UNIVERSITY OF CALIFORNIA,
IRVINE

From Understanding How Perturbations Affect Cardiac Tissue Function to Training the Next Generation of Researchers

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Chemical and Biomolecular Engineering

by

Jasmine Naik

Dissertation Committee:
Associate Professor Anna Grosberg, Chair
Assistant Professor Elizabeth Read
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2021

DEDICATION

To my parents and sister who have supported me and watched me grow. I wouldn't have made it this far without knowing you always have my back no matter how far I fall or where I travel to. To my friends who have lent an ear when and lifted me when I needed it most. To Ashwin who stuck with me through the hard times and kept reminding me how far I had come. And to the friends who became family on the west coast.

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B.S, Chemical Engineering, Rowan University, Glassboro NJ, 2015
Bantivoglio Honors Concentration
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RESEARCH EXPERIENCE

Graduate Student Researcher, University of California Irvine, 2015-Present
- Studied cardiac tissue stress strain properties and the effects of pollution on them
- Performed primary cell harvests to extract cardiac tissue from neonatal rats
- Studied structure of heart tissue to replicate for *in-vivo* work
- Mentored students on lab protocols

Research Assistant, Rowan University, 2014-2015
- Researched miscelles, nanoparticles and hydrogels for novel drug delivery system
- Developed and optimized drug delivery system for targeted therapy

National Science Foundation Research Fellow in Bioprocess Engineering, Virginia Technical Institute 2014
- Created vaccine for use against PRRSV virus affecting swine population
- Synthesized, collected and purified proteins to yield virus-like particles

Research Assistant, Rowan University, 2013-2014

- Determined best E. coli strain to optimize the production of ethanol for renewal biofuel source

National Science Foundation Research Fellow in Micro/Nano-Structured Material, Auburn University, 2013

- Designed, built and tested microfluidic device for uniform emulsion creation
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TEACHING EXPERIENCE

Pedagogical Fellow, University of California, 2019-Present

- Develop and improve pedagogical teaching skills
- Streamlined workshops for incoming teaching assistants to maximize class time
- Facilitate program to prepare incoming teaching assistants on duties and responsibilities

CardioStart Program Coordinator, University of California, 2018-Present

- Design and implement curricula teaching high school students tissue engineering
- Mentor students to become biomedical engineers through hands on experiments
- Adapt curricula for online learning environment
- Constructed budget for program supplies and tuition cost

Tutor – High school chemistry, biology, geometry, algebra, 2015-Present

- Create problem sets for students to establish conceptual learning
- Reinforce lab experiments through laboratory write-up assignments
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Teaching Assistant, University of California Irvine, 2015-Present

Courses: Momentum Transport, Biotransport Phenomena, Thermodyanmics, PCR lab

- Lead discussion sections utilizing active learning to solve problems
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- Improve skills through continuous student feedback

Teaching Assistant, Rowan University, 2014-2015

Courses: Energy Balances, Biology

- Lead discussion sections through problem solving
- Created lesson plans for students to apply class lectures
- Conducted small group discussions to promote collective learning

LEADERSHIP EXPERIENCE

E-Board Chemical Engineering Graduate Student Association, 2016-2020
University of California Irvine
Positions: Vice President, Faculty Liaison, Activity Coordinator

- Coordinated plans between president, faculty and students
- Planned multiple events throughout the academic year
- Provided student input into departmental changes and budgets for the year

Graduate InterConnect Peer Mentor, 2016-2019
University of California Irvine

- Guided incoming international students by providing important information before arrival at the University of California Irvine
- Facilitiated small meetings with students related to academics
- Fostered discussions to promote well-being in a new culture

Engineers without Borders Introductory Engineering Coordinator, 2013-2014
Rowan University

- Created lesson plans and experiments focused on students aged 6-12
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PUBLICATIONS

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Naik, Jasmine; Lundqvist, E., Grosberg, A (2019). "Analyzing Cellular Architecture and Contractile Forces using Angled Parquet Tiles." Poster presented at the National Society of Biomedical Engineering Conference.

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American Society of Engineering Education
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CERTIFICATIONS

Improv for Teaching Certificate 2018

- Improved teaching skills through improvisation techniques
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Mentor Excellence Certificate 2016

- Constructed foundation for mentoring other students of diverse backgrounds

Excellence in Public Speaking Certificate 2016

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ABSTRACT OF THE DISSERTATION

Consequences to Cardiac Function from Perturbations such as Structural Variations and COVID-19 Cytokines

by

Jasmine Naik

Doctor of Philosophy in Chemical and Biomolecular Engineering

University of California, Irvine, 2021

Professor Anna Grosberg, Chair

Heart disease remains the number one cause of death in the world leading many researchers to pursue fundamental research in understanding heart structure, cardiac disease, and healing. But even as more research is performed, patient treatment options are still limited and do not fully restore heart function. To provide better treatment options to patients, researchers have focused on understanding the structure of the heart and its relationship to cardiac output. As a pump providing nutrients to the body, the heart's structure is highly organized across multiple length scales ranging from the 3D organ, down to the force producing units inside cardiomyocytes, sarcomeres. Due to interactions between multiple, complex feedback mechanisms, understanding the structure-function relationship is not trivial. In this dissertation, we aimed to understand how perturbations such as structural changes and inflammatory cytokines affect force generated by cardiac tissue. By employing tools such as microcontact printing and muscular thin films, we were able to mimic 2D cardiac tissue structure and measure the force these tissues produced. Via the creation of novel tissues organized at different length scales, we began to determine whether the size of cellular organization affects the force these tissues can produce. We were also able to design

