Application of Human iPSC-derived Neurons for Phenotypic Screening and Disease Modeling

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The next great health crusade?

- **Huntington’s Disease (HD):** Affects >15,000 Americans and at least another 150,000 individuals have a 50% risk of developing the disease.
- **Amyotrophic Lateral Sclerosis (ALS):** 20,000-30,000 Americans have ALS; most die within 3-5 years from the onset of the symptoms.
- **Parkinson’s Disease (PD):** About 1M people in the US have PD; total cost of treatment/care is estimated to exceed $6B annually.
- **Alzheimer’s Disease (AD):** ~5.4M people in the US have AD; by 2050, it is estimated that 14M individuals will be affected; total cost of treatment/care is estimated at $200B/year.

An aging population means these numbers will grow

Most approved treatments of these diseases do not modify the course of the disease, but instead only offer temporary relief of some symptoms with limited effectiveness.
Current Disease Model Systems

<table>
<thead>
<tr>
<th>Primary Human Cells</th>
<th>Transformed Cell Lines</th>
<th>Animal Models</th>
</tr>
</thead>
</table>
| • Limited availability
  • Variable quality
  • Phenotypic instability
  • Donor variability
  • Limited characterization | • May not recapitulate relevant cell/tissue biology
  • May lack key functional characteristics
  • Cannot adequately represent human diversity | • May not represent relevant human biology
  • Resource intensive ($$ and labor)
  • Require significant quantities of compound
  • Animal welfare issues |

Existing cell sources for disease modeling, drug discovery, and toxicity testing are limited by availability, functionality, reproducibility, and translatability.
iPS Cell Technology
Revolutionary Access to Human Biology

Differentiate into all 208 cell types in the human body

Edit any gene in the genome

Represent any individual genotype

iPS cell technology revolutionizes life-science research & personalized medicine by enabling disease modeling in a biologically relevant system

iPS cell technology is a powerful tool to better understand and target human disease
iCell Neurons

### Key Neuron Characteristics

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Bipolar or multi-polar neurite outgrowths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Markers</td>
<td>βIII-Tubulin, Map2, Synaptophysin, Gephyrin, PSD-95, vGAT (GABAergic), vGLUT (Glutamatergic), Tau, A-Beta</td>
</tr>
</tbody>
</table>
| Functional Characteristics | - Neurotransmission: receptor and channel activation  
- Electrophysiology: patch clamp and MEA  
- Neurite outgrowth/sprouting |

### Purity and Morphology

![βIII Tubulin / Nestin / Hoechst Day 7 post-thaw](image)

- GABA/Map2/Hoechst
- vGLUT/vGAT/Hoechst

iCell Neurons are a mixed population of primarily GABAergic and Glutamatergic neurons

- 5 ml Pellet of Pure Neurons = ~4 Billion Neurons
- Day 1
- Day 8
- Day 21
iCell Neurons
Functional Endpoints

Neurite Outgrowth
- Control
- BDNF

Bioenergetics/
Mitochondrial Health

Disease Modeling
(Tau & β-Amyloid Proteins)

Transfection/Infectivity

Label-free Detection
- GABA (100 µM)
- Buffer (Vehicle Control)

Electrophysiology

Calcium Signaling
- FLIPTm Tetra System
Neurite Outgrowth Assay

Thaw and plate cells
(96-well, 384-well, or 1536-well plates)

Treat cells; then fix and stain

Image segmentation

Orthogonal assays

Data analysis

Log [K252a] (M)

Total Outgrowth

RLU (ATPliTe™)
Enhancement of neurite outgrowth is also possible

- Neurite Outgrowth (con’t)

- Control

- Control

- + BDNF

- Bar graph showing comparison between control and BDNF treatments.

