High-capacity microfluidic compartmentalized cell cultures for studying axonal regrowth in sporadic ALS iPSC-derived motor neurons

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INTRODUCTION

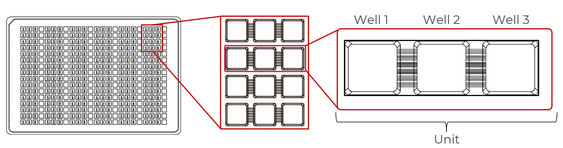
Neurological diseases are the leading cause of disability and the second most common cause of death globally, yet drug discovery in neurology remains highly failure-prone. This is largely due to the complexity of the human brain and limitations in current in vivo and in vitro models, resulting in a significant translational gap. Microfluidic in vitro systems offer improved physiological relevance and scalability.

Cellectricon has developed a high-capacity compartmentalized microfluidic co-culture plate that enables the study of complex neuronal interactions and targeted treatments. To demonstrate its utility, the plate was used to assess HER-096, a cerebral dopamine neurotrophic factor (CDNF)-derived peptide, in promoting axonal regrowth post-axotomy in ALS iPSC-derived motor neurons. While HER-096 is being developed for Parkinson's disease, CDNF has shown broad neuroprotective effects in models of neurodegeneration, including ALS, where motor neuron axonopathy is an early pathological hallmark.

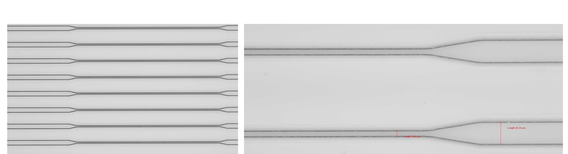
MATERIALS & METHODS

Cellectricon's high-capacity microfluidic co-culture plate

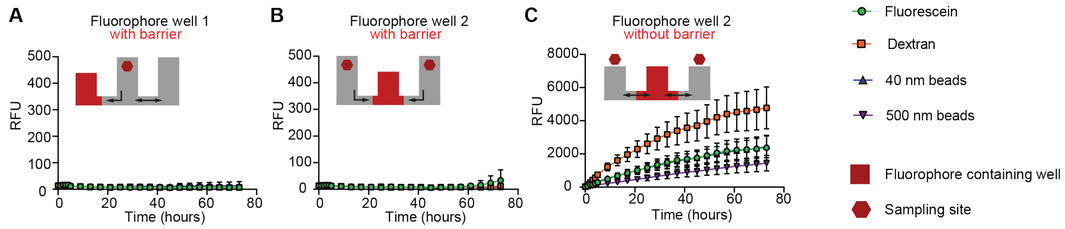
The plate is comprised of 96 experimental units compliant with American National Standard Institute of the Society for Laboratory Automation and Screening (ANSI/SLAS) standards of a 384-well plate [1]. Made entirely of cyclic olefin copolymer and assembled without adhesives, the plate offers key advantages over elastomeric or adhesive-based systems. Each unit consists of three wells connected by microchannels, that allow axons to pass through and enable their isolation from cell bodies and dendrites. The co-culture plate design offers scalability by enabling parallelization and automation of experiments, while minimizing the required volumes of cells or compounds. A hydrodynamic barrier enables long-term, targeted treatment of soma or axons, while the rigid plastic ensures superior flow control over elastomeric systems.



Overview of the high-throughput compartmentalized microfluidic co-culture plate, featuring 96 individual experimental units, each subdivided into three distinct compartments for parallel and controlled cell culture experiments.



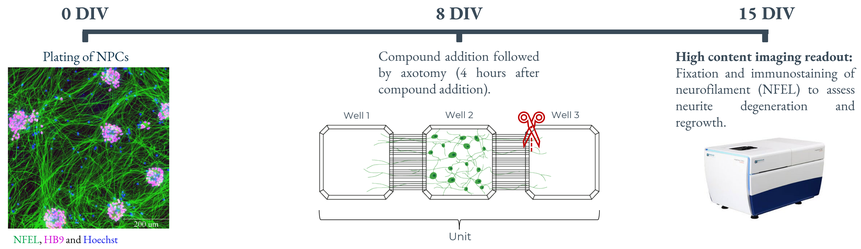
Representative light microscopy images showcasing the structural details of molded microfluidic channels.



The plate is a passive microfluidic system that operates without external pumps or pressure controls. Flow is gravity-driven, and by maintaining the fluid levels, the hydrodynamic barrier remains stable, supporting long-term experiments lasting several weeks.

