Investigating Neural Networks Using Human iPSC-derived Neurons on MEA
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Abstract

Motivation
Human cell types differentiated from induced pluripotent stem cells (iPSC) offer a unique source of cellular material for drug discovery and toxicity screening. As many of the underlying mechanisms in neuronal disorders and neurotoxicity affect the electrical properties of the brain, understanding neuronal circuit connectivity, physiology, and pathology under in vitro conditions plays an important role to advance drug development and toxicity screening.

Material and Methods
Differentiation into the various cell types was performed according to protocols developed at iCell. Donor iPSC cell lines were used either from normal healthy individuals (iCell) or from iPSC genetically engineered to model disease (MyCell DOP). High neuronal purity was measured by flow cytometry for all iPSC-derived neurons, identifying TUJ-1-positive (vHMSN) and nestin-negative cells. Neurons were seeded in high-density dots on 48-well MEA plates (Axion BioSystems) and cells were cultured in supplemented BrainPhys™ Neuronal Medium (STEMCELL Technologies) for 2-4 weeks. Electrical activity was recorded regularly on the Maestro MEA system (Axion) at 37 °C and 5% CO2. Data was analyzed using the Neural Matrix Tool (Axion) and complemented with MATLAB-based software tools generated internally.

Results
We observe that human iPSC-derived neurons are able to form synapses in culture over time and develop complex, macro patterns of spontaneous activity on MEA. A range of different baseline phenotypes were detected, but the degree of spontaneous bursting was generally dependent on the ratio of (E/I) excitatory to inhibitory cells within the culture. To help illustrate these findings, we tracked the evolution of a synthetically-driven culture on MEA over time and analyzed changes in numerous parameters that define neuronal activity. Predominantly excitatory neuronal cultures (e.g. glutamatergic neurons) display much more organized bursts (higher network bursting percentage) than inhibitory GABAergic neurons, and these events can be modulated with known blockers such as DMSO and A5P. Additionally, neurons derived from disease-specific iPSC (e.g. dopaminergic neurons harboring the alpha-synuclein SNCA A53T mutation) clearly present activity on the MEA that is distinct from the healthy (WT) isogenic control.

Discussion
Although primary rodent cortical neurons are the most widely-accepted cell source for MEA, we show that several different subtypes of human neurons derived from iPSC can be analyzed on this platform technology. Interpreting neuronal basal media and the various supplements have a dramatic effect on MEA assay performance, underscoring the importance of optimized culture conditions for implementing a new cell model into an established assay.

Conclusions
Currently, we utilize human cells on the MEA are limited in their ability to broadly address different human diseases in vitro. Data presented here demonstrate that human iPSC-derived neurons are rising to the challenge of becoming a reliable and predictive tool for use in drug discovery, disease modeling, and neurotoxicity applications.

Relevant Human Neuronal Cell Types

<table>
<thead>
<tr>
<th>Human Donor</th>
<th>iPSC Cells</th>
<th>iCell Induced Neurons</th>
<th>iCell DopamineNeurons</th>
<th>Cryopreserved Human Cells</th>
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We utilize iPSC technology to repopulate adult cells (from either skin or blood) back to the “stem cell” state. At this stage, iPSC cells can then be differentiated into virtually any cell type – including previously inaccessible human neuronal cell types. iPSC-derived neurons are produced in high purity with varying degrees of Excitatory (glutamatergic) and Inhibitory (GABAergic) cells in the population. Importantly, human neurons from CDI are provided as cryopreserved material that can be thawed and used any day of the week.

Use of MEA to Observe Neuronal Activity

Multi-electrode array (MEA) technology has become a widely-accepted tool for the electrophysiological, label-free assessment of in vitro activity of neuronal cell cultures. MEAs are grids of tightly spaced electrodes that are capable of directly sensing changes in voltage that are propagated down the membranes of excitable cells. MEA technology also makes it possible to detect network-level phenotypes through extracellular, single-unit recordings.

