Recapitulating Hypertrophic Cardiomyopathy in MyoPod® 3D Human Engineered Heart Tissues: Calcium, Contractility, and Therapeutic Efficacy

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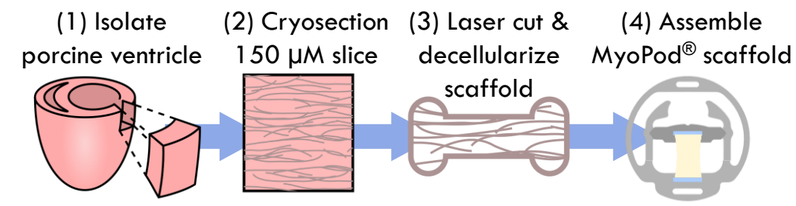
The Need for *in vitro* HCM Models

Hypertrophic cardiomyopathy (HCM), affects 1 in 500 people and over half of cases are linked to genetic mutations.[1-2] To develop effective therapeutics for HCM, it is critical to characterize the unique mechanism and phenotype of mutations. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) provide a means to study human cardiomyocytes in vitro, and CRISPR manipulation enables introduction of clinically relevant mutations. By incorporating these modified cells in 3D engineered heart tissues (EHTs), the functional profile of HCM mutations can be obtained and therapeutics can be assessed. In these studies, the contractile and calcium phenotype of EHTs made with the HCM-linked mutation, MYH7 R403Q[3], was characterized, and the dose response to acute therapeutic application was assessed.

MyoPod® Engineered Heart Tissues

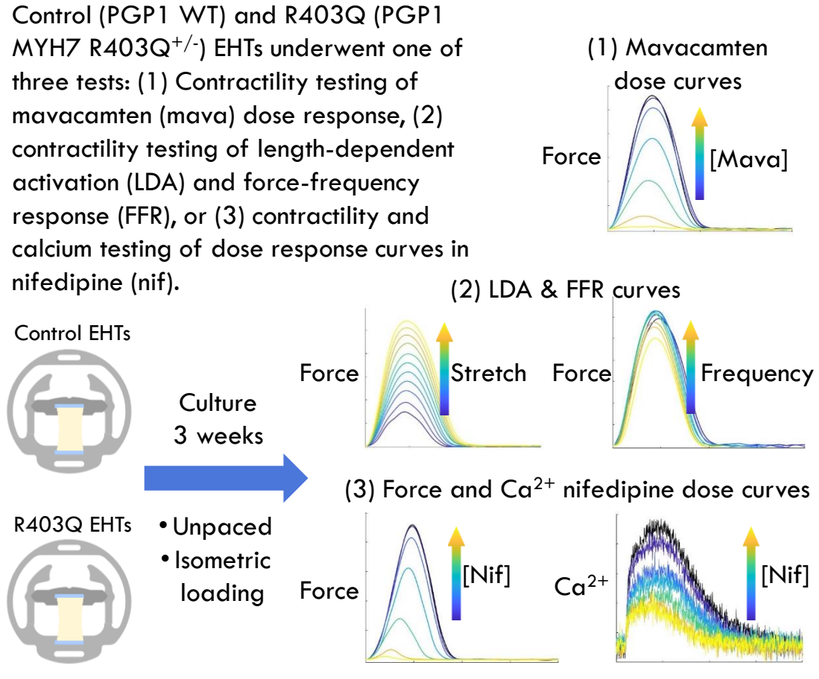
MyoPod® scaffolds are made from aligned, decellularized porcine left ventricular myocardium (steps 1-4).[4] MyoPods® are seeded with hiPSC-CMs and human cardiac fibroblasts (hCFs, PromoCell) at a 20:1 ratio to form EHTs (step 5). EHTs beat spontaneously in culture and are tested after 3 weeks.



(5) Seed MyoPod® EHT (scale bar: 2mm)

Study Design

Control (PGP1 WT) and R403Q (PGP1 MYH7 R403Q$^{+/-}$) EHTs underwent one of three tests: (1) Contractility testing of mavacamten (mava) dose response, (2) contractility testing of length-dependent activation (LDA) and force-frequency response (FFR), or (3) contractility and calcium testing of dose response curves in nifedipine (nif).



MYH7 R403Q$^{+/-}$ EHTs Possess HCM Phenotype

Baseline contractile behavior and the length and frequency dependent responses of EHTs with the MYH7 R403Q$^{+/-}$ mutation show a distinct HCM phenotype.

