Efficient Differentiation of Human Pluripotent Stem Cells to Sensory Neurons

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INTRODUCTION

Sensory neurons (SNs) are responsible for conveying signals from the periphery of the body to the central nervous system (CNS) to be experienced as sensations like pain and temperature. A variety of ligand, mechanosensitive, and thermosensitive receptors expressed on the surface of SNs contribute to cellular depolarization and subsequent neuronal firing. Therefore, the study of these receptors is crucial to the development of therapeutics that can alleviate pain, one of the most common causes of hospitalization worldwide. Current studies mostly rely on dissection of primary rodent dorsal root ganglia, which may not accurately represent the human pain response. To meet the need for a human-relevant pain model, we have developed STEMdiff™ Sensory Neuron Differentiation and Maturation Kits, which promote differentiation of human pluripotent stem cells (hPSCs) to SNs via a neural crest cell (NCC) intermediate. Here we describe the STEMdiff™ Sensory Neuron Differentiation and Maturation Kits and protocol, which promote differentiation of hPSCs to SNs.

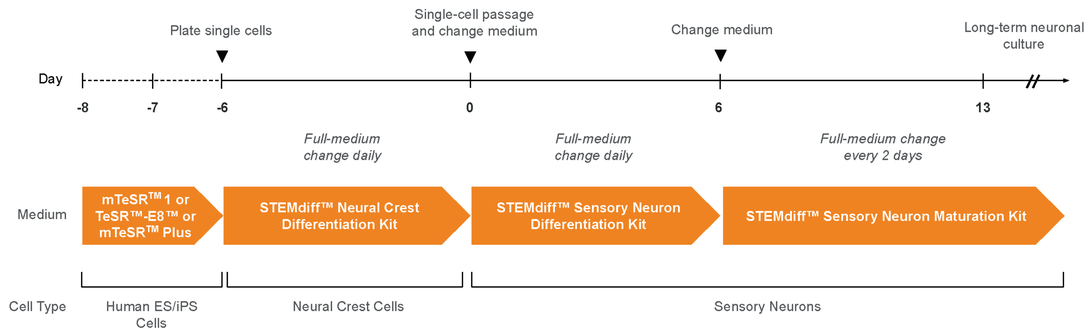

FIGURE 1. Workflow for STEMdiff™ Sensory Neuron Differentiation and Maturation Kits

hPSCs (embryonic stem [ES] and induced pluripotent stem [iPS] cells) are plated as single cells in STEMdiff™ Neural Crest Differentiation Medium. NCCs are generated after 6 days of culture. NCCs are passaged and plated into STEMdiff™ Sensory Neuron Differentiation Medium with daily full-medium changes for 6 days. Medium is changed to STEMdiff™ Sensory Neuron Maturation Medium, then cultured for an additional 6 days with full-medium changes every 2 days. SNs can then be analyzed or maintained for long-term neuronal culture.

METHODS

Differentiation to NCCs: Undifferentiated ES or iPS cells maintained in either mTeSR™1 (5 cell lines: 3 ES, 2 iPS), TeSR™-E8™ (2 cell lines: 1 ES, 1 iPS), or mTeSR™ Plus (3 cell lines: 2 ES, 1 iPS) were dissociated and plated at 2 x 10^5 cells/cm^2 on Corning® Matrigel®-coated plates in STEMdiff™ Neural Crest Differentiation Medium containing 10 µM Y-27632 (ROCKi) for one day, followed by daily full-medium changes (without ROCKi). After 6 days, differentiation was assessed by immunostaining for NCC markers SOX10 and neuroectodermal marker PAX6.

Differentiation to SNs: NCCs were dissociated and plated at 2 x 10^5 cells/cm^2 on Corning® Matrigel®-coated plates in STEMdiff™ Sensory Neuron Differentiation Medium and cultured for 6 days with daily full-medium changes. SNs were then matured by switching to STEMdiff™ Sensory Neuron Maturation Medium, then cultured for 6 days with full-medium changes every 2 days. On day 12, cultures were fixed and characterized by immunostaining for SN markers BRN3A, peripherin, TRPV1, and Nav1.7.

