A High-Throughput Contractility Assay for Human Cardiac Organoids: a Translational Platform for Cardiomyopathy and Drug Discovery

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SUMMARY

- Contractile dysfunction is a hallmark of **cardiomyopathies** that can be studied with hPSC-derived cardioids
- A **video-based method** was used to produce pixel displacement, **contraction, and velocity measurements** that can be used as proxies for **force readouts**
- We validated this model using **pacing frequency** and **isoproterenol** treatment to verify the pipeline's ability to track changes in frequency and contraction velocity
- This method meets the increasing demand for **high-throughput, large-scale, functional validation** in the study of cardiomyopathies at a **fraction of the cost** of current technologies

MODEL DEVELOPMENT

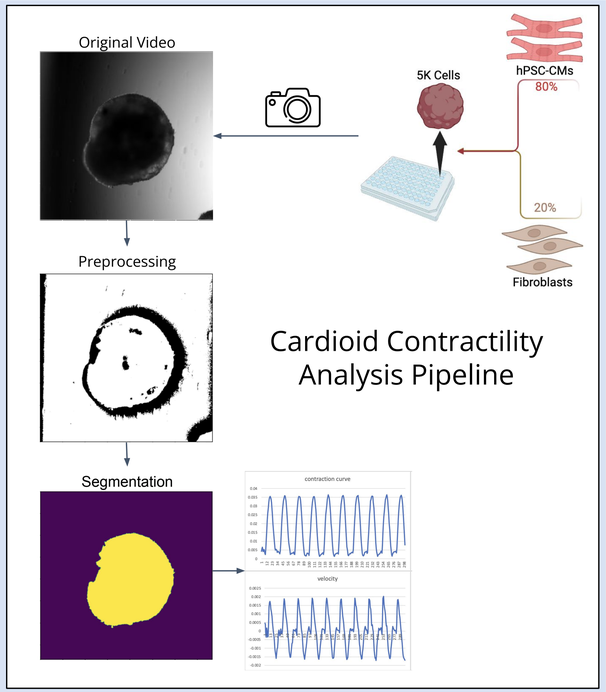

Figure 1. Process for generation of hPSC-derived cardioids (adapted from [1]), capture of cardioid videos, and steps of video-based analysis.

MODEL VALIDATION

Frequency-Dependent Contraction Measurements are Reliable and Reproducible

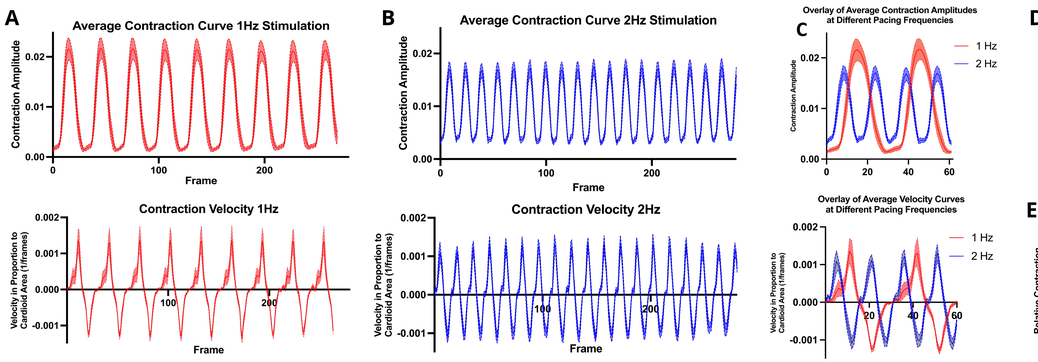

Figure 2. (A-C) Solid line indicates the average cardioid, shaded area indicates SEM. **(A)** Average contraction and velocity curves of cardioids paced at 1Hz. n=9. **(B)** Average contraction and velocity curve of cardioids paced at 2Hz. n=9. **(C)** Overlay of average contraction and velocity curves of 1Hz and 2Hz-paced cardioids. n=9. **(D)** Comparison of amplitudes of 1Hz and 2Hz paced cardioids. n=9, p=0.088 **(E)** Comparison of relative velocity between 1Hz and 2Hz-paced cardioids. n=8, $p_{contractile\ velocity}$=0.24, $p_{relaxation\ velocity}$=0.62.

