Human iPSC-derived sensory neurons and their potential as an alternative to primary DRG cells for drug discovery

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Sensory nerves transmit pain signals generated by trauma, temperature, or chemicals to the central nervous system. Primary afferent nerves, evoked by non-nociceptive and nociceptive stimuli, reside in the dorsal root ganglion (DRG) of the spinal cord. Primary DRG cells have been used as an *in vitro* model of nociceptive responses for decades. However, they are low-yielding, challenging to culture, and difficult to access. To negate accessability issues and improve ease of culture, REPROCELL has developed an alternative sensory neuron model using human-derived induced pluripotent stem cells (iPSC). This model was analyzed to confirm its phenotypical relevance and functionality. Immunocytochemistry and real-time PCR were used to compare the expression of sensory markers to primary DRG cells. Multi-Electrode Array (MEA) analysis was used to confirm whether these induced hiPSC-derived sensory nerves display the same functionality as primary DRG cells.

Conclusion: The sensory nerves we have developed in this study express sensory neuron-related proteins and genes, and responded to capsaicin, menthol, temperature, and bradykinin. We have therefore concluded that these iPSC-derived sensory neurons can replace primary DRG cells as a model for drug discovery research.

1 Expression of sensory neuron markers

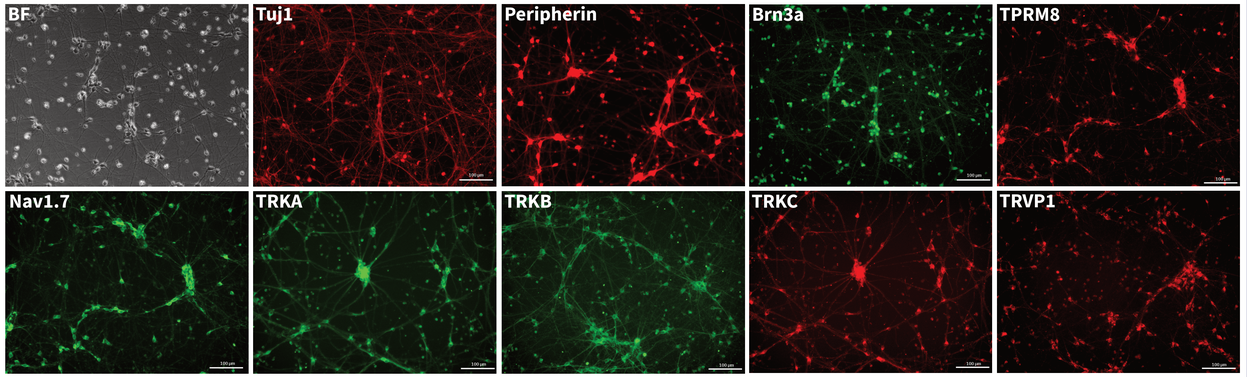
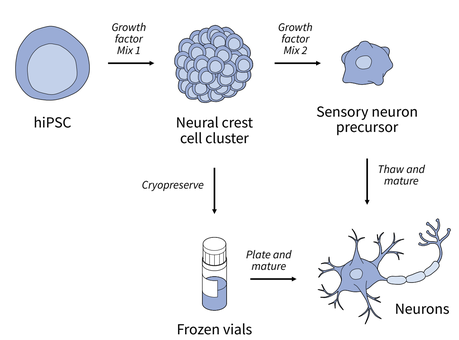


Fig 1. Immunofluorescence staining day 14 after thawing and seeding. Neurons were seeded at 1.5×10^5 cells/well (24 well plates) in Sensory Neuron Culture Medium. They were stained for neural markers, peripheral nerve markers, and various sensory nerve-related proteins including: Tuj1 (mature nerve marker), Peripherin (peripheral nerve marker), Brn3a (sensory nerve marker), TRPV1 (capsaicin receptor), TRPM8 (menthol receptor), Nav 1.7 (Na channel), TRKA (NGF receptor), TRKB (BDNF receptor) , and TRKC (neurorophin-3 receptor).

