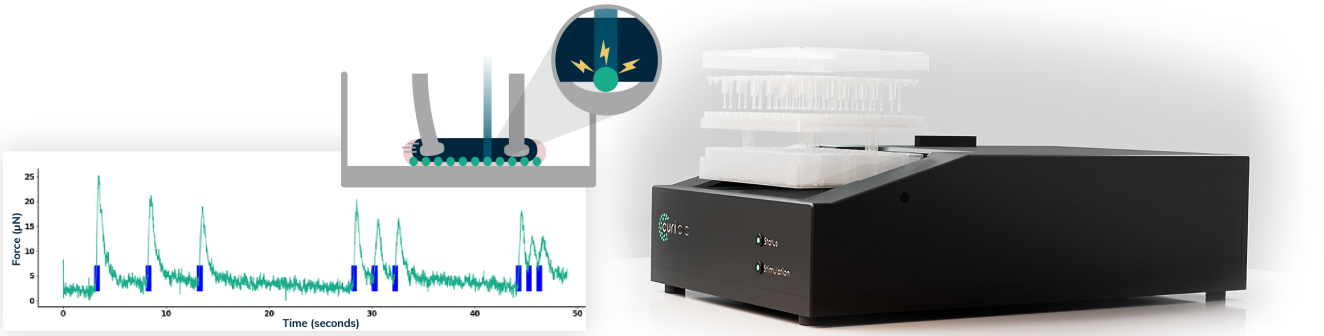
A Scalable Functional Model of 3D Human Neuromuscular Junctions Using the Mantarray Platform

Jacob Fleming *PhD*, **Christos Michas** *PhD*

Highlights

- Facile co-culture of iPSC-derived neurons & skeletal muscle into a 3D NMJ model.
- Scalable, reproducible & reliable formation of 3D human NMJs with long *in vitro* lifetimes.
- Turnkey, longitudinal collection of NMJ functional data, backed by histological evidence.



Summary

The neuromuscular junction (NMJ) converts nerve signals into muscle contractions and is at the epicenter of numerous disease pathologies and therapeutic interventions. Despite its importance, few scalable, easily adoptable and clinically relevant models of the human NMJ exist. Such a model would greatly accelerate our understanding of disease while maintaining the throughput, yield, and cost-efficiency demanded by the modern drug development paradigm. Here, we demonstrate the formation of a 3D in vitro model of the NMJ based on co-cultured human stem-cell-derived lineages. This new NMJ models is built on the foundation of Curi Bio's Mantarray platform and is rapidly assembled using commercially-available cells without complex tools or techniques. The model is suitable for highly-parallel compound assessment, as its 3D structure enables the automatic quantification of NMJ functionality through tissue contractile strength. Finally, we showcase the functionality of the NMJ model through muscle contraction in response to selective activation of the driving motor neurons. This platform will serve as the cornerstone of a variety of assays and is easily amenable to modeling human neuromuscular diseases.

support@curibio.com
+1 (800) 913-4403

Introduction

The NMJ is the specialized synapse between motor neurons and skeletal muscle (Fig. 1) which uses acetylcholine release to control voluntary muscle movement. Despite the tremendous effort to address devastating diseases that involve NMJ dysfunction[1], these indications remain largely underserved as prospective therapies fail to advance to the clinic[2]. A significant source of these failures is the unavailability of robust and relevant in vitro research models. This deficit is compounded by the poor utility of legacy animal models, as the NMJ function and structure greatly varies across species[3]. However, human iPSC-based models can provide functional outputs for direct insight into therapies and can enable modeling of population-level genotypic differences, including specific disease indications. To date, the scientific community has struggled to create iPSC-derived functional NMJ models that can be used rapidly and at a scale necessary for use in preclinical testing programs[4].

