Improving 3D Human Engineered Muscle Tissues and Tools: Quality, Stromal Cells, and Standardization / Democratization

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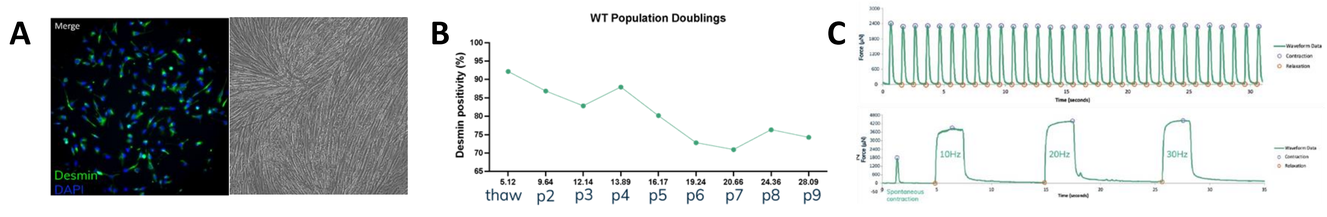
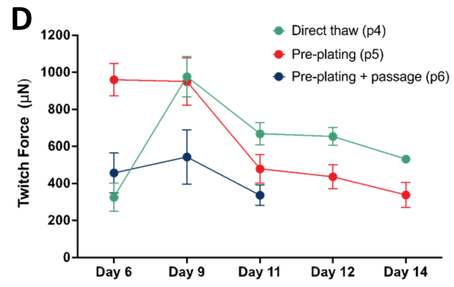
Problem

Preclinical research and development for muscular dystrophies has historically been hindered by a reliance on animal models that often fail to fully replicate human biology. This translational gap is a key contributor to the high clinical trial failure rate. Human in vitro cellular models enable testing in human cell environments, however, widespread adoption in the field has been slowed by limited translational phenotypes observed in the dish. Traditional 2D muscle cultures are plagued with developmental obstacles, such as random cellular organization, limited culture times, and immature developmental states. Moreover, contractile force measurements are largely impossible in 2D, limiting the ability to robustly stratify disease-relevant phenotypes. Engineering muscle tissues in 3D provides a solution to many of these limitations however, rigorous quality control is critical to ensure optimal tissue function. **Our data show that contractile force declines with increasing myoblast passages in 3D engineered muscle tissues (EMTs),** indicating myoblast purity and health may directly impact muscle performance. Furthermore, we show that **addition of stromal cells into EMTs, combined with electrical stimulation on the Mantarray™ contractility platform, improves and stabilizes contractile force and increases functional longevity of the muscle.** We have recently developed the MantaReady™ family of products designed to address numerous technical challenges in the 3D muscle field. The MantaReady lineup includes ready to cast iPSC-derived myoblasts, ready made engineered cardiac and skeletal muscle tissues, and skeletal muscle media optimized for 3D differentiation and long-term culture.

Skeletal Muscle Tissue Force Declines with Increasing Myoblast Passages



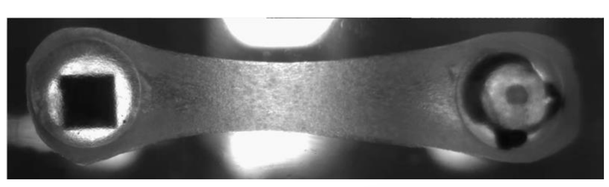
(A) Skeletal myoblasts were differentiated from iPSC WT lines, FACS sorted, expanded, and then frozen in large quantities as "passage 1 myoblasts". Cells were thawed and stained for the muscle specific marker, Desmin (left) to measure purity. Myotube fusion was confirmed for every passage (passage 6 pictured, right). **(B)** Desmin positive cells (as a percentage of total cell counts) decline with increasing population doublings. **(C)** Representative stimulated twitch and tetanic waveforms. **(D)** Myoblasts from the indicated passage numbers were used to engineer 3D skeletal muscle tissues. EMTs derived from higher passage myoblasts generate lower forces than those derived from low passage myoblasts, indicating lower purity likely impacts tissue development and corresponding contractile force. Tissues from myoblasts > 6 passages generated very low forces (not pictured).

