A Novel Functional In Vitro 3D iPSC-Derived Neuromuscular Junction Model for Investigating Botulinum Neurotoxin Activity and Neuromuscular Disease

Jacob Fleming[1], Kevin Gray[1], David Nash[1], Vincent Leung[1], Cameron Gelber[1], Christos Michas[1] , Shawn Luttrell[1], Mark Bothwell[2], Alec Smith[2], David Mack[2], Nicholas A. Geisse[1]
(1) Curi Bio, Inc., (2) University of Washington

Preclinical Models Are Not Predictive

- Many compounds fail during clinical trials due to insufficient early detection of ineffectiveness in preclinical testing.
- Animal-based preclinical models often do not accurately represent human physiology.
- Pre-release potency testing of complex biologics is becoming increasing important and complex with many unsuitable for animal models.

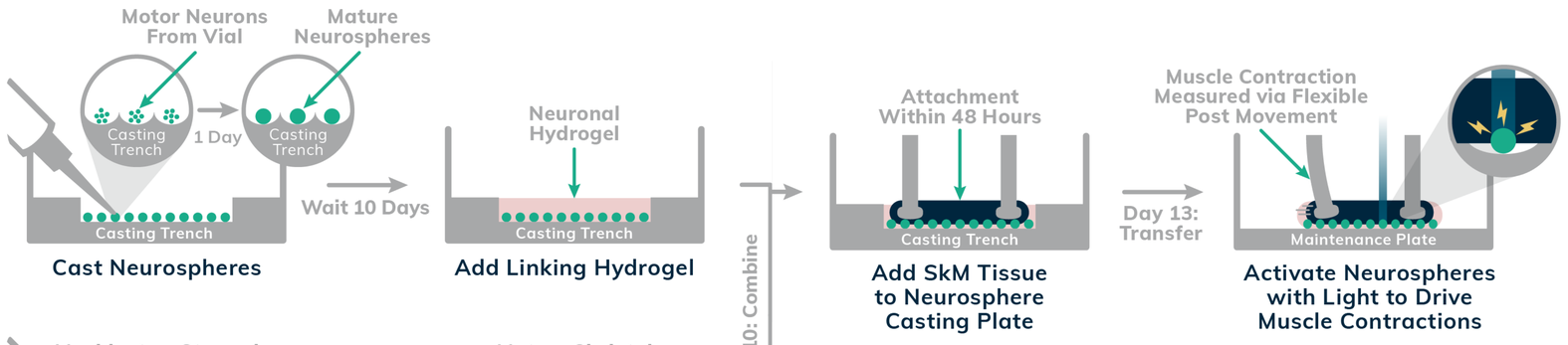
Opportunity of 3D NMJ Models

- 3D models of the NMJ exist but none offer reliable and high throughput generation of functional tissues.
- 3D in vitro models incorporate extracellular matrix (ECM) and cell-cell interactions, improving the in vivo fidelity of in vitro tests.
- Functional outputs from tissues allow direct assays of disease phenotypes or toxin action making them directly suitable for preclinical testing or potency assays.

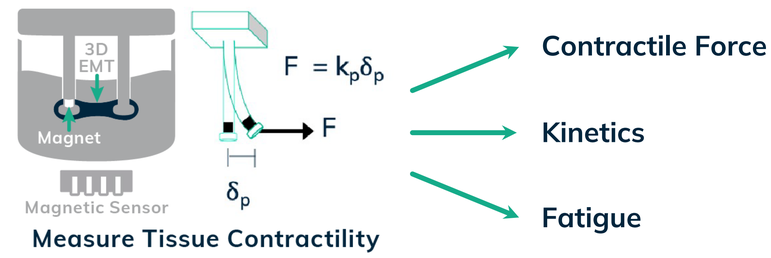
Our Objective – Demonstrate Utility

- Here, we present an iPSC derived model of the NMJ with controllable functional output and biologically relevant neurotoxin blockade.
- We show histological evidence of NMJs, important for many disease modelling applications.
- Furthermore, we demonstrate dose dependent loss of NMJ function in the presence of Botulinum toxin (BoT), acting as a proof of concept for a novel BoT potency assay.

Scalable and Simplified Creation of 3D NMJs



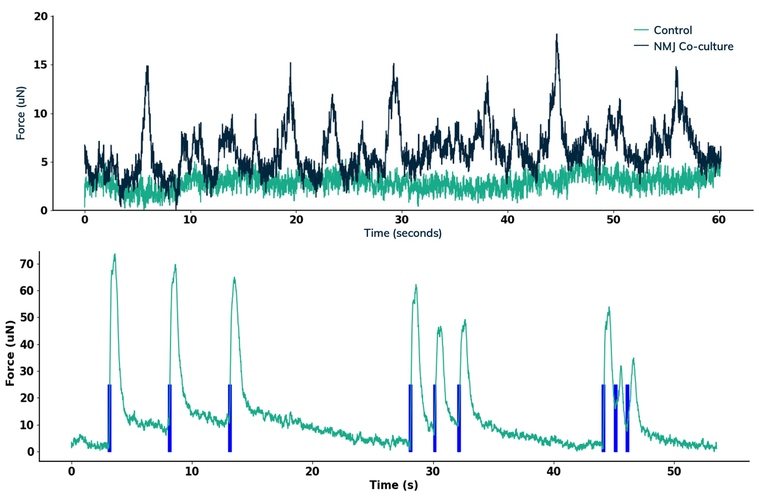
- In situ neurosphere creation removes need for direct spheroid handling.
- Blue light sensitive neurons allow specific activation of motor neurons independently of skeletal muscle.
- iPSC derived motor neurons and skeletal muscle.
- High success rate NMJ creation >95%.



