A model for high throughput therapeutic screening of peripheral neuropathy in a Human 3D Nerve-on-a-Chip microphysiological system

Abstract #152

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NerveSim® Powered by Nerve-On-A-Chip®

Introduction

Micro-physiological systems (MPS), including organs-on-chips, have emerged as promising screening platforms to bridge the gap between preclinical and clinical success. However, engineering 3D tissues relevant to the nervous system, especially peripheral nerves (PNs), is challenging because of the complex ultrastructure and necessity of functional outputs, including Schwann-cell myelination and electrophysiology. We have developed a nerve-on-a-chip construct to culture animal[1] and human[2] neural tissue in a dual hydrogel system that promotes axon growth analogous to mature nerve anatomy. In this study, we have engineered an all-human NerveSim® MPS, derived from human induced pleuripotent stem cell (iPSC) derived sensory neurons (hSNs) and human primary Schwann cells (hSCs). To demonstrate the resulting mature nerve anatomy, we analyzed neurite outgrowth and Schwann cell migration along neuronal axons, quantified functional electrophysiological responses, and conducted ultrastructure analysis of myelinated axons.

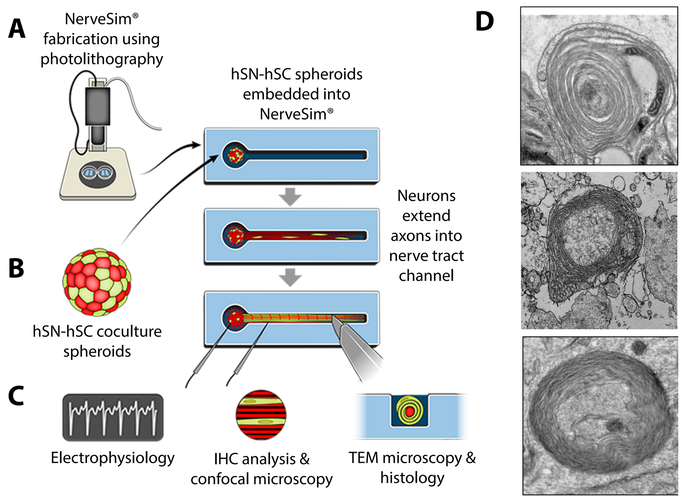

Figure 1. Schematic of creating a Nerve-on-a-Chip assay. (A) Fabrication: PEG hydrogel scaffolds are crosslinked onto permeable Transwell membranes using photolithography. **(B) Tissue culture:** Coculture hSN-hSC spheroids are inserted into the inner Matrigel-filled channel and are grown for 28 days in vitro, confined in a 3D space analogous to nerve fiber tract. **(C) Test metrics:** Endpoints include histological endpoints, including immunohistochemistry (IHC) analysis of neurite length and Schwann cell migration, as well as ultrastructure transmission electron microscopy (TEM) analysis of myelin structure. **(D) TEM:** TEM images of nerve cross-section stained with osmium tetroxide show Schwann cell-myelinated axon.

Coculture Density Selection

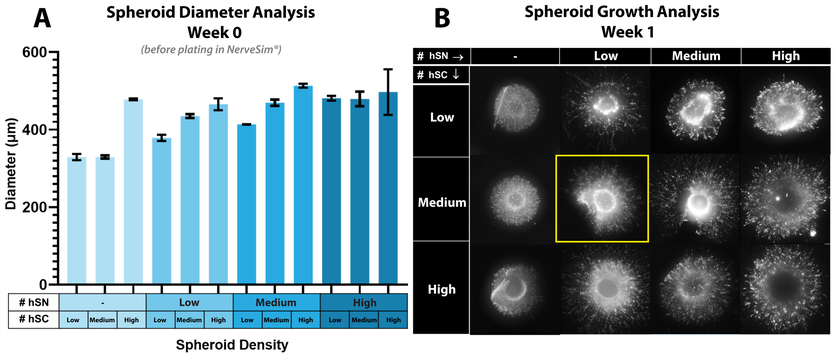

Figure 2. Coculture and monoculture spheroid density analysis and selection. (A) Monoculture (hSN) and coculture (hSN-hSC) spheroid diameter analysis at 3D DIV0, before plating in NerveSim® constructs. No significant trends were observed, and all spheroid conditions remained ≤ 500 µm in diameter. **(B)** IHC Analysis at 3D Week 1; DAPI (grey) staining shows Schwann cell migration out of spheroid body in an axonal pattern.