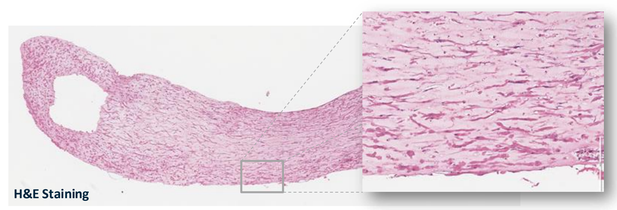


Mantarray Engineered Muscle Tissue Platform

The Mantarray system features a novel magnetic sensing technique that can detect the contraction of EMTs. This enables the user to measure contractility of 24 tissues in parallel and in real time. The system features user-friendly software that replaces tedious manual calculations of contractility, delivering contractile data at the click of a button.

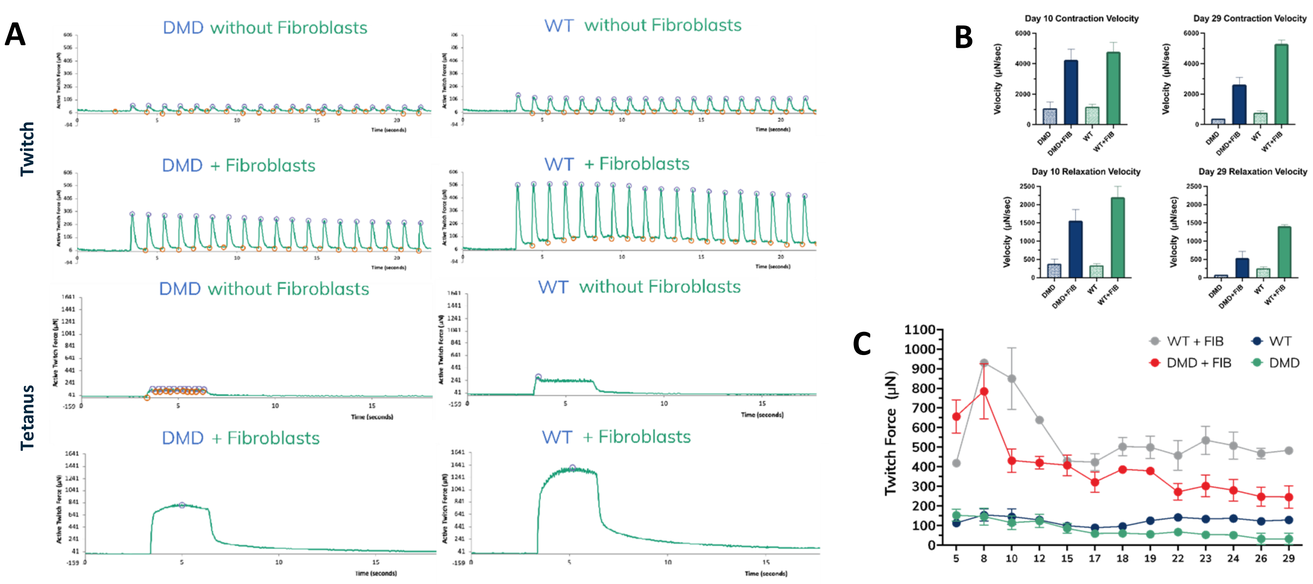
EMTs are fabricated in a consumable 24-well casting plate, in which tissues can be cast individually by hand or via automation. The Mantarray instrument can be operated inside of a standard cell culture incubator. All 24 wells are measured simultaneously at a bandwidth of >100Hz and controlled through custom-made software designed for simplicity and ease of use.





