# We need plenty of time to solve ALS

Axol Bioscience, working with <u>urgency</u> to create better *in vitro* models of human disease.



AXO

# NEW! axoCells<sup>™</sup> ALS Toolbox

Functional motor neurons from 6 iPSC lines to support ALS research

www.axolbio.com

# Manufacturing better human disease models. How iPSC technology is transforming drug discovery

In 2012, Professor Shinya Yamanaka and Sir John Gurdon were awarded the Nobel Prize "for the discovery that mature cells can be reprogrammed to become pluripotent." The ability to *reprogram* donated blood cells or fibroblast material from humans into stem cells and then the ability to *differentiate* these stem cells into end point cells is transforming the world of *in vitro* disease modelling.

With a consistent supply of functional end-point cells reflecting the phenotype of their donor and the ability to co-culture multiple cells, a new era of more human relevant and consistent *in vitro* models was born. With a strong tail wind of regulatory encouragement through the FDA Modernization Act, iPSC-derived model are now being used in the front-line of drug discovery for screening, mode-of-action and fundamental research.

#### Axol Bioscience, manufacturing functional cells, consistently and at scale

With over a decade of hard-earned experience, Axol Bioscience is recognised as a world leader in the quality manufacturing of functional iPSC-derived cells, at scale. Through this, we support the wider commercial life science industry with products, services, licensing, partnering and contract manufacturing.

We manufacture our **axoCells**<sup>™</sup> at our ISO 9001-accredited production facility in Roslin, allowing us to reach over 1,300 customers in 57 countries, including all top pharma companies.

To support urgent work to find adequate treatments for ALS, we continue to advance our range and characterization of iPSC derived motor neurons and microglia, two key cells involved in the pathology of this condition.

# In vitro models for ALS drug discovery

ALS (amyotrophic lateral sclerosis) is the most common form of motor neuron disease, where the progressive destruction of motor neurons leads to loss of muscular functions including walking, talking, swallowing and breathing. There is currently no cure, with treatment aimed at symptomatic relief and prolonging survival; most patients live only 3-5 years from the onset of symptoms<sup>1</sup>.

With limited treatment options and a predicted 69% increase in cases by 2040<sup>2</sup>, attention has turned to *in vitro* ALS models that use human iPSCs from healthy or ALS patient donors.

The cells generated from these iPSCs retain the characteristics of their donors, enabling researchers to generate *in vitro* ALS models to improve knowledge of the disease or to screen potential therapies on a more human-relevant platform.

1 Xu L et al. doi: <u>https://doi.org/10.1007/s00415-019-09652-y</u> 2 Arthur, K et al. doi: <u>https://doi.org/10.1038/ncomms12408</u>

# Key cell types involved in ALS:

- Motor neurons: Central to ALS pathophysiology is the progressive destruction of motor neurons, preventing communication between the brain/spinal cord and muscles.
  - axoCells Motor Neurons are functionally active in 10 days and demonstrate key marker expression (including HB9 and ChAT) and functional relevance in assays including electrophysiology and calcium imaging. Our ALSderived motor neurons (ax0074, C9orf72 mutation) demonstrate phenotypic and functional differences compared to healthy control-derived motor neurons, including more fibrous neurites and higher firing frequency.



**axoCells Motor Neurons** stained for TUJ-1 (red) at day 21. X20 magnification

- **Microglia:** There is an overlap between ALS and frontotemporal dementia (FTD) with several genes implicated including C9orf72, the most common cause of familial ALS. As the main neuroinflammatory cell of the brain, microglia can be used to model the ALS-FTD overlap
  - Our axoCells Microglia are assay-ready in 7 days and express key markers (including Iba1, TMEM119, CX3CR1 and P2RY12), with functional relevance in assays including phagocytosis, chemotaxis and cytokine release. ALSderived microglia (C9orf72) exhibit reduced phagocytosis of myelin basic protein compared to healthy control.



**axoCells Microglia** stained for CX3CR1 (red) and DAPI (blue) at day 7.

1 Badu-Mensah, A., Guo, X., McAleer, C.W. et al. Functional skeletal muscle model derived from SOD1-mutant ALS patient iPSCs recapitulates hallmarks of disease progression. Sci Rep 10, 14302 (2020). https://doi.org/10.1038/s41598-020-70510-3

# Using axoCells<sup>™</sup> Motor Neurons in ALS models



**Motor neurons** innervate muscle cells to control a range of voluntary and involuntary movements. The progressive destruction of motor neurons is central to neuromuscular conditions including **ALS**. axoCells Motor Neurons are in use in leading ALS research and drug discovery groups to fuel **advanced** *in vitro* **models of ALS**.



