Large-scale Generation of Human iPSC-derived Sensory Neurons for Pain Research

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Abstract

Sensory neurons of the peripheral somatic nervous system respond to a variety of impulses from sensory organs including touch, position in space (proprioception), temperature, and pain. Sensory neurons are of great value in pain research to aid in the development of better chemotherapy drugs with manageable side effects such as chemotherapy-induced peripheral neuropathy (CIPN) and hematologic toxicity. Differentiating human induced pluripotent stem cells (iPSC) enables access to authentic human sensory neurons, but challenges arise when attempting to consistently produce large quantities of these cells. Here we report the large-scale, directed differentiation of human sensory neurons from two (2) different iPSC lines, including both male and female donors. These iPSC-derived sensory neurons have high purity (>80% BRN3A$^+$/ UCHL1$^+$) and express hallmark nociceptive channels (i.e., Nav1.7 and Nav1.8) and receptors (i.e., TRPV1 and P2RX3). We characterized sensory function of these neurons using electrophysiology and calcium imaging. Notably, we confirmed the presence of P2RX3 and TRP channels and their response to different stimuli including capsaicin, menthol, and ATP using calcium imaging. Additionally, these sensory neurons display cytotoxicity to chemotherapeutic drugs (i.e., paclitaxel) in a dose-dependent manner and release substance-P and CGRP peptides in response to pain mediators. These data demonstrate a process for robustly generating iPSC-derived sensory neurons across different iPSC donor lines that result in expression of characteristic markers and respond appropriately to known pharmacology in functional assays. Large-scale production of such human sensory neurons will pave the road for studying sensory neuron properties and has the potential for use in high-throughput drug screening for pain research.

Methods

iCell Sensory Neurons were thawed and maintained in culture using iCell Sensory Neurons Maintenance Medium. Cells were evaluated at various timepoints for morphology, gene expression, and immunocytochemistry and morphology. Functional characterization of sensory stimuli was evaluated at 21 or 28 days of culture. Toxicity assays for chemotherapy-induced peripheral neuropathy were conducted on cells cultured for 4-7 days.

iCell® Sensory Neurons			
Catalog #	Donor	Sex	Vial Size
C1259	01279	Male	6M
C1260	01279	Male	1M
C1261	21527	Female	6M
C1262	21527	Female	1M

Characterization of iCell Sensory Neurons

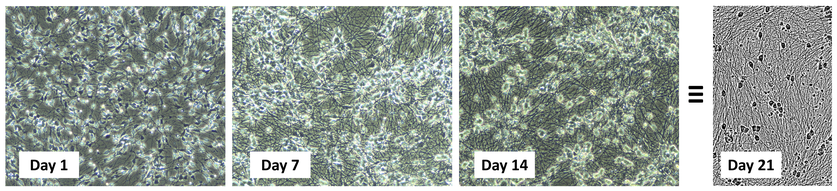


Figure 1. Neuronal Morphology. iCell Sensory Neurons were plated onto Geltrex-coated 6 well plate (1x10^6 cells/ well). Sensory neurons mature with a bright cell body and pseudo-unipolar morphology at 21 days post-thaw.

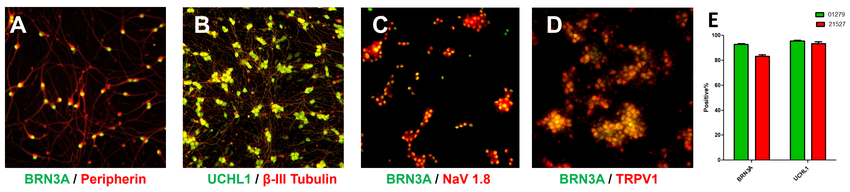


Figure 2. Neuronal Purity by ICC. iCell Sensory Neurons express pan-neuronal and sensory neuron markers. **A)** & **B)** Staining for BRN3A, Peripherin, UCHL1 and BIII Tubulin at day 7. Sensory neurons express mature sensory neuron markers **(C)** NaV1.8 and **(D)** TRPV1. **(E)** Quantification of BRN3a and UCHL1 % positive neurons across five (5) lots for each donor.