Histological Analysis

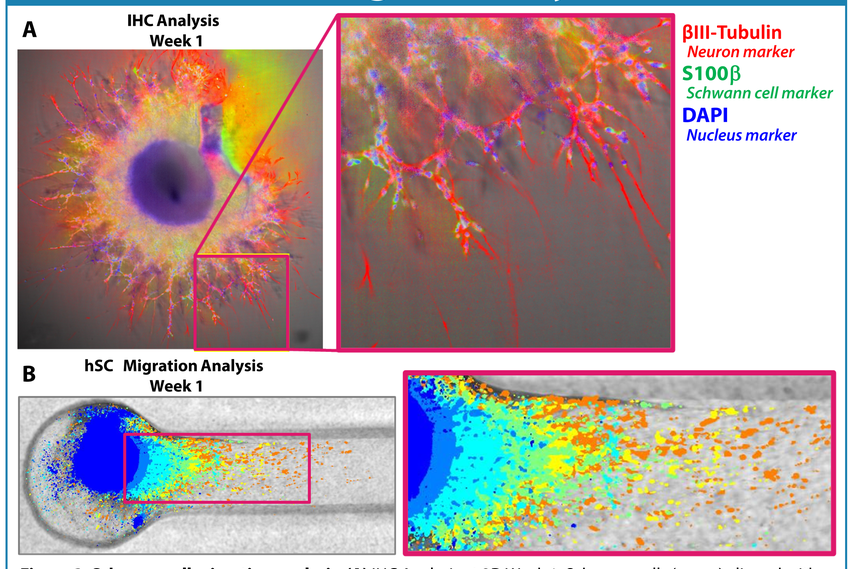

Figure 3. Schwann cell migration analysis. (A) IHC Analysis at 3D Week 1; Schwann cells (green) aligned with neuronal axons (red), necessary for the initiation of the myelination process. **(B)** Schwann cells were exclusively labeled with live cell tracking dye (Qdot 705) before coculture spheroid formation. Migration was tracked throughout 1 week of 3D growth in NerveSim® with daily fluorescent images. Pairwise images are shown stacked.

Histological Analysis

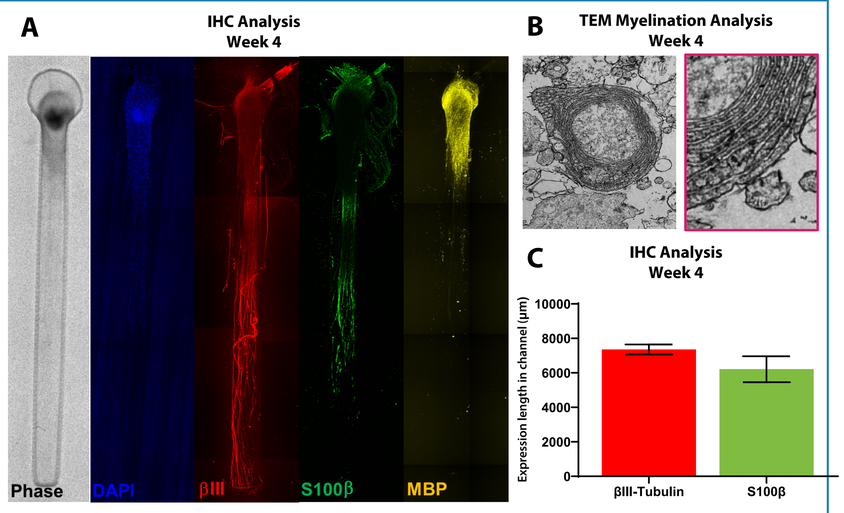

Figure 4. Analysis of NerveSim® samples at 4-weeks maturity. (A) Fluorescent images of immunostained human NerveSim® construct, showing neurite outgrowth (red) reaching the end of the axon channel, Schwann cells (green) migrating along axon tracks, and myelin (yellow) within the bulb and upper axon channel regions. **(B)** TEM images of nerve cross-section were stained with osmium tetroxide to show Schwann cell-myelinated axons. **(C)** Length of expression in immunostained NerveSim® axon channel was quantified using a custom developed MATLAB script. Results indicate robust neurite length (βIII-Tubulin), Schwann cell migration (S100), and myelination (MBP) in the NerveSim® axon channel. The total length of the channel is 7.5mm.