Assay ready from day 10



Express the **key markers** including HB9, MAP2, LIM3 and ChAT2



Demonstrated **functionality** in advanced *in vitro* models

### **Phenotypic characterization**

We've extensively characterized our axoCells Motor Neurons for phenotypic relevance including correct morphology (fig. 1) and expression of key cell markers via immunocytochemistry (fig. 2).



**Figure 1.** Phase contrast images of control axoCells Motor Neurons matured from progenitors over 21 days.



**Figure 2.** Immunocytochemistry of Day 21 mature axoCells Motor Neurons demonstrating presence of key markers (ChAT and TUJ1). Images captured on a Leica microscope x20 magnification.



**Accelerator:** We've developed an *in vivo* environment-mimicking supplement to reduce maturation times for our axoCells Motor Neurons from six weeks to just 10 days, with phenotypic and functional activity as assessed by morphology, immunocytochemistry and electrophysiology.

Scan the QR code to explore the data behind the development of our Accelerator Supplement.



## Access Kits which include cells, media and supplements

Our kits come with cells, motor neuron maintenance media, GDNF, BDNF, CNTF and Accelerator. Additional third-party reagents are required to complete the protocol.

## Use cells already validated on leading **MEA** platforms





Thawing and plating of cells in multi-well MEA plates.

AXO



Maturation of cells during cell maintenance in multi- well MEA plate. Feeding every second day.



Motor neurons are assay read at around day 10 with synchronised and regular burst firing soon after.



Day 10-20, acquisition of neural activity using the MEA. Noninvasive functional recording of neural networks and populations.



Download the protocol & contact <a href="mailto:support@axolbio.com">support@axolbio.com</a>



# INTRODUCING THE axoCells<sup>TM</sup> ALS TOOLBOX

An expanded range of iPSC-derived motor neurons to support ALS research

# NEW! axoCells ALS Toolbox for Drug Discovery

**4XO** 

Motor neurons derived from 6 donor lines

Unaffected donors iPSC-derived motor neurons



**ax0076:** From a male donor, 40-50 years old at time of donation

**ax0078:** From a male donor, 74 years old at time of donation

To support ALS research and drug discovery, Axol has developed an expanded set of 'ready to ship' motor neurons. With rapid 10 day to 'thaw to assay ready' protocols for axoCell motor neurons are backed with QC and functional QC. The new essential resource for ALS research.

#### ALS donors iPSC-derived motor neurons





**C9orf72 carrying donor** iPSC-derived motor neurons

**ax0073:** From a **C9orf72** male who asymptomatic at time of sample. He is the sibling to ax0074 donor. 62 years old at time of donation.





**ax0074**: From a female ALS donor with **C9orf72** mutation, 64 years old at time of donation

#### ax0735:

From a female ALS donor with **SOD1** mutation, 61 years old at time of donation

**ax0079**: From a female ALS donor with **TDP43** mutation, 62 years old at time of donation

# axoCells ALS Toolbox for Drug Discovery

Line and product information

## **Cells & Kits**







Kit contains cells, media and Accelerator, BDNF, GDNF, CNTF. Other third-party reagents may be required.

≥2 million cells, supplied frozen.

Donor	Age at sampling	Details	Mutation	Reprogrammin g method	Cells	Kit
Unaffected donor	40-50	Male	None	Sendai	ax0076	ax0186
	74	Male	None	Episomal	ax0078	ax0178
C9orf72 carrying donor	62	Male, sibling of ax0074. Caucasian / Ashkenazi Familial history of ALS and dementia	C9orf72 repeat expansion	Sendai	ax0073	ax0183
ALS donor	61	Female	SOD1 Het D109Y (G>T)	Sendai	ax0735	ax01835
	62	Female	TARDBP A382T	Sendai	ax0079	ax0189
	64 Onset of ALS at 60 - death at 65.	Female, sibling of ax0074. Caucasian / Ashkenazi Familial history of ALS and dementia	C9orf72 repeat expansion	Sendai	ax0074	ax0184

### **Media and Supplements**

Product Name	Product code	Quantity
axoCells™ Human CNTF Supplement, 20 μg	ax139888	20 µg
axoCells™ Human GDNF Supplement, 10µg	ax139855	10 µg
axoCells™ Human BDNF Supplement, 10 μg	ax139800	10 µg
axoCells™ Motor Neuron Maintenance Media, 200 ml	ax0072	200 ml
axoCells <sup>™</sup> Motor Neuron Accelerator Supplement, 1 ml	ax0179	1 ml

Additional third-party components may be required. Please refer to protocol for full list.

