In Vitro 3D Engineered Muscle Tissues Demonstrate Clinically-Relevant DMD Stratification

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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.
- Existing preclinical human models are typically 2D and overly simplistic, missing critical aspects of human body.

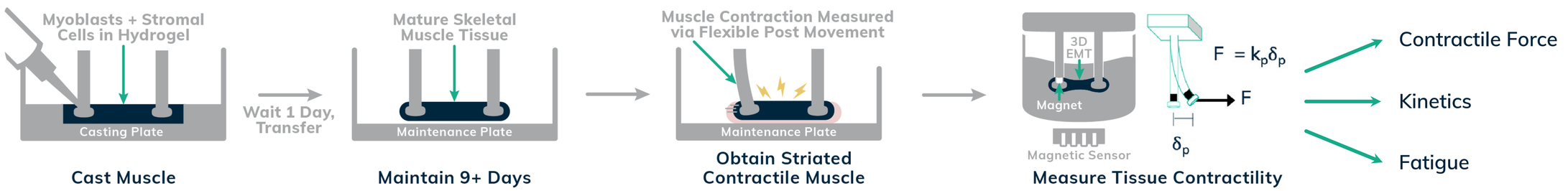
Advantages of 3D Tissues

- 3D in vitro models incorporate extracellular matrix (ECM) and cell-cell interactions, improving the in vivo fidelity of in vitro tests.
- iPSC technology allows for the use of patient-derived cell lines, better reflecting the diversity encountered in clinical trials.
- These models can provide detailed functional data to accurately stratify conditions with clinically-relevant experiments.

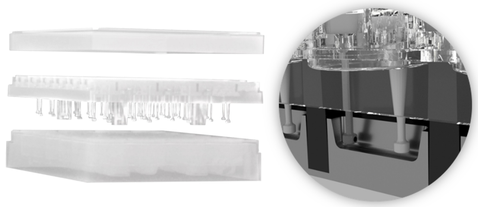
Our Objective – Demonstrate Their Utility

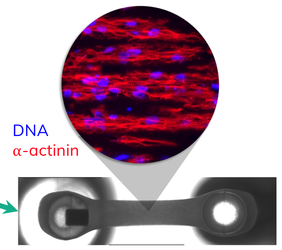
- Here, we develop isogenic lines of healthy (WT) and Duchenne Muscular Dystrophy (DMD) genotypes.
- We streamline the scalable fabrication and characterization of engineered tissues derived from these lines.
- We achieve histological and functional stratification of tissues, applicable in both skeletal muscle and cardiac contexts.

Scalable Fabrication and Functional Characterization of Engineered Muscle Tissues



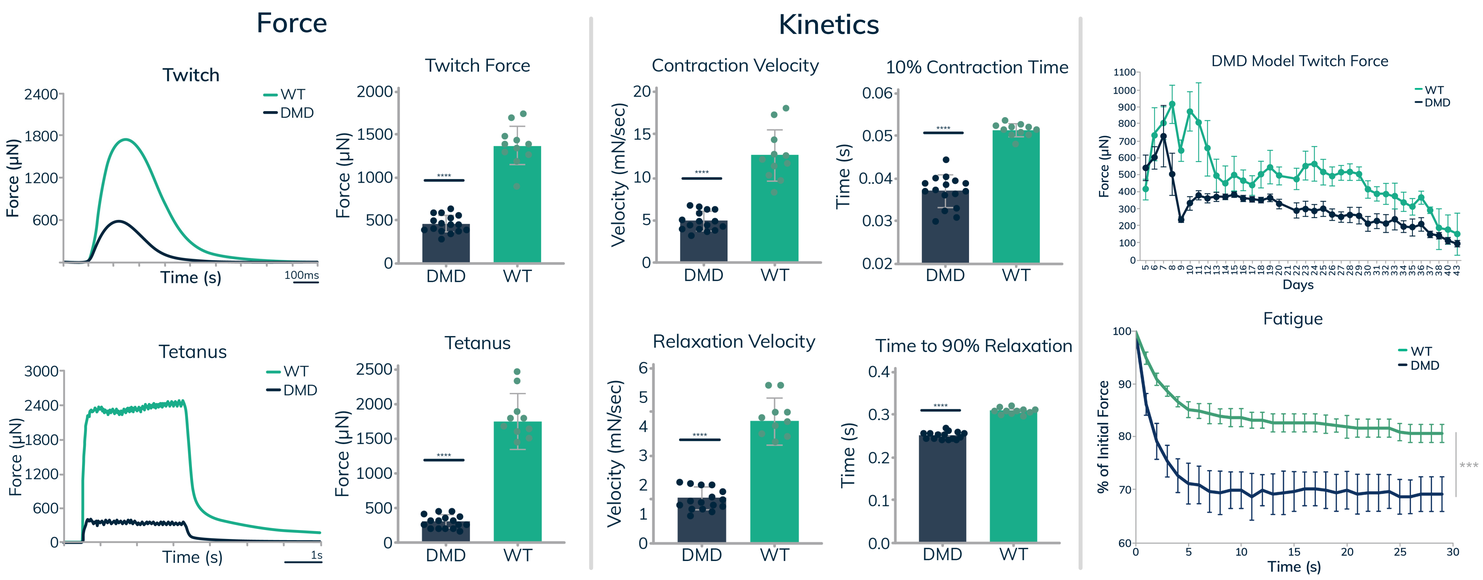
Cast Muscle | **Maintain 9+ Days** | **Obtain Striated Contractile Muscle** | **Measure Tissue Contractility**



