The role of extracellular matrix protein type on human induced pluripotent stem cell-derived cardiomyocyte's sarcomere function (Analysis Plan)

Beth Pruitt, Erica Castillo, Carina Yuen, Varsha Raju, Department of Mechanical Engineering, University of California, Santa Barbara, Stanford University

Abstract: Abstract: To improve the treatment of cardiomyopathies, we aim to understand more about the developmental biology of cardiomyocytes. This summer, I helped create a plan to help determine the role of extracellular matrix protein type on human induced pluripotent stem cell-derived cardiomyocyte's sarcomere function. We will record videos of sarcomeres on three different substrates: fibronectin, laminin and matrigel. As protein composition of the heart changes through development and disease, we can learn about the effects of each through this. These videos will be analyzed by SarcTrack to determine the effects of the substrate on sarcomere contractility. We will then exclude any outliers and compare the mean values for the number of observations for each contractility parameter.

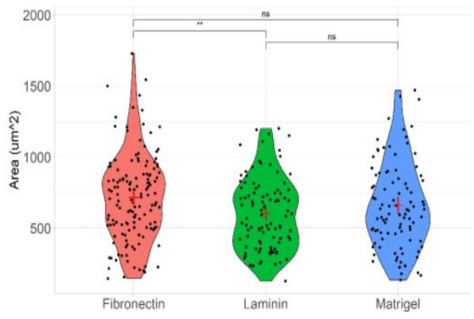
Background: Recapitulating In Vivo Heart Conditions

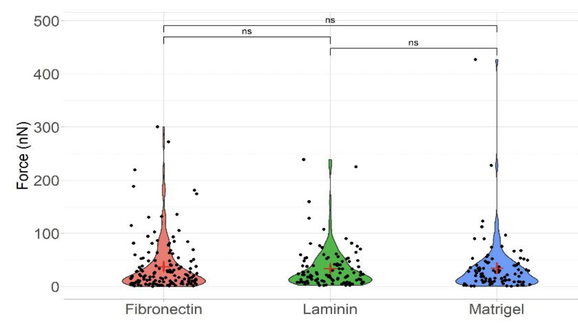
About 1 in 500 people inherit cardiomyopathies, heart disease related to the inability of the heart to circulate blood throughout the body. Our projects goal is to better understand the developmental biology of the cardiomyocytes. For some brief background, the myocardium is responsible for contracting and relaxing the heart, allowing for blood to be circulated throughout the body. Through the use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), we are able to study heart cells without the need to harvest the organ. Previous research by the Pruitt Lab has shown that extracellular matrix (ECM) proteins for cardiomyocytes change through development and disease and larger cell growth from different ECM proteins does not necessarily mean a larger contractile force created.

ECM protein	Embryonic	Fetal	Neonatal	Adult	Disease/injury
Fibronectin	■M (70)	■R (74) Fibronectin is abundantly expressed ↑	↓R (74) →M (70) Fibronectin is abundantly expressed	↓R (74) ↓	↑H, R, M (69, 76, 85, 87, 88) ↑
Elastin	■M (70)	1	→M (70)	■R (77)	→R (76, 77)

R = Rat
M= Mouse
H= Human

Castillo, et al.,, Annu. Rev. Biomed. Eng, 2020



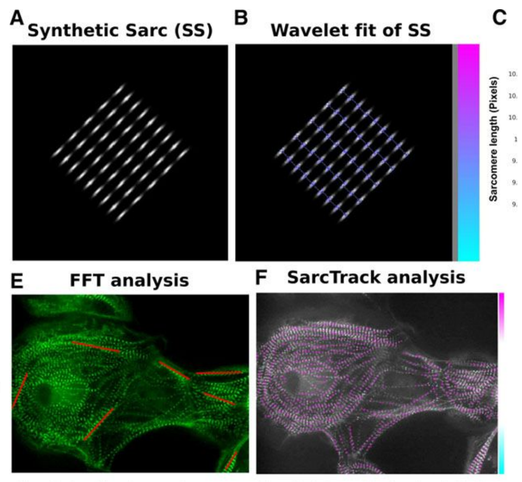


Given this information, our project aims to gain insight on how sarcomere contractility changes throughout development with upregulation of certain proteins. By observing the impact each protein has on contractility, we can hypothesized how contractility changes as a cardiomyocyte matures.

