Leveraging iPSC-derived Cortical Neurons Harboring Known Epilepsy Mutations to Advance Personalized Medicine

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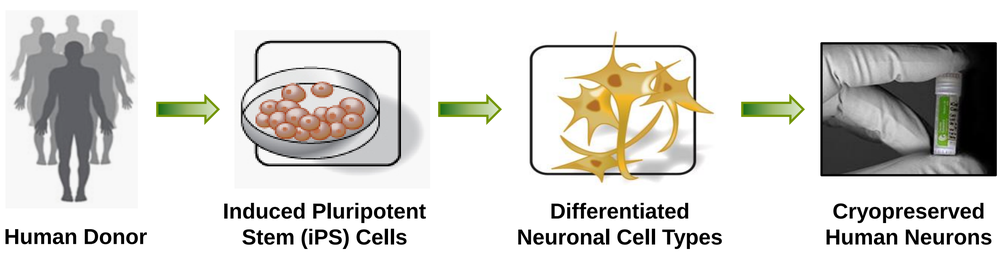
Abstract

Epilepsy is a neurological condition caused by disturbances in the electrical activity of the brain manifested through multiple etiologies. Over 65 million individuals suffer from epilepsy and one-third of these individuals live with uncontrollable seizures because there are no known pharmacological treatments to date. A portion of this population is accounted for by single-gene epilepsy disorders resulting from mutations within sodium, potassium, or inhibitory ion channels. With recent advances in personalized medicine, there is hope not only for diagnosis but also for treatment options for these individuals.

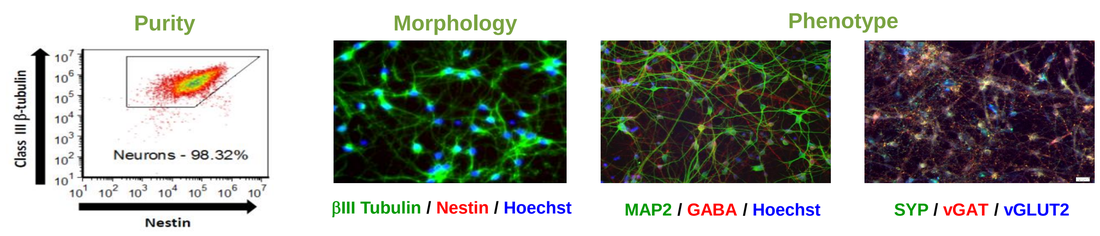
Central to this vision is induced pluripotent stem (iPS) cell technology, which provides a platform to increase our understanding of how single-gene mutations result in disease states. Here we illustrate how human iPS cell-derived cortical neurons can be used to highlight the "disease-in-a-dish" approach to drug development and can act as a springboard to discovering new therapies.

We have genetically engineered iPS cells with single-gene mutations that result in autosomal-dominant nocturnal frontal lobe epilepsy (*KCNT1* P924L), Dravet Syndrome (*SCN1A* knockout), or childhood absence epilepsy (*GABRG2* R43Q). Human cortical neurons were derived from these iPS cell lines and the resulting phenotypes were examined by various methods. Here we present morphological data (neurite outgrowth and branching) and functional data (electrophysiological MEA) comparing "healthy" (wild-type) vs. edited (KCNT1, SCN1A, or R43Q mutations) neurons illustrating hyperactive phenotypes correlated to the epileptic genotypes. Furthermore, we show examples of selective pharmacology that attenuates these observed "epileptic" phenotypes. The ability to engineer isogenic wild-type and disease-associated alleles by genome editing of human iPSC-derived neurons provides unprecedented access to in vitro models of neurological disorders. Collectively our results showcase how iPSC technology can be leveraged in the personal medicine space.

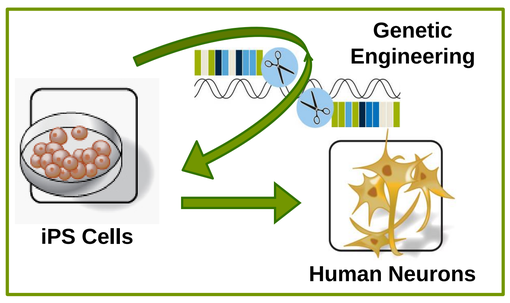
Human iPSC-derived Neuronal Cell Types



We have utilized iPSC technology to reprogram adult cells (from either skin or blood) back to the "stem cell" state. At this stage, iPS cells can the be differentiated into virtually any cell type – including previously inaccessible human neuronal cell types. Importantly, terminally differentiated neurons from CDI are provided as cryo-preserved material that can be thawed and used any day of the week.

