High-throughput Functional and Morphological Neurotoxicity Screening in Human NerveSim®

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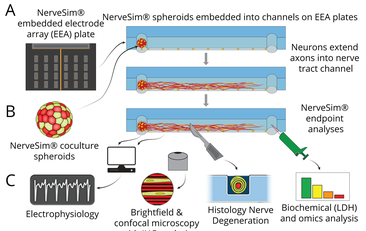
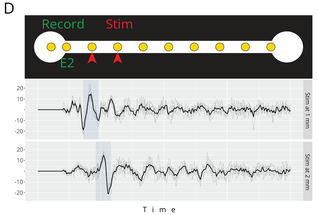
Overview

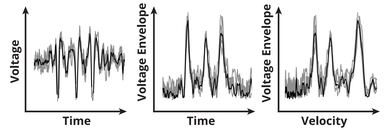
- AxoSim has developed a novel microphysiological human Nerve-on-a-Chip system, or NerveSim®, comprised of an iPSC-derived neuron and primary human Schwann cell 3D coculture system that forms a morphological and electrophysiological simulacrum of a human nerve.
- This system is employed in a 24-well Embedded Electrode Array (EEA) culture plate to provide high-throughput electrophysiological characterization amenable to testing multi-drug panels for neurotoxicity on a clinically relevant nerve model.
- We tested several chemotherapeutic compounds with known neurotoxicities by correlating changes in compound action potentials (CAPs) and axonal morphology for multidimensional non-invasive longitudinal functional characterization.



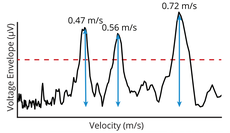


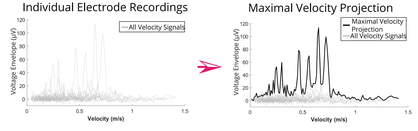
Coculture spheroids (B) are formed and placed into NerveSim® EEA plates plates (A). Neurites bundle and grow down the channel creating a three dimensional nerve model. Once developed, cultures are stimulated for evoked electrophysiology, imaged for degeneration measurement, and media is collected to estimate cytoxicity (C). Raw recordings from a human iPSC NerveSim® stimulated at electrodes 3 and 4 with 48 uA. The evoked responses on electrode 2 show latency shifts corresponding to stimulation distance with consistent velocities (D).

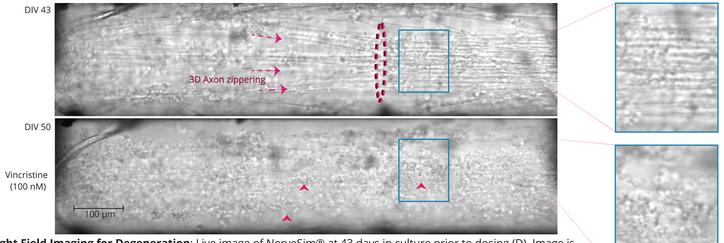
Methods



CAP Analysis (VDI): Raw signals were processed (A) and significant peaks were detected (B) for each electrode recording. Recordings from multiple electrodes in a single well were combined (C) into a maximal velocity projection (MVP) to quantify the population-level response to electrical stimulation for each individual NerveSim®. The area under the curve of the MVP was calculated to create a velocity density index (VDI).