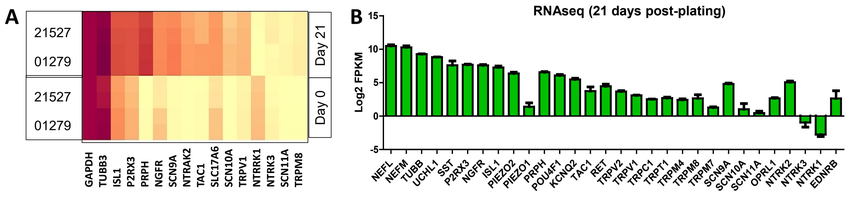


Figure 3. Neuronal Gene Expression. A) iCell Sensory Neurons (01279 and 21527 donor) mRNA was collected at day 0 and day 21 post-plating. qPCR shows consistent expression of pan-neuronal genes (TUBB3) and sensory neuron-specific genes (P2RX3, TRPV1,NaV1.7) across donors and increased expression at day 21. **B)** RNASeq data (Log2FPKM) collected from sensory neurons at day 21 post plating corroborate qPCR data in panel A and confirm expression of additional neural markers (NEFL, NEFM) and the presence of additional sensorineural TRP channels, sodium channels (SCN), and mechanoreceptor receptors (PIEZO1/2).

Response of iCell Sensory Neurons to Various Sensory Agonists

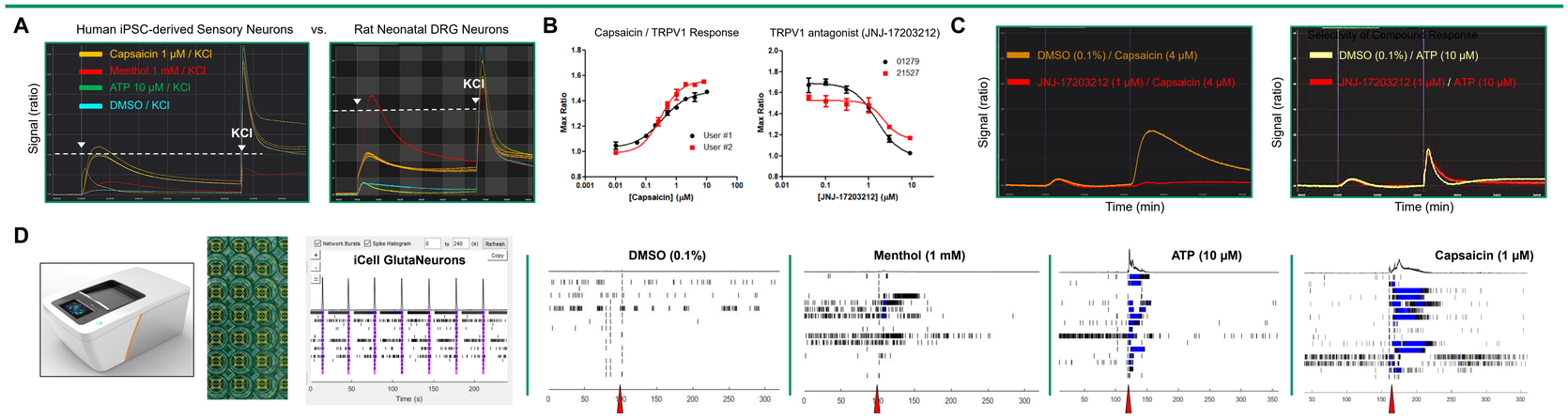
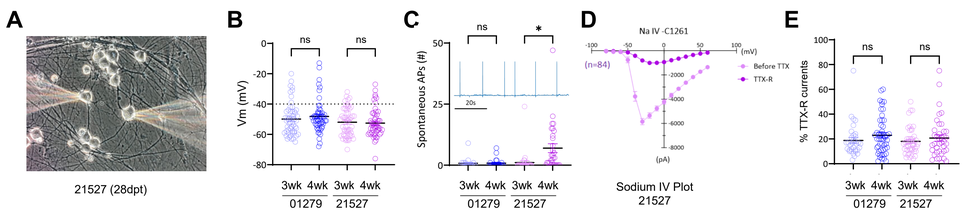
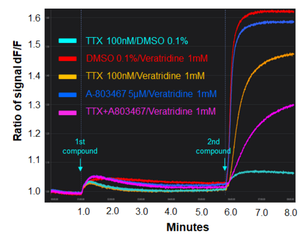
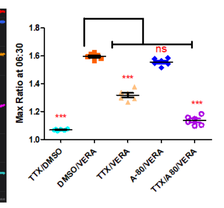