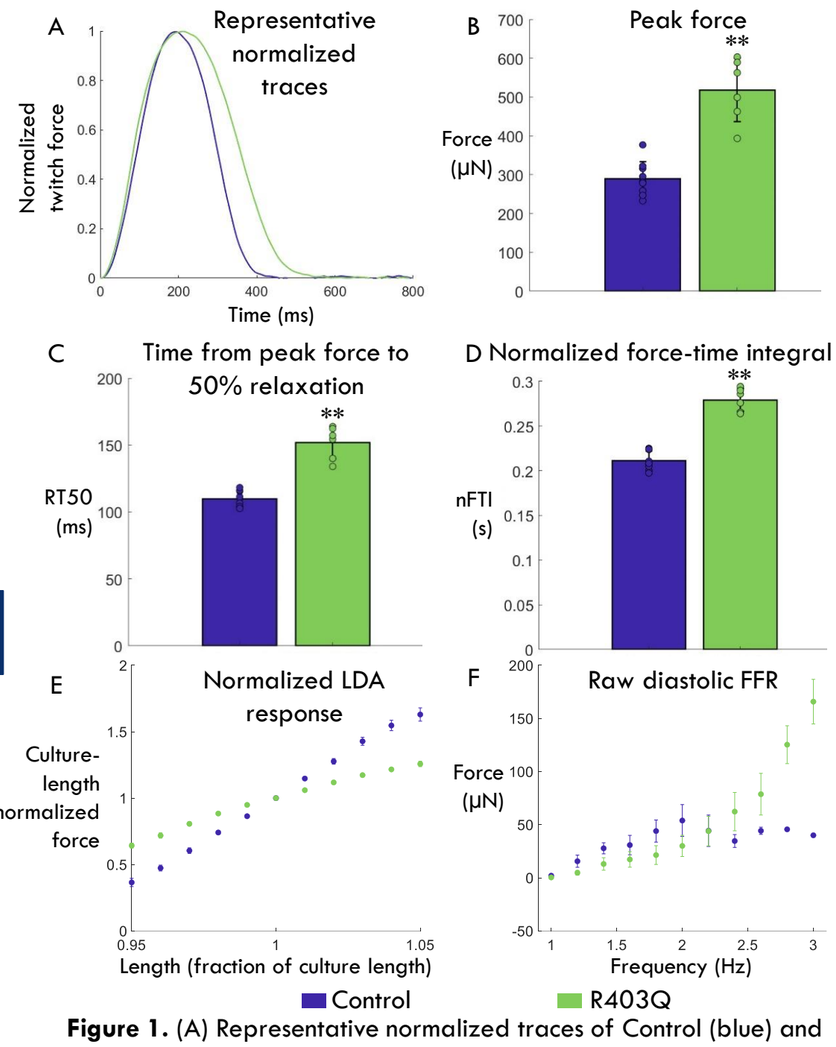


Figure 1. (A) Representative normalized traces of Control (blue) and HCM (green) EHTs at baseline. (B) Peak twitch force, (C) time from peak force to 50% relaxation (RT50), and (D) normalized force-time integral (nFTI) of EHTs at baseline. Error is standard deviation, **P<0.01 vs Control by Student's unpaired t-test. Control: n=9, R403Q: n=6. (E) LDA response of EHTs stretched from -5% to 5% of culture length (length=1). (F) Diastolic FFR of EHTs paced from 1 to 3 Hz. Error is standard error. Control: n=8, R403Q: n=4.

HCM Mutation Alters Sensitivity to Mavacamten

Sensitivity of EHTs to mavacamten was shifted rightward in EHTs with the MYH7 R403Q$^{+/-}$ mutation. (Control: n=8, R403Q: n=5; p<0.01).

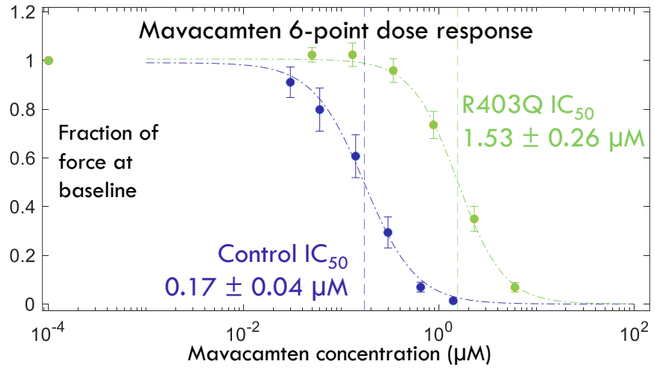


Figure 2. Hill fits of 6-point dose response curves in mavacamten in Control (blue) and R403Q (green) EHTs. Dotted vertical lines indicate IC_{50}. Error is standard deviation.