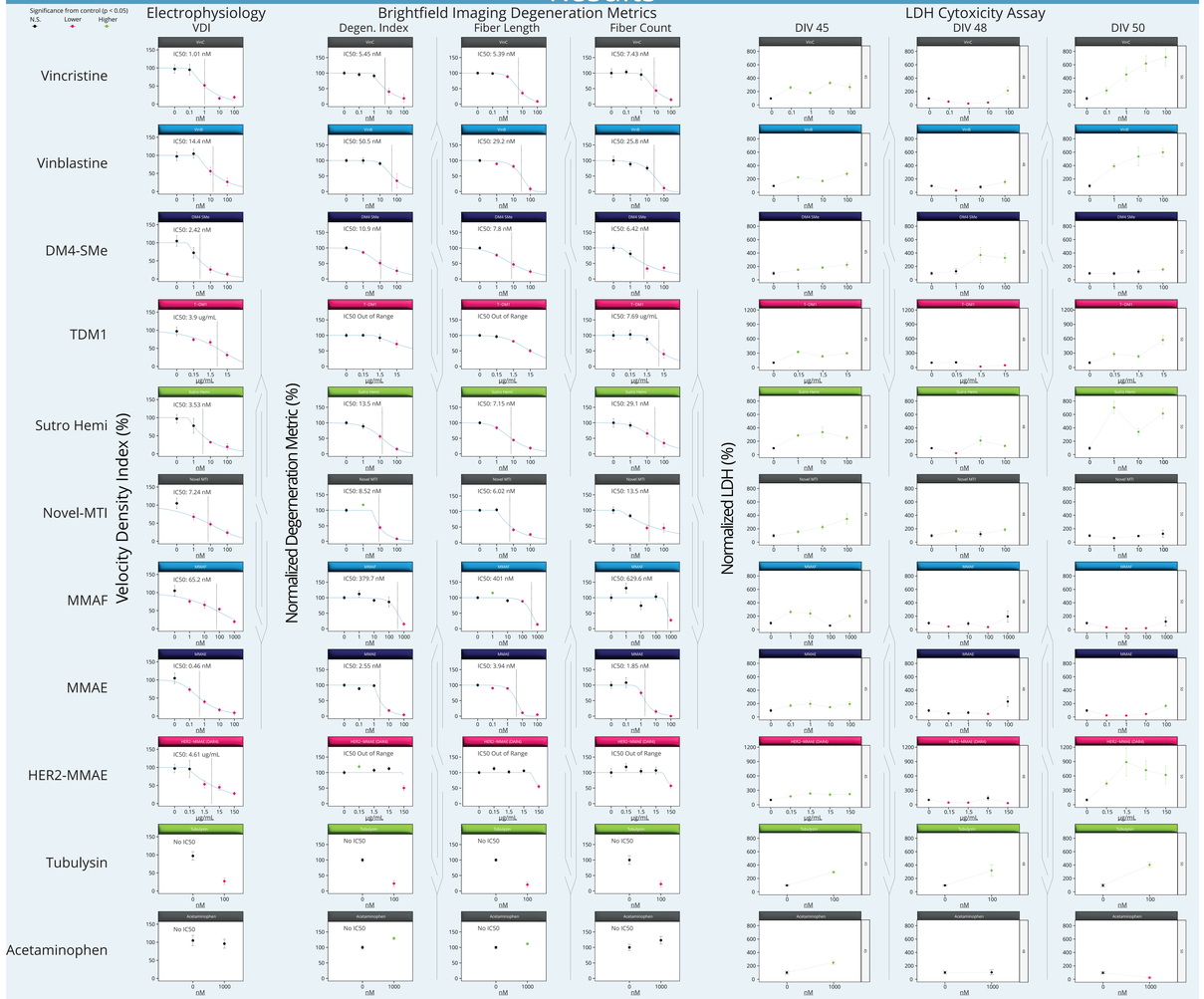




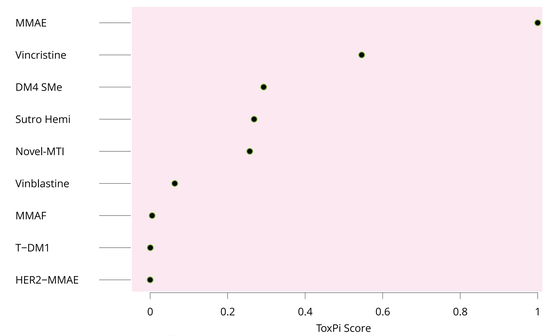


Bright Field Imaging for Degeneration: Live image of NerveSim® at 43 days in culture prior to dosing (D). Image is one slice of a z-stack. There is axon bundling and aligned neurite growth down the channel (arrows). After 7 days of dosing with 100 nM Vincristine, the bundles are no longer visible and significant fragment of neurites is present (arrowheads, E). Quantification of degeneration is done by comparing changes to the same slice, well, and plate combination over time using a mask to highlight edges of processes.