Figure 4. Functional Responses in Calcium and Multi-electrode Array (MEA) Assays. General protocol for testing iCell Sensory Neurons in a kinetic Ca^{2+} assay was to culture the cells for 21 days in 96- or 384-well format, load cells with a calcium indicator dye for ≥ 2 hours and then expose cells to sensory stimuli. Signal ratio changes were recorded using the FDSS/µCell. Potassium chloride (KCl, 50 mM) was applied following compound treatment as a positive control for total neuronal response. **(A)** Representative Ca^{2+} traces in human iPSC-derived sensory neurons following exposure to Capsaicin (1 µM; TRPV1 agonist), Menthol (1 mM; TRPM8 agonist), and ATP (10 µM; P2RX3 agonist). iCell Sensory Neurons showed similar responses to those observed in rat neonatal dorsal root ganglion (DRG) cells. **(B)** iCell Sensory Neurons show a dose-response curve to capsaicin with an EC_{50} ≈ 0.3 µM, which was repeatable between two different users. Importantly, pre-treatment with a known TRPV1 inhibitor (JNJ-17203212) was shown to reduce the capsaicin-induced response (with 1 µM) with cells from both donors. **(C)** Selectivity of JNJ compound was demonstrated by stimulating cells with either capsaicin or ATP (P2RX3 agonist) in the presence of inhibitor. **(D)** Axion Maestro Pro MEA system is routinely used to evaluate the electrical activity of iPSC-derived neurons. iCell Sensory Neurons are relatively "quiet" as compared to excitatory neurons that show high spontaneous spike activity. Cells were cultured on a 48-well MEA plate and stimulated on Day 35 with Menthol (1 mM), ATP (10 µM), and Capsaicin (1 µM). Representative MEA raster plots showed acute response (dosing indicated by red arrow) and electrophysiological network burst activity to known sensory agonists vs. DMSO control.

Sodium Channel Characterization in iCell Sensory Neurons







Veratridine:
Na$^+$ channel activator
[1 mM]

Tetrodotoxin (TTX):
Na$^+$ channel inhibitor
[100 nM]

A-803467:
Nav1.8 inhibitor
[5 µM]

Figure 5. iCell Sensory Neurons Exhibit TTX-r and TTX-s Sodium Currents. iCell Sensory Neurons from 01279 male donor and 21527 female donor were cultured using iCell Sensory Neurons Medium and patched manually at 3 weeks and 4 weeks. **(A)** Representative brightfield image of patched sensory neuron. **(B)** Resting membrane potential (Vm) is ~50mV for both donors at 3 & 4 weeks. **(C)** Recording of spontaneous action potentials (APs) shows that the majority of neurons are silent for both donors at 3 & 4 weeks. **(D)** Representative current traces (Na IV plot) from 21527 iCell Sensory Neurons showing sodium currents pre- and post-TTX treatment. **(E)** Quantification of TTX-sensitive (TTX-s) and TTX-resistant (TTX-r) currents show ~20% of sodium current is TTX-r for both donors and at both 3 & 4 weeks in culture.

Figure 6. Measuring TTX-s and TTX-r Sodium Influx in iCell Sensory Neurons using ING-2. iCell Sensory Neurons were cultured for 21 days in culture, then pre-treated with Na$^+$ channel inhibitors (TTX, A-803467) or DMSO before stimulation by veratridine, a Na$^+$ channel activator. DMSO→ veratridine and TTX → DMSO treatments served as controls and results quantified based on the max ratio of the signal. Sodium influx was measured using ING-2 (ION Biosciences) a fluorescent sodium indicator. Pre-treatment with TTX alone or in combination with A-803467 showed inhibition of Na$^+$ currents. TTX + A-803467 combo showed increased inhibition over TTX alone, suggesting the contribution of Nav1.8 channels to the overall Na$^+$ current.

