Mantarray 3D Engineered Heart Tissue Platform for In Vitro Pharmacological Screening and Disease Modeling of Duchenne Muscular Dystrophy

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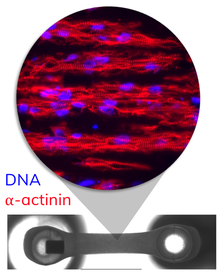
Problem

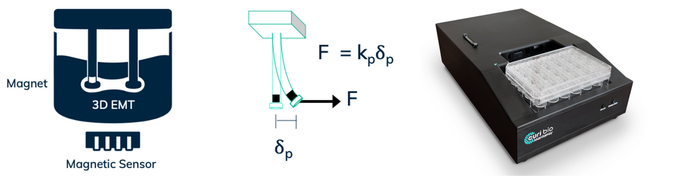
For cardiac and skeletal muscle diseases, direct assessment of contractile output constitutes a reliable metric to assess overall tissue function, as other 'proxy' measurements are poor predictors of muscle strength. 3D engineered muscle tissues (EMTs) derived from iPSCs hold great potential for modeling contractile function. However, the bioengineering strategies required to generate these predictive models are oftentimes out of reach for many investigators. Here, our goal was to design, build, and validate an EMT platform that uses 1) facile and scalable bioengineering approaches to generate tissues from a variety of cell sources, and 2) a label-free measurement technique capable of direct contractility assessment across several tissues in parallel.

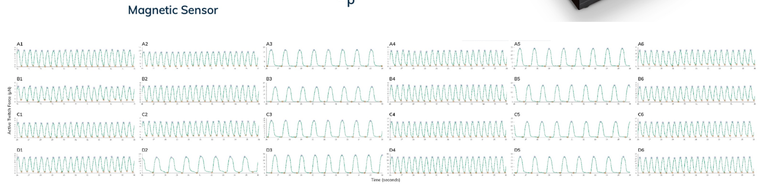


Approach

Tissue-specific Biosystem for Measurement of Tissue Contractility

The tissue casting approach uses chemically functionalized silicones, which improves EMT casting to >95% success rates across a variety of cell lines using both human and robotic fabrication approaches. The detection instrument (Mantarray) utilizes magnetic sensors that can measure contractility with good concordance to optical means. Sensors in the instrument detect the position of a magnet embedded in the flexible post of an EMT. As the tissue contracts and bends the post, the magnetic field changes in a linear and measurable fashion. The platform integrates individual, well-based control of electrical stimulation and is coupled with automated assessment of muscle contraction, providing an inclusive, high throughput platform to measure contractility across 24 tissues in parallel.

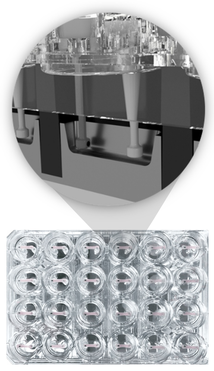




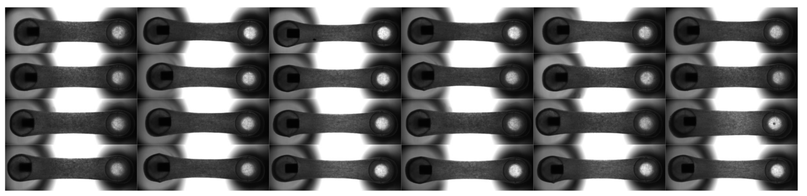
Electrical pacing data measured from 24 wells simultaneously (above).

Design

We have extended the single-well prototype to a 24-well, self-contained system that can be operated inside of a standard cell culture incubator. All 24 wells are measured simultaneously at a bandwidth of >100Hz. Measurements are collected via USB to a PC for analysis in custom-made software. Furthermore, we have designed a novel, modular casting system that facilitates the formation of EMTs. The design is compatible with robotic liquid handling systems. The casting trench is made of a chemically passivated silicone that prevents tissue binding. The posts are made of cell-compatible silicone that facilitates EMT binding. Posts can be removed and replaced with other designs to tune the system for specific forces or afterloads. Using this system, we have cast cardiomyocytes, skeletal myoblasts, and stromal cells using both iPSC and primary cell lines. In each case, casting success (measured as tissue survival to >7 days) is >95%. Tissue width and cross-sectional areas varied <8% across individual plates.

