Bioengineered Human Skeletal Muscle as a Platform to Model Injury, Regeneration and Drug Response

MYOMAKER BIO
Bespoke screening on complex muscle organs

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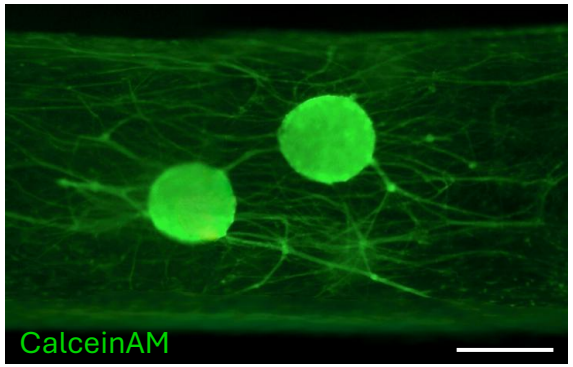
Overview Engineered human skeletal muscle tissues provide a powerful *in vitro* model for studying injury, regeneration, and pharmacological modulation in a physiologically relevant setting. Using 2D monolayer, 3D muscle tissues, and 3D neuromuscular co-cultures, Myomaker Bio has tested various compounds spanning injurious, anabolic, metabolic, inflammatory, and neuromuscular functions. Each integrated model can support translation between traditional 2D cell culture and *in vivo* muscle physiology.

Methods 3D human skeletal muscle tissues were engineered in 50 µL collagen–Matrigel hydrogels (4 × 10^6 cells/mL), which self-aligned within 3D-printed moulds. At day 14, iPSC-derived motor neuron spheroids were added in a type I collagen overlay to generate innervated neuromuscular tissues. Contractile function was assessed by evoked electrical tissue contraction, while immunostaining, confocal imaging, and molecular assays quantified myotube structure and pathway activation. Mouse and human myoblast cell line cultures were used in parallel for mechanistic or metabolic analyses.

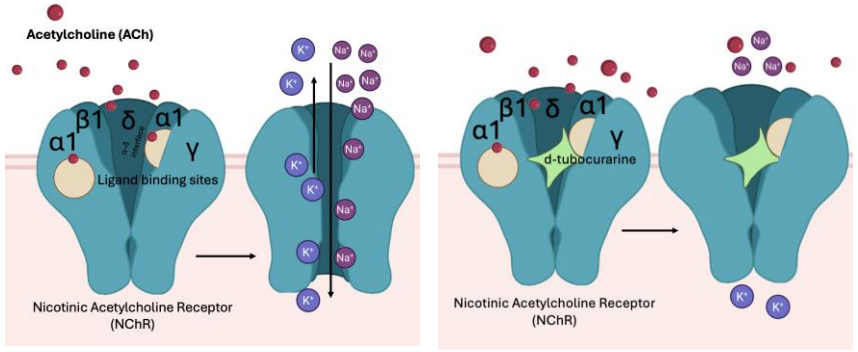


Fluorescence microscopy. *Two iPSC-derived motor neuron spheroids cultured on 3D human skeletal muscle tissue. Scale bar: 200 µm.*

D-Tubocurarine (d-TC): Antagonism of the Muscle nAChR

1. Molecular Structure & Receptor Binding

Tubocurarine is the primary active alkaloid component of **curare**, derived from South American plants, which was previously used as **arrow poison** and later as a **muscle relaxant** in anaesthesia. It competitively blocks acetylcholine (ACh) binding at **nicotinic receptors** in the neuromuscular junction (NMJ).



2. Competitive Antagonism & Mechanism of Action

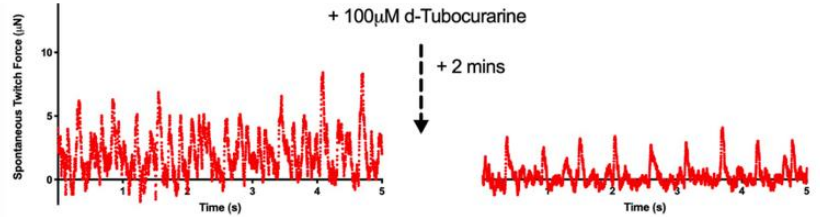
Normal NMJ function:
1. ACh is released at the neuromuscular junction
2. ACh binds both α subunits of the nAChR
3. Receptor changes shape and opens
4. Na^+ influx, K^+ efflux
5. Muscle depolarises and muscle contraction

d-Tubocurarine treated:
1. d-Tubocurarine binds one α subunit of the nAChR
2. ACh cannot bind both sites
3. Receptor does not open
4. No Na^+ influx / K^+ efflux
5. No depolarisation → no muscle contraction

4. Utility Within Neuromuscular Junction Validation

Applied to innervated neuromuscular tissues, 100 µM d-tubocurarine produces:
- Ablation of spontaneous twitch activity
- Selective blockade of nerve-driven contractions
- Functional confirmation of pre- and postsynaptic coupling

5. Experimental Findings
- **Innervated tissues**: Spontaneous contractions increased 4-fold above baseline (P≤0.001), indicating **functional NMJ formation.**
- **Post-tubocurarine:** Inhibition of spontaneous activity, confirming **motor nerve-driven contractions.**
- **Enhanced physiology:** Time to peak twitch (P≤0.001) and half-relaxation time (P≤0.01) significantly decreased following innervation.



Functional neuromuscular validation. *Inhibition of the acetylcholine receptor in spontaneously contracting neuromuscular tissues via the addition of 100 µM d-Tubocurarine.*

6. Consequences

Selective nAChR blockade without muscle damage, reversible inhibition enabling dynamic studies, **pharmacological proof of functional synaptogenesis and validation of bioengineered neuromuscular junctions.**

Conclusion Bioengineered muscle (and neuromuscular) systems reproduce human injury, repair, and pharmacological responsiveness. Distinct classes of compounds, injury agents, anabolic nutrients, anti-inflammatory mediators, and metabolic stressors elicit predictable, quantifiable outcomes in tissue function. The Myomaker Bio tissue platform enables mechanistic screening of therapeutics targeting muscle health, regeneration, and disease.