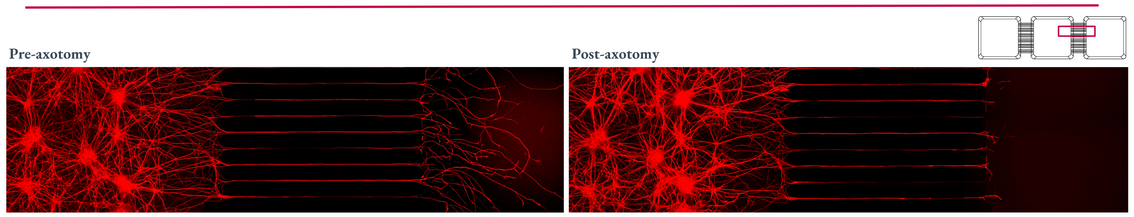
The axotomy/neurodegeneration model

Distal axonopathy is an early hallmark of ALS, making studies of motor axon regeneration using hiPSC-derived neurons highly relevant. To model this, human iPSCs (30HU-004) derived from patients with sporadic ALS were differentiated into neural progenitor cells (NPCs) and seeded into the middle compartment (well 2). At DIV 8, either medium control, NGF or HER-096 was added in a concentration-dependent manner. Four hours post-treatment, axotomy was performed in the axonal compartment (well 3) by pipetting, while well 1 remained untouched as a reference. At DIV 15, cultures were stained, imaged (ImageXpress), and axonal length was quantified - allowing assessment of regeneration.



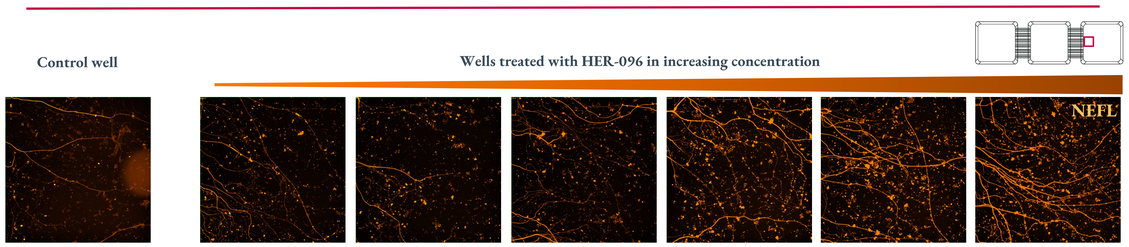
RESULTS

Successful axotomy confirmed in compartmentalized motor neuron cultures



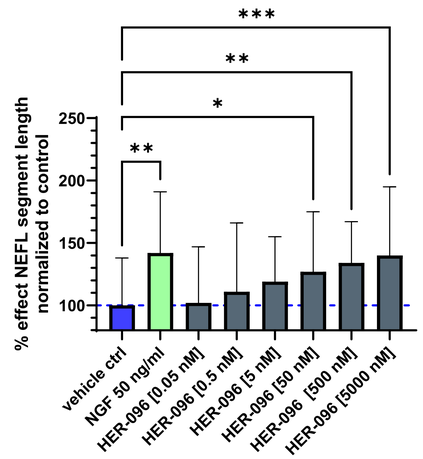
Successful axotomy was verified via live-cell imaging using a Beta III tubulin-based fluorescent tracer and further validated through high-content imaging analysis (data not shown).

HER-096 promotes dose-dependent axonal regeneration in sporadic ALS iPSC-derived motor neurons in the microfluidic model



HER-096 promotes dose-dependent axonal regeneration in sporadic ALS iPSC-derived motor neurons cultured in a microfluidic model. Representative images show NEFL-labeled axons in control well and wells treated with increasing concentrations of HER-096, 7 days after axotomy.

HER-096 significantly promotes axonal regrowth post-axotomy:



Normalized NEFL segment length in axonal compartments of axotomized cultures at 15 DIV. Treatments include vehicle control, NGF (50 ng/ml), and increasing concentrations of HER-096 (0.05–5000 nM). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. Significance levels: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

CONCLUSIONS

Cellectricon's high-capacity microfluidic co-culture plate enables the establishment of physiologically relevant in vitro models for studying complex neurological processes. Its compartmentalized design allows for axon-specific analysis, targeted treatment, and high-throughput experimentation. Using motor neurons derived from sporadic ALS iPSCs, we demonstrated the plate's utility by evaluating HER-096, a CDNF-derived peptide.

HER-096 significantly promoted axonal regrowth post-axotomy, highlighting its potential as a neurorestorative agent and validating the platform's relevance for neurodegenerative drug discovery. This model offers a powerful tool for bridging the translational gap in neurological research.

REFERENCES

[1] Moll L et al.. ACS Chem Neurosci. 2024 Jan 17;15(2):236-244. doi: 10.1021/acschemneuro.3c00409. PMID: 38150531; PMCID: PMC10797611