experiments to determine the effect that inflammatory cytokines have on force production. Even with these advances, many mysteries remain, leaving decades of work to be done as heart disease becomes more prevalent due to an aging population. As a result, more scientists and engineers will be needed to take on research roles to provide better treatment options to patients. Therefore, to encourage a new generation of students, a high school tissue engineering summer program, CardioStart, was created in the hopes of inspiring students to pursue degrees in STEM. An in-person summer program was modified to create an online course with the aims to increase student access and engagement. While the in-person program included hands-on activities to supplement presentations, students learned the same material through the online platform overall knowledge at the end of the program was comparable. Furthermore, the online platform provided more accessibility to students with five-fold the enrollment rate over the in-person program. In the future, this program can be expanded by working with other researchers in different fields for students to further their knowledge. With this increased knowledge, more students would be interested in pursuing STEM and taking over research roles to further fundamental cardiac research. This would include further studies in understanding cardiac remodeling and incorporating more complexity back into the simplified systems used in this dissertation.

Chapter 2 : Introduction

Heart disease is the number one cause of death in the world, but despite the great need for treatments there are still many questions left unanswered in heart disease progression and healing [1-3]. Indeed, more than $2 billion is spent on cardiovascular research annually and is expected to increase as the world's population continues to age; however, this is insufficient compared to funding for other disease research in order to generate better treatment options [4, 5]. While treatment options exist to reduce and prevent cardiovascular symptoms, they are still limited as none cure heart disease [4, 6]. The biggest hurdle to providing a cure is recreating native heart tissue since it cannot repair itself once damaged, thus leading researchers to pursue tissue engineering as a possible solution [2, 3, 7]. To better help these patients, more research needs to be conducted in the field of cardiac tissue engineering, which will require recruiting more researchers to assist.

The field of cardiac tissue engineering begins with understanding the heart's complex structure and its important functions in daily life. The heart is an essential organ in the human body as it pumps blood to peripheral tissues to provide oxygen and nutrients. Its main structure consists of 4 chambers, 2 atria and 2 ventricles, which act as pumps. The ventricle walls are thicker than those of the atria as the ventricles' contractions pump blood to the lungs and body whereas the atria are receiving chambers [8]. The walls of the ventricles contain many layers of 2 dimensional tissue sheets comprised of aligned cardiomyocytes. These aligned cardiomyocytes are able to produce a uniaxial force due to the crystalline structure of their contractile unit, the sarcomeres [9]. Sarcomeres are comprised of a highly organized thin actin filament and a thick myosin filament which glide

over one another causing cardiomyocytes to shorten or contract [9-11]. Due to this intricate organization at a multi-scale level, the heart's structure plays an important role in its ability to function, thus making understanding this relationship a vital goal in cardiac tissue engineering.

The heart's organized structure is of importance as diseased cardiac tissue has a different structure than healthy tissue leading to a decline in cardiac function. Understanding this structure-function relationship is vital as many perturbations such as structural variations or cytokines can affect cardiac function due to its effect on cardiac structure [6, 10]. To begin to unravel the structure-function relationship, methods for measuring cardiac stress have been developed. Traction force microscopy (TFM) is useful to measure the force an object exerts on a surface when it adheres or moves. When cardiomyocytes contract, bead displacement is tracked, thus measuring the force a single cardiomyocyte produces when contracting [12-14]. However, using deformations to calculate force requires specific equipment and deformations may not fully describe force generation [14, 15]. Another method utilizes micropillars, instead of beads, to measure traction forces of single cardiomyocytes [15]. However, these techniques are difficult when measuring stresses of full tissues [14]. To better understand forces generated in a tissue, muscle strips can be employed [16]. A muscle strip can be excised from the heart wall, the papillary muscle, vessel ring, or trachea wall. It is then suspended in a bath with a force transducer attached, which allows for direct measurement of developed force [17]. This technique is ideal for measuring force produced by muscle fibers, but fibers cannot be altered to mimic changes encountered in a diseased tissue which is vital for understanding how changes in organization affect force production. Because muscle strips are not sufficient to relate organizational changes in heart

tissue to force produced, heart on a chip devices were examined. Unlike previous methods mentioned, muscular thin films are used to calculate the force of engineered tissues [17, 18]. This is achieved using an elastic thin film that acts as a cantilever where the deformations can then be used to calculate the radius of curvature at every time point [17]. The force produced is observed by monitoring deformations of the elastic thin film and the stress is then calculated based on film deformations [17-19]. This tool is useful to study how various perturbations effect the heart's ability to contract. While uncovering how these perturbations will contribute to understanding heart disease, there are still many research questions that will remain unanswered that require more research to be done in the decades to come.

