MYOMAKER BIO

axoCells® iPSC-Derived Motor Neurons as a Platform for Human Neuromuscular Junction Modelling

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Abstract

This study demonstrates the use of Axol Bioscience's human-induced pluripotent stem cell (iPSC)-derived motor neurons within a high-throughput, human-relevant skeletal muscle platform for preclinical modelling in muscle biology testing. When generating methods for human neuromuscular organs, spheroids formed from iPSC-derived motor neurons showed enhanced motor neuron maturity compared to monolayer cultures (increased OLIG2, ISLET1, and SMI32). With spheroids, the neuromuscular organ exhibited functional twitch and tetanic responses, and enhanced acetylcholine receptor clustering. Spontaneous activity modulated by d-tubocurarine confirmed functional innervation through neuromuscular junctions (NMJs), demonstrating the potential of neuromuscular organs for preclinical screening assays.

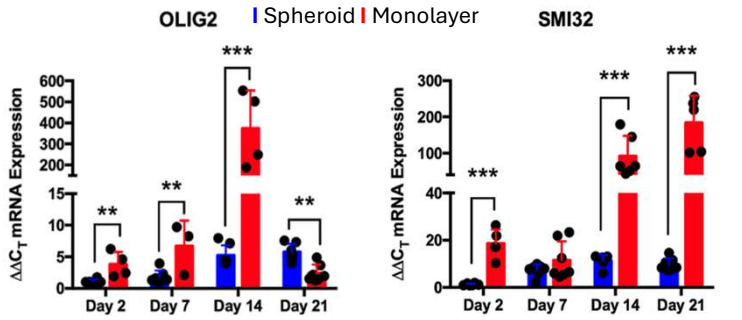

Figure 1. Progenitor marker OLIG2 and mature motor neuron marker SMI32, comparative axoCells spheroids and monolayer.

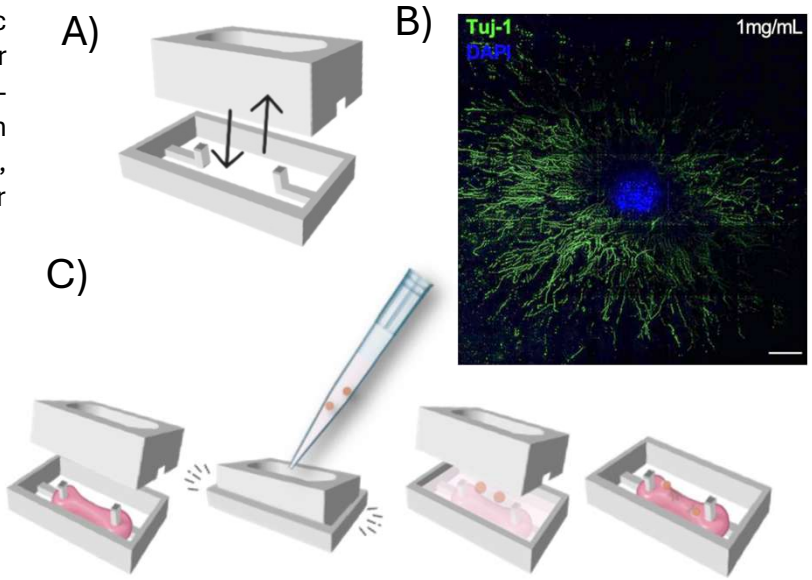

Figure 2. A) 2-part 50 µL mould. B) Confocal tile of spheroids within 1 mg/mL collagen, extending axons over 5 days. Scale Bar: 200 µm. C) Neuromuscular Model Preparation.

Methods

Spheroid Preparation

Human iPSC-Derived Motor Neuron Progenitors **(ax0078)** were seeded in 96-well U-bottom spheroid microplates (MoBiTec, MS-9096UZ) at 1×10^5 cells/cm^2 in motor neuron maintenance medium (MM, Axol) with 0.5 µM RA, 5 ng/mL BDNF (PeproTech), and 10 ng/mL CNTF (Axol).

Neuromuscular Organ Preparation

3D Human Skeletal Muscle (hSkM) organs were engineered in 50 µL collagen hydrogels (65% v/v TIRatT collagen, 20% Matrigel, 10% v/v 10X MEM, neutralised with NaOH). Cells were seeded at 4x10^6 cells/mL in 5% v/v media into 3D printed moulds (Fig. 2A; 37 °C, 5% CO_2). At day 14 of individual cultures, the motor neuron spheroids were placed onto the muscle in a type I collagen (1 mg/mL) matrix (Fig. 2C).

Functional Assessment

Neuromuscular organs were washed and attached to a force transducer. Electrical stimulation was applied via parallel wire electrodes in Krebs–Ringer-HEPES buffer. Antagonistic blocking of AChR was achieved using d-tubocurarine. Data acquisition and analysis were performed with a PowerLab system and LabChart software.

RNA Extraction and Quantification

RNA was extracted using TRIzol, quantified by Nanodrop, and 5 ng was used per SYBR Green RT-PCR on a ViiA™ 7 system for target genes, with expression normalised to RPIIβ and calculated by the ΔΔCT method.

Immunocytochemistry (ICC)

Incubations of primary and secondary antibodies, followed by imaging with confocal and fluorescence microscopes, and analysis using ImageJ/Fiji software.

