The multiplatform utility of human iPSC-derived neuronal models to provide complex biological *in vitro* systems for drug discovery

Dr Stuart Prime (stuart.prime@axolbio.com), Dr Jessica Tilman (jessica.tilman@axolbio.com)
Axol Bioscience, Roslin Innovation Centre, Charnock Bradely Building, Easter Bush Campus, Easter Bush, Edinburgh EH25 9RG, United Kingdom.

Abstract

Human induced pluripotent stem cells (iPSCs) can be used to model complex diseases in human-relevant *in vitro* platforms. Human iPSC lines can be reprogrammed from patient blood cells and fibroblasts, and then differentiated into endpoint cells covering the central and peripheral nervous systems, the cardiovascular system and skeletal muscle.

These iPSC-derived cells can be produced from patients with complex diseases including Amyotrophic Lateral Sclerosis (ALS), Alzheimer's Disease and Parkinson's Disease, and incorporated into *in vitro* models for research and drug discovery[1,2]. Combining these cells with micro-physiological systems (MPS) can better mimic the relationship between human cell types *in vivo*, providing more complex *in vitro* models of human disease.

Here we demonstrate the use of multiple MPS platforms with human iPSC-derived neuronal cell types to build models of neurodegenerative disease and pain. The compatibility of iPSC-derived neuronal cell types and MPS platforms is key to the success of establishing complex biological interactions; we outline here a variety of critical parameters for creating a successful culture within the MPS platforms. Targeted and established bio-compatibility is needed to develop methodologies that measure cell-to-cell interactions via multimodal endpoints, including immunocytochemistry, cytokine release, neurite outgrowth and multi-electrode array (MEA).

We also provide examples of complex human iPSC-based models. These include 2D and 3D neuromuscular junction (NMJ) models using iPSC-derived skeletal muscle and motor neurons (to study specific disease-relevant targets in ALS) and simple monoculture systems using iPSC-derived motor neurons or sensory neurons for axotomy, pain and itch models for drug discovery.

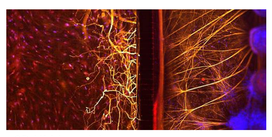
Microphysiological systems and 3D organoid models

Several systems now exist to construct a chosen tissue model. We have focused on the use of 3 different systems: standard microfluidic molded devices, 3D bio-printed scaffolds to work in conjunction with multi-electrode array instruments and complex microfluidic systems with integrated MEA technology.

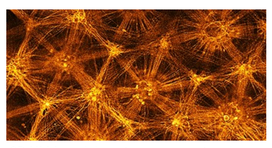
2D Model - Xona Microfluidics®
Uses existing microfluidic technology, plating fluidically isolated cells across a microchannel system.

3D Model - Axion BioSystems Maestro Pro + Scaffold
Uses existing state-of-the-art MEA technology combined with 3D PP2 bio-printed scaffolds

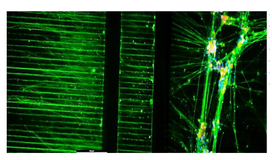
2D Model - NETRI MEA Microfluidics
Uses existing microfluidic technology combined with Axion Maestro Pro MEA system.



2D NMJ model



3D NMJ model- motor neuron network



2D sensory neuron model

Figure 1. Overview of MPS platforms we currently use: **2D models** using standard microfluidic commercial devices, **3D MEA** using Axol Bioscience's proprietary cell scaffold in combination with the Axion Maestro Pro MEA system, and **2D MEA**, using the NETRI Neurofluidics™ DuaLink device with the Axion Maestro Pro MEA system.

Key considerations for bio-compatibility

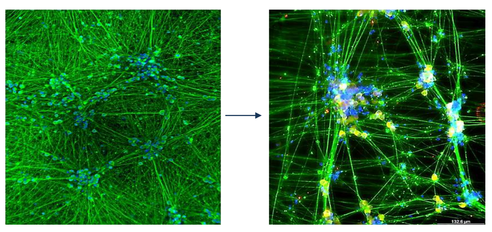
Bio-compatibility of the MPS and specific cell types is an important factor to consider. It is crucial in fine-tuning the iPSC-derived neuronal cell types and can enhance the reproducibility and consistency of *in vitro* models.