In the first half of this dissertation, we aimed to discover how structural variations in cardiac tissue and how the addition of cytokines to cardiac tissue affect the heart's function. Structural variations in cardiac tissue occur in many cardiovascular diseases. As cardiomyocytes are damaged, the myocardium alters in attempts to fix itself by forming scar tissue and rearranging cell alignment [2, 3, 7, 20]. To understand the fundamentals of how rearrangement of cells affects tissue force generation, chapter 2 details how heart-on-a-chip assays were utilized in combination with engineered cardiac tissues to elucidate the relationship between the local organization of cardiac cells in a tissue and the force produced by the tissue [17, 18, 21]. This will yield a better understanding of how local organization of cardiac cells directly affects global tissue force production. Along with structural changes, cytokines have also been thought to alter cardiac function [22, 23]. This has been especially apparent in hospitalized COVID-19 patients presenting with heart failure [24, 25]. Although the mechanism behind COVID-19 and heart failure is not yet known, a proposed mechanism

involves an immune cascade causing a cytokine storm [25-27]. Through the utilization of muscular thin film assays, chapter 3 elucidates a connection that can be made between cytokine concentration and cardiac stress generation. While we have uncovered some of the relationships between cellular reorganization and cytokine effects on heart functionality, there are still many unknowns in how to best overcome these diseased states and the need for researchers in this field is of utmost importance.

While expanding the field of cardiac tissue engineering is important in understanding the heart; researchers share another role in disseminating knowledge to inspire the next generation of scientists as well as to educate the public on important findings. For the next generation to become interested in STEM fields to fill the need for job growth, more scientists and engineers need to make their research more approachable to students. This has become more crucial than ever as biomedical engineering jobs are predicted to grow steadily in the future [28-30]. Previously, STEM exposure has been achieved through afterschool and summer programs run through local schools, community centers and universities [31-33]. While these programs exist, engineering programs are scarce, costly, and are unable to provide students an in-depth knowledge of all fields due to the breadth of each field [34-37]. Specifically, biomedical engineering programs are designed to cover a wide variety of topics during the summer to expose students to the entire field and while this is great for some students, many others would like to explore each topic in greater detail [33, 38]. Therefore, as a second part of this dissertation, we present a pedagogical approach to provide more students the opportunity to learn about tissue engineering. CardioStart, a high school tissue engineering summer program, underwent a variety of iterations to determine the most robust program conducive to student learning. The program had to be cost effective while

engaging students and therefore, students' responses and overall learning were accessed to determine the most efficient program. CardioStart will continue to educate students about the field of tissue engineering as new and innovative ideas are required to answer challenging questions remaining in understanding cardiac diseases.

In combination, the dissertation details both cardiovascular research with aims to understand more about the relationship between structure and function of the heart as well as a pedagogical approach to inspire the next generation to contribute to the field of cardiac tissue engineering.

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Chapter 3 : Understanding Cardiac Remodeling Through Parquet Tissues

Abstract

Organization within the heart is extremely important as the 3D organ is composed of 2D sheets that are comprised of cardiomyocytes that house the force contractile units of the heart, sarcomeres. It has been shown that any change to the organization greatly impacts the force generated by the heart, leading to detrimental effects to a patient's heart. To better understand the relationship between cellular organization and function, we created parquet tissues that are organized locally at a scale of 100 μm while the tissue organization at a scale of 1 millimeter remained varied. This local organization scale mimics spontaneously generated local organization in isotropic tissues. By measuring the tissue organization through a muscular thin film assay, we were able to compare tissue organization and function. By calculating a single model parameter for a previous empirical model, we were able to determine that the small parquet tiles resemble isotropic tissue stress generation more so than aligned tissue. More parquet tissues with different sizes of local organization should be tested to create a more robust model to better understand the role local organization plays in stress generation. This will then lead to a better understanding of the relationship between cardiac structure and function.

Introduction

The heart is organized across many length scales ranging from 2D sheets that wrap around one another creating the 3D organ to the force producing unit within cardiac cells, sarcomeres [1-3]. This multiscale organization is vital to create a uniform, coordinated

electrical signal and an efficient unified heart contraction [4, 5]. Heart disease that causes remodeling of this architecture has detrimental effects on patient's heart function causing arrhythmogenesis and decreased cardiac output [1, 2, 6]. However, due to highly complex feedback mechanisms between cellular organization and force generation, relationships between them have not been fully elucidated [7, 8]. Therefore, one of the standing challenges in the field is to understand how the organization of the sarcomeres at different scales affects tissue force generation.

To unravel the relationship between cardiac tissue structure and function, many platforms have been developed to measure cardiac stress from patterned tissues [1, 2, 4]. One such platform, a muscular thin film assay, measures the force of a cardiac tissue that can be engineered to align the cells through microcontact printing [1, 9]. Previously, this assay was used to create a simple empirical force model relating actin orientational order on a millimeter scale to the force produced [10]. This was demonstrated by engineering tissues to be organized at a length scale of 250 μm while the overall tissue organization (1 mm scale) was varied [10]. In retrospect, the fact that isotropic tissues produced significantly less force than that predicted by the empirical model was surprising because isotropic tissues also have spontaneous patches of organization [10, 11]. The reduction in force could be explained by either the fact that isotropic tissue patches are three times smaller than those tested by the original model or it could be due to a fundamental difference resulting from spontaneous tissue formation.

By considering the local size of organization in isotropic tissues, we hypothesize that the area of local organization determines the net force production potential of the tissue.