Known resources include the SarcTrack software (MATLAB based script that can analyze the parameters listed above)[2]. In addition, it is known that there ideal conditions such as parallel sarcomere alignment, and ideal length that impacts total force produced[3].

Experimental Design/Methods: Utilizing GFP Labeling and SarcTrack Software

First, we plan to prepare the sarcomeres by marking the titin, the protein on the Z-discs of the sarcomeres, with green fluorescent protein. Next we will place the sarcomeres on their respective substrates and collect videos with 100x objective fluorescent microscope with a framerate of at least 30 frames per second. These videos are inputted into SarcTrack, a MATLAB based software that analyzes contractility parameters of sarcomeres. SarcTrack uses a set of double wavelets to model each sarcomere, allowing it to process hundreds of sarcomeres in each cardiomyocyte. The program outputs three Excel Files: DWDists, DWStats, and DWPrdFrq . DWDists displays the length of different sarcomeres throughout the frames of the video, DWStats shows contraction time, relaxation time, and minimum and maximum wavelet distances for each sarcomere. We will place each cardiomyocyte on three different substrates: matrigel, fibronectin, and laminin. We will input our single-cell videos into the SarcTrack DWProcess Frame script first as a calibration step, and then into the DWProcess Folder to process all the frames.



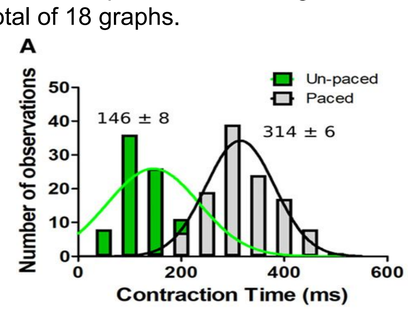
Data: Fixing Misreadings

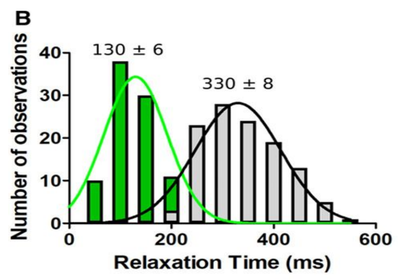
Though Sarctrack is effective at analyzing sarcomeres, it does occasionally output misreadings our outliers that we know are not physiologically possible (large differences in length). We hypothesize that these errors may come from low video resolution and can be minimized by ensuring high quality videos are collected.

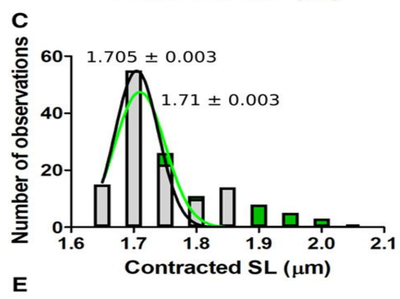
What are Patterns in Contractility Parameters?

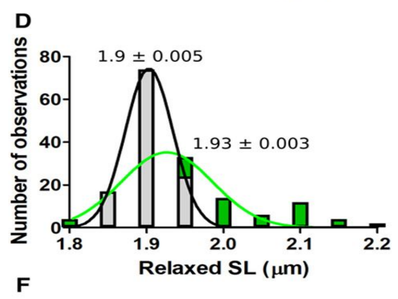
The Excel sheets output different parameters such as contraction and relaxation time, sarcomere percent shortening and relaxing that will be graphed into histograms. We will produce similar graphs to the ones shown below for each protein, creating a total of 18 graphs.