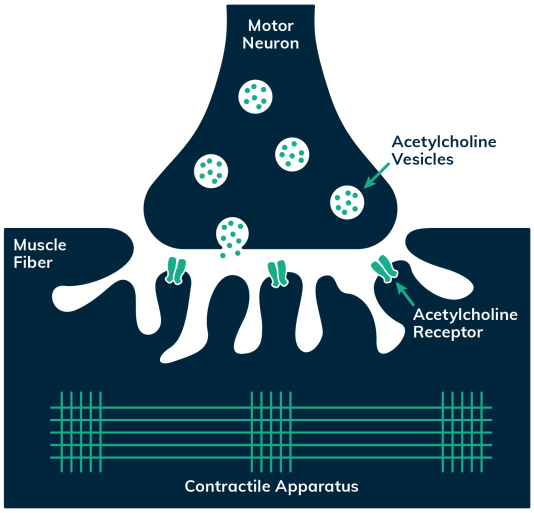


Figure 1: *The NMJ is the specialized synapse between motor neurons (top) and skeletal muscle (bottom).*

In this application note, Curi Bio has designed, built, and tested a functional human 3D model of the neuromuscular junction within its established Mantarray™ engineered tissue platform. The model features contractile 3D skeletal muscle tissues that are innervated with neuronal cells; the design of the Mantarray enables the automated, parallel, and label-free measurement of muscle contractility due to stimulation from the neurons[5]. The NMJ is fabricated with routine laboratory tools, while collection of functional data requires minimal user interventions. The formation of functional NMJs is demonstrated by measurement of elevated spontaneous muscle activity, and by the consistent muscle contractions when the motor neurons are selectively activated. In addition, the model is compatible with standard assays such as histological examination, which shows clear colocalization of presynaptic and postsynaptic markers at the junction of the two tissues.

Methods

3D engineered neuromuscular tissues were generated using the Mantarray casting kits (Fig. 2) and analyzed using the Mantarray instrument (CAT# MANTA-24). Briefly, iPSC-derived myoblasts (CAT# MRC-SkM-WT) were combined with stromal cells and were assembled into skeletal muscle tissues in a Mantarray casting plate following the standard Mantarray casting protocol[6]. Separately, iPSC-derived, blue-light sensitive neurons were assembled into neurospheres using the NMJ-specialized Mantarray casting plate. Starting on culture day 1, the two tissue plates were maintained separately in media specialized for either skeletal muscle (CAT# SKM-MED-IPS-M) or motor neurons respectively. On culture day 10, 3D skeletal muscle tissues and neurospheres were combined in situ through the addition of an acellular hydrogel. The resulting co-cultures were maintained for up to 39 days in NMJ-optimized media.

support@curibio.com
+1 (800) 913-4403

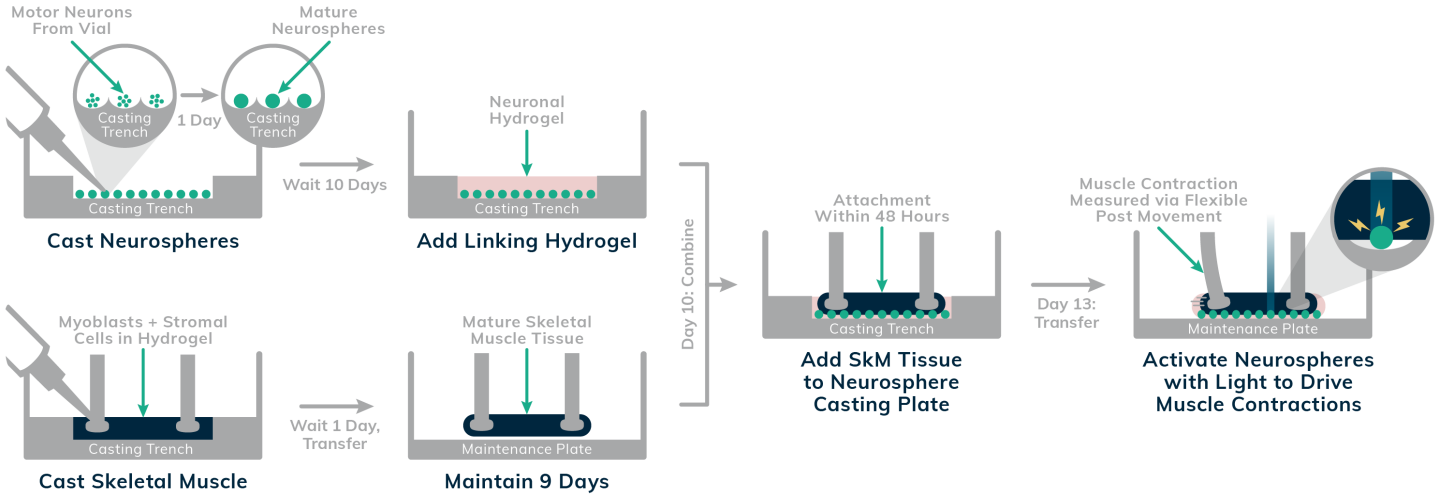


Figure 2: *The Mantarray™ technology enables the easy assembly of a functional NMJ model with automated functional data collection.*