Multi-Electrode Array (MEA) Recording and Analysis: NCCs were plated and differentiated to SNs directly in a 48-well CytoView MEA plate (Axion Biosystems; M768-tMEA-48B), with 16 recording electrodes in each well. A 5-minute recording of spontaneous neuronal activity was acquired at 37°C under a 5% CO_2 atmosphere using an MEA system (Maestro, Axion Biosystems). To measure neuronal activity, mean firing rate (MFR) was calculated based on the activity recorded from 16 electrodes for each condition. For nociceptive stimulus testing, neural activity was recorded throughout a temperature increase to 42°C for 5 minutes followed by a 5-minute recovery at 37°C, or upon addition of 100 nM capsaicin (Sigma M2028) into wells. For comparison to CNS neurons, midbrain neurons were differentiated using STEMdiff™ Midbrain Neuron Differentiation Kit. For all recordings, a Butterworth band-pass filter (200 - 3000 Hz) was applied, and the adaptive threshold spike detector was set at 6X standard deviation. Recordings were analyzed using AxIS (2.5.1) analysis software.

RESULTS

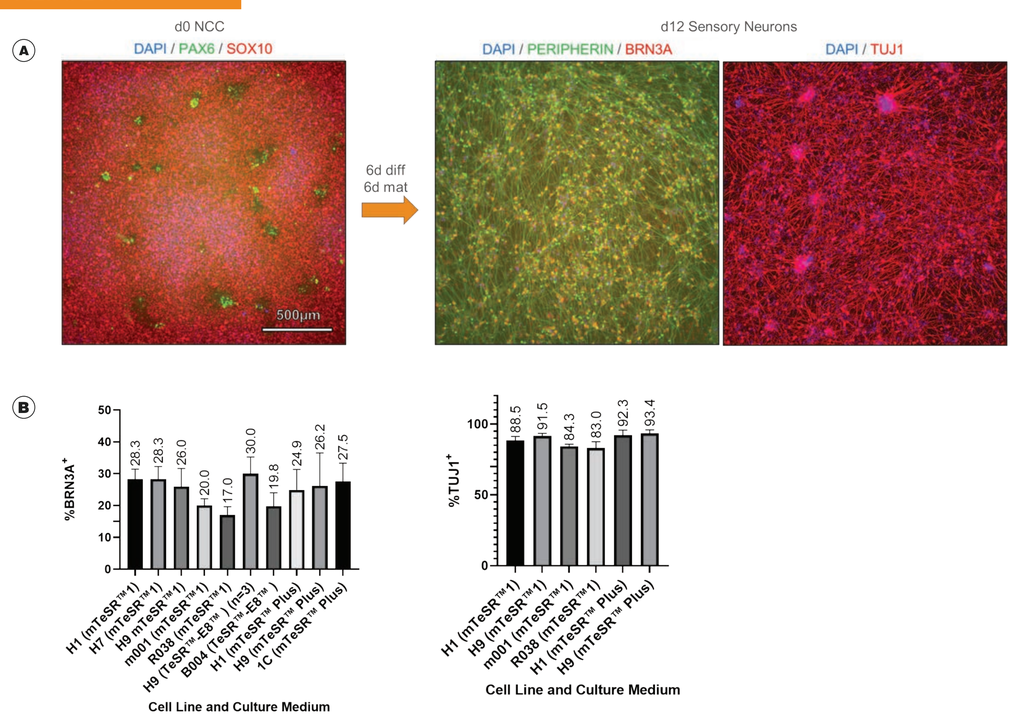

FIGURE 2. STEMdiff™ Sensory Neuron Kits Promote SN Differentiation Across Multiple ES and iPS Cell Lines and Media

(A) Representative immunocytochemistry image showing an NCC culture (derived from H1 cell line maintained in mTeSR™ Plus) positive for NCC marker SOX10, with few PAX6$^+$ neuroectodermal cells. Representative immunocytochemistry images showing resulting SN cultures positive for SN markers peripherin and BRN3A and neuronal marker TUJ1. **(B)** Quantification of the percentages of BRN3A$^+$ and TUJ1$^+$ cells. Human ES and iPS cell lines maintained in either mTeSR™1, TeSR™-E8™, or mTeSR™ Plus generated BRN3A$^+$ SNs (25.3% ± 6.9%; mean ± SEM; n=7 cell lines, 3 - 23 replicates per condition) that expressed neuronal marker TUJ1 (90.3% ± 4.1%; mean ± SEM; n=4 cell lines, 3 - 12 replicates per condition). Numbers are % positive over total DAPI in a tiled image.