# Exploring the phenotype and function of the axoCells ALS Toolbox motor neurons

# Characterization of iPSC-derived motor neurons Immunocytochemistry

Donors	Key characteristics
Unaffected donors ax0076: Unaffected From a male donor, 40-50 years old at time of donation	<ol> <li>Large uniform cell-body clusters</li> <li>Thick cabling between cell bundles.</li> <li>Few fibrous thin neurites</li> </ol>
<b>ax0078: Unaffected</b> From a male donor, 74 years old at time of donation	<ol> <li>Large uniform cell-body clusters</li> <li>Thick cabling between cell bundles.</li> <li>Few fibrous thin neurites</li> </ol>
C9orf72 carrying donor	
<b>ax0073: C9orf72 unaffected</b> From a male unaffected donor, sibling to ax0074 donor, 62 years old at time of donation	<ol> <li>Smaller irregular cell-body clusters that are dispersed</li> <li>Thinner cabling between cell bundles.</li> <li>More fibrous thin neurites</li> </ol>
ALS donors	
<b>ax0074: C9orf72</b> From a female ALS donor with C9orf72 mutation, 64 years old at time of donation	<ul> <li>ax0074</li> <li>200µm</li> <li>200µm</li> <li>3. More fibrous thin neurites</li> </ul>
<b>ax0079: TDP43</b> From a female ALS donor with TDP43 mutation, 62 years old at time of donation	<ol> <li>Smaller irregular cell body clusters</li> <li>Thinner cabling between cell bundles.</li> <li>More fibrous thin neurites</li> </ol>
<b>ax0735: SOD1</b> From a female ALS donor with SOD1 mutation, 61 years old at time of donation	<ol> <li>Large, irregular cell body clusters</li> <li>Thick cabling between cell bundles</li> <li>A mix of thin and thick neurite cabling network</li> </ol>

sibling

# **Characterization of iPSC-derived motor neurons Spontaneous Neuronal Activity**



# **Characterization of iPSC-derived motor neurons Multi-Electrode Array**



## **EDITORIAL:**

# Observing hyperexcitability, a classic trait of ALS, in iPSC-derived motor neurons.

A critical aspect of ALS pathophysiology is the hyperexcitability of motor neurons, a phenomenon that has garnered significant attention in the study of this disease. Hyperexcitability refers to an increased responsiveness of motor neurons to synaptic inputs, leading to an abnormal increase in firing rate. In the long term this increased electrical activity can lead to excitotoxicity which is believed to contribute to the degeneration of motor neurons and the progression of ALS.

#### Mechanisms of Hyperexcitability in ALS Motor Neurons

The hyperexcitability of motor neurons in ALS can be attributed to several underlying mechanisms. One key factor is the altered function of ion channels that regulate neuronal excitability. Motor neurons express a variety of ion channels, including voltage-gated sodium (Na+), potassium (K+), and calcium (Ca<sup>2+</sup>) channel, all of which play critical roles in the generation and propagation of action potentials. Another factor is that ALS enhances motor neuron vulnerability to glutamatemediated excitotoxicity via AMPA receptors leading to calcium overload. In ALS, there is evidence that the function of these ion channels is dysregulated.

#### How to read hyperexcitability in MEA electrode traces:

 Higher frequency - more bursts (synchronised events) happening in the same time scale
 More activity ('noise') in between the bursts
 Lack of synchronization of bursts

S. Lack of Synchronization of Dursts

# How hyperexcitability is observed in *in vitro* studies using multielectrode array (MEA).

MEA enables non-invasive measurement of electricallyactive cells within a familiar multi-well cell culture format. MEA measurements are based on the detection of extracellular field potentials, which are the electrical signals generated by the activity of neurons or other excitable cells. These potentials arise due to the flow of ions (such as sodium, potassium, and calcium) across the cell membrane during action potentials or other electrical events. 'Raw' data is seen in real-time as an electrode trace. A simple way to visualize this neural network behavior is with a raster plot. Each tick mark represents the time, on the x axis, that a spike occurred. Each row of tick marks represents the spikes from a single electrode. Several consecutive spikes make a burst (shown in blue) and when multiple electrodes burst at the same time, this is represented as network event (pink box).

Differences between cells can be observed in connection to the overall firing rate, the synchronization of firing, the length of bursts and the amplitude of bursts.

#### 1a. Electrode trace from ax0078



Overview data presented in form of raster plot captures the difference between the synchronised burst firing of ax0078 unaffected donor motor neurons (1b, left) and the increase in firing rate and loss of synchronisation in ALS-affected ax0079 TDP43-mutant motor neurons (1c, right.)