Results



Summary



Toxicological Prioritization Index (ToxPi) score that ranks all compounds which were tested via a dosing series. This plot combines the IC50s from VDI and Fiber length dose response curves to rank order compounds where the most toxic compound has a score of 1 (MMAE in this case). ADCs were converted to nM concentration by taking into account the molecular weight and the Drug/Antibody Ratio (DAR).

Description of metrics

VDI - Velocity Density Index, area under the curve of electrophysiology responses (maximal velocity projection)
Degeneration Index - Number of small objects in mask, increases with fragmentation.
Fiber length - Length of continuous tracts of neurites in mask (fibers).
Fiber count - Number of continuous fibers in mask.
LDH - Lactate dehydrogenase cytoxicity assay measures enzyme concentration released from the cytoplasm into the media as membrane permeability increases.

	Class	Modality	VDI IC50	Deg.In IC50	Fib.L IC50	Fib.C IC50
Vincristine	Vinca	Small molecule	1.01 nM	5.45 nM	5.39 nM	7.43 nM
Vinblastine	Vinca	Small molecule	14.4 nM	50.5 nM	29.2 nM	25.8 nM
DM4-SMe	Maytansinoid	Payload	2.42 nM	10.9 nM	7.8 nM	6.42 nM
TDM1	Maytansinoid	ADC	3.9 µg/mL	OoR	OoR	7.69 µg/mL
Sutro Hemi	Hemiasterlin?	Payload	3.53 nM	13.5 nM	7.15 nM	29.1 nM
Novel-MTI	NA	Payload	7.24 nM	8.52 nM	6.02 nM	13.5 nM
MMAF	Auristatin	Payload	65.2 nM	379.7 nM	401 nM	629.6 nM
MMAE	Auristatin	Payload	0.46 nM	2.55 nM	3.94 nM	1.85 nM
HER2-MMAE	Auristatin	ADC	4.61 µg/mL	OoR	OoR	OoR
Tubulysin	Tetrapeptide	Payload	NA	NA	NA	NA
Acetaminophen	Analgesic	Control	NA	NA	NA	NA

Summary of IC50s for the tested metrics and compounds. VDI - velocity density index, Deg.In - degeneration index, Fib.L - Fiber length, Fib.C - Fiber count, OoR - Out of range. All tested chemotherapeutics are microtubule inhibitors (MTI).

Conclusions

- VinC also showed high neurotoxicity and had ~10-fold lower electrophysiology IC50 and ~6-fold lower growth IC50 than VinB. LDH showed expected dose response curves at DIV50.
- T-DM1 (trastuzumab emtansine) electrophysiology IC50 was ~ 10 fold higher than the maytansinoid payload DM4-SMe (3.9 µg/mL or 26.8 nM vs 2.42 nM). Growth IC50 for T-DM1 exceeded dose range while DM4-SMe growth IC50 was 7.8 nM.
- Sutro Hemi had a potent electrophysiology IC50 of 3.53 nM. Degeneration index and fiber length were comparable at 13.5 nM and 7.15 nM respectively. Fiber count was ~10 fold higher than electrophysiology at 29.1 nM which may reflect less impairment of fiber number.
- Novel-MTI was also neurotoxic, with an electrophysiology IC50 of 7.24 nM and comparable potency via degeneration metrics.
- MMAE show high neurotoxicity and had ~100 fold lower electrophysiology IC50 and growth IC50 than MMAF. LDH was elevated for both 2 days after dosing. MMAE at its highest concentration was significantly elevated in LDH at DIV 50.
- HER2-MMAE had a similar electrophysiology IC50 to T-DM1 (4.61 µg/mL and 3.9 µg/mL respectively) with both having growth IC50s that exceeded the tested dose range.
- Tubulysin nearly abolished all electrophysiological activity at 100 nM and caused a significant decrease in fiber length.
- Acetaminophen had no significant effect on electrophysiology at 1000 nM. It had significantly increased fiber length and LDH (first time point only) which could be due to biological variability.