Addition of Fibroblasts Increase Force Output & Tissue Longevity

The Mantarray platform enables well-based stimulation control. A custom cooling unit maintains temperature at 37°C +/-0.7 at all times, enabling long term pacing without deleterious temperature shifts. To demonstrate the importance of stromal cells, **(A)** EMTs (iPSC derived myoblasts) from either wild type or cells bearing a dystrophin knockout (DMD) were co-cultured with and without exogenously added fibroblasts (bottom and top panels, respectively). Tissues were stimulated with a 100mA current at 1Hz to induce twitch contractions or 50Hz to induce tetanus. **(B and C)** Elicited force measurements were sampled up to day 29. There was an increase in both active twitch force and tetanic force in the presence of fibroblasts. The low signals in the "without fibroblast" samples and high output in the + fibroblast samples demonstrate the dynamic range of the biological system and instrument. Data compiled over 29 days show functional differences as early as day 5 that persisted over the course of the month-long experiment.



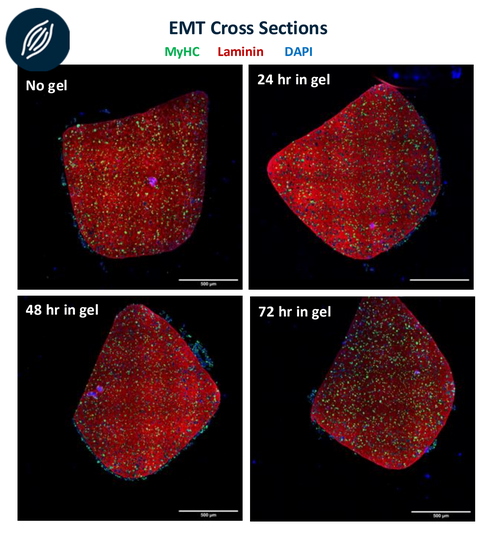
Democratization & Standardization of 3D Tissue Models

Incorporating EMTs into a real-world therapeutic discovery workflow requires significant resources and expertise. Engineered muscle tissues require substantial cell numbers demanding tightly controlled production workflows to maintain high purity and quality. Furthermore, 3D muscle tissues require medium to support development and maintenance in a complex microenvironment including different cell types. To address these limitations, we have manufactured iPSC-derived skeletal and cardiac cell lines and banked them in "ready-to-cast" cryovials. Cells have undergone extensive QC and are designed to thaw and cast directly into tissues. Secondly, we have developed a method to embed tissues in a biocompatible gel that permits shipment and transfer of EMTs at ambient temperatures. Tissues remain viable and fully functional upon gel dissolution for direct interrogation with therapeutic compounds, eliminating the need to fabricate 3D tissues in-house. Finally, we have developed a media formulated to meet the metabolic needs of 3D EMTs.