#### 1b. **ax0078**

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1c. ax0079 TDP43



#### **Intrinsic Properties of ALS Motor Neurons**

ALS motor neurons exhibit intrinsic properties that contribute to their hyperexcitability. These include changes in the expression and function of ion channels, as previously mentioned, but also involve alterations in other cellular components, such as the cytoskeleton, which may affect the structural and functional integrity of the neurons.

For example, ALS motor neurons may show a reduction in the expression of small conductance calcium-activated potassium (SK) channels, which normally act to hyperpolarize the membrane following an action potential and prevent excessive firing. The downregulation of SK channels can therefore lead to prolonged depolarization and increased excitability. Additionally, mutations in ALSlinked genes, such as SOD1, TDP-43, and FUS, can directly or indirectly affect the expression and function of these channels, further contributing to the hyperexcitability phenotype.

Moreover, motor neurons in ALS may also exhibit changes in the expression of synaptic proteins, which can alter synaptic strength and plasticity. These alterations can lead to increased synaptic input onto motor neurons, further enhancing their excitability. The loss of inhibitory inputs, such as those mediated by GABAergic or glycinergic interneurons, can also contribute to the hyperexcitability phenotype, as the balance between excitation and inhibition in the motor neuron circuitry becomes disrupted.

#### **Genetic and Environmental Contributions**

The hyperexcitability of motor neurons in ALS is influenced by both genetic and environmental factors. Mutations in several genes associated with familial forms of ALS, such as SOD1, C9orf72, TARDBP (encoding TDP-43), and FUS, have been linked to hyperexcitability in motor neurons. For example, SOD1 mutations are known to cause oxidative stress and mitochondrial dysfunction, which can lead to the dysregulation of ion channels and calcium homeostasis, contributing to hyperexcitability. The expansion of GGGGCC repeats in the C9orf72 gene, the most common genetic cause of ALS, has been associated with the formation of toxic RNA foci and dipeptide repeat proteins, which can disrupt normal cellular function and contribute to neuronal hyperexcitability. Similarly, mutations in TARDBP and FUS, which encode RNA-binding proteins involved in RNA processing and transport, can lead to the accumulation of toxic protein aggregates and dysregulation of gene expression, further exacerbating the hyperexcitability of motor neurons.

Environmental factors, such as exposure to toxins, oxidative stress, and inflammation, can also contribute to the development of hyperexcitability in ALS motor neurons. For instance, chronic exposure to environmental toxins like pesticides and heavy metals has been linked to an increased risk of ALS, potentially through their effects on neuronal excitability and synaptic function. Oxidative stress, which is a common feature in ALS, can lead to the modification of ion channels and other proteins involved in maintaining neuronal excitability, further promoting hyperexcitability.

# Observations from the ALS iPSC-derived motor neuron toolbox

In the characterization of this expanded set of iPSCderived motor neurons, we used MEA, SNA and imaging to observable key traits. By building in this characterization into the manufacturing QC and functional QC processes we recorded multiple lines and multiple production runs (differentiations) to ensure the traits were reproducible and were not just a consequence of a single differentiation run.

In both the 'unaffected control' lines we saw synchronization, short burst events and constant signal amplitude. In comparison, motor neurons from the ALS lines and the unaffected sibling control motor neuron harbouring the C9orf72 mutation, demonstrated a reproducible loss of regular synchronous firing and different degrees of a hyperexcitability phenotype. This is in accordance with the understood ALS clinical pathology and supports the case for the use of these cells in experimental *in vitro* models.

#### Read more:

 Wainger BJ, Kiskinis E, Mellin C, Wiskow O, Han SS, Sandoe J, Perez NP, Williams LA, Lee S, Boulting G, Berry JD, Brown RH Jr, Cudkowicz ME, Bean BP, Eggan K, Woolf CJ. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep. 2014 Apr 10;7(1):1-11. doi: 10.1016/j.celrep.2014.03.019. Epub 2014 Apr 3. PMID: 24703839; PMCID: PMC4023477. https://oubmed.ncbi.nlm.nih.gov/24703839/

Xie, M., Pallegar, P.N., Parusel, S. et al. Regulation of cortical hyperexcitability in amyotrophic lateral sclerosis: focusing on glial mechanisms. Mol Neurodegeneration 18, 75 (2023). <u>https://doi.org/10.1186/s13024-023-00665-w</u>

