COUPLING COMPARTMENTALIZED MICROFLUIDIC PLATFORMS WITH MEA FOR ADVANCING NEUROMUSCULAR JUNCTION MODELING

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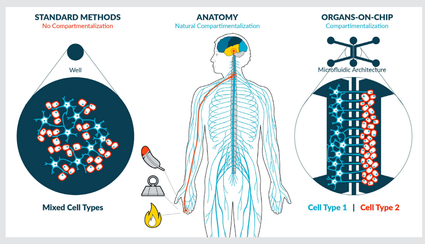
BOOTH #317

BACKGROUND



Neuromuscular diseases such as amyotrophic lateral sclerosis (ALS) remain challenging to study due to the limited scalability and translatability of current human neuromuscular junction (NMJ) models. To this end, we developed a physiologically relevant compartmentalized *in vitro* NMJ platform by integrating NETRI's DuaLink MEA microfluidic device with human iPSC-derived motor neurons and skeletal muscle myotubes.

This proof-of-concept provides a scalable, human-relevant model for studying NMJ development, disease mechanisms, and therapeutic interventions—including pharmacological and dermato-cosmetic compounds. Its versatility enables real-time monitoring of neuronal activity, muscle contraction, and localized compound delivery, opening new avenues for efficacy and toxicity assessment.

RESULTS

COMPARTMENTALIZED NMJ – VISUAL MORPHOLOGIC CHARACTERIZATION

Each cell type is seeded into a separate microfluidic channel, maintaining physical separation of the neuron's somas. Over the course of six days, hiPSC-derived motor neurons extend their axons through the microchannels to reach and innervate the skeletal muscle cells, enabling the formation of neuromuscular junctions.

Consistent morphological differentiation of human Skeletal Myoblasts.

- Differentiation of human skeletal muscle is consistent based on morphological observations under phase microscopy as seen in figure B.

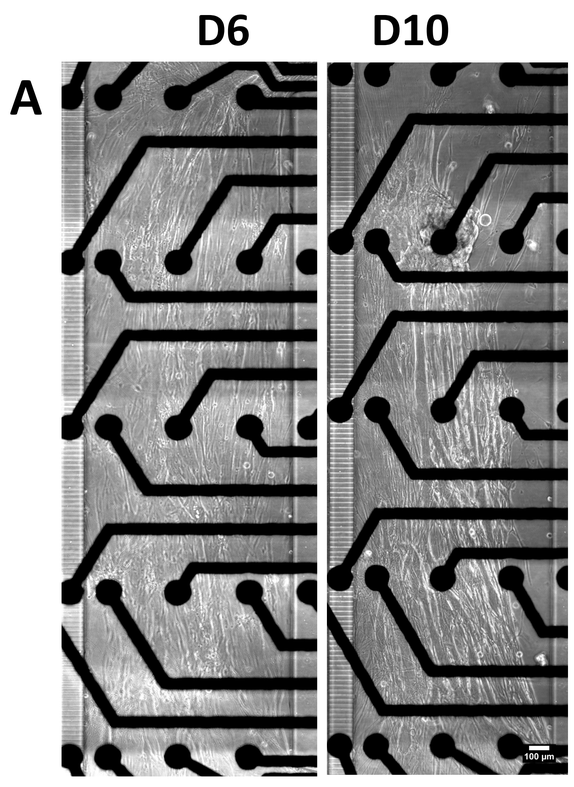
Coculture day 17.

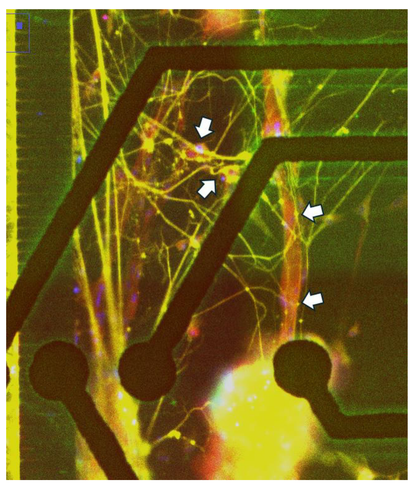
- Longitudinally aligned muscle fiber
- α-Bungarotoxin staining indicating NMJ formation

Immunofluorescence of coculture.