To quantify the maximum contractile force of tissues independently from neuronal activation, tissues were electrically stimulated via Curi Bio's multi-well stimulation lid (CAT# MA-STM), with electrical impulses driven by contractile strength measured by the Mantarray instrument. Next, to determine the degree of skeletal muscle contraction solely via neuronal activation, the optogenetically modified neurospheres were selectively excited with 250ms blue-light pulses using Curi Bio's laser-based optical stimulation system. In all cases, contractile force was measured using Mantarray and was processed using a specialized feature of Curi Bio's Pulse™ data analysis platform (CAT# PULSE-B2). Histological analysis was performed using standard cryosectioning techniques producing complete co-culture tissue cross sections for immunohistochemical analysis.

Results

Co-culture of neurospheres with 3D skeletal muscle tissues resulted in the establishment of functional neuromuscular junctions (NMJs), evidenced by both functional NMJ-mediated contractility (Fig. 3) and histological proximity (Fig. 4). In terms of functionality, co-cultures exhibited spontaneous contractions (Fig. 3A), a marked departure from the quiescent behavior observed in matured mono-cultured skeletal muscle tissues. Furthermore, when 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 (Fig. 3B). Stimulation of co-cultured tissues at 0.2 and 0.5 Hz elicited responses in 100% of cases (Fig. 3E), while stimulation at 1 Hz saw a reduction in stimulation fidelity caused by the appearance of unfused tetanus in some tissues. Concurrently, a slight reduction in both absolute contraction force (Fig. 3D) and force relative to the maximum contractile force (Fig. 3E) was observed with the escalation of illumination frequency, justified by the partial relaxation of tissues at higher stimulation frequencies.

Further evidence for NMJ formation is provided through histological examination of co-culture tissues. Immunohistochemistry for the neuronal marker (TuJ1) and muscle marker (MYH) showed cells independently localized within the 3D tissue cross-section (Fig. 4B,C). Axonal extensions visualized with TuJ1 staining could also be identified crossing between neuronal and muscle populations (Fig 4E, red arrow). Finally, staining of

support@curibio.com
+1 (800) 913-4403

the acetylcholine receptor with a bungarotoxin conjugate (Fig 4D) showed characteristic punctate staining on myotube peripheries. This staining often colocalized with TuJ1 staining (Fig. 4E, white arrows) indicating the presence of NMJ-like interactions at these sites. Large numbers of such colocalization sites were identified in cross sections, consistent with the observed functional innervation and allowing potential future studies to look in more detail at key pre- and post-synaptic components of the NMJ.

