A 3D Human Model for Preclinical Drug Screening using a Myelinated Nerve-on-a-Chip Micro-physiological System

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Overview

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 electrophysiological measurements. 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. This platform has been shown to exhibit crucial aspects of PN physiology and function, displaying robust neurite outgrowth, Schwann cell myelination, and measurable electrical activity, acting as a promising screening platform for improving pre-clinical success.

In this study, we have engineered an all-human NerveSim® MPS, derived from human 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 and conducted ultrastructure analysis of myelinated axons.

Methods

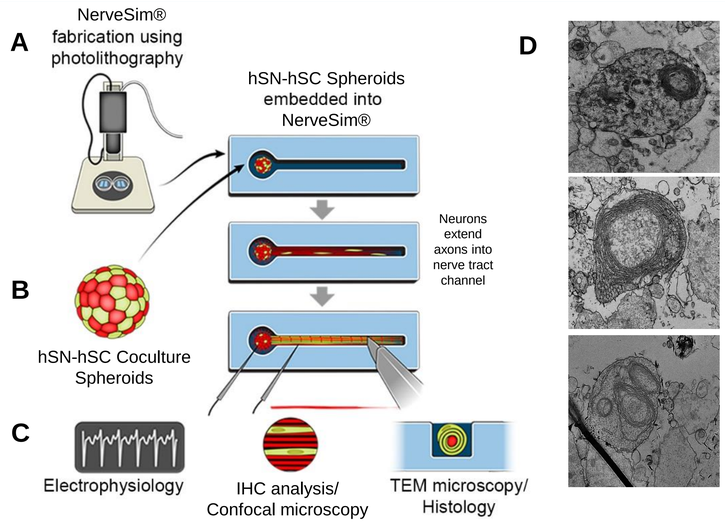

Figure 1. Schematic of creating a Nerve-on-a-Chip assay. (**A**) **Fabrication**: PEG hydrogel scaffolds are crosslinked onto permeable Transwell membranes using photolithography. (**B**) **Tissue culture**: Coculture hSN-hSC spheroids are inserted into the inner Matrigel-filled channel and are grown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

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

Results

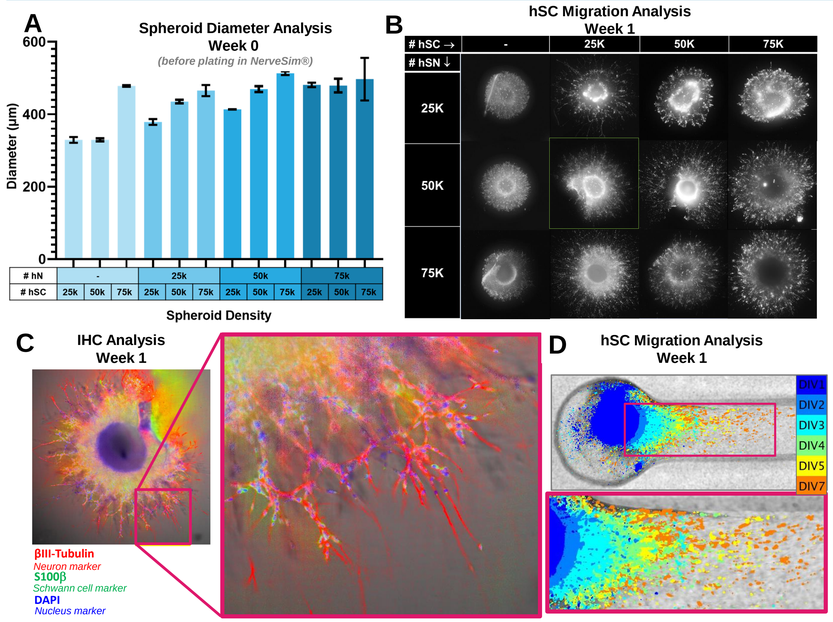

Figure 2. Coculture and monoculture spheroid density analysis and selection. (**A**) Monoculture (hSN) and coculture (hSN-hSC) spheroid diameter analysis at 3D DIV0, before plating in NerveSim® constructs. No significant trends were observed, and all spheroid conditions remained ≤ 500 µm in diameter.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