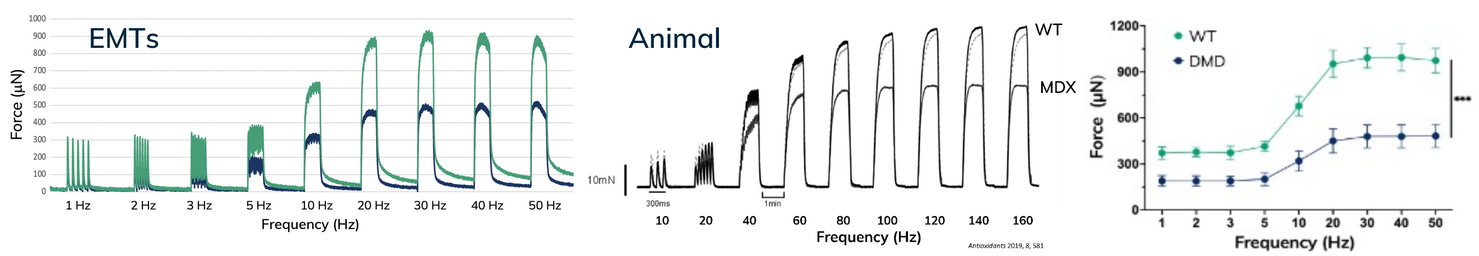


- Easy rapid casting.
- Over 95% casting success rate.
- Label-free tracking of 24 tissues.
- Well-based e-stimulation of 24 tissues.
- Clinically-Relevant protocols.
- Automated metric extraction.

Functional DMD Stratification – Skeletal Muscle

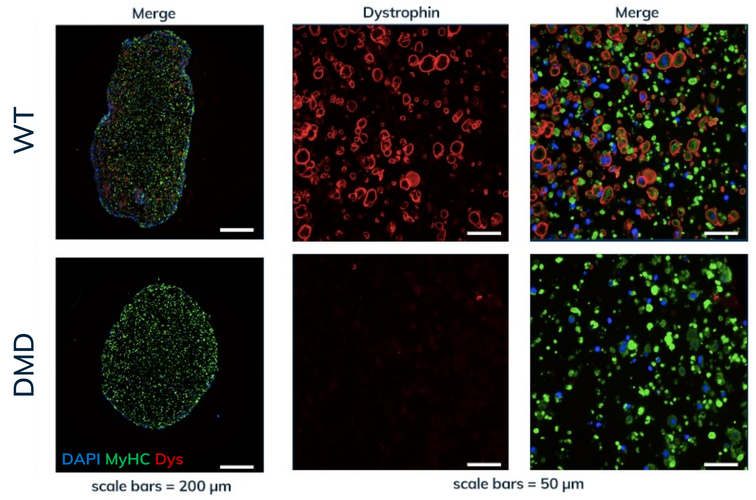


Force – Frequency Relationship



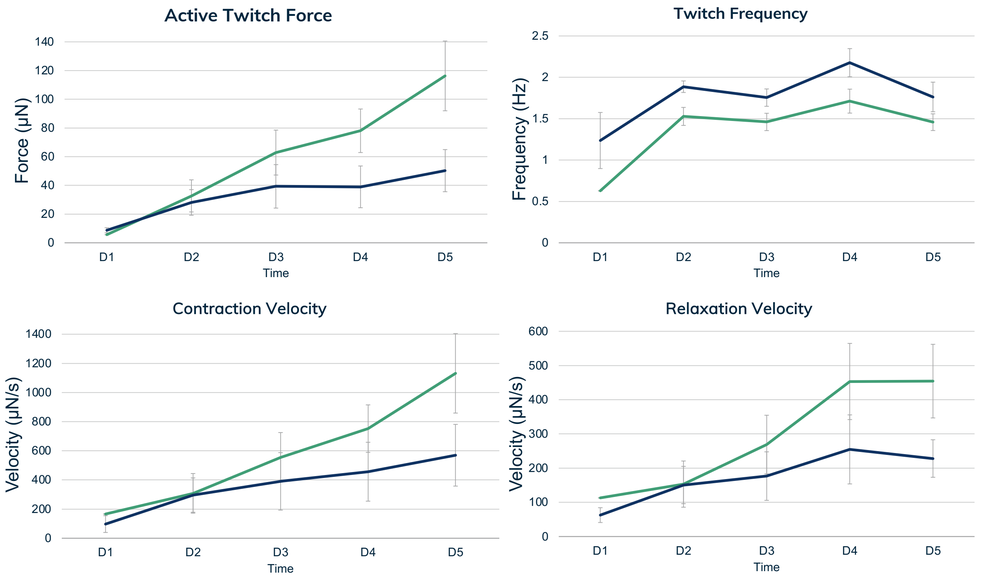
DMD Engineered Muscle Tissues (EMTs) exhibit significant contractile deficiencies during twitch and tetanic contractions compared to their isogenic WT counterparts. The deficiencies are characterized by reduced contractile force and slower contraction dynamics. The impaired contractility is maintained over an extended culture period of 35 days and is consistent across various stimulation frequencies, from isolated twitches to fully fused tetanic contractions. Additionally, the DMD phenotype demonstrates significantly accelerated fatigue rates when subjected to repetitive 1Hz twitch contractions for 30 seconds. These results recapitulate the clinical presentation of DMD, and data collected through standard animal tests such as electromyography, fatigue and grip tests.

Histological DMD Stratification - Skeletal



The WT tissues exhibit dystrophin localization on the myotubular membrane, whereas dystrophin is fully absent in DMD tissues.

Functional DMD Stratification – Cardiac



Preliminary results in cardiac tissues recapitulate the reduced contractile force and slower contraction kinetics observed in skeletal muscle tissues.