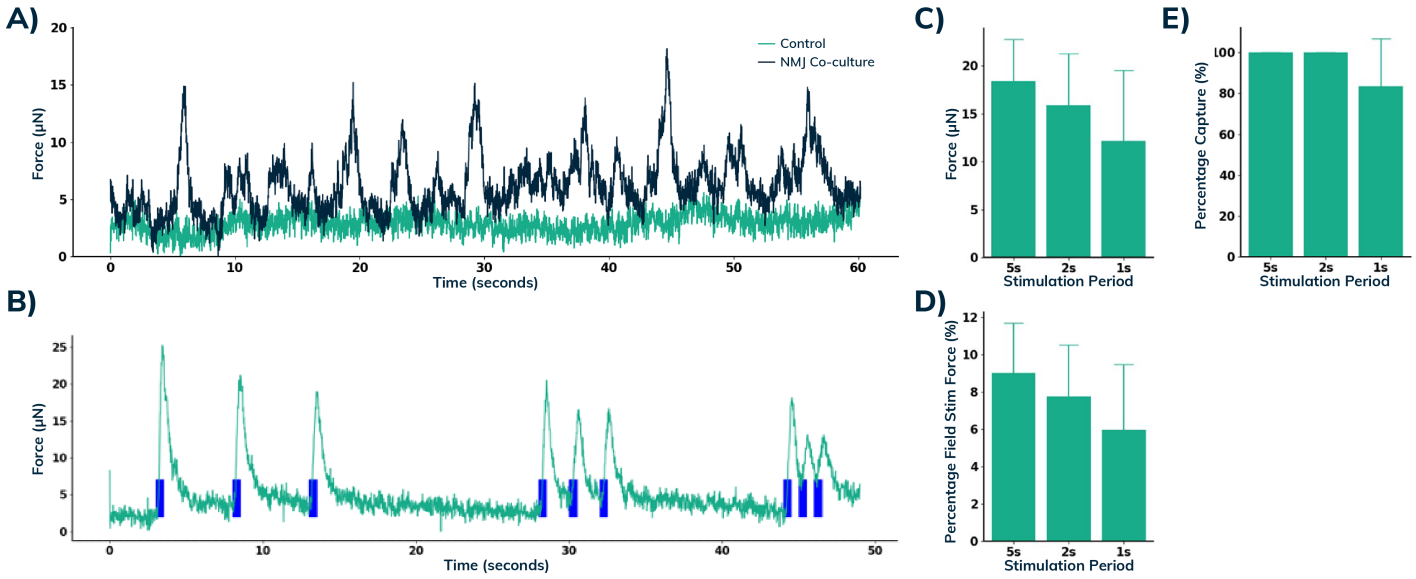


Figure 3: *Functional evidence of NMJs: The neuromuscular coculture (A) features spontaneous muscle activity and (B-E) exhibits muscle contractions in response to selective light-induced neuronal activation.*