Action potentials fired by neurons are recorded by the instrument as a “spike,” which can then be graphically represented as a raster plot (example above) or spiking histogram (example below). Yes, the mean firing rate of a neuron is a good indicator of its health, but interpretation of neuronal activity is most meaningful when it is centered around timing. For example, the time interval between two spikes or the pattern of a series of spikes recorded within a high frequency neuronal circuitry (that electrophysiologists expect to see from their experiments).

Synchronous Bursting in Human Neurons

Primary cultures of rodent neurons have a long history in neuro-electrophysiology as they display well-characterized bursting patterns and generally respond to compound treatment with the appropriate pharmacology. Implementation of highly pure cultures of human iPSC-derived neurons that were not recently isolated from an adult brain is challenging. Here we show some examples of the factors that affect network-level bursting behavior with these cells.

Media Matters

We have observed that cell culture media, neuronal supplements, and the basic protocols for changing media make a significant impact on the phenotype of every human iPSC-derived neuron we have tested. In these example data with iCell DopaNeurons and BrainPhys® Neuronal Medium (STEMCELL Technologies), we see improved cell health / neurite outgrowth (ICC staining), detect more organized bursting patterns (raster plots), and quantify more synchronous bursts (grahs).

Addition of CellAstrocytes into a culture of iCell DopaNeurons on the MEA can impact functionality and promote network-level synchronicity. 75K DopaNeurons were held constant and increasing amounts of Astrocytes were added from left to right. *These experiments were done without BrainPhys medium.

Highly pure populations of iPSC-derived neurons can be utilized to customize the features of a neuronal culture on MEA. iCell Neurons (predominantly inhibitory culture) have measurable activity on the MEA, butumble at only ~1-3 Hz. Altering the E/I ratio by mixing in a more excitatory population of cells (iCell Induced Neurons) in a 1:3 ratio generates a totally different MEA phenotype producing amplified, synchronous network burst intensities. These “true” Maestro trace examples are voltage traces from each of the 16 electrodes within a given well; more synchronous bursting activity denotes connected neuronal circuitry. Furthermore, mixing in too much excitation (4:1 ratio) results in a seizure-like phenotype evident by the hyper-intense, longer-lasting bursts that disrupt the regular bursting pattern. This experiment highlights the importance of a proper balance of neuronal subtypes (E/I ratio) on the functional MEA activity.

Evolution and Analysis of Network Formation

MEA is a powerful tool because it is a label-free measurement of neuronal activity. This enables the scientist to watch the evolution of a network connected culture. There is a great deal of data generated in these experiments (~1GB per minute) and properly analyzing it is a major challenge. Shown above are raster plots taken from iCell DopaNeurons on the Maestro MEA system at Day 6, 14, and 20. White ticks are action potentials, red ticks are “Poisson” channel bursts, and magenta boxes indicate wide network bursts. The organization of the white marks into groups of red and ultimately lined up in the boxes is clearly evident. These well-wide network bursts can be assessed for “peak intensity,” as shown in the upper-right all-points histograms of the raster plots for iCell DopaNeurons and iCell DopaNeurons harboring the A53T mutation (DIV 20). Note that the average peak burst intensity (Hz) for the MyCell SNCA A53T cells (~1500 Hz) are ~4X larger than that of the isogenic iCell DopaNeurons control (~400 Hz).

Summary and Future Directions

• Using iPSC technology to produce highly pure neuronal cell types, we have generated previously inaccessible human neurons of specific brain regions that represent particular populations of in vivo cells.

• We have relied on MEA technology to understand the functional differences between the cells, testing them both in mono-cultural and as co-cultured tissue.

• While the standard cell models in this space are primary rodent cultures, they do not enable modeling of human disease like the cell types presented here do. We are actively expanding our catalog offering to include neurons and astrocytes from diverse backgrounds (i.e. more healthy / normal donors) and disease-relevant backgrounds (i.e. patient-derived samples).