Investigating the Impact of Increased Afterload on Hypertrophic Cardiomyopathy

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Background

Hypertrophic cardiomyopathy (HCM) is the most common inherited heart condition[1]. HCM is caused by mutations in genes encoding sarcomere proteins and leads to pathologic left ventricular hypertrophy and fibrosis. At the cell level, these mutations are linked to increased contraction, increased energy usage, and decreased relaxation[3]. The mechanisms linking sarcomere mutations to the extensive myocardial remodeling observed in HCM are not well understood. This gap in knowledge has limited the discovery of druggable targets for therapeutic development. HCM bears striking resemblance to pressure-overload hypertrophy induced by hypertension, and the sarcomere modulates activity of several mechanosensitive signaling pathways. Therefore, we hypothesize that mechanical stress may play an important role in the development of symptomatic HCM. The goal of our research is to determine the impact of biomechanical loading on HCM progression.

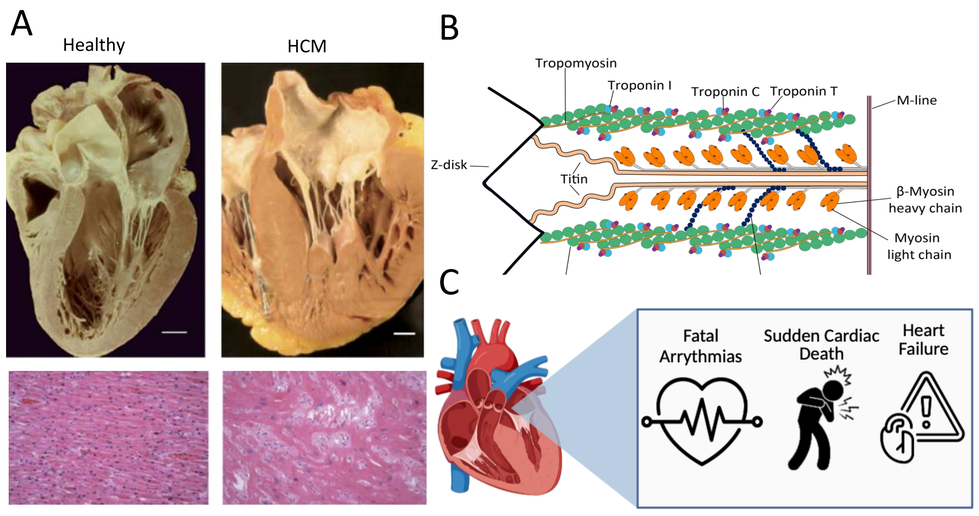

Fig 1. A. Explants and H&E staining of section from myocardium from a heart from a healthy donor and from a patient with HCM[2]. B. Sarcomere proteins, of which ~60% of HCM patients have a mutation in either β-myosin heavy chain (*MYH7*) or myosin binding protein C (*MYBPC3)*[2]. C. Clinical outcomes of HCM patients who suffer from ventricular hypertrophy.

Methods

To examine the impact of increased afterload on HCM in 2D and 3D, we engineered cardiac microtissues consisting of stem cell-derived cardiomyocytes (hiPSC-CMs) housing HCM-causing mutations or healthy (WT) hiPSC-CMs with ventricular cardiac fibroblasts (vCFs) and cultured them in a 3D device with tunable pillar stiffness to modulate applied afterload. Tissues were cultured for 7 days then harvested to quantify force production and metrics of fibrotic remodeling including collagen and α-smooth muscle actin.
We also assessed the impact of substrate stiffness on HCM cardiomyocytes, we seeded single cells onto polyacrylamide gels with elastic moduli ranging from 1-22kpa and performed traction force microscopy to determine single-cell force production.