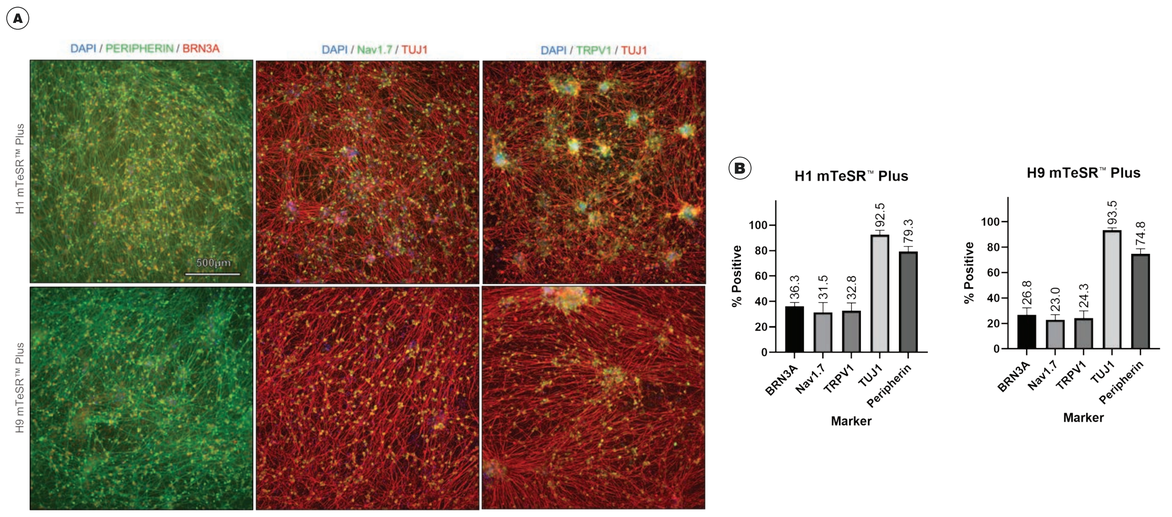

FIGURE 3. SNs Derived Using STEMdiff™ Sensory Neuron Differentiation and Maturation Kits Express Typical SN Markers

(A) Differentiation was assessed by immunostaining for SN markers peripherin, BRN3A, Nav1.7, TRPV1, and neuronal marker TUJ1 at day 12. Representative 10X images are shown from the differentiation of H1 and H9 cell lines cultured in mTeSR™ Plus. Rows shown are the results of the same experiments. **(B)** Quantification of the % positive cells for each of the displayed markers (mean ± SEM; 3 replicates per line). Numbers are % positive over total DAPI in a tiled image.