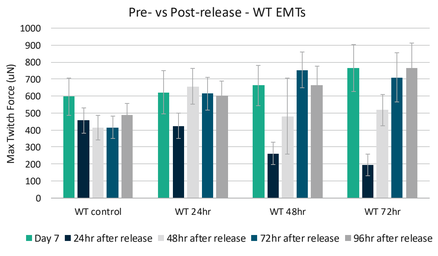
Engineered for 3D

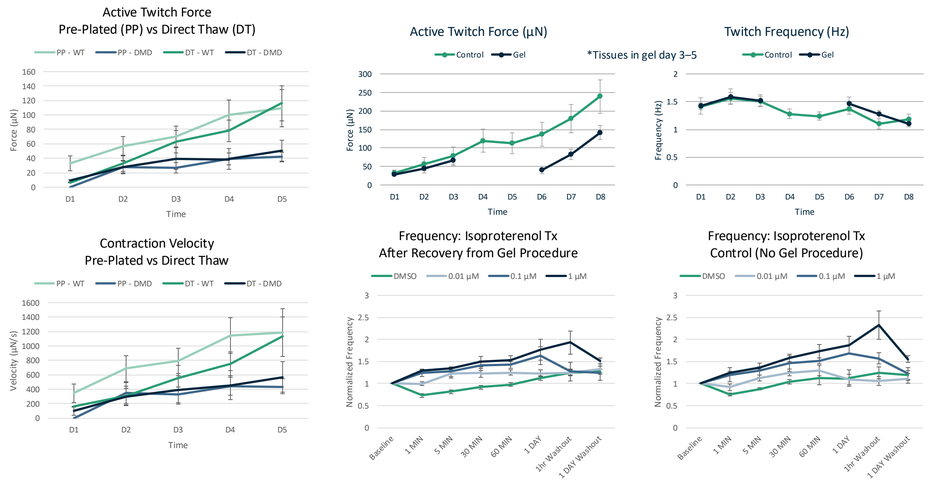
MantaReady

Cells, Media, and Ready-Made Tissues



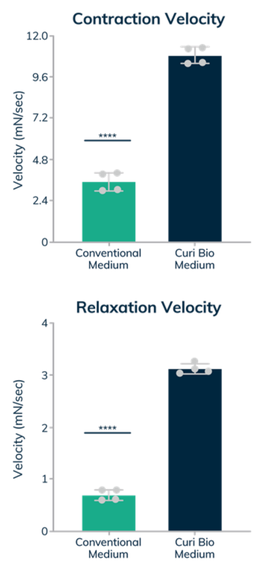
Engineered heart tissues (EHTs) comprised of healthy iPSC-Cardiomyocytes (~75% viability) and human ventricular fibroblasts thawed on day of casting (~95% viability) were cast using an Integra robot pipetting system. Twitch force and frequency were measured every day for up to 8 days. Tissues labeled "Gel" were embedded in a biocompatible gel on day 3 that slows/stops metabolic activity during shipping. Tissues were reanimated following dissolution of the gel on day 5 and EHTs were returned to fresh media. Functional measurements resumed on day 6 followed by treatment with Isoproterenol. Tissues undergoing the gelation/shipment procedure fully recovered with no detectable changes in contraction behavior and responded to Isoproterenol (ISO) similarly (lower panels).

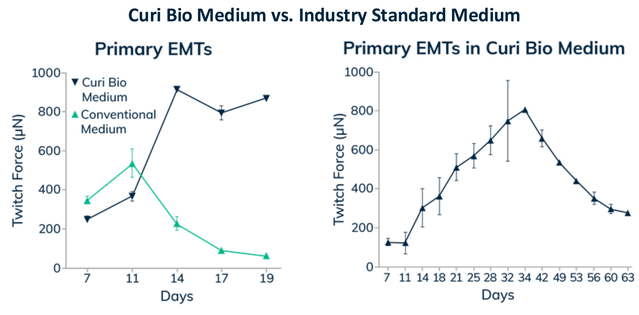




3D Tissues Require Enriched Media

Primary skeletal muscle tissues were cultured in either a proprietary serum free medium engineered by Curi Bio or a conventional "industry standard" formula (DMEM + glucose 4.5 g/L / 2% horse serum / 10 ng/mL IGF-1) which has been published in over 1000 articles (mostly featuring 2D applications). Contractile force declined markedly after 11 days in conventional medium, while EMTs in Curi Bio medium continued to get stronger over 19 days in culture. Contraction and relaxation velocities were also significantly faster in Curi Bio medium, indicating improved development. Delayed contractile kinetics may indicate impaired function. In a separate study, primary skeletal tissues were cultured in Curi Bio medium long-term and remained contractile for 63 days in culture.





Future Directions

The Mantarray platform and growing ecosystem offer a highly scalable and translatable platform for functional potency assessment of numerous therapeutic modalities. We are currently developing stretch in the Mantarray 3D platform to model eccentric contractions and injury. We are seeking collaborators and partners and help develop and expand the Mantarray platform into the neuromuscular junction space, as well as other organ systems, like smooth muscle, lung, gut, and cancer biology.

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Contact Curi Bio