Isoproterenol Treatment on Cardioids Recapitulates its Inotropic Effect on Contraction

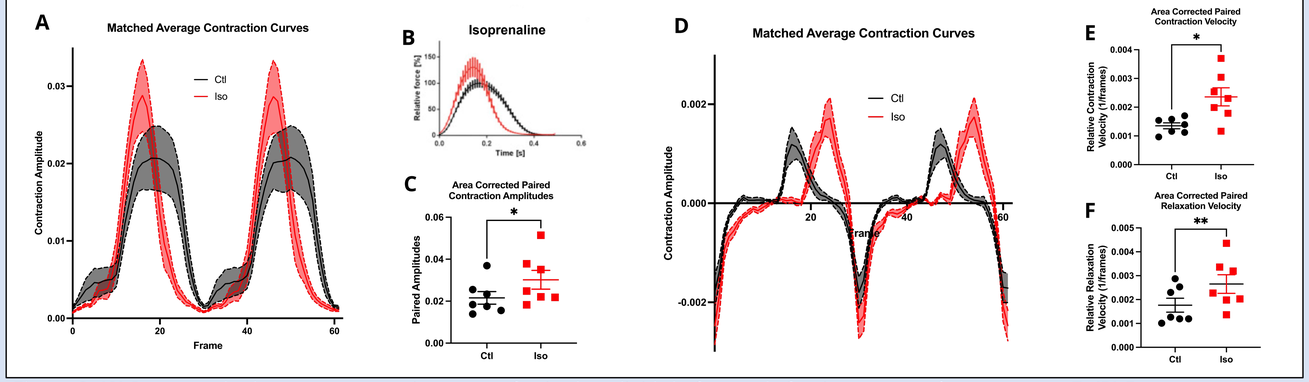

Figure 3. (A) Pace-matched average contraction curve of paired cardioids before and after isoproterenol treatment. n=7. **(B)** Force measurement of isoprenaline-treated & WT EHT tissue [2]. **(C)** Comparison of average amplitude between control and isoproterenol-treated cardioids. p=0.038, n=7. **(D)** Average velocity curve of paired cardioids treated with isoproterenol. n=7. **(E)** Paired comparison of contraction velocity between control and isoproterenol-treated cardioids. n=7, p=0.024. **(F)** Paired comparison of average relaxation velocity between control and isoproterenol-treated cardioids n=7, p=0.0042.

FUTURE DIRECTIONS

Knockdown of ML-Predicted Core Regulatory Gene for Phenotype Reversion of Dilated Cardiomyopathy

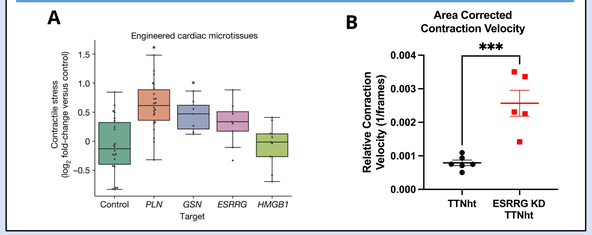

Figure 4. (A) Force measurement of ESRRG knockdown EHTs in a DCM phenotype line. p=0.069 [3]. **(B)** Relative contraction velocity of ESRRG knockdown in TTN-heterozygous cardioids. n=6 and n=5, p=0.00082

Potential Applications

- Develop mutant cardiods to model cardiovascular disease phenotypes such as dilated and hypertrophic cardiomyopathy.
- Compound screening using cardioids as a low-cost functional validation technique
- Integration of more than two cell types into biologic model to understand contractility in more complex contexts

HIGHLIGHTS

- High-throughput generation of hPSC-derived cardioids lead to reproducible contractility readouts
- Development of a custom analysis pipeline for analysis of cardioid contractility
- Model has been validated and can consistently reproduce contractility at different pacing frequencies and after isoproterenol stimulation
- This method replicates direction of effect of core regulatory gene knockdown in ESRRG as suggested by Geneformer.

REFERENCES

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