2 Creation of Sensory Neurons



3 Expression of sensory neuron genes (n= 1)

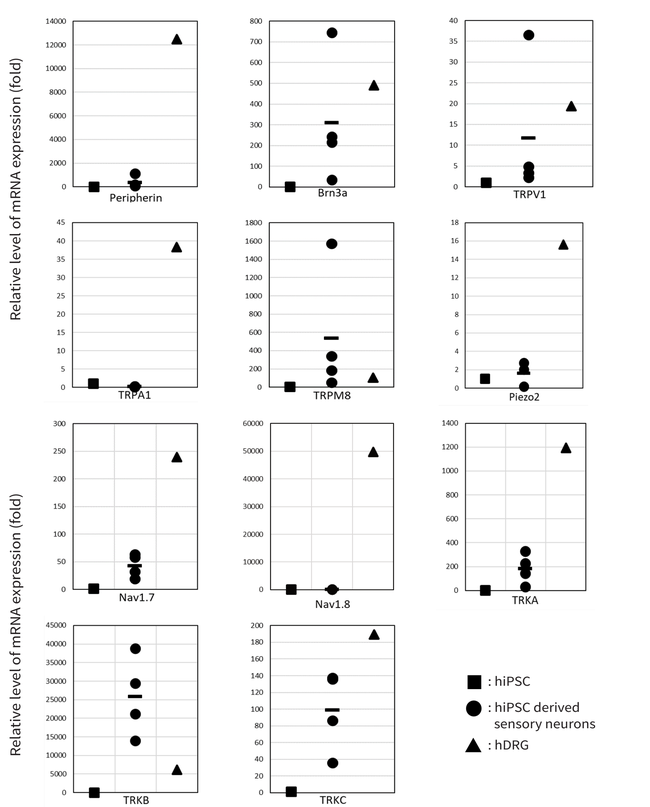


Fig 3. Real-time PCR analysis on day 14 after thawing and seeding. Gene expression of Peripherin, Brn3a, TRP channel, and high-affinity neurotrophin receptors was increased significantly in DRG compared with hiPSCs. There was no marked difference in Brn3a, TRPV1, TRPM8, and TRKB gene expression.

4-8 Electrophysiological analysis by MEA.

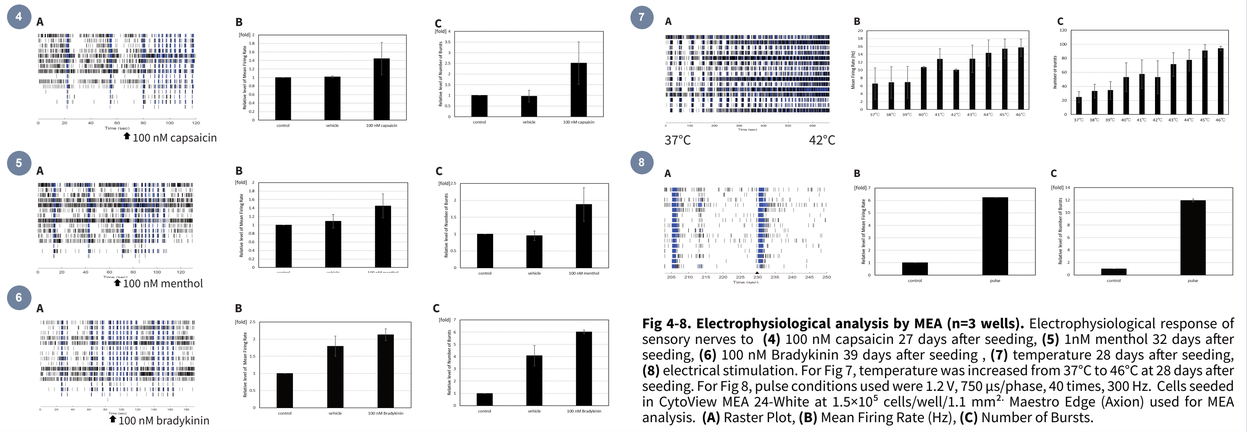


Fig 4-8. Electrophysiological analysis by MEA (n=3 wells). Electrophysiological response of sensory nerves to **(4)** 100 nM capsaicin 27 days after seeding, **(5)** 1nM menthol 32 days after seeding, **(6)** 100 nM Bradykinin 39 days after seeding , **(7)** temperature 28 days after seeding, **(8)** electrical stimulation. For Fig 7, temperature was increased from 37°C to 46°C at 28 days after seeding. For Fig 8, pulse conditions used were 1.2 V, 750 µs/phase, 40 times, 300 Hz. Cells seeded in CytoView MEA 24-White at 1.5×10^5 cells/well/1.1 $mm^{2.}$ Maestro Edge (Axion) used for MEA analysis. **(A)** Raster Plot, **(B)** Mean Firing Rate (Hz), **(C)** Number of Bursts.

9 Electrophysiological response of sensory nerves to ProTX-II

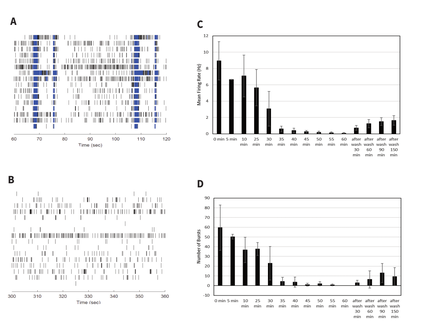


Fig 9. Electrophysiological response of sensory nerves to 1 µM ProTX-II (n=3 wells). Cells seeded in CytoView MEA 24-White at 1.5×10^5 cells/well/1.1 $mm^{2.}$ Maestro Edge (Axion) used for MEA analysis. (A) Raster plot before addition, (B) Raster plot 30 min after addition when exposed to 1 µM ProTX-II at 43 days after thaw seeding, (C) Mean Firing Rate (Hz), (D) Number of Bursts.