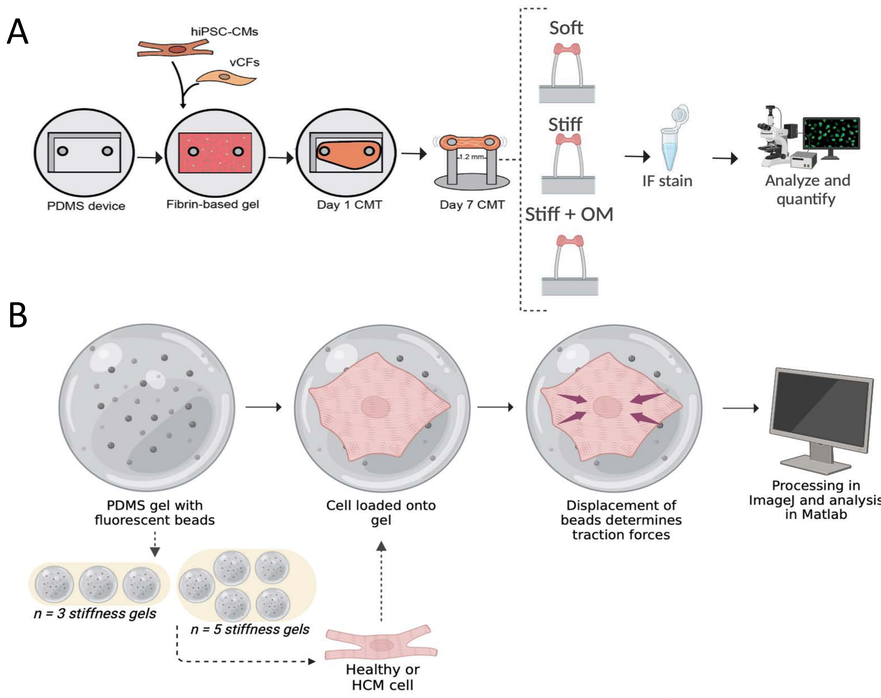

Fig 2. A. using hiPSC-CMs with a mutation in *MYBPC3* or a healthy WT control and healthy vCFs, we created 3D cardiac microtissues to study HCM *in vitro.* The platform can be tuned to modulate applied afterload via casting with PDMS of different base to crosslinker ratios. All tissues were cultured for 7 days before analysis. B. Healthy or mutant hiPSC-CMs were seeded onto polyacrylamide gels containing fluorescent beads with elastic moduli ranging from 1-22kPa. Videos of cell contraction were collected and bead displacement was used to calculate single cell contraction stress.

Increased Afterload Exacerbates Hypercontractility and in HCM Microtissues

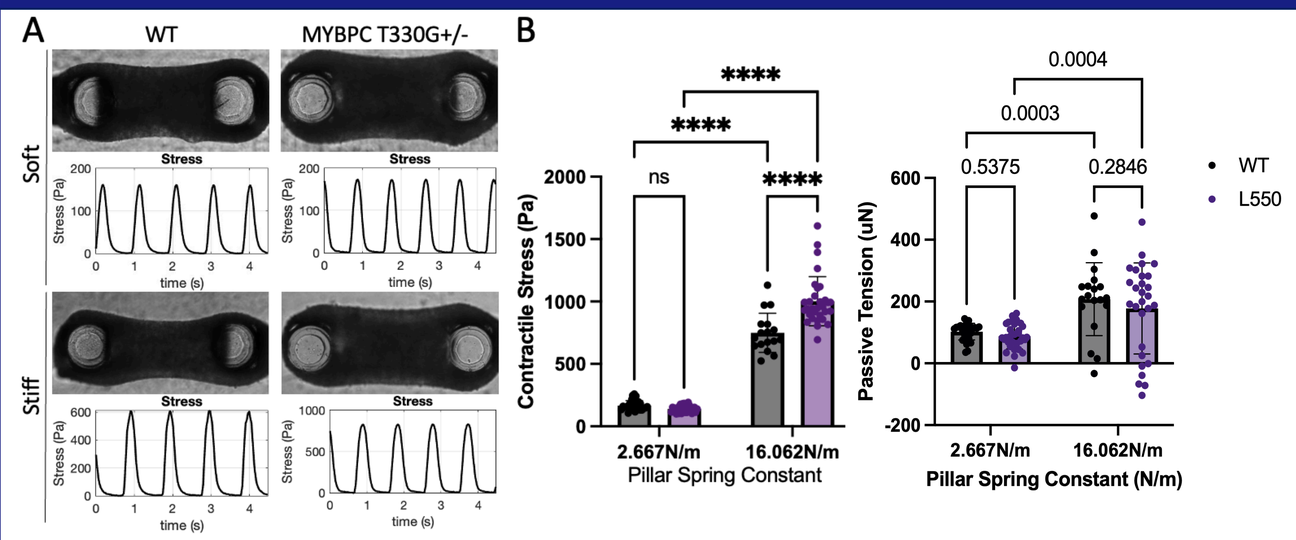

Fig 3. A. Brightfield images of microtissues with representative force traces, calculated from displacement of the two PDMS pillars. B. Contractile stress of microtissues. C. Passive tension of microtissues calculated by quantifying pillar displacement in diastole.