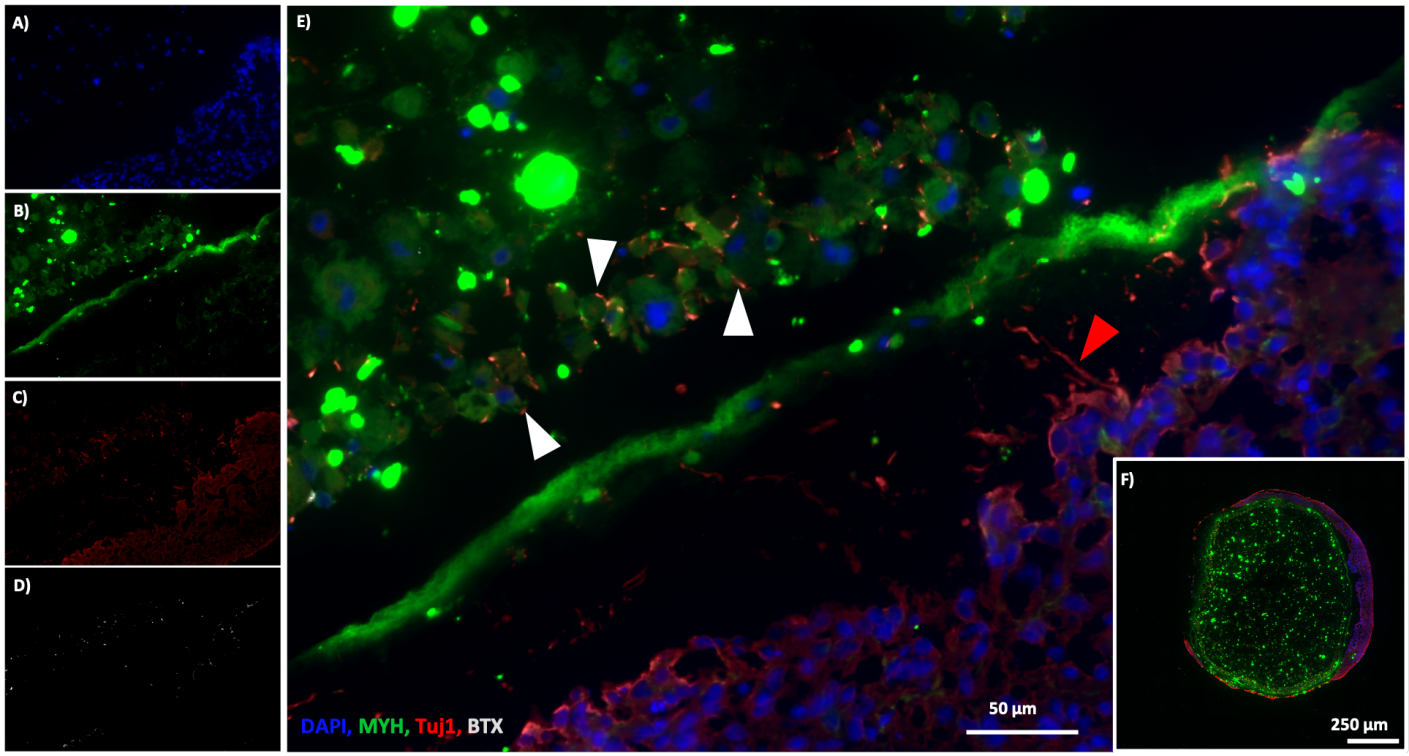


Figure 4: *Histological evidence of NMJs: (A-E) Motor neurons (TuJ1) extend axons (red arrow) from the periphery of the tissue (shown in F) to synapse with the myotubes (MYH) in the core. The NMJ-identifying Bungarotoxin conjugate (BTX) aggregates at the interface of neurons and the skeletal muscle (white arrows).*

support@curibio.com
+1 (800) 913-4403

Conclusions

The Mantarray™ platform was used to measure functional contractions from a novel 3D in vitro model of the NMJ. The NMJ model is assembled with minimal manual interventions and incorporates motor neurons, myoblasts and stromal cells via direct casting into mass-produced 3D co-culture plates. This model not only demonstrates histological evidence of muscle innervation, but also shows clear muscle contraction in response to selective neuronal activation via optogenetic approaches. The scalability and ease-of-use of the Mantarray platform enabled the repeatable formation of NMJs and can serve as a testing platform for broadly adopted neuromuscular disease models and therapeutics development.

References

[1] National Institutes of Health, "Estimates of funding for various research, condition, and disease categories (rcdc)." https://report.nih.gov/funding/categorical-spending#/, 2023. Accessed: 2024-02-01.

[2] D. Petrov, C. Mansfield, A. Moussy, and O. Hermine, "Als clinical trials review: 20 years of failure. are we any closer to registering a new treatment?," *Frontiers in Aging Neuroscience*, vol. 9, 3 2017.

[3] I. Boehm, A. Alhindi, A. S. Leite, C. Logie, A. Gibbs, O. Murray, R. Farrukh, R. Pirie, C. Proudfoot, R. Clutton, T. M. Wishart, R. A. Jones, and T. H. Gillingwater, "Comparative anatomy of the mammalian neuromuscular junction," *Journal of Anatomy*, vol. 237, pp. 827–836, 11 2020.

[4] Y. Leng, X. Li, F. Zheng, H. Liu, C. Wang, X. Wang, Y. Liao, J. Liu, K. Meng, J. Yu, J. Zhang, B. Wang, Y. Tan, M. Liu, X. Jia, D. Li, Y. Li, Z. Gu, and Y. Fan, "Advances in in vitro models of neuromuscular junction: Focusing on organ-on-a-chip, organoids, and biohybrid robotics," *Advanced Materials*, vol. 35, 10 2023.

[5] A. S. Smith, S. M. Luttrell, J.-B. Dupont, K. Gray, D. Lih, J. W. Fleming, N. J. Cunningham, S. Jepson, J. Hesson, J. Mathieu, L. Maves, B. J. Berry, E. C. Fisher, N. J. Sniadecki, N. A. Geisse, and D. L. Mack, "High-throughput, real-time monitoring of engineered skeletal muscle function using magnetic sensing," *Journal of Tissue Engineering*, vol. 13, 1 2022.

[6] B. J. Berry, S. M. Luttrell, and et al., "Preclinical Drug Testing in Scalable 3D Engineered Muscle Tissues," *JoVE Journal (J. Vis. Exp.)*, vol. 194, p. e64399, 2023.

PUBLISHED BY

Curi Bio, Inc.
3000 Western Avenue,
Seattle, WA 98121, USA

www.curibio.com