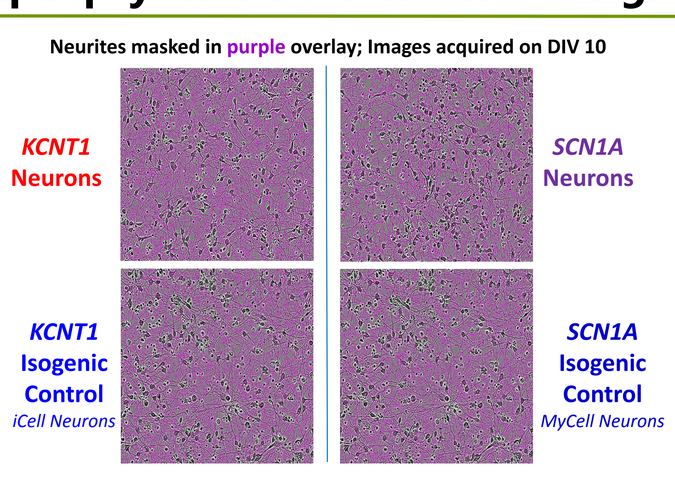


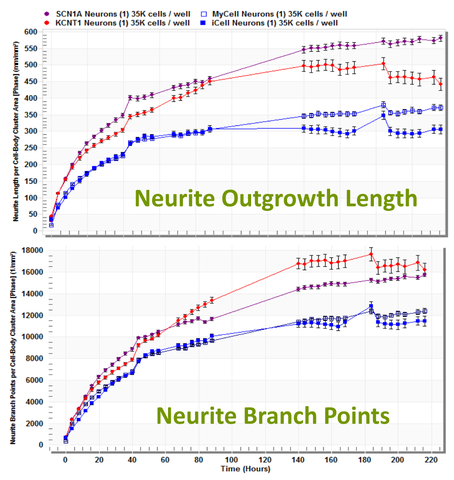
iCell Neurons area highly pure population (>95%) of human iPSC-derived cortical neurons. They possess the classical neuronal morphology. These cells are a mixture of both inhibitory (GABAergic) and excitatory (glutamatergic) neurons evident by gene expression and phenotypic analysis for characteristic markers.



Genetic engineering enables iPSC-based models of epilepsy. To examine the effects of single-gene mutations in human cortical neurons, current nuclease-mediated genome editing technologies were used to introduce specific alterations into a normal healthy "control" iPS cell line (with no family history of neurological disorders). Both the WT and mutant iPS cell lines were differentiated into cortical neurons and cryopreserved. Human neurons were thawed and used in subsequent in vitro experiments.

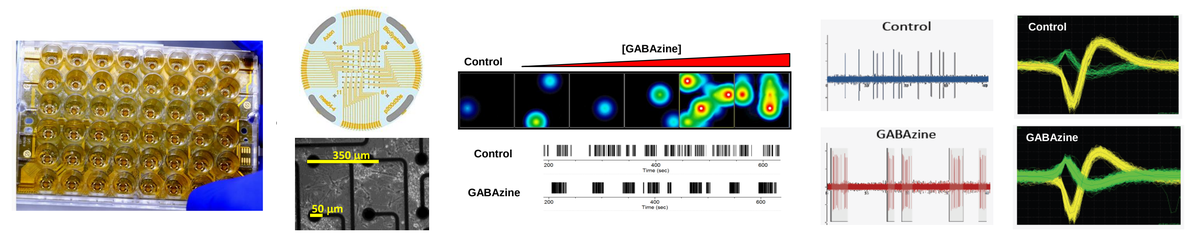
Epilepsy-in-a-Dish: Neurite Outgrowth





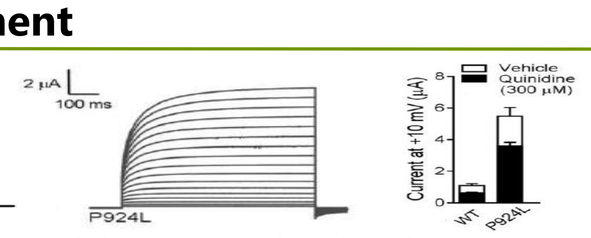
Engineered MyCell Neurons harboring a KCNT1 P924L mutation or a heterozygous SCN1A knock-out mutation display an aberrant morphological phenotype. Neurons were plated in 96-well format and analyzed after DIV 10 for various neurite outgrowth properties by high-content imaging. Mutations in the sodium-activated potassium channel (**KCNT1**) present in clinic with autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE), whereas sodium channel, voltage-gated, type 1, alpha-subunit (**SCN1A**) mutations present with a variety of human epilepsies, including the devastating Dravet syndrome (severe myoclonic epilepsy of infancy) and febrile seizures Plus (GEFS+). Both MyCell neuron epilepsy cell cultures display increased neurite outgrowth length and neurite branch points compared to the isogenic control neurons.