G Lorenzo Odierna, Steve Vucic, Marcus Dyer, Tracey Dickson, Adele Woodhouse, Catherine Blizzard, How do we get from hyperexcitability to excitotoxicity in amyotrophic lateral sclerosis?, Brain, Volume 147, Issue 5, May 2024, Pages 1610–1621, <a href="https://doi.org/10.1093/brain/awae039">https://doi.org/10.1093/brain/awae039</a>

Nathan Pavey, Andrew Hannaford, Mehdi van den Bos, Matthew C Kiernan, Parvathi Menon, Steve Vucic, Distinct neuronal circuits mediate cortical hyperexcitability in amyotrophic lateral sclerosis, Brain, Volume 147, Issue 7, July 2024, Pages 2344–2356, <a href="https://doi.org/10.1093/brain/awae049">https://doi.org/10.1093/brain/awae049</a>

# **Consistently different.**

# Unaffected vs ALS on multiple manufacturing runs showing functional consistency, batch to batch.

The ability to manufacture cells of consistent functionality and performance, and cells from different lines that capture their inherent phenotypes, are the hallmarks of a quality iPSC manufacturing process. At Axol we have spearheaded quality manufacturing systems in the iPSC arena to support the development of better models for human disease.

In line with our approach to ISO 9001 accreditation, we compared the phenotype and MEA read out from 3 runs from 3 healthy donors to examine run to run control and line to line difference. All runs passed QC criteria for commercial release.

## QC data from 3 manufacturing ('differentiation') runs

Donors	ICC - confirmation of key markers MEA - observation of phenotype	- observation of phenotype		
ax0076: From a male	Run 1	PASSED		
years old at time of donation	e Run 2	PASSED		
		PASSED		
ax0078: From a male	Run 1	PASSED		
donor, /4 years old at time of donation	Run 2	PASSED		
	Run 3	PASSED		
	Both unaffected donor lines demonstrated consistent ICC results and regular fin	ring MEA cellular analysis		
ax0074: C9orf72 From a female	2 Run 1	PASSED		
ALS donor with C9orf72 mutation, 64	Run 2	PASSED		
years old at time of donation	e Run 3	PASSED		

C9orf72 cells consistently display a hyperexcitable phenotype.

Using these fQC readouts, we are able to consistently see clear phenotypic differences between healthy and ALS motor neurons. Healthy lines (ax0076 and ax0078) show very regular burst firing which is synchronised. Motor neurons derived from an ALS patient (ax0074) show much more irregular burst firing with more activity between bursts.

# axoServices

Outsourced services for model building, assay development and compound screening

# axoServices<sup>™</sup> Outsourced services to support iPSC drug discovery and research



### iPSCs? What can we do to help?

Through axoServices, we partner with drug discovery groups, from the very largest to the very earliest in assay development, model creation and compound testing. Each project is unique but built on the shoulders of giants. Our project managers look to maximize efficiency and success always through the best spirit of collaboration and partnership.



Discussion NDA Design Agreement



**Modelling** Which lines and cells Mono- or Co-Characterization



**Experiment** Compound modalities Treatment regimes Endpoint, midpoint assays



Data management Quality control Data analysis Data mining

**Communication** Regular updates Final reports & data

# **axoServices**<sup>™</sup> Differentiation

We offer a QC-rich full-service differentiation process from human iPSCs to endpoint cells. Potential endpoint cells include cortical excitatory neurons, cortical inhibitory interneurons, striatal neurons, astrocytes, microglia, sensory neurons, motor neurons, myotubes (as made-to-order), atrial cardiomyocytes and ventricular cardiomyocytes.

## What does an axoServices Differentiation project look like?



#### Step 1: Kick-off meeting with the Axol iPSC experts

- Decades of experience in the room
- Open, two-way communication
- Rapid follow-up with competitive quote



#### Step 2: Extensive iPSC characterization

- Flow cytometry to confirm pluripotency-associated marker expression
- Ensure high-quality starting material to maximize project success



#### Step 3: Directed differentiation and optimization

- Our proprietary differentiation process uses small molecules to generate footprint-free endpoint cells
- Built-in optimization provides safety net for project challenges
- Robust target cell populations for QC, custom assays and shipping



#### Step 4: QC and custom assays

- · Rigorous checks for contaminants and optimal post-thaw viability
- ICC to confirm expression lineage-specific markers
- Custom assays capabilities to match project requirements
- · Desired end-point cells are shipped with media and instructions



#### Step 5: Ongoing support

- · Continued communication and advice from iPSC experts
- · Ease the adaptation of cells into your experimental system
- · An open channel for future projects, collaborations and partnerships

