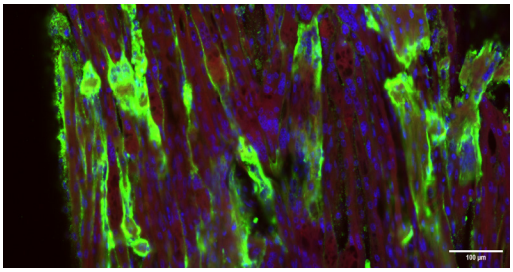
The 3D Neuromuscular Junction in a Dish:
Could this new assay made using human stem cells revolutionize ALS research?

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing and devastating disease, characterised by the loss of function of motor neurons, which then fail to activate the skeletal muscle, meaning patients lose control of their limbs, the ability to swallow or breathe, resulting in terminal paralysis and death. There is no cure for ALS and to date limited therapies have been approved for its treatment.

Riluzole, the most common treatment, extends life expectancy by as little as three months on average, outlining the urgent requirement for new treatments for patients affected by this disease.



Fluorescence microscopy image of 3D human skeletal muscle tissue. Credit: Rowan Rimington

To date, the majority of complex cellular models of pre-clinical research into the condition has been undertaken in animal models that have been engineered to express ALS-linked genes, enabling them to develop key aspects of the disease as seen in patients.

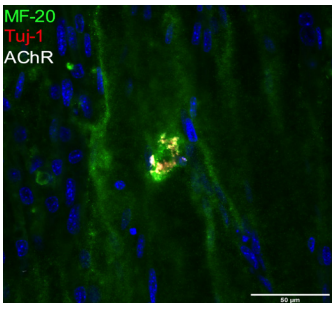
However, It is becoming apparent that intricate differences exist between humans and model animals in some of the key physiological processes that are affected in ALS, such as the neuromuscular junction (NMJ).

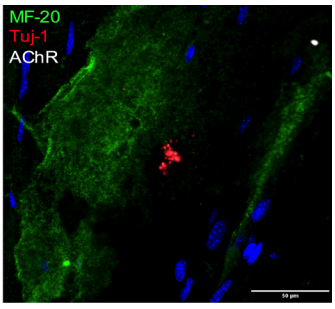
The NMJ is the functional connection between motor neurons and skeletal muscle fibers where the neurons form synapses with the fibers in order to activate the muscle and cause contraction, enabling the movement of our bodies. This process has been identified as a critical component of ALS, although little is known about its role in human disease progression.

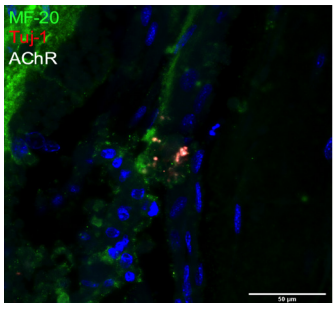
The impact of using these non-human models is that many candidate drugs identified in animals have failed to translate into effective therapies for ALS patients in clinical trials.[1,2] While candidate drugs that have no beneficial effect in animal models may be prematurely removed from the therapeutic process without evaluating their influence in humans.

Now, scientists at Loughborough University have created a 3D cell culture model of the human NMJ.[3] Grown in a dish and made from human stem cell motor neurons and muscle obtained from primary needle biopsy, this model recapitulates the human physiology of this process, and consequently could be applied to both better understand the disease as well as develop therapies to treat it.

In this article we speak to Dr Rowan Rimington, Lecturer at Loughborough University and Ashley Barnes, Chief Scientific Officer of Axol Biosciences about how Rowan's group have been able to model the human NMJ using Axol's iPSC-derived motor neurons.







NMJ synapses in 3D motor unit tissue. Close apposition of markers for muscle fibres (MF 20, green), motor neurons (Tuj 1, red) and Acetylcholine Receptors (AChR, white) are indicative of synapses. Credit: Rowan Rimington

Dr Rowan Rimington
Lecturer in Neuromuscular Cellular Physiology
Loughborough University
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Dr Rimington has a background in Applied Sport Science, Exercise Physiology and Bioengineering of skeletal muscle tissue.