Modeling Chemotherapy-induced Neurotoxicity

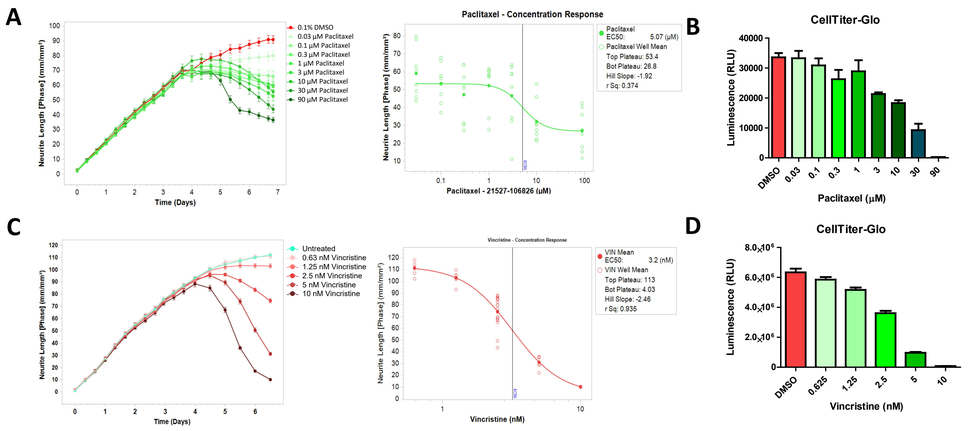
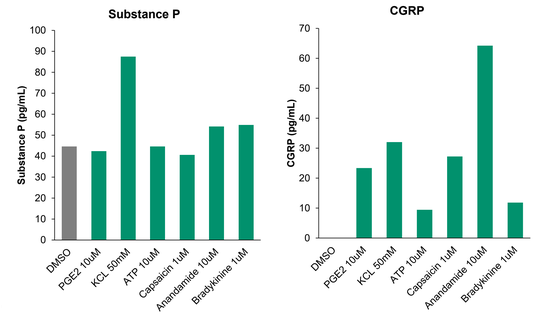


Figure 7. iCell Sensory Neurons Demonstrate CIPN-mediated Toxicity to Paclitaxel and Vincristine. iCell Sensory Neurons were cultured for 7 days. Treatment with chemotherapeutic agents were applied at 4 days and neurite outgrowth and toxicity monitored out to day 7. **(A, B)** Paclitaxel neurotoxicity. **(A)** Increasing doses of Paclitaxel results in truncation of neurite outgrowth or neurite degeneration. Paclitaxel EC_{50} = 5 µM. **(B)** Dose-dependent cell death in response to Paclitaxel (CellTiter-Glo). **(C, D)** Vincristine neurotoxicity. **(C)** Increasing doses of Vincristine results in truncation of neurite outgrowth or neurite degeneration. Vincristine EC_{50} = 3.2 nM. **(D)** Dose-dependent increase in cell death (CellTiter-Glo).

iCell Sensory Neurons Release CGRP and Substance P

Figure 8. Sensory Neuron Stimulation Results in Substance P and CGRP Secretion. iCell Sensory Neurons cultured for 14 days prior to stimulation with a selection of pain modulators or with potassium chloride (KCl; positive control) or DMSO (vehicle control). **(Left)** iCell Sensory Neurons responded by secreting Substance P as measured using ELISA on day 14 post-plating. **(Right)** iCell Sensory Neurons were stimulated at day 14 and measured for CGRP release using ELISA. In neurons treated with Papain, the response surpassed the high range of kit detection and is not reported here.



Conclusions

We have developed a robust differentiation process for large-scale manufacturing of iPSC-derived sensory neurons.

iCell Sensory Neurons:

- Can be produced in large batches with minimal lot-to-lot variation
- Recapitulate biological and functional properties of human sensory neurons, providing a human-relevant model for drug discovery research.
- Displays sensitivity to known neurotoxic drugs, indicating their relevance for modeling chemotherapy induced peripheral neuropathy (CIPN).
- Release Substance P and CGRP peptides in response to pain modulators demonstrating utility for developing new pain killers
- Male and female cell lines enable studies on sex differences
- Serum-free post-thaw maturation medium supports consistent performance