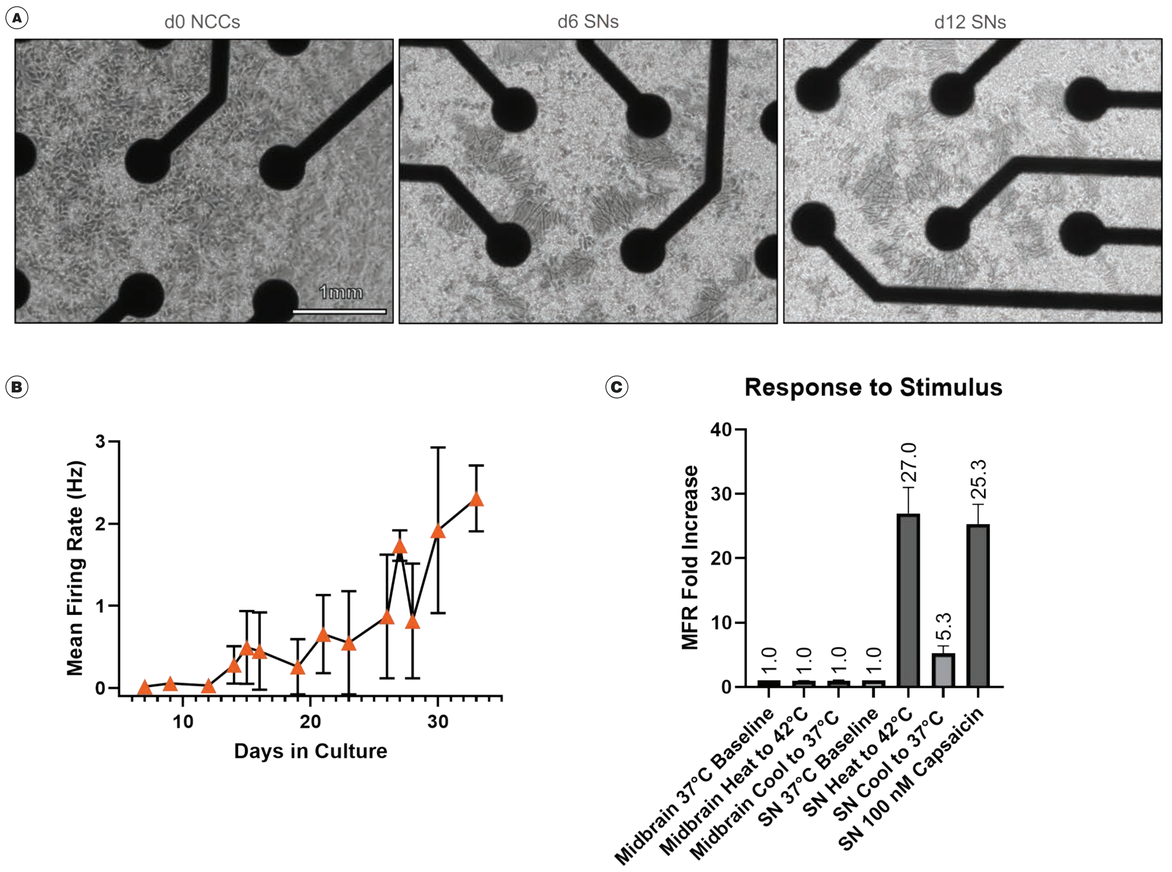

FIGURE 4. SNs Derived Using STEMdiff™ Sensory Neuron Differentiation Kit Develop Spontaneous Neuronal Activity Over Time and Respond to Nociceptive Stimulus

(A) Representative images showing H1 mTeSR™ Plus culture-derived differentiation of NCCs to SNs directly in an MEA plate. NCCs were plated at 2 x 10^5 cells/cm^2 and differentiated as described in Methods. SNs grew directly on top of the recording electrodes. **(B)** Mean firing rate (MFR) was assessed following SN differentiation. There was a gradual increase in MFR for SNs cultured over time, from 0.018 ± 0.008 Hz on day 7 to 2.3 ± 0.4 Hz on day 33 (n=3; mean ± SEM). **(C)** MFR response to nociceptive stimulus was assessed for midbrain neuron and SN cultures. Day 21 SN cultures displayed a 27 ± 4-fold (mean ± SEM, n=3) increase in MFR in response to temperature increase, which gradually decreased upon recovery. In contrast, day 50 midbrain neurons that displayed spontaneous neuronal activity at 37°C did not display any fold increase in MFR upon temperature increase. Addition of 100 nM capsaicin to SN cultures on day 21 resulted in a similar fold increase in MFR (25 ± 3-fold increase in MFR; mean ± SEM, n=3).

Summary

STEMdiff™ Sensory Neuron Differentiation and Maturation Kits:

- Generate BRN3A$^+$, peripherin$^+$, TRPV1$^+$, Nav1.7$^+$, TUJ1$^+$ sensory neurons from neural crest cells in 12 days
- Convert multiple human ES and iPS cell lines maintained in either mTeSR™1, TeSR™-E8™, or mTeSR™ Plus into sensory neurons, when used downstream of STEMdiff™ Neural Crest Differentiation Kit
- Sensory neuron cultures develop spontaneous neuronal activity over time, and display an increase in MFR in response to nociceptive stimulus such as temperature increase or exposure to capsaicin