Expected markers indicating:

- Motoneurons in the "spine" compartment
- Skeletal Muscle cells in the "muscle" compartment
- presence of NMJ



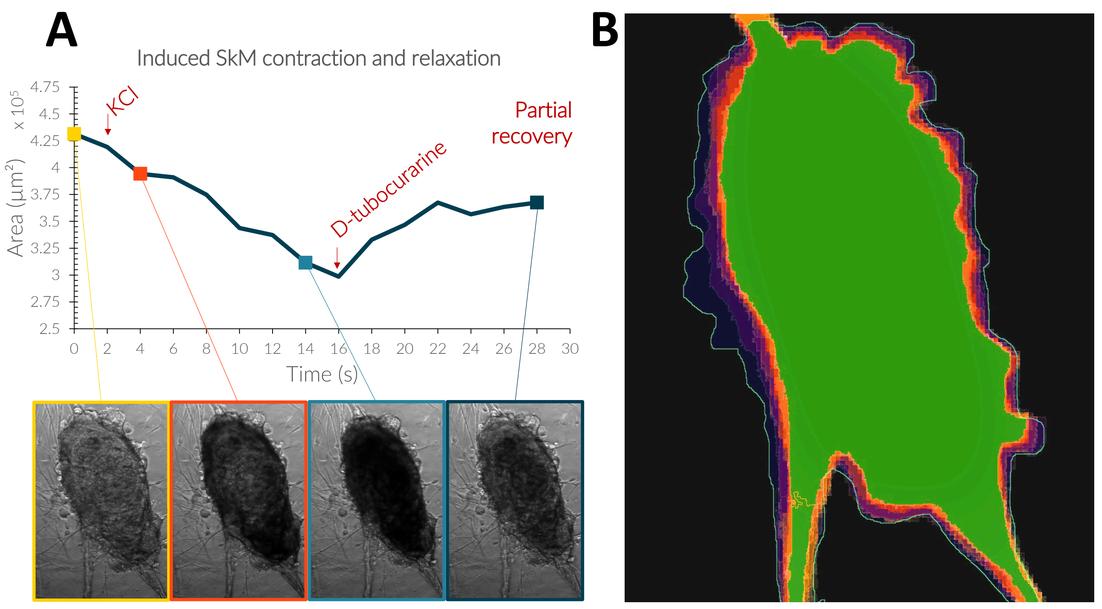


RED - SkMcells;
GREEN – α- Bungarotoxin on NMJ;
Orange – β3tubulin
INTENSE YELLOW= overlap green-orange, NMJ buttons

Bright field and immunofluorescence images of coculture of hiPSC motoneurons and skeletal muscle cells in Dualink MEA devices. (A) Motoneurons axons reaching SkMCs compartment at day 6 of coculture. At day 10 striated muscle fibers are present and perpendicular to the motoneurons axons axis. (B) Higher magnification showing motoneurons axons distribution connected to differentiated myotubes (yellow arrows) in the muscle channel. (C) Immunofluorescence staining of Motoneurons, Skeletal Muscle cells and NMJ. (D) Zoom on a longitudinally aligned muscle fiber. Dual bright field image and green (488 nm) light imaging of live cells for the staining with α-Bungarotoxin, which specifically couples with NMJs (yellow arrows confirming NMJs). Microchannels are visible to the lefthand side of the bright field images. The black stripes are MEA electrodes.