#### Timelines are usually >4 weeks but differ based on specific endpoint cell.

# Differentiation of multiple iPSC lines to different cell types to enable cohort analysis in neurodegeneration: challenges and learnings

Scan the QR code to explore this poster where we demonstrate our differentiation expertise. Here we describe the development of differentiation procedures for multiple axoLines<sup>™</sup> iPSC lines simultaneously, including 2 control lines and 6 from individuals with Alzheimer's Disease, with mutations in presenilin 1, homozygous ApoE4 or heterozygous ApoE4/ApoE3 genotype. By optimizing the differentiation of astrocytes, cortical excitatory neurons and microglia, we can better standardize the quality and consistency of cells used to power advanced *in vitro* models, driving better therapies for complex neurodegenerative diseases.



# axoServices<sup>™</sup> Compound Screening

We combine our **iPSC expertise** with a **comprehensive suite of custom assays** to deliver efficient compound/phenotypic screening, with a specific emphasis on the quality and communication of assay data. We can help you to generate **robust pre-clinical data** to validate compounds, or narrow down a large compound library for further **in-depth experimental analysis**.

## What does an axoServices Compound Screening project look like?



#### Step 1: Kick-off meeting with the Axol iPSC experts

- Decades of experience in the room
- Open, two-way communication
- Rapid follow-up with competitive quote



#### Step 2: Assay design and optimization

- In-house expertise to guide design and maximize project success
- · Small-scale pilot experiments to optimize assay selection
- Progression to higher throughput screening to enhance reproducibility



#### Step 3: Data analysis and reporting

- Across the service project, all raw data is stored and lab notebooks are maintained in keeping with data management requirements
- Full written report produced upon project completion with data-sharing meeting for full transparency



#### Step 4: Ongoing support

- Continued communication and advice from iPSC experts
- · Ease the adaptation of cells into your experimental system
- · An open channel for future projects, collaborations and partnerships

# Validation of a cortical tri-culture axoModel<sup>™</sup> for in vitro compound screening a blinded compound study

Scan the QRD code to read this poster demonstrating our expertise in compound screening.

In partnership with Sumitomo Pharma America, Inc., we performed a blinded study testing eight reference compounds on an isogenic cortical tri-culture axoModel, using an Axion multi-electrode array (MEA) system to measure electrophysiological response.

Here we demonstrate robust identification of reference compound mode of actions, validating this axoModel for use in human-relevant drug discovery and neurotoxicity screening.



# Resources

## **Characterisation of iPSC-derived motor neurons** using an accelerated maturation protocol



11

Steven Broadbent, Stuart Prime, Signe Springe, Joe Lyndsay, Sian Humphreys, Ashley Barnes - Axol Bioscience Ltd, Cambridge, UK

Results ctd.



1





www.axolbio.com

Abstract Understanding neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) requires a supply of cells for in vitro modelling and Orderstanding her obegenerative controllions sech as anipytorphic lateral sciences (kLS) requires a supply or lease for *in vico* introdening and drug screening. **Motor neurons derived from induced pluripotent stem cells** (RS) provide an unlimited source of such cells and can potentially facilitate precision medicine approaches to compound testing when derived from somatic cells from patients. Furthermore, in co-culture with IPSC-derived muscle cells, a fully human preclinical screening model of the Neuromuscular Junction is possible. However, IPSC-derived motor neurons traditionally required up to six weeks maturation to exhibit relevant functional phenotypes.

We demonstrate use of an *in vivo* environment-mimicking supplement, to produce a more physiological, modified growth medium for maturation, which reduces the time to reach functional maturity from six weeks to 10 days. Cells showed typical morphology with abundant neurities and were positive for expression of the mature motor neuron marker SM-32 along with HB9, Ch4T and IsI-1, as assessed by immunocytochemistry

Recordings using a 48-well multi-electrode array (MEA) demonstrate synchronised spontaneous firing at a similar timepoint. Motor neurons matured using the accelerated protocol formed junctions with muscle cells in a microfluidic co-culture system, as demonstrated by staining with fluorescencify labelled alpha-bungarotoxin. The phenotype of motor neurons from an ALS patient was also investigated.

These rapidly matured cells provide an invaluable tool for research into neuromuscular disease and a model allowing a higher throughput nound screening for co

METHODS

Cell Jourse of JPSC progenitor cells to mature cell types was performed using Axol Bioscience user protocols and media (ax0072) for motor neurons (ax0078 control and ax0074 ALS C90RF72, greater than 145 GGGGCC repeats). Initial studies used Motor Neuron Accelerator (ax1078) with a poly-D-lysine and SureBond XT matrix (day 13 plus). Subsequently the process was accelerated further using a vitromecht matrix (day 13 plus).