Thus, we aimed to create a new pattern in which the local area of organization remained constant at $1x10^4$ μm^2 while keeping the global tissue area of $1.5x10^7$ μm^2 unorganized. The force generated by these tissues were then measured using a muscular thin film assay and compared to the previous empirical model. Through understanding the fundamentals of cardiac cell alignment and net force generation at smaller scales, we hope to improve current models and gain insight into the consequences of cardiac remodeling.

Materials and Methods

Substrate Fabrication

For structural studies, a large cover glass (Brain Research Laboratories) was sonicated in 95% ethanol for 30 minutes and incubated in a 60°C oven for 30 minutes. PDMS (polydimethylsiloxane, Ellsworth Adhesives, Germantown, WI) was made using a 10:1 base/curing agent. Cleaned coverslips were then spun coat in the PDMS and cured overnight in a 60°C oven. Once cured, the cover glass was cut into individual coverslips using a diamond scriber (Musco Sports Lighting, Oskaloosa, IA) based on custom template for 12-well culture plates.

For functional studies, coverslips were created as described by Grosberg et al. [9]. Briefly, a large cover glass (Brain Research Laboratories) was sonicated in 95% ethanol for 30 minutes and incubated in a 60°C oven for 30 minutes. The cleaned cover glass was then covered with protective film (static cling; Grafix Plastics, Cleveland, OH), which was cut according to custom templates to provide 1 cm strips of exposed cover glass. Next, a 1 mg/ml solution of PIPPAm (poly(*n*-isopropylacrylamide); Polysciences, Warrington, PA) was created by dissolved 1 gram of PIPPAm in 10 ml of 1-butanol (Macron Fine Chemicals, Center Valley,

PA). The PIPPAm solution was then spincoated onto the protected cover glass and placed in an 60°C oven overnight to cure. The remaining protective cling was removed and the entire cover glass was then spun coat in PDMS that had been cured at room temperature for five hours. The cover glass was then allowed to cure overnight in a 60°C oven. Lastly, the cover glass was cut into individual coverslips using a diamond scriber (Musco Sports Lighting, Oskaloosa, IA) based on custom template for 12-well culture plates.

Extracellular Matrix Patterning

Patterns were created in Adobe Illustrator (Adobe Systems, San Jose, CA). Stamps were made to by 1.5 cm by 1.5 cm to produce extracellular matrix lines of 20 μm wide with 5 μm gaps between lines to produce an anisotropic tissue. Parquet patterns were designed to produce 100 μm by 100 μm squares of 20 μm wide with 5 μm gaps. Each square had a unique orientation to control the tissue organization globally. Patterns were generated to range from isotropic tissues to anisotropic tissues.

The Illustrator designs were then etched into a 5" by 5" chrome with soda-lime glass mask by a third-party vendor (FrontRange Photo Mask, Lake Havasu City, AZ). The glass masks were then used to make silicon wafers via SU-8 deposition in the Bio-Organic Nanofabrication Facility (University of California – Irvine). Once the wafers were made, PDMS was poured onto the wafers to create stamps. To microcontact print, a similar method to that used by Tan et al was utilized [12]. Briefly, the stamps were sonicated for 15 minutes in 95% ethanol and were dried in a biosafety cabinet using compressed nitrogen to maintain a sterile environment. Next, stamps were coated with 0.025 mg/mL concentration of fibronectin (FN) (Sigma-Aldrich, St. Louis, MO) and were left to incubate for one hour. After

the incubation period, extra FN was removed using compressed nitrogen and stamped onto 8-minute UV treated (Jelight Company, Irvine, CA) coverslips coated in PDMS. The stamped coverslips were then submerged in 1 mg/ml solution of Pluronics (5g Pluornics F-127, dissolved in 500 ml of sterile water, Sigma-Aldrich, St. Louis, MO) for 5 minutes. Coverslips were then rinsed with room temperature PBS (phosphate-buffered saline; Life Technologies, Carlsbad, CA) three times.

Isotropic tissues were created by incubating a 8-minute UV treated PDMS coated coverslips with a 200 μL drop of 0.025 mg/mL concentration of FN for 10 minutes. The coverslips were then rinsed three times with room temperature PBS.

Myocyte harvest, seeding and culture

Neonatal rat ventricular myocytes were isolated from 2-day-old neonatal rats (Charles River Laboratories, Wilmington, MA) [13]. Briefly, ventricular tissue was removed in sterile conditions and placed in Hanks balanced salt solution (HBSS; Life Technologies, Carlsbad, CA) to rinse the tissue. Ventricular tissue was then incubated in 1 mg/mL trypsin solution (Sigma-Aldrich, St. Louis, MO) dissolved in HBSS at 4°C for 12-14 hours overnight. The trypsin solution was then removed and tissue was neutralized in warmed M199 culture medium (Invitrogen, Carlsbad, CA) supplemented with 10% heat inactivated fetal bovine serum, 10 mM HEPES, 20 mM glucose, 2 mM L-glutamine (Life Technologies, Carlsbad, CA), 1.5 μM vitamin B-12 and 50 U/mL penicillin (Sigma-Aldrich, St. Louis, MO). Without disturbing the tissue, media was removed. The tissue was then dissociated with 1 mg/mL collagenase dissolved in HBSS over multiple washes. The collagenase cells were then centrifuged at 1200 rpm for 10 minutes. The supernatant was then aspirated, and cells were

resuspended in cold HBSS before re-centrifugation at 1200 rpm for 10 minutes. Supernatant was then aspirated, and cells were resuspended in warmed 10% M199.