Electrophysiology

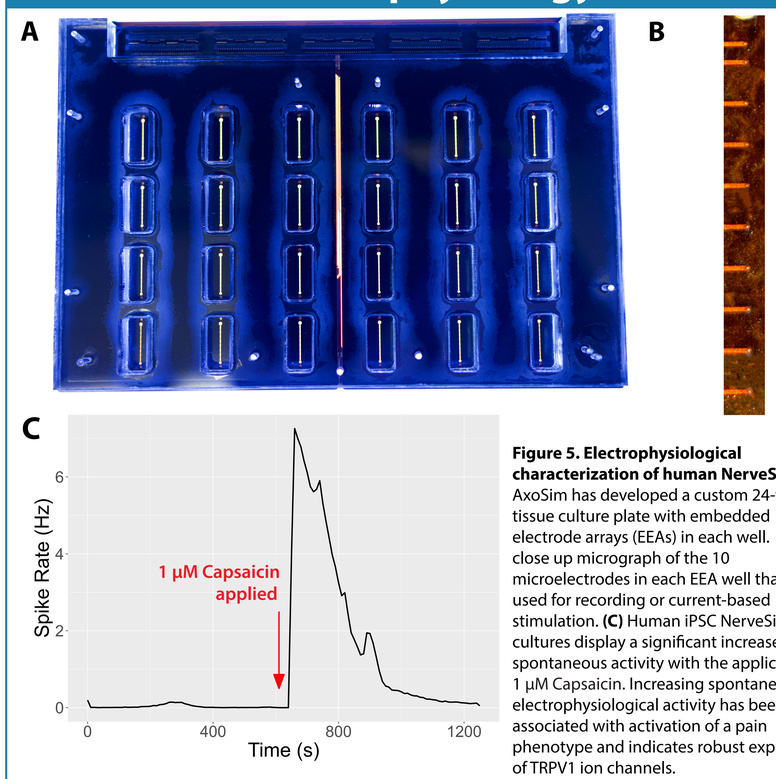

Figure 5. Electrophysiological characterization of human NerveSim®. (A) AxoSim has developed a custom 24-well tissue culture plate with embedded electrode arrays (EEAs) in each well. **(B)** A close up micrograph of the 10 microelectrodes in each EEA well that can be used for recording or current-based stimulation. **(C)** Human iPSC NerveSim® cultures display a significant increase in spontaneous activity with the application of 1 µM Capsaicin. Increasing spontaneous electrophysiological activity has been associated with activation of a pain phenotype and indicates robust expression of TRPV1 ion channels.

Microelectrode Recordings

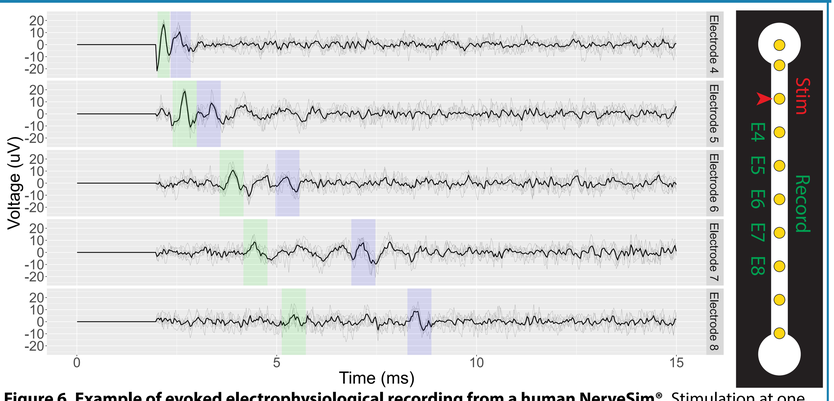

Figure 6. Example of evoked electrophysiological recording from a human NerveSim®. Stimulation at one electrode evokes two distinct time-delayed CAPs, highted in blue and green, on nearby electrodes as the signals travel at different conduction velocities.

Longitudinal Electrophysiology

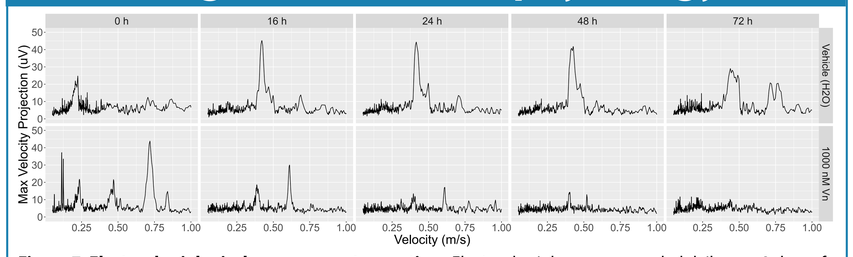

Figure 7. Electrophysiological measurements over time. Electrophysiology was recorded daily over 3 days of dosing with a vehicle (top) and 1000 nM vincristine (bottom). Population level responses are shown using maximal velocity projection (MVP) traces pooling responses across all electrodes for each NerveSim® sample.