Rowan's research aims to further enhance the physiological relevance of these bioengineered neuromuscular systems, identify new therapeutic targets by elucidating the underpinning physiology/pathophysiology of the human NMJ, and investigate how this synapse responds to therapeutic interventions.

Ashley Barnes
CSO, Axol Biosciences

Ash has over 21 years of experience in the pharma industry with skills sets in molecular biology, gene editing and assay pharmacology and screening.

He has extensive experience in leading teams in both target identification and validation as well as developing novel compounds in the respiratory, immunology and neuroscience disease areas.

In the last five years he has been focused on the iPSCs as a platform for developing better translatable models for diseases.

What drives you in your work to create these cellular models?

Rowan Rimington:

My research interests are in neurological disease and how, when that disease pathology progresses, breakdowns in communication with the muscle tissue occur. I'm particularly interested in how that then results in a lack of function in the musculoskeletal system, such as in ALS, where it creates a strikingly visual degrading pathology.

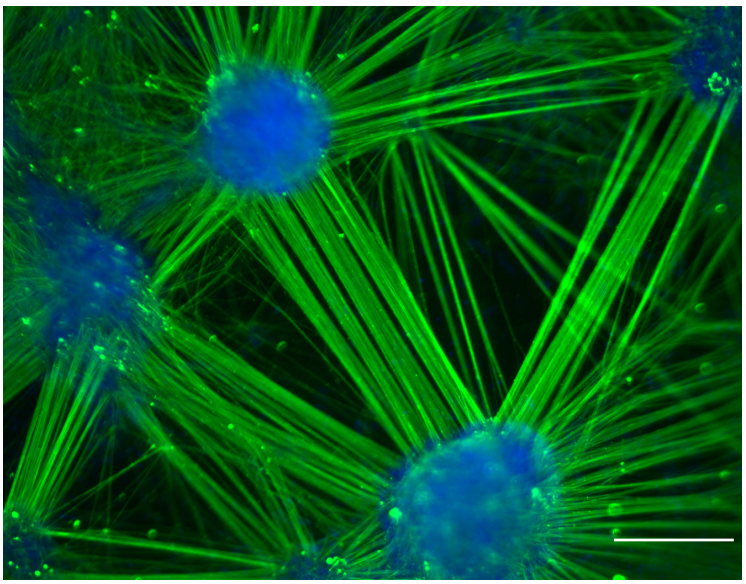
As a physiologist with the molecular biology skills and bioengineering knowledge to build tools and models for the research community, I feel compelled to help research efforts to better understand and treat this devastating disease. I see it as a big opportunity to bring all my expertise into one space, to try and create a piece of technology that could then be used to help people affected by the disease.

Ashley Barnes:

At Axol we are an iPSC platform company. Our focus is very much on using iPSCs to develop reagents, products and to provide custom services to various clients for drug discovery and for model development.

I've been working in Pharma and biotech for 25 years, and with the advent of iPSC technology, it fast became clear to me that this area was a big opportunity to provide better models than those currently used in drug discovery, particularly in neuroscience applications.

iPSCs have pushed the pre-clinical research realm into the human space because we're taking samples from patients and pushing those into neuronal cell types where we can start to really do drug discovery and target work on. That's what excites me about the work we're doing at Axol, working with others in this field to deliver better value models for drug discovery.



Human iPSC-derived motor neurons in monoculture. Green staining is for SMI-32 a marker of mature motor neurons. Credit: Rowan Rimington

> "Axol were very supportive, and the experience of using the iPSC motor neurons for the model development was great. In fact, they have been invaluable in enabling what we've been able to achieve today with our model."
>
> **Dr Rowan Rimington**,
> Lecturer, Loughborough University

How did this partnership begin?

Rowan Rimington:

Shortly after my PhD, I was awarded a highly competitive Doctoral Prize Fellowship from Loughborough University to establish my own line of research investigation. One of my senior mentors had a previous connection with Axol and put me in touch with the company.

For my line of investigation, I needed a robust, reproducible and highly differentiated line of stem cell derived motor neurons to create a 3D *in vitro* model of the human NMJ or motor unit, which could then be applied to neurological diseases, such as ALS.