Cells were purified through a series of 3 consecutive pre-plates of 50, 50 and 45 minutes in cell culture flasks (Fisher Scientific, Waltham, MA) and were seeded at a density of 450,000 cells per 2 mL.

24 hours after seeding, coverslips were washed three times with warmed PBS to remove dead cells. After washing, warmed 10% M199 media was added and coverslips were placed back in the incubator. 24 hours later, 10% M199 was replaced with warmed 2% M199 media.

Contractility experiments

Heart-on-a-chip experiments were completed four days after cell seeding. Experiments were conducted in a warmed normal Tyrode's solution of 5mM HEPES (Arcos Organics, Thermo Fischer Scientific, Bridgewater, NJ); 1 mM magnesium chloride (Santa Cruz Biotechnology, Dallas, TX), 5 mM glucose, 1.8 mM calcium chloride, 5.4 mM potassium chloride, 135 mM sodium chloride, and 0.33 mM sodium phosophate (Sigma-Aldrich, St. Louis, MO). First, seeded coverslips were placed into a 60 mm petri dish with warmed normal Tyrode's solution. Then, coverslips were cut using a razor to create thin films as previously described in Grosberg et al. [9]. The coverslips were previously cooled to below 37 °C to dissolve the PIPPAm and release the films from the surface of the coverslip, thus releasing the thin films. Coverslips were then transferred to a 35 mm petri dish containing warmed normal Tyrode's solution. This 35 mm petri dish was placed inside of an INUL-MS2 stage top incubator (Tokai Hit, Fujinomiya-shi, Shizuoka-ken, Japan) for temperature control. A MyoPacer field

stimulator (IonOptix, Milton, MA) was then used in conjunction with customized electrodes attached to the 35 mm petri dish to pace the films with 15-30 volts at 1-3 Hz.

Contractility experiments were acquired on a model no. SZX-ILLB2 Stereoscope (Olympus America, Center Valley, PA) mounted with a model acA1300-200μm camera (Basler, Exton, PA). Five second video clips were recorded for each coverslip and analyzed using a custom ImageJ and MATLAB software as previously described in Grosberg et al [9]. Briefly, film shortening was tracked for each thin film where systole (film shortest) and diastole (film longest) were recorded. Each video was labeled with initial film length. Active stress was calculated and defined as the difference between systole and diastole for each film.

To accurately calculate muscular thin film contractility, cell and substrate thickness were taken into account [14]. Cell thickness was averaged across multiple coverslips for each tissue type and used in the data analysis. Substrate thickness was measured for each chip preparation using a DektakXT profilometer (Bruker, Tucson, AZ).

Fixing and Immunostaining

Warm 4% paraformaldehyde (Fischer Scientific, Hanover Park, IL) supplemented with 0.001% Triton-X-100 (Sigma-Aldrich, St. Louis, MO) in PBS was used to fix cells. Once fixed, cells were washed three times in warmed PBS.

Cells were immunostained for actin (Alexa Fluor 488 Phalloidin; Life Technologies, Carlsbad, CA), sarcomeric α-actinin (Mouse Monoclonal Anti-α-actinin; Sigma-Aldrich, St. Louis, MO), nuclei (4',6'-diaminodino-2-phenylinodole (DAPI; Life Technologies, Carlsbad, CA), and FN (polyclonal rabbit anti-human fibronectic, Sigma-Aldrich, St. Louis, MO). Secondary staining was done using tetramethylrhodamine- conjugated goat anti-mouse IgG antibody (Alexa

Fluor 633 Goat Anti-Mouse, Life Technologies, Carlsbad, CA) and tetramethylrhodamine-conjugated goat anti-rabbit IgG antibody (Alexa-Fluor 750 Goat Anti-Rabbit, Life Technologies, Carlsbad, CA).

Imaging and image analysis

Fixed and stained coverslips were imaged on an IX-83 inverted motorized microscope (Olympus America, Center Valley, PA) mounted with a digital charge-coupled device camera ORCA-R2 C10600-10B (Hamamatsu Photonics, Hamamatsu City, Japan) using an UPLFLN 40x oil immersion objective (Olympus America, Center Valley, PA). To acquire images for one coverslip, 10 fields of view were acquired. ImageJ was then used to process images while custom MATLAB software (The MathWorks, Natick, MA) was used to analyze orientational order parameter previously described by Grosberg et al [9] and Feinberg et al [1].

Statistics

One-way ANOVA with the Tukey Test were used to determine differences between anisotropic, parquet and isotropic tissue stresses as well as confluency between parquet types. Significance was considered for an unadjusted p-value of less than the critical level. Non-linear regression dynamic fitting was done using the empirical force model to determine the 95% confidence intervals of both the isotropic and aligned models.