Factors for consideration include:

Impacting factors	Affected outputs
Extracellular matrix or matrices used	Cell adherence, function, cell longevity
Platform surface chemistry and adaptation	Cell adherence, cell distribution, neurite outgrowth
Plating processes and cell density	
Post thaw viability of cells	

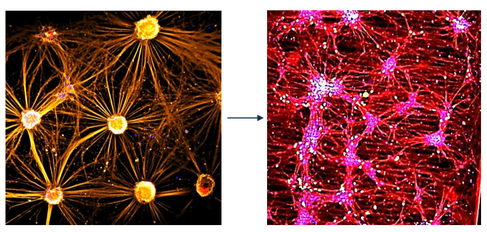
Extracellular matrix evaluation

axoCells™ Human iPSC-derived sensory neurons



PDL+ Surebond XF **PLO + Surebond XF**

axoCells Human iPSC-derived motor neurons

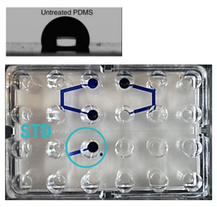


PDL + Vitronectin **PLO + Vitronectin**

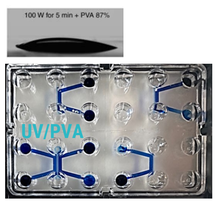
Figure 2. Comparison of different matrix coatings for both axoCells sensory neurons and motor neurons, displaying how different coatings can influence neuronal morphology and formation. PDL= Poly-D-Lysine, PLO= Poly-L-Ornithine

Model surface chemistry

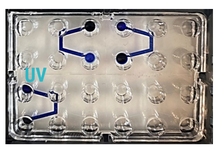
PDMS Standard treatment



PDMS PVA / UV treatment



PDMS UV treatment



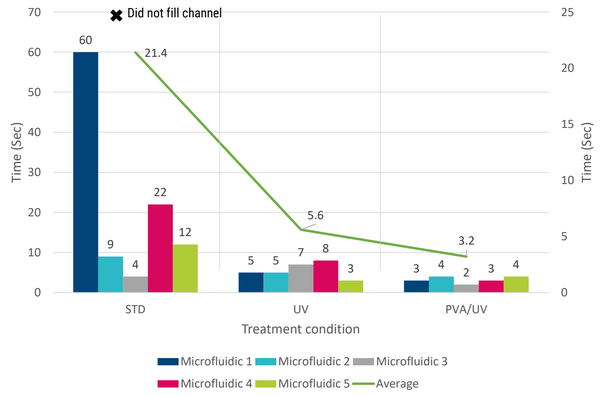
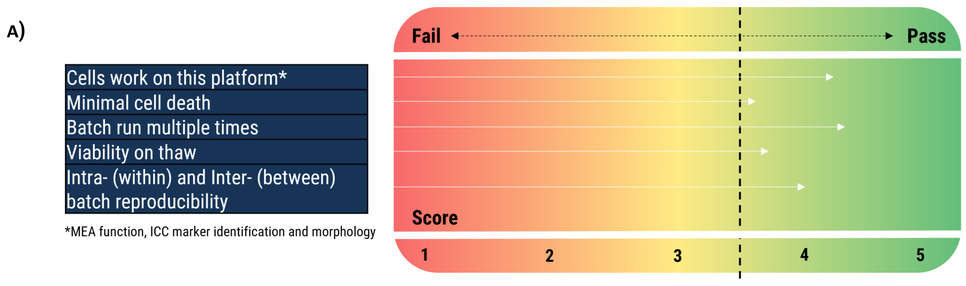


Figure 3: Specific treatments to the surface chemistry of an MPS system can change the flow dynamics and surface tension of a cellular solution inserted into the device. Treatment with UV and or PVA solution on PDMS microfluidic molds completely alters the surface tension and flow of a solution by converting a highly hydrophobic surface to hydrophilic. PDMS = polydimethylsiloxane

Assessment of cell performance: MPS compatibility



Establishment of measurable criteria

B)