Immunostainine

Immunostaining was conducted using standard PFA fixing, 0.3% Triton, using commercially available antibodies. For visualisation of the neuromuscular junction, cultured motor neuron progenitor or skeletal muscle progenitor cells were seeded into a

icrofluidic chamber with 450um channels

#### MEA Recording:

Extracellular field potentials were acquired at 37°C, 5% CO<sub>3</sub>using the Axion Maestro Pro high-throughput MEA platform. 48-wells plates with a 350µm separation between each well-floor mounted electrode were used. This enabled simultaneous recording of extracellular potentials from 16 electrodes per well at a sampling rate of 20kHz/channel. Cultured motor neuron progenitor cells were spotted onto the array in a 10µ volume of media. These were then differentiated in-situ to form their mature cell types.

offerentiated in-situ to form their mature cell types. Figure 1. MEA Configuration. Figures A, B and C show the axion MEA system, well plate and MEA array that resides in wells of the plate. Figure D, playes contrast J of MEA coated motor neuron culture.



Motor neurons were transfected at day 2 and day 7 post-thaw with Incucyte\* Neuroburst Orange Lentivirus, encoding a calcium reporter driven from the synapsin promoter. Spontaneous activity was recorded using Incucyte\* Neuronal Activity Analysis Software on an Incucyte\* S3.

#### RESULTS

Motor Neuron Activity Measurement Via MEA Detection Synch



In reution activity in 60 well inter Adout http://document.com/adout/inter/adout/i ess the motor neuron crus supplement, without vitro elopment of mature neurites v

#### 2-Dimensional Neuromuscular Junction Model



#### 1. DAPI (blue) - nuclear stain) 2. Alpha-Bungarotoxin (green) – synaptic acetylcholine binding marker

NeuN (yellow) –
 Titin (red) – skel
 Composite image

Figure 3. Motor Neuron/Skeletal Muscle – co-Culture. Microfluidic co-culture of human iPSC motor neurons and skeletal muscle. The images show development of the neuromuscular junctic neuron nerve (yellow, NeuN) completely overlaps the postsynaptic acetylcholine receptors (green, fluorescent a-bungarotoxin conjugar direct abia). (red. titin)

etal mu

#### Expression of markers by immunocytochemistry: WT and C9Orf72 mutant

iPSC used were from a healthy donor (ax0078) and from a donor with C90RF72 hexanucleotide expansion (greater than 145 GGGGCC repeats) and diag with anyotrophic lateral sclerosis (ALS) (ax0074). Both lines were matured to motor neurons for 10 days with characterization by immunocytochemistr





Expression of markers by immunocytochemistry: WT and C9Orf72 mutant

WT ax0078 D10 ALS ax0074 D7





otor neuron characterization by electrophysiology ings were made from day 10-matured iPSC-derived motor neurons from a healthy donor (left) and day 7-matured from a donor with ALS Figure 5. Mo MEA recordi

Motor Neuron Activity Measurement Via Neuroburst® Orange and Incucyte® S3







ure 6a. Analysis from red ording of spont us neural activity eous neuronal firing in iPSC-derived motor neurons from a healthy donor

Incucyte® Neuroburst Orange lentiviral reagent was used to image sp (left) and one with ALS (right), after 11 days maturation Control



differentiated neurons from a do n an ALS donor. e evenly distributed and far less so in m

The ALS *in vitro* model recapitulates results from SOD1A4V/+ mutation To prove the model correlated with clinical findings, the authors? used MEA to evaluate iPSC-derived ALS neurons from two patients with the SOD1<sup>4</sup> mutation. • ALS neurons showed increased neural firing compared to control cells. Maestro recordings confirmed clinical findings of neural hyperexitability compared to controls.

Below are representative recordings from four out of 64 MEA electro cultured for 28 days on the arrays. MEA recordings revealed increase neurons, confirming previous clinical findings and further validating les in control (11a, 18a) and SOD1<sup>A4VJ</sup> I spontaneous firing in the mutant-de mutant (39b, RB9d)-derived motor neurons ived neurons compared to control-derived



1. Video available to view at Axol's booth no. 617

2. Wainger BJ. et al. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep. 2014 Apr 10;7(1):1-11.

#### CONCLUSION

Notor neurons matured for 10 days with a more physiological medium with Motor Neuron Accelerator (ax0179) show typical expression of HB9 and ChAT and a high level of neuraxis. Synchronized spontaneous firing is also evident after only 10 days of maturation, recorded using a multiple electrode array, o using the Incuryce<sup>®</sup> Neurobust Compacie Lentivins.