Results

Tissues were patterned using 100 μm patterns in the same orientations as the larger parquet tiles, or sharp angle parquets. These tissues did not follow the predicted z-line OOP; indeed, the z-line OOP values had no trend as the sharp angle parquet pattern became

more globally aligned (Figure 3-5). The sharp angle parquet tiles also yielded a stress lower than that of the isotropic tissues and showed no pattern due to cells not fully following the pattern (Figure 3-5 and 3-6). Because the above data suggested that the cells were not able to form confluent monolayers on sharp angle small parquet tiles, new 100 μm patterns were generated with more gradual angle changes (Figure 3-1). Confluency of tissues was compared between the sharp angle parquets and gradual angle parquets; the difference in confluency explained why the sharp angle parquet tissue stress was low (Figure 3-7). The gradual angle parquets templates (Figure 3-1 A-C (i)) include more angles to guide the cells appropriately into the smaller areas (Table 3-1), thus leading to a higher confluency (Figure 3-7). The microcontact fibronectin pattern (Figure 3-1 A-C (ii)) follows the template pattern and the cells orient themselves to fit into the pattern (Figure 3-1 A-C (iii)). To quantify this alignment, orientational order parameters (OOP) were calculated for both Actin (Figure 3-1D) and Z-line (Figure 3-1E). The actin OOP was predicted based on the stamped pattern as actin OOP get very close to perfectly organized. The predicted z-line OOP was calculated based on the assumption that z-lines always have some disorganization and therefore used the pattern OOP and co-orientational order parameter (COOP) calculated for isotropic tissues, $COOP_{iso}$ [13]. The COOP value was calculated using previously acquired actin and z-line organization data from isotropic tissues [10, 11]. The OOP of each individual parquet pattern was then multiplied by the $COOP_{iso}$ value to better predict the relative levels of z-line organization for the parquet patterns. The OOP measured for z-lines follows the predicted OOP values, thus the cells are following the pattern correctly unlike the sharp angle parquet tissues. The OOP measured for actin does

follow the predicted line, and the slight deviations could be explained by measurements of actin found in fibroblast cells instead of cardiomyocytes and were expected in any tissue.

Table 3-1: Sharp angle vs Gradual Angle patterns and global OOP values

Parquet Type	Angles	Global OOP
Sharp Angle Pattern	45-90-45-0	0
	10-50-90-130-170	0.1
	30-60-90-120-150	0.38
	40-65-90-115-140	0.53
	50-70-90-110-130	0.68
	60-75-90-105-120	0.81
	80-85-90-95-100	0.98
Gradual Angle Pattern	10-30-50-70-90-110-130-150-170	0.05
	30-45-60-75-90-105-115-130-145	0.3
	40-50-65-75-90-105-115-130-140	0.47
	50-60-70-80-90-100-110-120-130	0.64
	60-65-75-80-90-100-115-125-130	0.79

