARE) Hepatocytes

- Expanded hepatocytes that retain many characteristics of primary human hepatocytes
- Metabolically functional & express cytochrome P450 (CYP) enzymes

Comparison of the Phase I CYP enzyme activity between ARE Hepatocytes, ARE Hepatocytes (CYP2D6 Overexpressing) & HepaRG cells
Expression of hepatic transporter genes in primary hepatocytes, ARE hepatocytes & HepG2 cells

Reproducible CYP induction & inhibition in a donor-specific manner by prototypical inducers and inhibitors, for example, Naphthoflavone inhibition (N=2)
Assay-Ready Expanded (ARE) Hepatocytes

Genotoxicity studies

Increasing cyclophosphamide concentration affects the percentage of cells with MicroNuclei (% MN) & cell viability

Hepatotoxicity studies

Sensitivity to hepatotoxic compounds
Hepatotoxicity Summary

- ARE Hepatocytes display a primary liver cell phenotype
- ARE Hepatocytes are metabolic-competent cells expressing liver specific transporters and metabolizing enzymes
- Large batch sizes from the same donor for consistency in toxicity & high-throughput screening
- Sensitivity to hepatotoxic compounds & reliable genotoxicity testing
- ARE Hepatocytes can be co-cultured with liver sinusoidal endothelial cells (data not shown)
Conclusions

Our aim is to provide physiologically relevant \textit{in-vitro} disease models for toxicity & drug discovery campaigns

**Axol iPSC-derived NSC**
- Express neural markers at gene and protein level
- Excellent neurite outgrowth
- Electrophysiologically functional
- Capable of synaptic plasticity

**Axol iPSC-derived Cardiomyocytes**
- Expressing definitive cardiac markers and form organized sarcomeres
- Synchronous beating monolayers, electrophysiologically functional,
- Functional on xCelligence & for calcium imaging

**ARE Hepatocytes**
- Display a primary liver cell phenotype
- Metabolic competent cells expressing liver specific transporters and metabolizing enzymes
- Sensitivity to hepatotoxic compounds & reliable genotoxicity testing
Thank you!

… your discovery stems from here

For more information please contact us at: support@axolbio.com

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