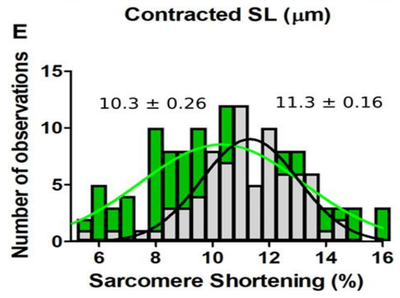
Toepfer, et al.,, Circulation Research, 2019

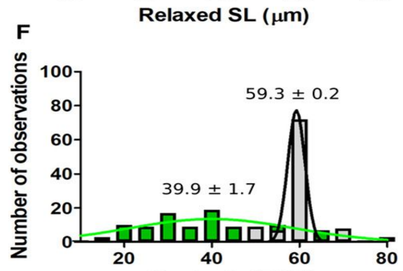












Each column represents a sarcomere

Sarcomere lengths for SarcTrack Sample Data Set (pixels)

36	11.8	12.2	12	12	13	12.2	11.6	11.4	12.2	11.8	13.4	13.4	13.4
37	11.8	12.2	12	12	13	12.2	11.6	11.4	12.2	11.8	13.4	13.4	13.4
38	12	11.4	12	12.2	12.4	12.4	11.4	11.4	11.6	13.4	13.4	13.4	12.2
39	11.6	11.4	13.4	12	12.6	13.4	11.4	11.4	11.4	13.4	13.4	13.4	13.4
40	11.6	11.8	12.2	11.8	13	12.2	11.4	11.4	11.4	13.4	13.4	13.4	11.8
41	12	11.4	13.4	13.4	13.2	12.2	11.4	11.4	11.2	13.4	13.4	13.4	12.4
42	12	11.4	13.4	13.4	13.2	12.2	11.4	11.4	11.2	13.4	13.4	13.4	12.4
43	11.6	11.4	12.2	12.6	12.8	12.4	11.6	11.6	11.6	13.4	13.4	13.4	13.4
44	11.6	11.4	13.4	12	13.2	12.4	11.4	11.4	11.4	13.4	13.4	13.4	12.2
45	11.4	11.4	13.4	12.2	13.2	13.4	11.6	11.4	11.8	13.4	13.4	13.4	13.4

Sample1_DWDists

Each row represents a frame within the video

Key Observations and Future Research:

Once we are able to collect data physically in the lab (September 2021), we will use this proposed method of data analysis to interpret results and explore the following questions.

How does sarcomere contractility change throughout cardiomyocyte development? Because protein composition changes throughout development, we can take note of how the sarcomeres behave depending on varying substrates with the ultimate goal of learning more about the developmental biology of the sarcomeres.

Are there more, misaligned sarcomeres through aging and disease? Though data still needs to be collected for our current project, once we understand the effects that certain ECM proteins have on sarcomere contraction, we aim to further explore what is actually happening with sarcomere count and alignment using the software ImageJ. If we notice that certain substrates lead to slower or faster contraction times, beat rate, or contracted and relaxed length, we can confirm our hypothesis that larger cell growth may lead to more, but misaligned, sarcomeres, resulting in the same amount of force being produced.

Acknowledgments: I would like to thank Dr. Beth Pruitt, our principal investigator, for giving me the amazing opportunity join her lab. I would also like to thank Erica Castillo and Cheavar Blair for being incredibly helpful and supportive mentors throughout this process. Special thanks to Dr. Samantha Davis,Timnit Kefela and everyone at CSEP for funding and giving me this opportunity to be a part of EUREKA. Last but not least, I would like to thank my peers Varsha Raju, Joseph Campos, and Janae Gayle for their help throughout the project.

References

[1]Castillo, et al.,, Annu. Rev. Biomed. Eng, 2020
[2]Lieber, Ward, Skeletal muscle design to meet functional demands. Philos Trans R Soc Lond B Biol Sci. 2011
[3]Toepfer, et al.,, Circulation Research, 2019