Calcium Handling is Unaltered in MYH7 R403Q$^{+/-}$ EHTs

EHTs with the MYH7 R403Q$^{+/-}$ mutation did not display altered calcium handling kinetics and had the same sensitivity to the L-type calcium channel blocker, nifedipine, as Control.

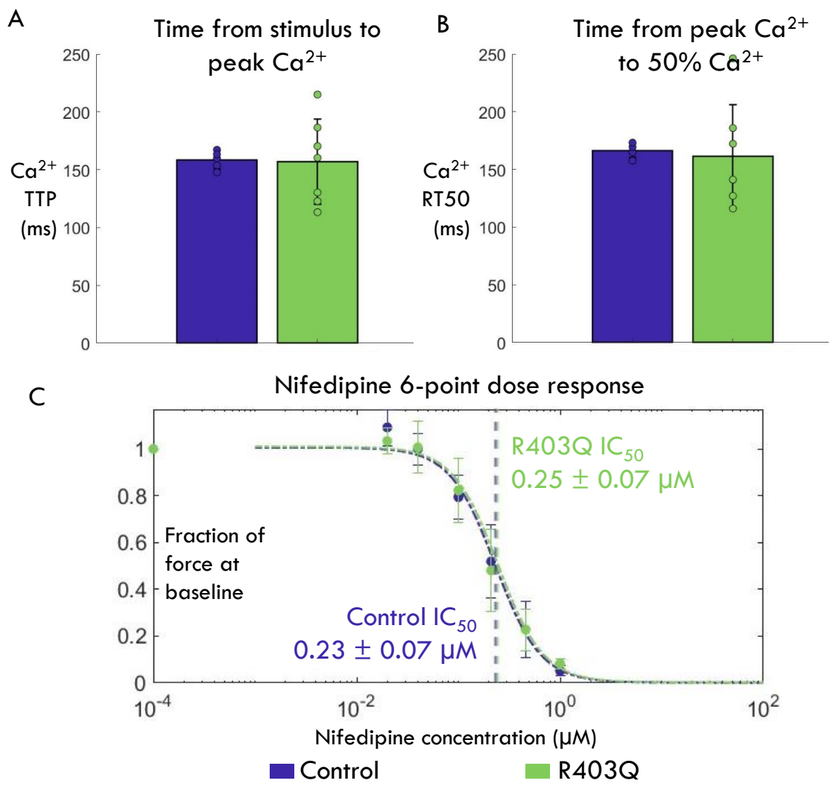


Figure 3. (A) Time from stimulus to peak Ca^{2+} transient amplitude and (B) time from peak to 50% Ca^{2+} transient amplitude of EHTs at baseline. Control: n=5, R403Q: n=6. (C) Hill fits of contractile response to 6-point dose curves in nifedipine. Control: n=4, R403Q: n=5. Error is standard deviation.

Conclusions

In this study, we analyzed the contractile and calcium phenotype of isogenic control and MYH7 R403Q$^{+/-}$ EHTs. Our results suggest:

1. R403Q EHTs possess a distinct contractile phenotype, exhibiting hypercontractility, slowed contraction kinetics, and a blunted LDA response (Fig 1).
2. R403Q EHTs have a decreased sensitivity to negative inotrope, mavacamten (Fig. 2).
3. Calcium handling at baseline and the response to L-type calcium channel blocker, nifedipine, is the same in isogenic control and R403Q EHTs (Fig. 3).

In summary, we demonstrate that Propria's 3D MyoPod® EHT model of the HCM-linked mutation MYH7 R403Q$^{+/-}$ recapitulates an HCM phenotype in vitro, providing a test bed for evaluating therapeutic efficacy.

References & Disclosures

[1]Lopes et al., J. Med. Genetics, 2013

[2]Mosqueira et al., Trends in Mol. Med., 2019.

[3]Geisterfer-Lowrance et al., Cell, 1990.

[4]Schwan et al., Sci. Rep., 2016.

Disclosures: All authors are equity shareholders and/or employees of Propria LLC. SC is faculty at Yale University.

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