- Line graph showing neurite length over time with different cell densities.
Basics of Epic® Label-Free Technology

• Cell-based assay that measures the movement of mass (mostly proteins) inside the cell [Dynamic Mass Redistribution (DMR)]

• Truly a “whole cell” response in a phenotypic assay

• Cells are plated on microplates incorporated with Epic® biosensor technology

• Sensor is illuminated with broadband light, and the Epic® Label-free module measures the differences in reflected wavelengths based on the cellular response
Label-Free Analysis of Endogenous Signaling Through GABA$_A$ Receptor

Cell-based assay development is straightforward

- GABA is a neurotransmitter
- GABA$_A$ receptor is a ligand-gated ion channel
- Binding of GABA to its receptor results in activation and opening of the channel to allow flow of Cl$^-$ or K$^+$ ions in and out of the cell
- GABAzine is an antagonist of the receptor complex and prevents channel opening
Label-Free Analysis for Screening

**Activation**

Log [GABA] (M) vs. Response (pm)

- EC$_{50}$ = 1.8 µM

**Inhibition**

Log [GABAzine] (M) vs. Response (pm)

- IC$_{50}$ = 0.65 µM (in presence of 2 µM GABA)

- Z' = 0.61
- 45 data pts

Response (pm)

- GABA (100 µM)

- Buffer (Vehicle Control)

Well #
Neuronal Network Assessment by MEA

Multi Electrode Array

GABAznine Concentration

GABAznine Dose Response
### Compound-Induced Changes in Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Class</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picrotoxin</td>
<td>GABA antagonist</td>
<td>25 µM</td>
</tr>
<tr>
<td>GABA\textsubscript{A} antagoni st</td>
<td>Na\textsuperscript{+} Ch neurotoxin</td>
<td>10 µM</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>Na\textsuperscript{+} Ch blocker</td>
<td>50 µM</td>
</tr>
<tr>
<td>Domoic Acid</td>
<td>Glu antagonist</td>
<td>0.1 µM</td>
</tr>
<tr>
<td>GABA</td>
<td>GABA agonist</td>
<td>3 µM</td>
</tr>
</tbody>
</table>

**Mean % Change in MFR**

- **MFR = mean firing rate**
• Bead-based proximity assay
• Donor and Acceptor beads are brought into proximity thru biomolecular interactions
• Donor beads are excited with a laser at 680 nm resulting in the release of singlet oxygen
• This initiates an amplified fluorescent signal cascade in the Acceptor beads
• Emission only occurs if the beads are within 200 nm
• Magnitude of light emission is proportional to the number of biomolecules interacting
Alzheimer’s Disease Biomarker Detection

Alzheimer’s Disease biomarkers detectable in iCell Neurons

AlphaLISA kit for Amyloid Beta (1-40)

AlphaLISA kit for Human TAU (total)

AlphaLISA kit for Human sAPP α

Alzheimer’s Disease biomarkers detectable in iCell Neurons
Gamma secretase inhibitor modulates extracellular amyloid beta levels
Sensitivity of iPSC-derived neurons to Aβ1–42 was demonstrated using a cell viability assay (top) and high content imaging of neurite outgrowth (bottom).


Large batches of iPSC-derived neurons with consistent quality enabled a successful library screen for compounds that prevented Aβ1–42-induced toxicity.

Rescue of toxicity induced by Aβ1–42 was shown by shRNA knockdown (left) and small-molecule inhibition of CDK2 (right).
Summary – the Promise of iPSC Technology

- The complexity of the nervous system makes it difficult to understand the underlying processes of neurodegeneration.
- iPSC technology is changing the way we study human disease – especially in the brain (with previously inaccessible cell types).
- Disease modeling should significantly advance the understanding of these diseases ultimately leading to new therapeutics.
- Develop applications with normal cells – focused around phenotypic assays – and then be poised to compare patient-specific cells.
- “Human brain in a dish” and “in vitro clinical trials”
Acknowledgements

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  • Molecular Devices
  • PerkinElmer
  • Axion BioSystems

• Neuron R&D and Production Teams at CDI
• Application Development Team at CDI

Thank you!
Epigenetics in Neurological Diseases

The Role of Epigenetics in Neurodegenerative Diseases

Enhancing Histone Acetyl Transferase Activity as a Therapeutic for Alzheimer’s Disease

Epigenetic modulations can be detected in iCell Neurons