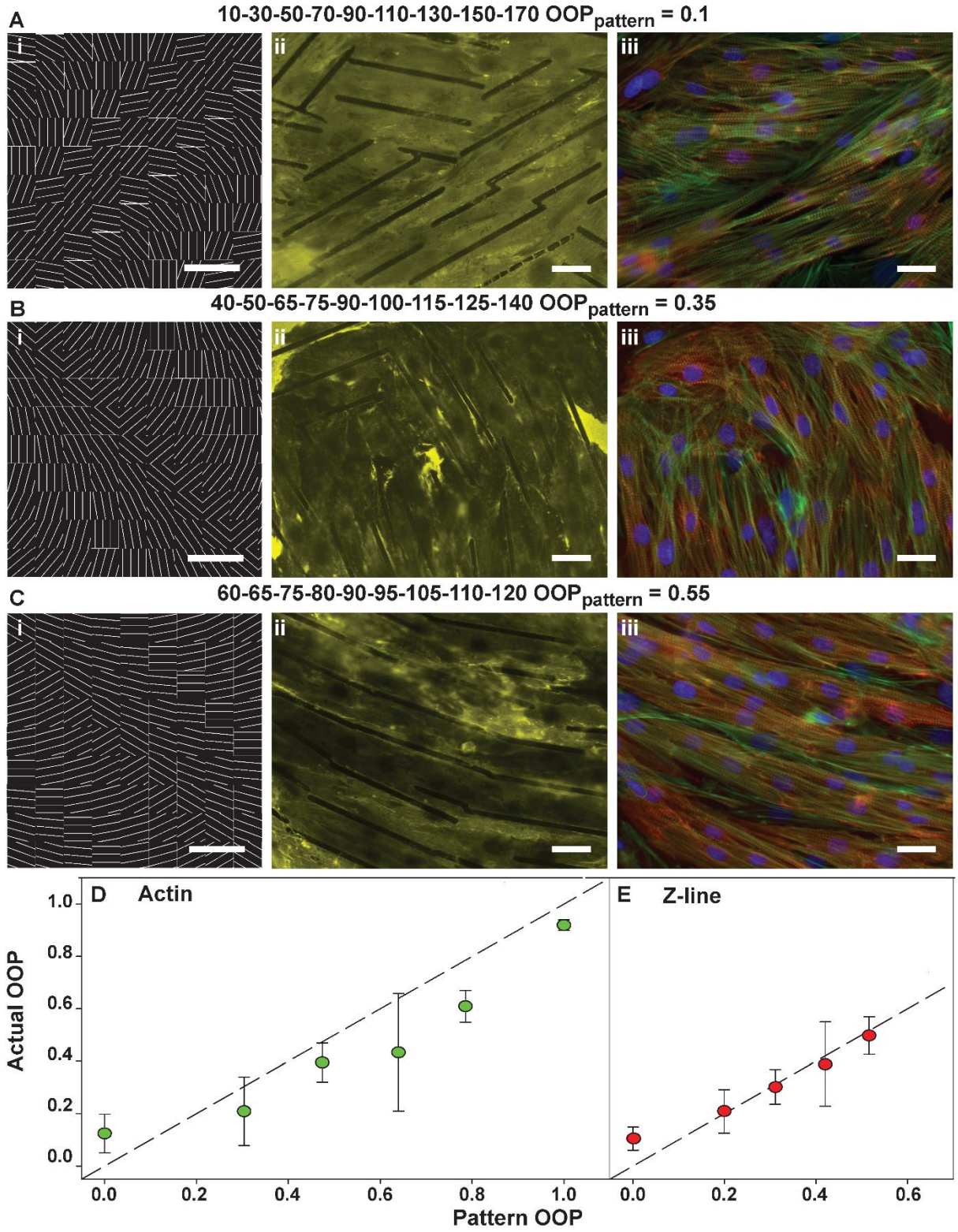

Figure 3-1: Gradual Angle Parquet Tissues *((i)'s)* Stamp Patterns, scale bar 200 μm (*(ii)'s*) Fibronectin Patterns, scale bars 25 μm (*(iii)'s*) Cardiac Tissues red- zlines, green - actin, blue – nuclei, scale bars 25 μm (D) Actin OOP of Pattern vs Actual OOP (E) Zline OOP of Pattern vs Actual OOP.

Tissue stresses were then measured using a muscular thin film assay. Systolic stress (Figure 3-2), Diastolic stress (Figure 3-3), and Active stress (Figure 3-4) were measured for isotropic tissues, gradual angle parquet, and aligned tissues. Stresses were also normalized by z-line fraction calculated through a custom matlab code to account for differences in tissue confluency [15]. To determine whether stress of the gradual angle parquets could be predicted based on actin or z-line orientation, a previous empirical model was recreated [10]. This model is centered on a single parameter that is calculated based on the actual stress of a tissue and the organization of that tissue. This parameter was calculated for both isotropic tissues and aligned tissue using both actin and z-line organization. The error from averaging the stresses of these tissues to create the single parameter was then used to calculate the 95% confidence intervals of the model prediction.

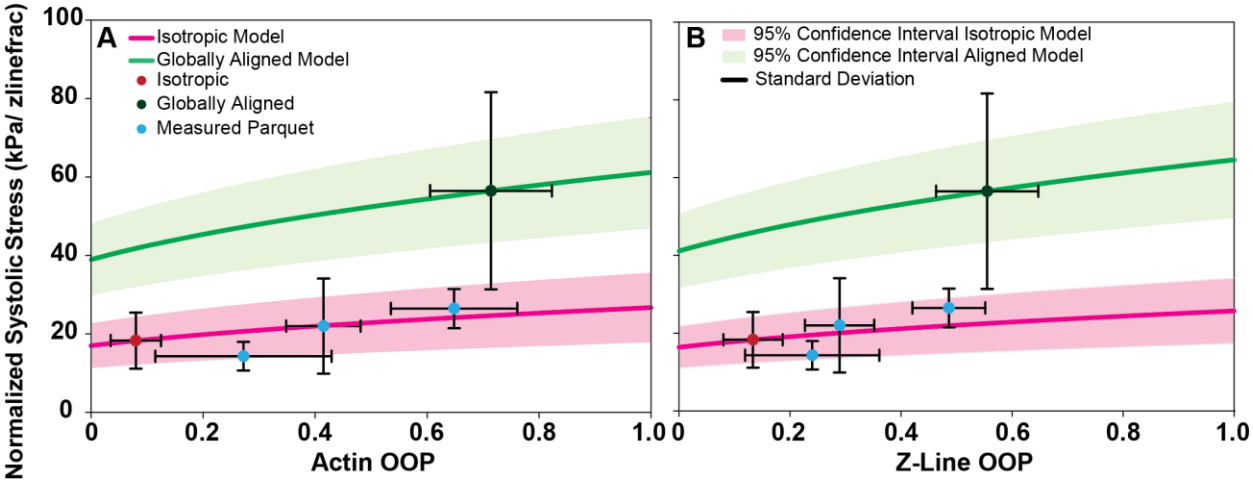

Figure 3-2: Normalized systolic stress values as a function of Actin OOP(A) and Z-line OOP (B) predicted by the model based on calculated parameter σ0(isotropic – thick pink line, aligned – thick green line). The mean systolic and diastolic stress for each parquet tissue (light blue circles) fall within the 95% confidence limit of the isotropic model (light pink, shading) and none fall within the 95% confidence limit of the aligned model (light green, shading). The mean isotropic stress (red circle) was used to calculate the isotropic model while the mean aligned stress (dark green circle) was used to calculate the aligned model. Error bars (black) represent stand deviation. *preliminary data