Epilepsy-in-a-Dish: Network Excitability

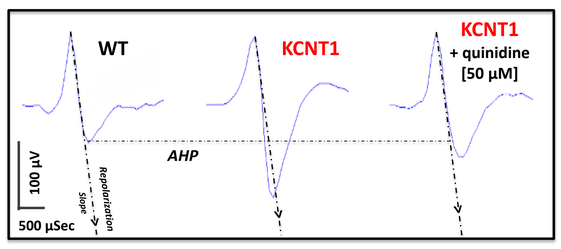


Multi-electrode arrays (MEAs) provide a direct voltage sensing platform to assess neuronal activity within iPSC-derived neuronal cell cultures. 48-well plates, with 16 high-resistance electrodes per well (768 total electrodes allowing for single-unit recordings), provides the simultaneous evaluation of neuronal activity from many cells from different cell cultures, which can express various cell types and/or genotypes.

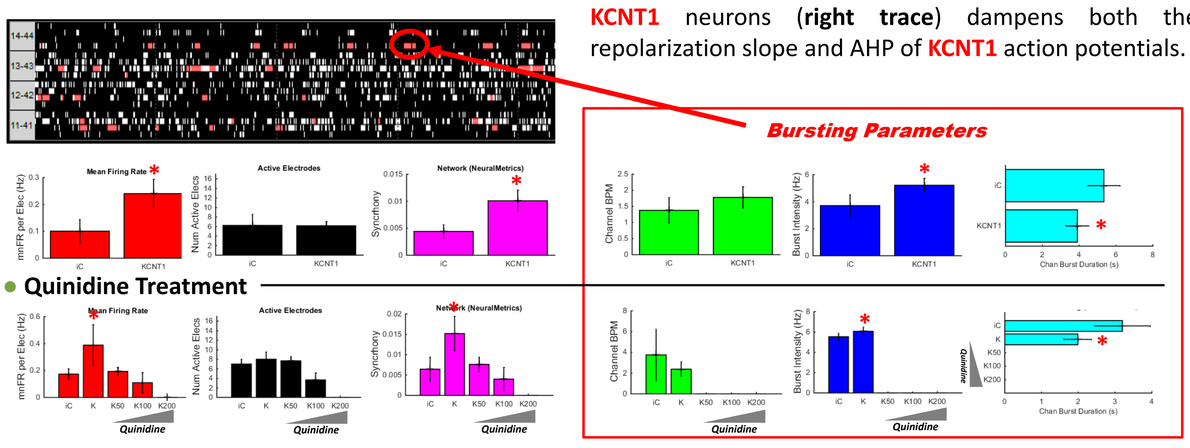
MEA Analysis & Drug Treatment



KCNT1 P924L is a gain-of-function mutation. Expression of the Na^+-activated K^+ channel (KCNT1) P924L mutation in Xenopus oocytes shows an increase in current amplitude compared to wild-type KCNT1 channels. The authors in this study also described a sensitivity of KCNT1 channel to inhibition with quinidine, delaying repolarization and helping prevent "fast" or "repetitive" firing behavior. Quinidine is an FDA approved drug for treatment of cardiac arrhythmias. The figure above was adapted from **Milligan et al. Ann. Neurol. 2014**.

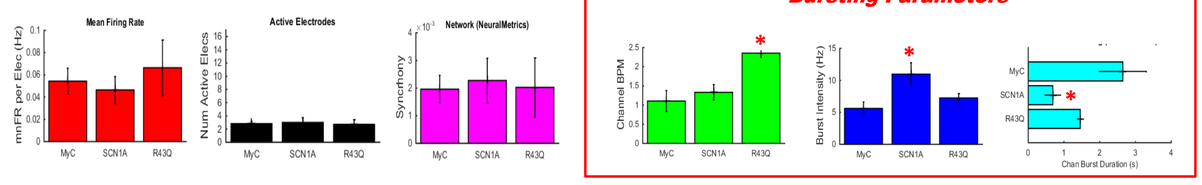


High-resistance (single-unit recording) electrodes allow individual waveforms to be captured, parsed (PCA), and compared across different conditions, before and after drug treatment. Untreated **KCNT1** neuronal action potential waveforms (**middle trace**) display a quickened repolarization slope and a larger after-hyperpolarization (AHP) compared to WT (**left trace**) (average of ≥80 action potentials per trace). Treatment of 50 µM quinidine onto **KCNT1** neurons (**right trace**) dampens both the repolarization slope and AHP of **KCNT1** action potentials.