Cell type	Matrices	Morphology	Marker expression	Function
Sensory neuron Morphological and functional performance uniform across both ECM.	PDL + SXF		ß3 Tubulin ✓ Nav1.7 ✓ Nav1.8 ✓ VR1 ✓	MEA
	Poly-Ornithine + SureBond-XF			
Motor neuron Matrices induce both different morphological and functional characteristics in the motor neuron culture.	Vitronectin + SureBond-XF		ß3 Tubulin ✓ Hb9 ✓ CHAT ✓ ISL-1 ✓	MEA <Synchronicity <Network burst firing
	Poly-Ornithine + SureBond -XF			

Figure 4: (A) Strategic approach to measuring cell performance across cell culture platforms or MPS devices. On this matrix, a score of 3.5+ is needed to pass each criterion.
(B) A potential model for measurable criteria associated with the neuronal cells and the platform to validate a batch of cells against.

Established MPS platforms with platform-specific, functionally validated neuronal systems

Well-validated and highly functional cell types can be used to build complex *in vitro* models with multifaceted end-point readouts. These can be built as mono-, co-or even tri-culture with multi-platform utility. At Axol Bioscience, we have used this approach to develop biological models across several different MPS platforms with rapid maturation.

A) B)

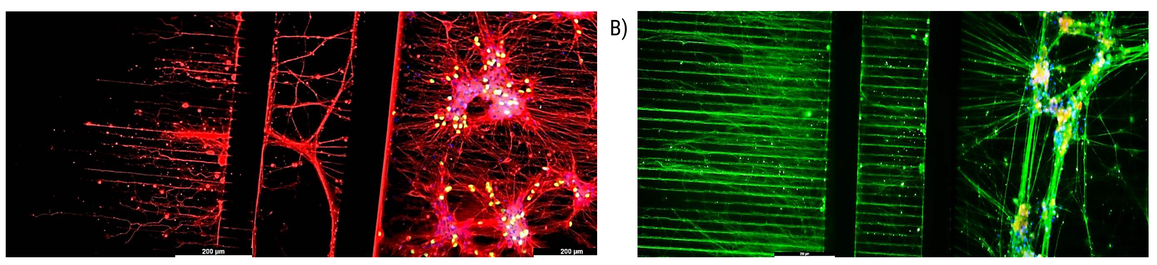
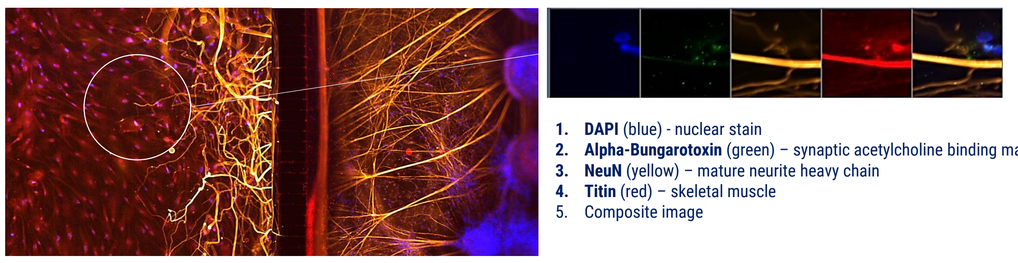


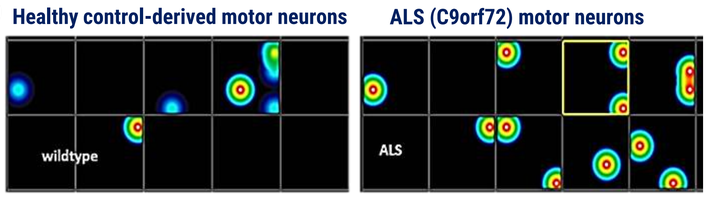
Figure 5. 2D model: NETRI Microfluidic devices built with axoCells motor neuron and sensory neuron cultures
(A) axoCells iPSC-derived motor neurons at day 10. Red = Beta tubulin marker, yellow = ChAT marker **(B)** axoCells iPSC-derived sensory neurons at day 20. Green = Beta tubulin marker, yellow = Nav 1.7 marker, blue = Dapi



1. **DAPI** (blue) - nuclear stain
2. **Alpha-Bungarotoxin** (green) – synaptic acetylcholine binding marker
3. **NeuN** (yellow) – mature neurite heavy chain
4. **Titin** (red) – skeletal muscle
5. Composite image

Figure 6. 2D model: Neuromuscular junction (NMJ) built with axoCells motor neurons and skeletal muscle in co-culture
Xona microfluidics co-culture of axoCells human iPSC-derived motor neurons and skeletal muscle as an NMJ model. The motor neurons (stained yellow using NeuN) completely overlap the postsynaptic acetylcholine receptors (stained green using fluorescent α-bungarotoxin conjugates) and skeletal muscle (stained red using Titin).