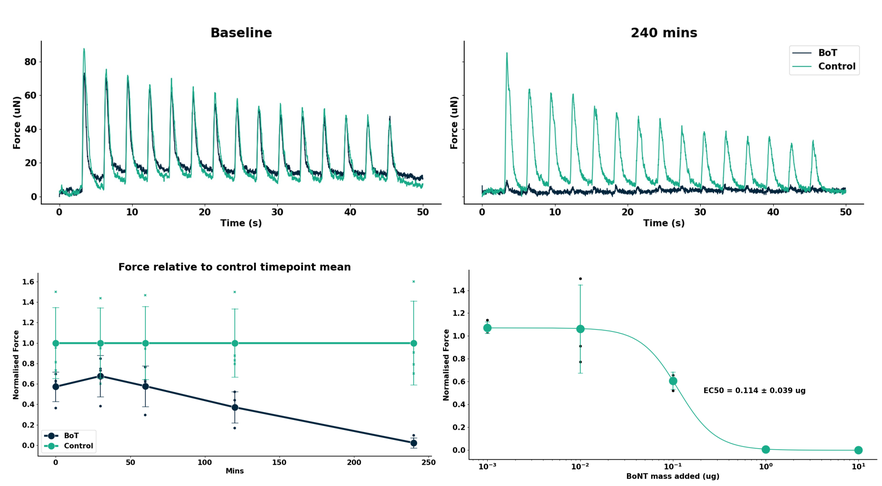
- Easy rapid casting.
- Over 95% casting success rate.
- Label-free tracking of 24 tissues.
- Well-based e-stimulation of 24 tissues.
- Clinically-Relevant protocols.
- Automated metric extraction.

Functional Characterization of NMJs

Baseline NMJ Function



Botulinum Toxin Blockade of NMJ Transmission



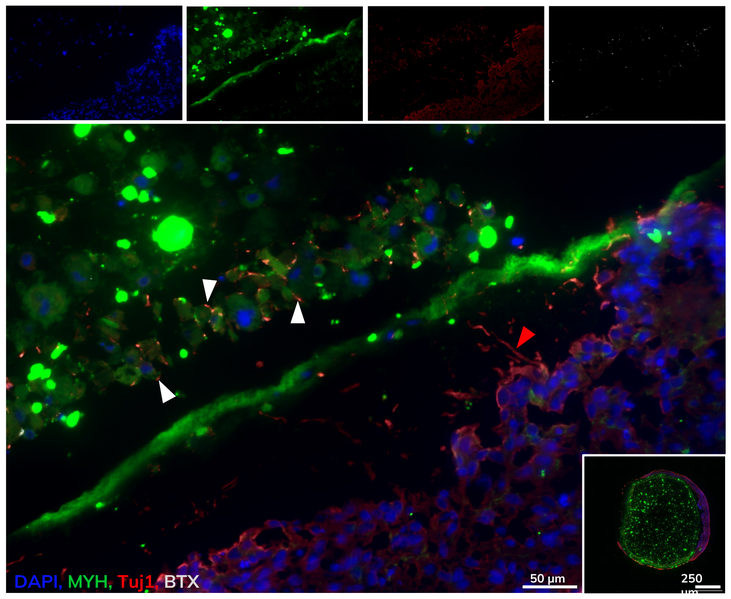
Baseline NMJ Function

Co-cultures exhibited elevated spontaneous contractions, compared to the relatively quiescent behavior observed in mono-cultured skeletal muscle tissues. Light-sensitive neurospheres were selectively activated in the coculture through 250 ms blue-light pulses, the skeletal muscle exhibited synchronized contractions within 100 ms of the applied light pulses. The contractile force of neuronally elicited contractions averaged 13.9±4.5% of electrical field stimulation of the bulk tissue (1 Hz, 100 mA, 10 ms biphasic). **This demonstrates the ability to activate specifically the muscle tissue through the neuronal component.**

Botulinum Toxin Blockade of NMJ Transmission

Research grade *Botulinum Neurotoxin* (BoT) complex A was added to culture media at 5 µg/mL, and the light-induced contractile response was measured across the following 4 hrs. Force was normalized to the control condition, with a reduction in function to 0.03±0.05% in BoT tissues after 4 hrs of treatment. Furthermore, a dose response curve was generated and an EC50 value of 0.114 µg (of BoT mass added) was determined following 24 h of exposure. Electrically-induced contractile force remained stable in both control and treated tissues following BoT treatment at all doses (data not shown). These data demonstrate that **the NMJ co-culture tissues are sensitive to BoT synapse blockade and that this effect is specific to the neuronal component**, as the skeletal muscle remains functionally competent.

Histological Characterization of NMJs



Conclusions

Curi Bio has designed, built, and tested a functional human 3D model of the neuromuscular junction within its established Mantarray engineered tissue platform. We present this platform as a tool for preclinical drug discovery and potentially potency testing for biological products such as Botulinum neurotoxins.

Data presented within was supported by NCATS under award number U44TR004795.

Contact Curi Bio