Comparison of a 3D Nerve-on-a-Chip peripheral nerve model to 2D neuronal assays for clinical translation with antibody drug conjugate (ADC) toxicity screening

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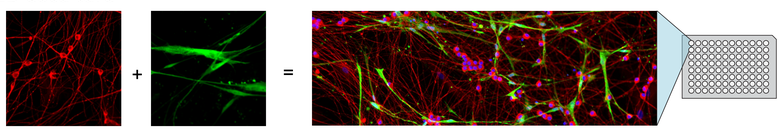
Background and Purpose

Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating side effect of anticancer therapies, often resulting in chronic pain and loss of sensation as peripheral sensory and motor neurons degenerate from chemotherapy exposure. CIPN ranges in severity from mild annoyance through life-threatening and is a leading cause for patients to switch or discontinue treatments. Many compounds have passed preclinical testing demonstrating minimal toxicity but were found to cause peripheral neuropathy and other side effects in clinical testing, leading to low clinical trial success rates and limited use of approved compounds. Antibody-drug conjugates (ADCs), highly toxic payloads linked to tumor-specific monoclonal antibodies, are a recent example of compounds designed to have excellent specificity through preclinical testing but unfortunately have shown unexpected peripheral neuropathy in patients. Complex *In Vitro* Models (CIVMs) offer a promising alternative, bridging the gap between 2D cell cultures and human clinical data by providing an *in vitro* model with *in vivo*-like characteristics. We have developed a CIVM incorporating human iPSC-derived sensory neurons and human primary Schwann cells to assess toxicity in 2D and 3D formats.

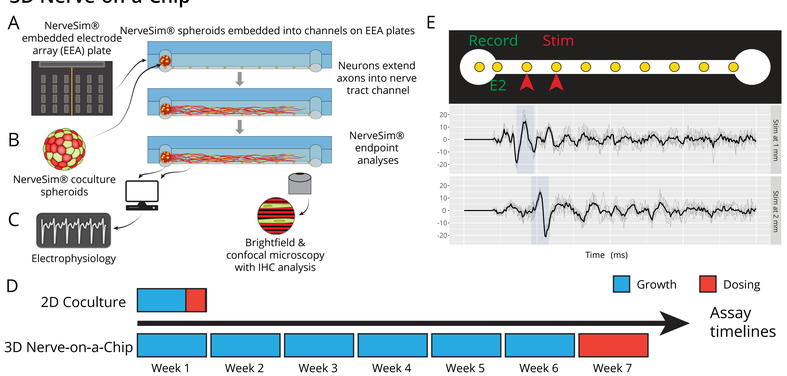
Methods

2D Coculture



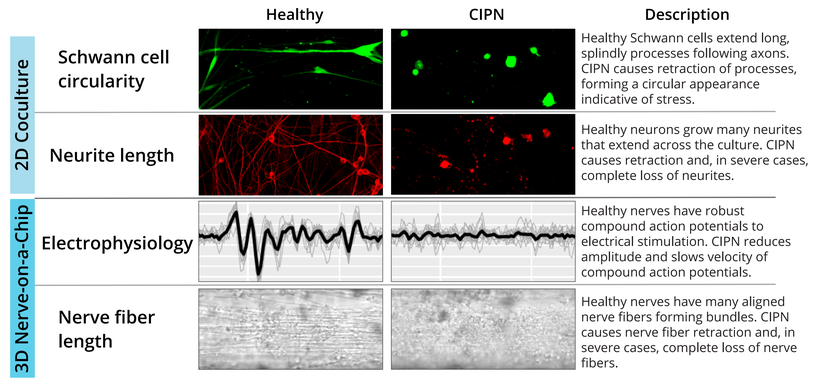
Human iPSC-derived sensory neurons are combined with human primary Schwann cells in 96-well microplates to allow high throughput screening with 2D cocultures. Samples are stained via immunohistochemistry and imaged in a high content imager (Molecular Devices) to quantify neuron (BIII Tubulin) and Schwann cell (S100) count and morphology metrics.