A) **Healthy control-derived motor neurons** **ALS (C9orf72) motor neurons**



B) **Healthy control-derived motor neurons** **ALS (C9orf72) motor neurons**

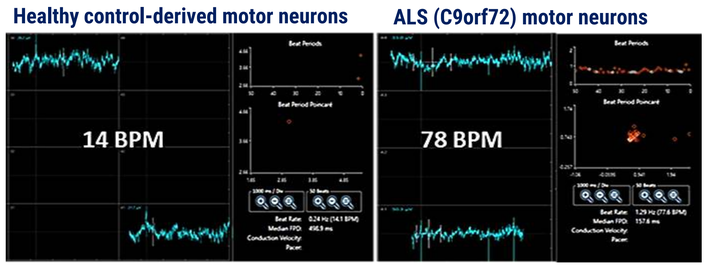
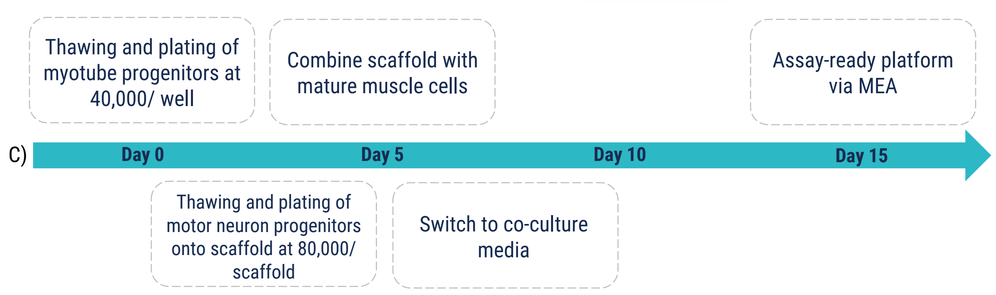


Figure 7. 3D Model: MEA NMJ model measuring motor neuron-induced skeletal muscle contraction for ALS.

(A) The MEA platform measures skeletal muscle contraction driven by innervated motor neurons from the scaffold above, where neurites span a 50um fluidic space between the cell types.

(B) The C9orf72 hyperexcitability phenotype of the ALS motor neurons is demonstrated here by the increased number of driven contractions ("beats") per minute, BPM, compared to the healthy control-derived motor neurons.

(C) Schematic timeline of the protocol used to generate the assay-ready platform in 15 days



Here we constructed a 3D model comprising functional axoCells skeletal muscle (assay ready in 5 days), ALS (C9orf72) motor neurons and healthy control motor neurons (assay ready in 10 days).

Overall, the C9orf72-derived motor neurons exhibited hyperexcitability, driving over 5x more contractions per minute compared to healthy control motor neurons.

Conclusion

Building useful iPSC–based *in vitro* models across multiple platforms requires well-validated cell types that have been tested against multiple parameters for optimal performance in the chosen platform. Here we have demonstrated important parameters to consider when validating human iPSC-derived neuronal cells, and data on potential *in vitro* models that can be built with these cells.

The development of isolated 2D and 3D culture environments offers unique methodologies for measuring cell-to-cell interactions, including an *in vitro* neuromuscular junction model. By fuelling these culture environments with functional human iPSC-derived cell types, researchers can produce more human-relevant advanced *in vitro* models for research and drug discovery, driving better therapies for patients worldwide.

References

1 Nicholson, M., Ting, C., Chan., *et al.* Utility of iPSC derived cells for disease modelling, drug development, and cell therapy. Cells 11, 1853 (2022). https://ncbi.nlm.nih/pmc/articles/PMC9180434/
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www.axolbio.com
operations@axolbio.com