So, I visited Axol's labs, described my research plans and how I thought they could be mutually beneficial for both Axol and the models I wanted to create. Axol were very supportive, and the experience of using the iPSC motor neurons for the model development was great. In fact, they have been invaluable in enabling what we've been able to achieve today with our model.

Ashley Barnes:

We are experts in iPSCs and understanding iPSCs and driving iPSCs to different cell types. But we are not experts in ALS or in other diseases that we work on and the samples and iPSC lines that we use.

At Axol we work with a number of different companies and a number of different groups who are experts and use our expertise to complement theirs. That's why it's really important that we work with pioneering scientists like Rowan who are pushing the envelope of disease modeling.

What are the barriers or challenges that this work has overcome?

Rowan Rimington:

In terms of the neuromuscular space and with regard to analyzing how neurological disease progresses and affects synaptic communication, we needed a model capable of representing the synapse in 3D. The synapse is not a monolayer single Z plane biological event. It's a highly organized biological process of protein complexes which reside across the Z dimension. And the only way you can recreate that is in 3D. But, at the point we began this collaboration, there was a real lack of 3D human models.

We set out to create a 3D human model that should in theory recreate the complex architecture of the synapse. And hence that should be able to recapitulate its functional capability to contract the muscle tissue and also create representative physiology in there.

The second main barrier which iPSCs have addressed is the species aspect. Lots of the previous models around motor neuron diseases involved transgenic animal models. But there has been a lot of data recently that shows the actual neuromuscular physiology and the cellular makeup of the synapse between animals and humans is significantly different.[4,5]

So, the two main barriers we overcame were the technological one in terms of development of functional and physiologically relevant 3D human neuromuscular junctions and doing that from entirely human derived material. Now we hope we can improve therapeutic translation from human to human, as opposed to an animal to human.

> "Using this model to screen compounds will really move things forward quite significantly for drug screening and for understanding targets in this space."
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> **Ash Barnes**,
> CSO of Axol Biosciences

Ashley Barnes:

Translation of therapies from the dish to the clinic is a key challenge for every drug developer. iPSCs have come to the fore in enabling scientists to work with human cells to create better disease models for testing and developing therapies that will better translate into the clinic. That's why scientists are looking at these to move drug discovery and therapies forward and translate them into humans because they have been discovered in human cells.

This model sits more in the translational space than it does in a high-throughput screening space, but that is important. To overcome the failure rate in drug discovery we need more valuable models that better recapitulate the disease. It comes down to the idea of what is effectively a clinical trial in a dish. Can you get to a point where you're not having to spend millions and millions to triage compounds which are going to fail in the clinic? Can you triage them much earlier on with highly relevant disease models that facilitate significantly better clinical translation? These models do that.

Spheroid culture of human iPSC-derived motor neurons (ax0078). Credit: Rowan Rimington

What are the therapeutic benefits that could be realized from this partnership?

Rowan Rimington:

There are two ways therapeutic benefits can be gained from using this model. 1) You can learn more about the disease and find a really specific target to develop therapies against. 2) The functional readout provided by this model when it is translated using patient samples is a key differentiator. So, you can manifest the disease pathology in the system and drug screen against the function of the entire motor unit, as opposed to a single cell type. That's important.

It means you can screen against things which improve that function. Meaning we might be able to find some therapies which could delay the disease onset and improve the function of that connection. This could give patients a few more years of a better-quality life. That's notwithstanding the exciting possibility of finding a treatment that functionally cures the disease.

Ashley Barnes:

It's well documented that there are a number of genetic changes with respect to different proteins in motor neuron disease and ALS. We can actually see some changes in the behavior of the motor neurons in a 2D monoculture model just by taking an electrophysiological approach.

Now, if we take some of the iPSC lines that we have from patients who have motor neuron disease or ALS and plug those into Rowan's 3D system, one would hope that we would see a significant change in the behavior of the skeletal muscle functional readout. Using this model to screen compounds will really move things forward quite significantly for drug screening and for understanding targets in this space.

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