3D Nerve-on-a-Chip

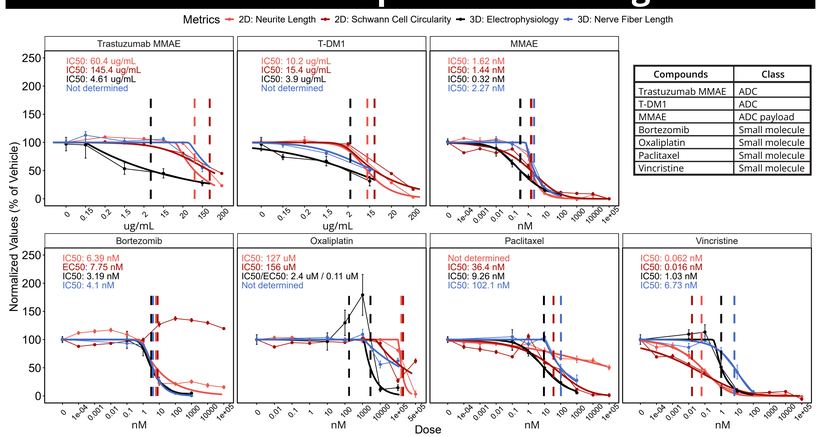


Coculture spheroids (B) are formed and placed into NerveSim EEA plates plates (A). Neurites bundle and grow down the channel creating a three dimensional nerve model. Once developed, cultures are stimulated for evoked electrophysiology and imaged for degeneration measurement (C). The 3D Nerve-on-a-Chip requires 6 weeks for growth and 1 week of compound dosing compared to the 4 days of growth and 2 days of dosing in the 2D coculture (D). Raw recordings from a 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 (E).

Endpoint Metrics



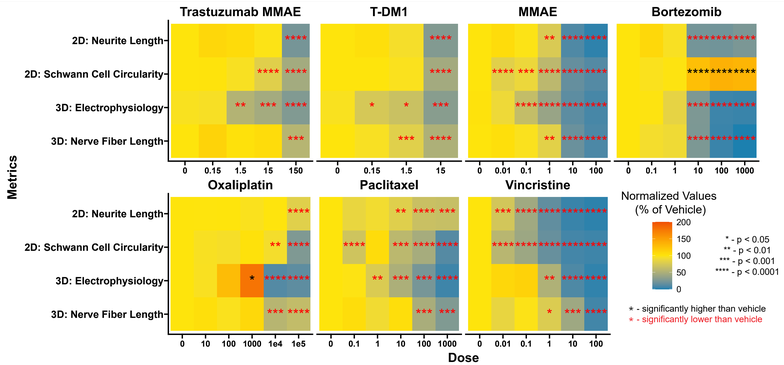
Chemotherapeutic Screening



Compounds	Class
Trastuzumab MMAE	ADC
T-DM1	ADC
MMAE	ADC payload
Bortezomib	Small molecule
Oxaliplatin	Small molecule
Paclitaxel	Small molecule
Vincristine	Small molecule

Seven chemotherapeutics (see table in upper right) were tested in both the 2D and 3D peripheral assays. CIPN was evaluated using 4 metrics (2 for each assay) through the estimation of IC50 values. In all but one case (vincristine), the functional electrophysiology endpoint from the 3D Nerve-on-a-Chip assay provided the most sensitive detection of neuropathy with lower estimated IC50 values compared to other metrics. Most striking were the 2 ADCs and oxaliplatin, that showed toxicity via electrophysiology 1-3 orders of magnitude below the other metrics.

Statistical Analyses



Heat map comparing the 4 metrics across the different compounds. Significant differences (t-test) for each concentration compared to the vehicle control (Dose = 0) are indicated by black or red asterisks, indicating significantly higher or lower values respectively. The 2D metrics were well aligned with the 3D metrics for the standard small molecule chemotherapeutics (e.g. bortezomib, paclitaxel, vincristine). However, the 3D metrics, especially the functional electrophysiology, detected toxicity at lower concentrations compared to the morphological 2D metrics for the ADCs and oxaliplatin.

Conclusions

Compounds	2D Metrics		3D Metrics		Clinical Free Plasma (max rec dose)
	Neurite Length	Schwann Cell Circularity	Ephys	Nerve Fiber Length	
Trastuzumab MMAE	60.4 ug/mL	145 ug/mL	4.61 ug/mL	n.d.	N/A
T-DM1	10.2 ug/mL	15.4 ug/mL	3.9 ug/mL	n.d.	85 ug/mL ‡
MMAE	0.87 nM	2.39 nM	0.46 nM	3.94 nM	5.57 nM
Bortezomib	6.39 nM	7.75 nM †	3.19 nM	4.1 nM	53 nM
Oxaliplatin	127 uM	156 uM	0.111 uM †	n.d.	0.496 uM
Paclitaxel	n.d.	36.4 nM	9.26 nM	102.1 nM	213 nM
Vincristine	0.062 nM	0.016 nM	1.03 nM	6.73 nM	1.75 nM

† - Reported EC50 values instead of IC50 ‡ - Max serum concentration only as protein binding not available

- Both 2D and 3D assays detected neurotoxicity in a panel of positive controls consiting of antibody-drug conjugates and small molecule chemotherapeutics.
- The 2D assay had similar or better performance for standard small molecule chemotherapeutics known to cause widespread cell death.
- The functional electrophysiology from the 3D assay had better performance for antibody-drug conjugates and compounds that have an impact on function at concentrations below the threshold for cell death.

References

(1) Liston, D. R.; Davis, M. Clinically Relevant Concentrations of Anticancer Drugs: A Guide for Nonclinical Studies. Clin Cancer Res 2017, 23 (14), 3489–3498. https://doi.org/10.1158/1078-0432.CCR-16-3083.