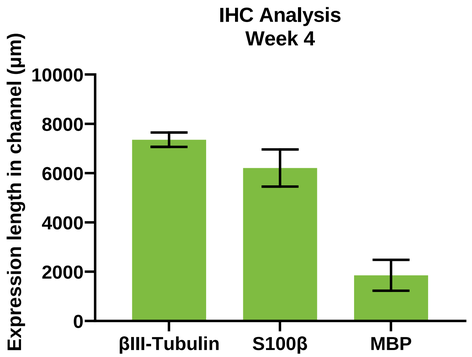

Figure 3. IHC analysis of NerveSim® samples at 4-weeks maturity. Length of expression in immunostained NerveSim® axon channel was quantified using a custom developed MATLAB script. Results indicate robust neurite length (βIII-Tubulin), Schwann cell migration (S100), and myelination (MBP) in the NerveSim® axon channel. The total length of the channel is 7.5mm.

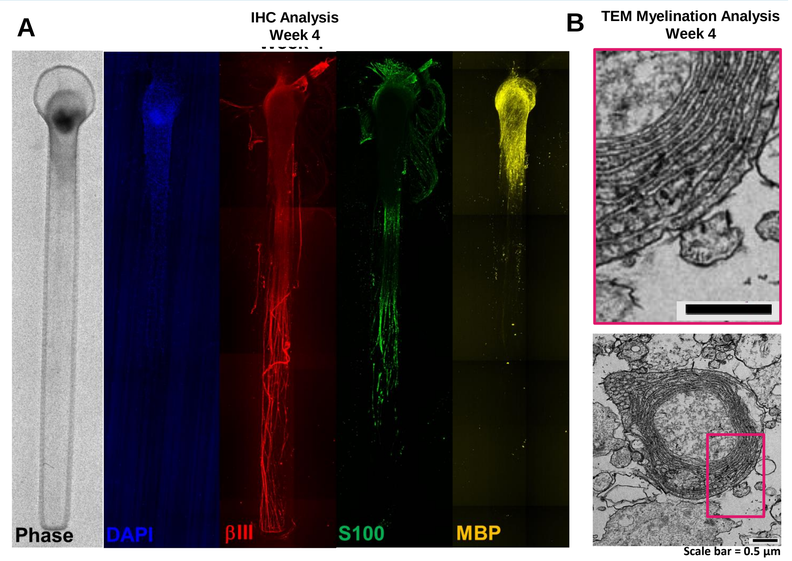

Figure 4. Analysis of NerveSim® samples at 4-weeks maturity. (**A**) Fluorescent images of immunostained human NerveSim® construct, showing neurite outgrowth (red) reaching the end of the axon channel, Schwann cells (green) migrating along axon tracks, and myelin (yellow) within the bulb and upper axon channel regions. (**B**) TEM images of nerve cross-section were stained with osmium tetroxide to show Schwann cell-myelinated axons.

Summary

We have developed a new human sensory neuron-based NerveSim® model, which shows 3D robust neurite length (>7mm), with Schwann cell proliferation and migration (>6mm) into the NerveSim® channel along axon tracks. Schwann cell-axon alignment is observed throughout the length of the channel, a necessary pre-requisite for the myelination process. Myelination is observed near the NerveSim® bulb region, and as far as 2mm into the axon channel, with both IHC analysis and TEM ultrastructure analysis.

In the next study, to demonstrate the efficacy of the NerveSim® assay as a preclinical screening tool, the same metrics will be investigated after mature 4-week NerveSim® samples undergo a 7-day exposure to chemotherapy drugs known to cause peripheral neuropathy. In addition, extended culturing timelines of up to 8-weeks, may be explored in efforts to extended myelination processes further down the NerveSim® axon channel.

Finally, we are validating electrophysiology (EPHYS) as an additional metric for preclinical screening. Custom embedded electrode arrays (EEA) have been designed for automated, longitudinal EPHYS studies. Through a closed loop system with multiple recording sites, additional clinically relevant metrics are enabled, providing higher content metrics and further insights into mechanism of action. The EEA NerveSim® assay has been validated with rat-derived spheroids, while validation with human cells is currently ongoing.