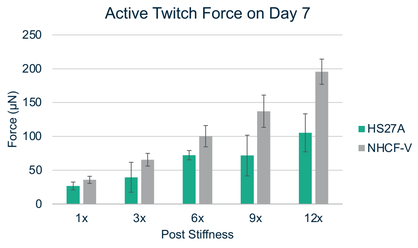


Engineered Heart Tissue Model

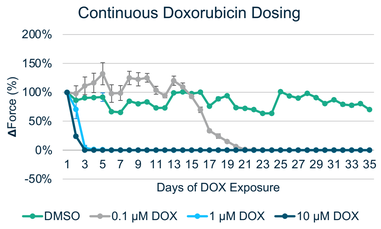


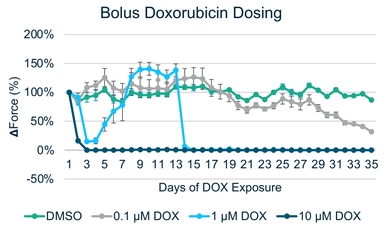
Engineered heart tissues (EHTs) consisting of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) and a supporting exogenous fibroblast population were constructed direct from thaw in a 24-well casting plate. Tissue compaction is rapid within the first 5 days of culture and EHTs begin to spontaneously contract with the first few days after casting.

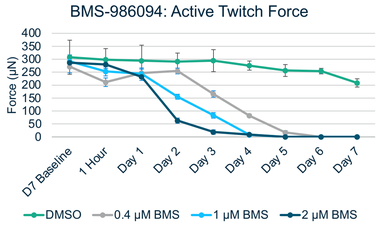
The flexible post stiffness can be modulated to simulate increasing afterload on the EHTs. In accordance with previous findings, increasing afterload promotes maturation of the EHTs. EHTs cast on 12x stiffness posts generate the most active twitch force. The source of the fibroblast population also plays a critical role in EHT function. Compared to bone marrow derived HS27A stromal cells, primary normal human cardiac fibroblasts from the left ventricle (NHCF-V) significantly improve tissue force generating capacity.

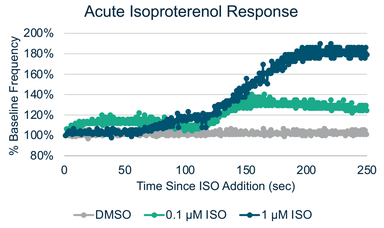


We tested the pharmacologic response of the EHTs by exposing the tissues to known cardiotoxic agents and beta-adrenergic stimulation.



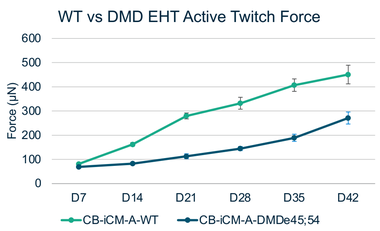


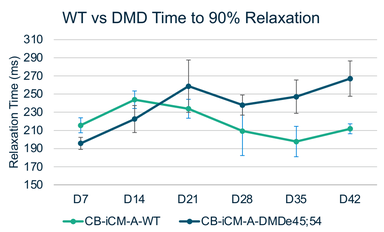




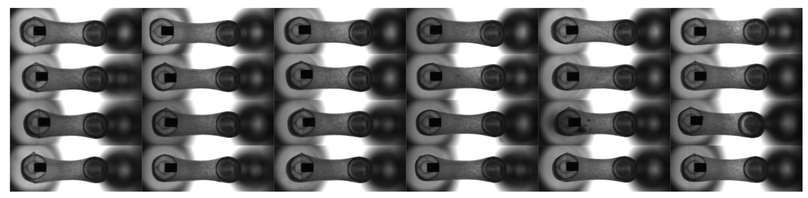
Doxorubicin and BMS-986094 both exhibit a dose-dependent reduction of EHT force generation. EHTs exposed to a 3-day bolus dose of doxorubicin, which better represents clinical exposure, showed a recovery of function at 1 μM but were unable to recover after a second bolus, indicating a compounding of tissue damage with repeated exposure. EHTs respond within minutes to isoproterenol exposure in a dose-dependent positive chronotropic response.