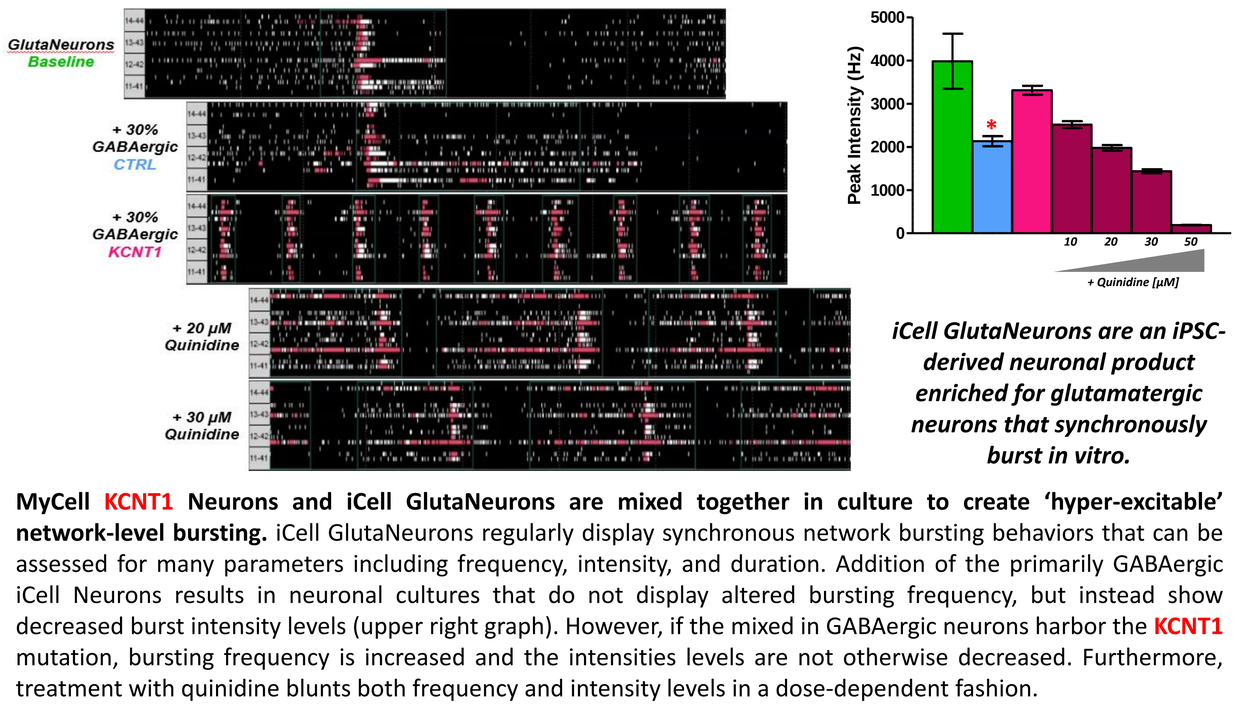


MyCell KCNT1 Neurons display more intense and shorter 'Poisson' bursting behaviors compared to WT. Neuronal cell cultures of both WT iCell Neurons (iC) and MyCell **KCNT1** Neurons (KCNT1) (10 DIV) display spontaneous activity (raster plot) and bursts (colored tick-marks) that can be measured via MEA. Quinidine treatment of **KCNT1** cultures ameliorates the increased mean firing rate (red bars) and synchrony (purple bars) observed in these cultures. Furthermore, quinidine eliminated 'Poisson' bursting in **KCNT1** neurons.

MyCell Neurons harboring the SCN1A or R43Q mutation also display epileptic phenotypes when cultured on and measured via MEA. MyCell **SCN1A** Neurons showcase a similar epileptic phenotype to **KCNT1** cultures, matching their neurite outgrowth profiles. MyCell $GABA_A\gamma2$ (**R43Q**) Neurons, in comparison, display a different but still hyper-bursting phenotype in culture.



Mixing in 'Excitatory' Cell Types on MEA



iCell GlutaNeurons are an iPSC-derived neuronal product enriched for glutamatergic neurons that synchronously burst in vitro.

MyCell KCNT1 Neurons and iCell GlutaNeurons are mixed together in culture to create 'hyper-excitable' network-level bursting. iCell GlutaNeurons regularly display synchronous network bursting behaviors that can be assessed for many parameters including frequency, intensity, and duration. Addition of the primarily GABAergic iCell Neurons results in neuronal cultures that do not display altered bursting frequency, but instead show decreased burst intensity levels (upper right graph). However, if the mixed in GABAergic neurons harbor the **KCNT1** mutation, bursting frequency is increased and the intensities levels are not otherwise decreased. Furthermore, treatment with quinidine blunts both frequency and intensity levels in a dose-dependent fashion.

Conclusions

- Human iPSC-based disease models for "epilepsy-in-a-dish"
  - KCNT1 *(P924L),* SCN1A *KO, and* GABRG2 *(R43Q)*
- Unique and measurable epileptic phenotypes on MEA
  - *'Poisson' burst analysis*
- Advancing drug discovery and personalized medicine
  - *Quinidine ameliorates* KCNT1 *bursting phenotype*