INDUCED MUSCLE TETANY AND ITS REVERSIBILITY

Primary human skeletal muscle cells respond to KCl (100mM) stimulation by displaying a tetanic contraction, for matter of seconds. This action can be counteracted by addition of D-tubocurarin that leads to a time-dependent partial recovery (85.17% area recovery).



(A) Area measurements of the SkM cell clumps after the addition of KCl 100mM, with representative images at the bottom. (B) Tetanic contraction induced by KCl resulted into skeletal muscle cell clump shrinking and asymmetrical perimetral changes.

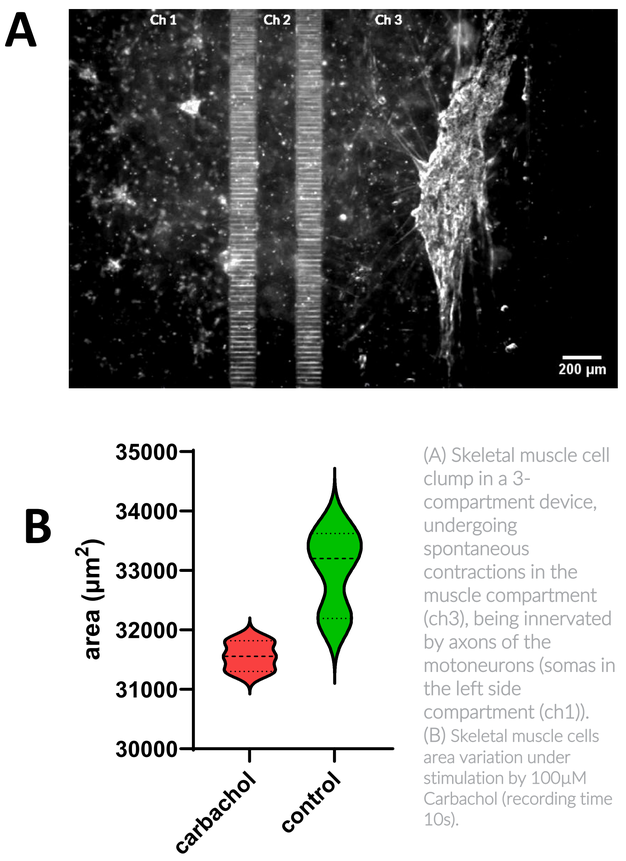
INDUCED AND SPONTANEOUS MUSCLE CONTRACTION

Spontaneous contractions

- Displayed since day 10 in culture.
- Occurrence in the presence of motoneurons axons.

Induced contractions

- Reversible skeletal muscle cells contraction induced by Carbachol (100µM).
- Contractions were limited in time and of low amplitude, involving peripheral areas of the myotube aggregates.



(A) Skeletal muscle cell clump in a 3-compartment device, undergoing spontaneous contractions in the muscle compartment (ch3), being innervated by axons of the motoneurons (somas in the left side compartment (ch1)). (B) Skeletal muscle cells area variation under stimulation by 100µM Carbachol (recording time 10s).

CONCLUSION & PERSPECTIVES

This proof-of-concept demonstrates that our flexible MEA-compatible microfluidic platform reliably supports the formation of neuron–muscle junctions. It paves the way for the expanded development of scalable, human-relevant *in vitro* NMJ models for pharmaceutical and dermocosmetic research.

By combining hiPSC-derived motor neurons and skeletal muscle cells in a compartmentalized system offers a high-impact tool suitable for disease modeling, drug screening, and potential integration of patient/disease-specific cells.

- Aligned, striated myotubes differentiate robustly within the muscle chamber.
- Spontaneous muscle contractions occur only after motor neuron axons reach the muscle, indicating neuromuscular coupling.
- α-Bungarotoxin–positive AChR clusters co-localize with motoneuron terminals, confirming NMJs.
- Integrating contractility assays, real-time electrophysiology, and molecular read-outs, this platform has the potential to accelerates efficacy testing for ALS and other neuromuscular therapeutics as well as for Botox-like applications.

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