We investigated a Duchenne Muscular Dystrophy disease model using a WT and a dystrophin-null isogenic iPSC pair. Cardiomyocytes were derived from the isogenic pair and cast into EHTs. Compared to the WT control, DMD EHTs display a marked reduction in force production and slower relaxation kinetics with extended time in culture.



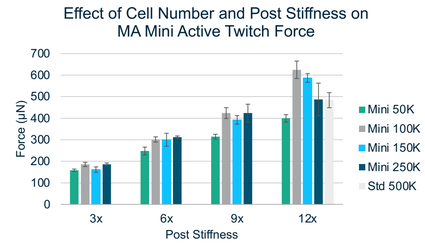


Mantarray Mini EHT Model

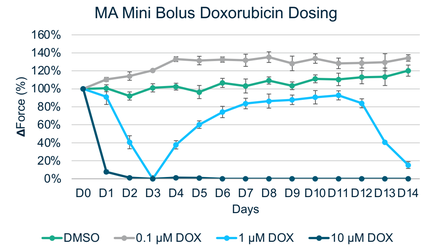


As a means to improve the throughput and efficiency of the EHT model, we developed a miniaturized casting system called Mantarray Mini (MA Mini). MA Mini EHTs are roughly 50% the volume of the standard sized EHTs but have equivalent functional characteristics.

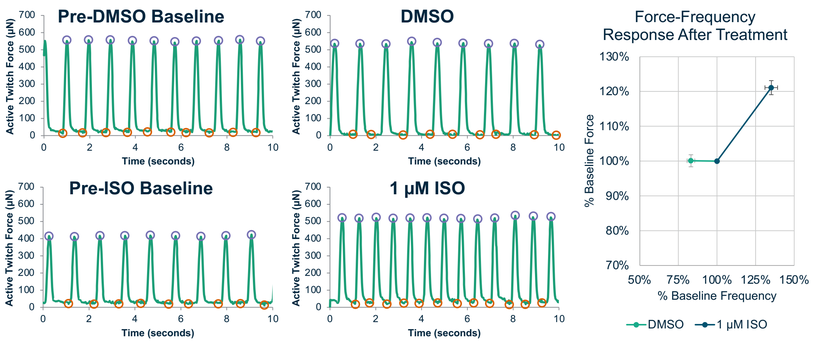
MA Mini tissues display the same enhanced maturation with increasing afterload as standard sized EHTs, with only 30% the number of total hPSC-CMs. MA Mini EHTs with 100K cells produced more force than standard sized tissues on the same post stiffness. This improved cell utilization efficiency allows for experiments with larger sample sizes and greater statistical power from the same pool of cells.



MA Mini EHTs also display the same pharmacological response to doxorubicin as full sized EHTs. Bolus doxorubicin dosing at 1 μM resulted in complete cessation of beating followed by a slow recovery phase. Higher 10 μM bolus dosing results in a total loss of function while lower 0.1 μM doses have no functional impact for the first 2 weeks.



MA Mini tissues also display a positive force frequency relationship in response to isoproterenol treatment. Compared to DMSO carrier control, which showed no change in force and a slight reduction in beat frequency, MA Mini EHTs exposed to 1μM isoproterenol significantly increased in both force and frequency. This positive force-frequency behavior, along with the expected response to doxorubicin and increasing afterload, highlight the functional utility of the MA Mini EHTs for drug screening and disease modeling applications.



Conclusion

EMTs are a promising 3D cell model for measuring contractility. Our casting device results in successful fabrication of EMTs across a variety of cell lines, including primary and iPSC lines. Our magnetic detection modality can replace optical contractility measurements and can be performed in a parallel fashion. This platform can be used to measure drug-induced contractile changes as well as to model contractile dysfunction in diseases.

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