HCM hiPSC-CMs Exhibit Lower Tolerance for Substrate Stiffness

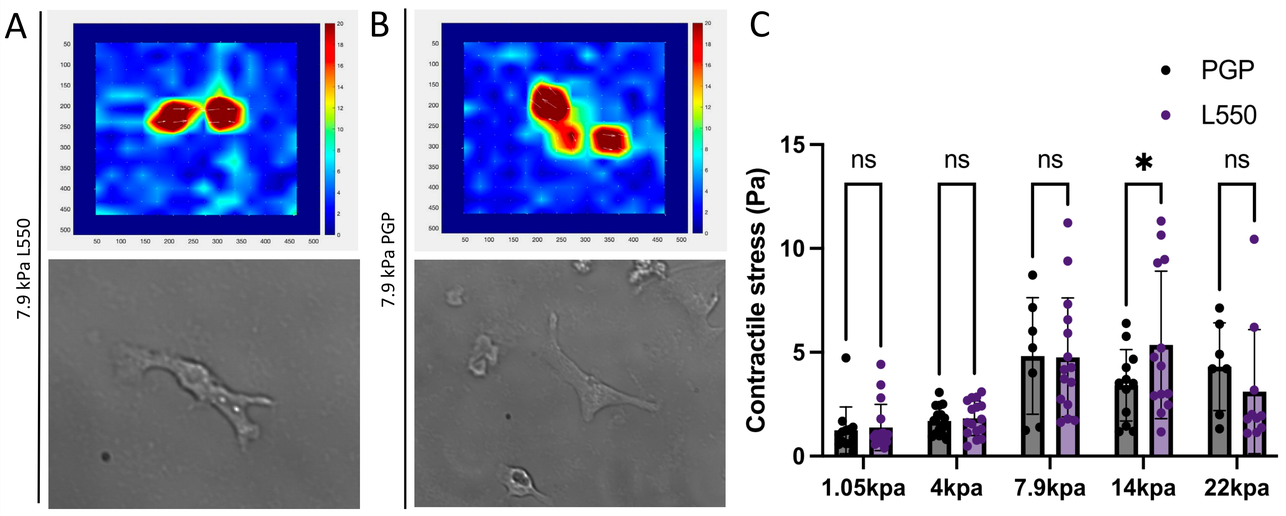

Fig 4. A. Force traces and brightfield images of healthy and mutant cells on 7.9kPa polyacrylamide gel substrates. B. Contractile stress of hiPSC-CMs, quantified from single-cell TFM on 1.05 kPa, 4kPa, 7.9 kPa, 14 kPa, and 22 kPa polyacrylamide gels.