Results and Discussion

3D spheroid cultures of axoCells® iPSC-Derived Motor Neurons accelerate development and increase SMI32 mRNA transcription by 200 times compared to monolayers and extend axons over 1000 µm at 14 days, of which 90% are SMI32 positive (Fig. 1.). Using a gel concentration of 1 mg/mL collagen enhances the mRNA expression of SMI-32, VAChT, and MAPT compared to 0.5, 1.7 mg/mL and monolayered motor neurons at day 19 (Fig. 2B; 3A). Faster maturation time points enhance the throughput capacity of these organ models.

Placing these spheroids onto the muscle (Fig. 2C) demonstrated pre- and postsynaptic TuJ-1/AChR colocalization, along myofibers positive for myosin heavy chain (MyHC) (Fig. 3B); this reflects the development of a maturing neuromuscular junction (NMJ). The addition of axoCells Motor Neuron spheroids to muscle increases tetanic force and demonstrates spontaneous twitch profiles compared to skeletal muscle only organs (Fig. 3C; 3D). This spontaneity increased fourfold above baseline (Fig. 3D).

Inhibition of the acetylcholine receptor in spontaneously contracting neuromuscular organs, achieved by the addition of 100 µM d-tubocurarine (Fig. 3E), demonstrates the blockade of acetylcholine receptors and suppression of spontaneous neuromuscular contractions.

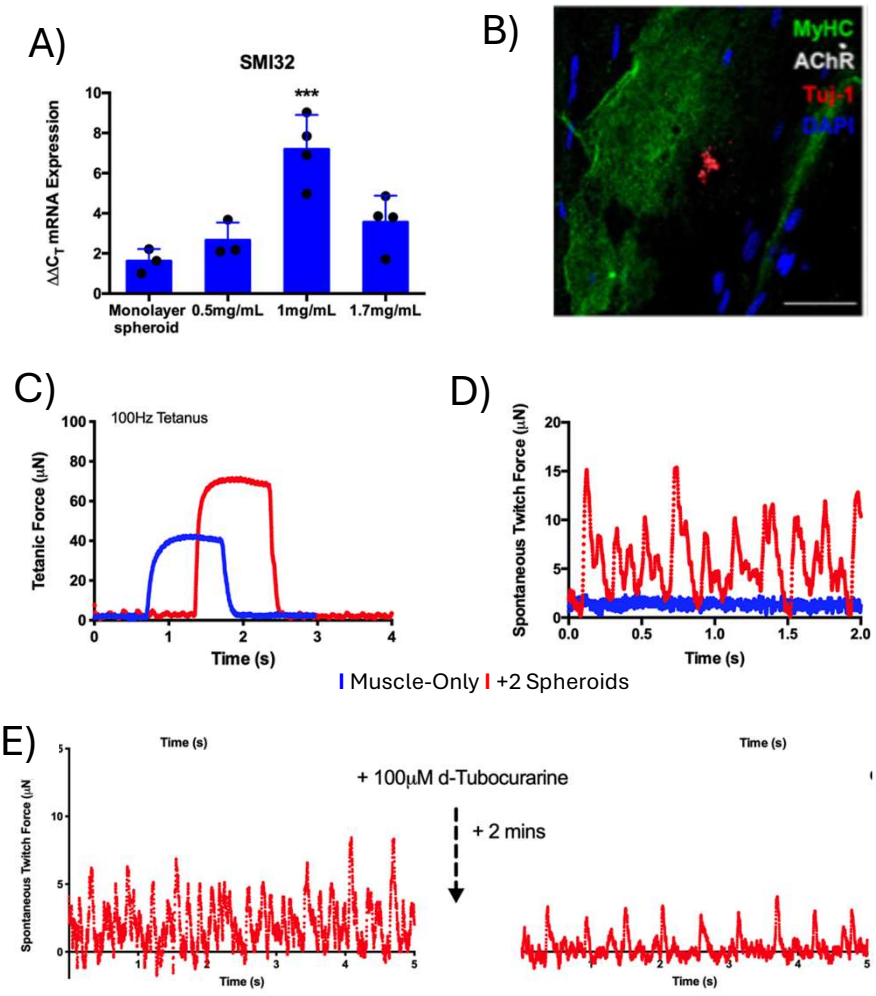

Figure 3. A) 1mg/mL loading gel enhances maturity. B) Pre- and post-synaptic Tuj-1/AChR colocalization. Scale Bar: 50 µm. C) The addition of Motor Neurons creates higher tetanic force profiles. D) Spontaneous twitch profiles compared to skeletal muscle only tissues. E) Inhibition of the acetylcholine receptor in spontaneously contracting neuromuscular tissues via the addition of 100 µM d-tubocurarine.

Conclusions

The findings from this work highlight the unique advantages of axoCells® iPSC-Derived Motor Neurons as a robust and scalable platform for building physiologically relevant human neuromuscular models. By integrating 3D neuronal spheroids with engineered muscle tissues, the system achieved accelerated neuronal maturation, extended axonal outgrowth, enhanced acetylcholine receptor clustering, and functional twitch and tetanic responses.

Pharmacological validation using d-tubocurarine further confirmed the presence of active neuromuscular junctions, highlighting the model's value as a robust assay for drug screening and mechanistic research. This work showcases the strength of Myomaker Bio's expertise in skeletal muscle engineering, offering researchers a powerful, reproducible, and translationally relevant platform. With applications ranging from disease modelling and target discovery to toxicology and precision medicine, the next generation of human in vitro neuromuscular models will bridge the gap between fundamental research and clinical translation.