Vincristine Dose Response

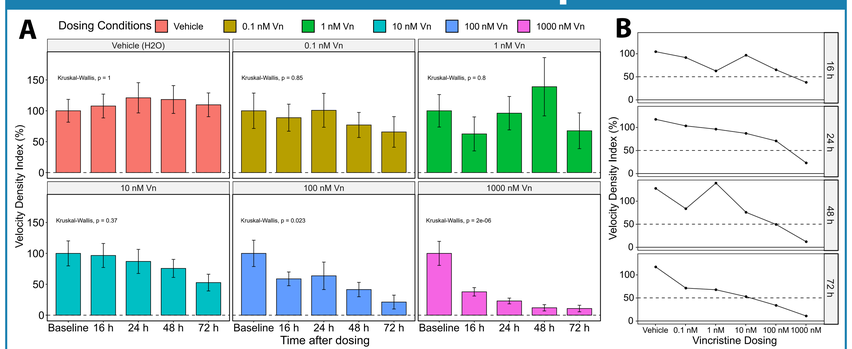

Figure 8. Vincristine dose response over 3 days. (A) Human iPSC NerveSim® cultures were grown for 42 days before dosing with 5 levels of vincristine as well as a vehicle control (water). Electrophysiology was measured daily and the total electrophysiological activity was quantified between 0 and 1 m/s as a velocity density index (VDI) at each timepoint, showing dosage dependent decreases at concentrations at 10 nM vincristine and greater. **(B)** Vincristine dose response curves at each timepoint showing progressively larger dose dependent decreases in activity compared to the vehicle control.

Functional Electrophysiology

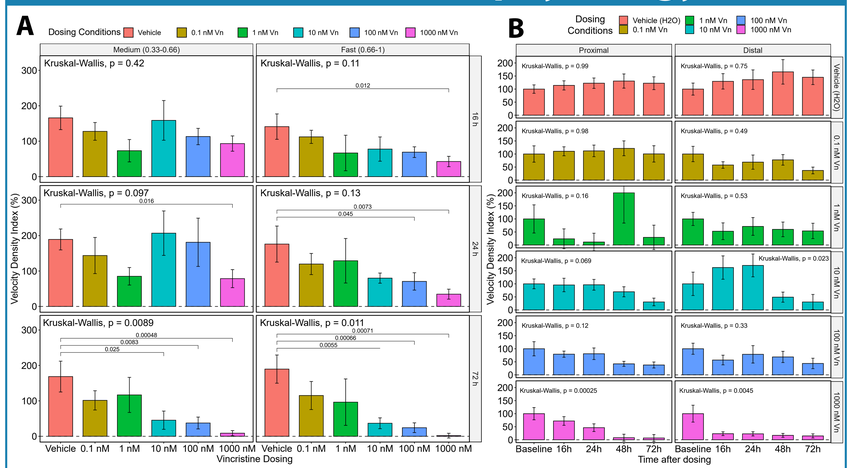

Figure 9. Functional electrophysiological metrics change with vincristine dosing (A) The VDI metric was calculated for velocity bins of 0.33-0.66 m/s (medium speed) and 0.66-1 m/s (fast speed) and compared across vincristine dosing conditions and time. High doses (> 1 nM) caused progressive loss of faster responses over the course of 3 days. **(B)** The VDI metric was calculated based on the distance separating the stimulation electrode from the spheroid, breaking data into two groups: proximal (<2.5 mm) and distal (>2.5 mm) groups. High doses of vincristine caused rapid loss of distal responses consistent with axonopathy while proximal responses persisted for longer time periods.

Conclusions

- Histologically, the NerveSim® platform has been shown to exhibit crucial aspects of PN physiology and function, displaying robust neurite outgrowth of >7mm, Schwann cell axonal alignment, and Schwann cell myelination
- Functionally, the NerveSim® platform provides collection of data-rich electrophysiological metrics that can provide insights into neurotoxicity, neuroprotection, and neurorehabilitation
- Functional electrophysiological measurements provide the same quantitative metrics as clinical electrophysiology
- Future screening of multiple compounds with different mechanisms will generate a database for predicting compound mechanisms based on high-throughput electrophysiology

For more information:

1. Kramer L, et al. ALTEX 2020;37(3):350-364
2. Sharma, et al. Scientific Reports 2019 Jun 20;9(1):8921