neurons derived from iPSC carrying a C9ORF72 hexanucleotide expansion (greater than 145 GGGGCC repeats) exhibit earlier (day 7) activity, but with burst trains and decreased synchronization. The distribution of firing across the well, as visualized on the Incucyte®, is uniform in the wildtvoe metror s, but heterogenous in the AIS motor neurons lecreased synchronizatio ous in the ALS motor ne

-derived motor neurons have been shown to exhibit a clinically relevant phenotype by electrophysiology or using the In ity module. Using a medium supplement whose components minic the physiological cellular environment, motor neur enforts in only 10 days, providing a disease-relevant model for medium throughput compound screening or mechanisti



ALS



# Case study: iPSC-Derived Motor Neurons and Microglia From ALS Background Display Disease Phenotype

The overlap between **ALS and FTD** (frontotemporal dementia) has led researchers to investigate the role of neuroinflammation in ALS development. This has led to increasing interest in the role of microglia, the main neuroinflammatory cell, in the development of ALS. In collaboration with **Sartorius**, we investigated the **morphology and functional performance** of ALS-derived motor neurons and microglia compared to healthy control cells. Here we present key highlights from the project.



#### Spontaneous neuronal activity analysis on day 18 axoCells Motor Neurons

**Figure 5**. The IncuCyte® Neuronal Activity Software Module was used to monitor and analyze calcium signaling. Statistical significance was assessed using a non-parametric T-test, \*p<0.05, \*\*p<0.01. ALS motor neurons exhibited significantly higher burst rates compared to the control line, along with lower burst duration and lower mean correlation (hence less synchronized burst firing).

#### Phagocytosis of myelin basic protein (MBP) by axoCells Microglia.



**Figure 6.** Phagocytosis of pHrodo® for IncuCyte® labeled MBP was assessed on an Incucyte® Live-Cell Analysis System up to 24h using fresh and cryopreserved microglia. Cytochalasin D (10  $\mu$ M) was used as a control. Statistical significance was assessed using a one-way ANOVA, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.001

This indicates that ALS microglia are affected by the C9orf72 expansion in ALS with decreased phagocytosis, demonstrating a useful assay to identify an ALS-like phenotype.

In this project, we demonstrated distinct **ALS-like phenotypes** in axoCells Motor Neurons and microglia derived from ALS iPSCs, which can be quantified and used to inform future drug discovery purposes. As part of this project, we also looked into **morphology**, **immunocytochemistry** and **electrophysiology** (data available in the full whitepaper), demonstrating the wide range of useful assays for investigating ALS-like phenotypes in motor neurons and microglia. Scan the QR code to access the full whitepaper





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# **axoCells ALS Toolbox** for Drug Discovery Product information

Axol Bioscience manufacturers specialist precursor cells, media and supplements to achieve high performance *in vitro* models.

Use our motor neuron precursor cells + specialist iPSC media + Accelerator supplement + growth factors to achieve functional motor neurons.

## How to make high performing axoCells motor neurons

#### **Cells and Kits**

Product Name	Quantity	Cells only	Bundle Kit (Cells + media + supplements)
Human iPSC-Derived Motor Neurons (unaffected male 1)	>2M cells	ax0076	ax0186
Human iPSC-Derived Motor Neurons (unaffected male 2)	>2M cells	ax0078	ax0178
Human iPSC-Derived Motor Neurons (male, C9orf72 carrying)	>2M cells	ax0073	ax0183
Human iPSC-Derived Motor Neurons (female, ALS, SOD1)	>2M cells	ax0735	ax01835
Human iPSC-Derived Motor Neurons (female, ALS, TDP43)	>2M cells	ax0079	ax0189
Human iPSC-Derived Motor Neurons (female, ALS, C9orf72)	>2M cells	ax0074	ax0184



#### **Specialist Media and Supplements** – available separately or included in kit

Product Name	Product code	Quantity
axoCells™ Human CNTF Supplement, 20 µg	ax139888	20 µg
axoCells™ Human GDNF Supplement, 10µg	ax139855	10 µg
axoCells™ Human BDNF Supplement, 10 µg	ax139800	10 µg
axoCells™ Motor Neuron Maintenance Media, 200 ml	ax0072	200 ml
axoCells™ Motor Neuron Accelerator Supplement, 1 ml	ax0179	1 ml

For a quotation, email operations@axolbio.com or contact your local distributor.

# iPSCs? How can we help?

operations@axolbio.com

support@axolbio.com

www.axolbio.com