Myosin Activity Alone Does Not Drive Fibrotic Response to Increased Afterload

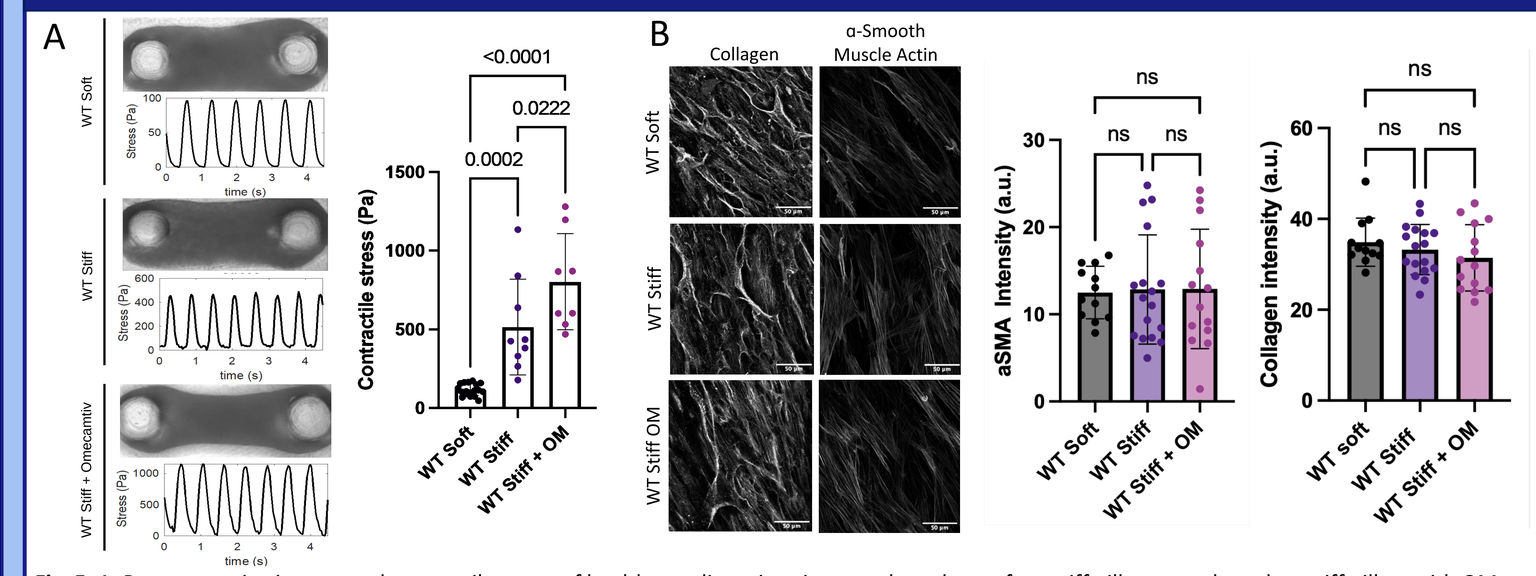

Fig. 5. A. Representative images and contractile stress of healthy cardiac microtissues cultured on soft or stiff pillars, or cultured on stiff pillars with OM. B. Maximum intensity z-projections of microtissues stained for collagen type I and αSMA. Collagen and αSMA intensity were quantified and compared between conditions.

Conclusions

In this work, we built on our previously developed model of HCM to show that HCM cardiomyocytes are hypersensitive to changes in afterload. We found that microtissues formed from hiPSC-CMs housing an HCM-causing mutation in MYBPC3 exhibit hypercontractility when exposed to increased afterload; however, there was no observed statistical difference between passive tension generated between HCM and healthy cell conditions. We found through single-cell traction force microscopy that HCM cells appear less tolerant to seeding on high substrate stiffnesses, showing peak traction force at a lower substrate stiffness than healthy cells. However, further experimentation will be needed to validate this conclusion. Based on our finding that increased afterload exacerbates hypercontractility in HCM microtissues, and building from previous data showing that when exposed to high afterloads HCM microtissues exhibit elevated levels of collagen type I and αSMA, we sought to understand whether increased myosin activity drives the HCM fibrotic response in high mechanical stress conditions. We found that increasing myosin activity with omecamtiv mecarbil augmented contractile stress but did not elevate markers of fibrotic activation in our tissues. Therefore, we posit that increased myosin activity alone is not sufficient to provoke fibrotic activation. Thus, a mechanism other than myosin activity, such as metabolic state, may contribute to stress observed in HCM cells. We hope that this work can provide insight into the impact of mechanical loading on HCM and contribute to the development of new HCM druggable targets.

References and Acknowledgements

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2. Teekakirikul et al. *Journal of Cell Biology* 2012
3. Seidman & Seidman. *Circulation Research* 2011

Figures created with BioRender.com

I would like to thank Miranda Wang for her guidance and ceaseless dedication to mentoring this project. I would also like to thank Xining Gao who has been instrumental in this research. Next, I extend my gratitude to Dr. Christopher Chen for supporting this research and introduction to biomedical engineering and mechanobiology. Thank you to all members of the Chen Lab and those at the Biological Design Center. Thank you to researchers at the Wyss Institute and all the members of CELL-MET for providing expertise in the field of cardiac tissue engineering.

NSF ERC EEC-1647837