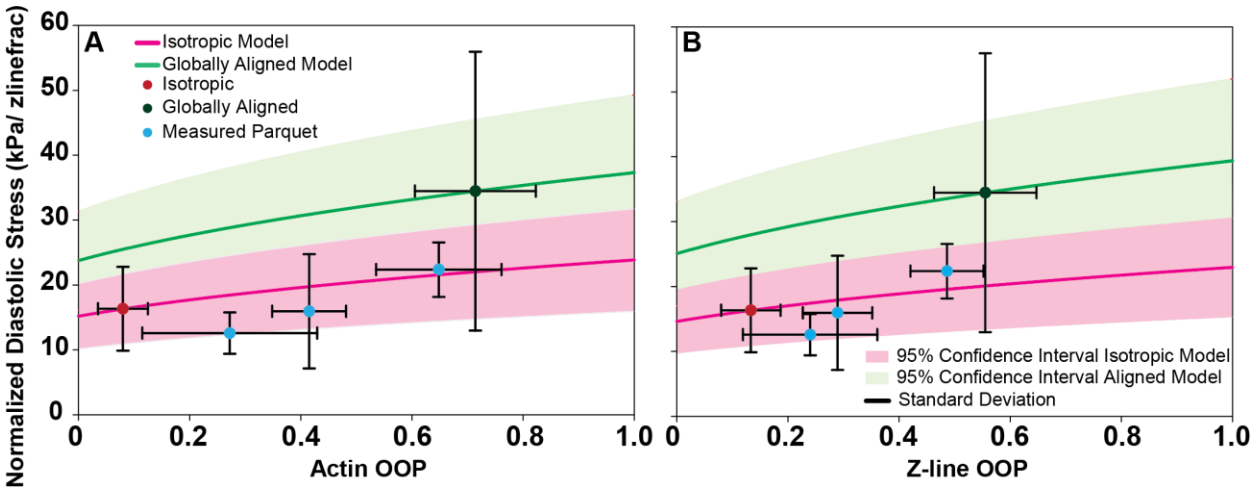

Figure 3-3: Normalized diastolic stress values as a function of Actin OOP(A) and Z-line OOP (B) predicted by the model based on calculated parameter σ0(isotropic – thick pink line, aligned – thick green line). The mean systolic and diastolic stress for each parquet tissue (light blue circles) fall within the 95% confidence limit of the isotropic model (light pink, shading) and none fall within the 95% confidence limit of the aligned model (light green, shading). The mean isotropic stress (red circle) was used to calculate the isotropic model while the mean aligned stress (dark green circle) was used to calculate the aligned model. Error bars (black) represent stand deviation. *preliminary data

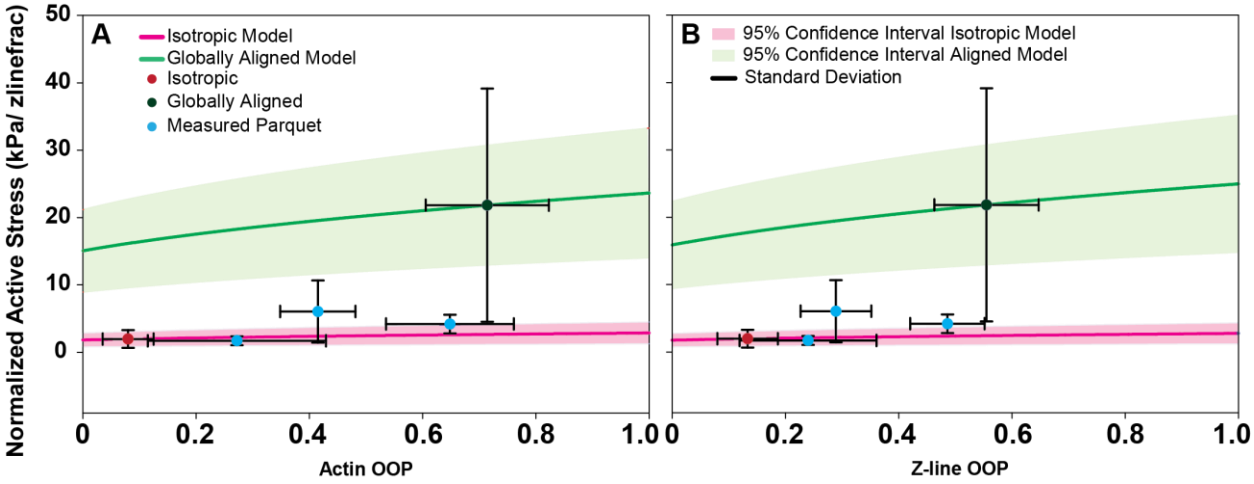

Figure 3-4: Normalized active stress values as a function of Actin OOP(A) and Z-line OOP (B) predicted by the model based on calculated parameter σ0(isotropic – thick pink line, aligned – thick green line). The mean systolic and diastolic stress for each parquet tissue (light blue circles) mostly fall within the 95% confidence limit of the isotropic model (light pink, shading) and none fall within the 95% confidence limit of the aligned model (light green, shading). The mean isotropic stress (red circle) was used to calculate the isotropic model while the mean aligned stress (dark green circle) was used to calculate the aligned model. Error bars (black) represent stand deviation. *preliminary data

Discussion

In this paper, we aimed to uncover whether the three-fold smaller patches than those tested by the original model produce less force or whether fundamental differences in spontaneous tissue formation account for a lower stress generation. To test this, parquet patterns were designed with an organization at 100μm compared to the original parquet tiles with an organization of 250 μm. Organization was quantified by calculating the OOP and stresses of these tissues were measured by using a MTF assay.

The gradual angle parquet tissues created follow the designated pattern and produce confluent tissues unlike the parquet tissues created using the same angle orientation as the larger parquet tiles (Figure 3-1D, Figure 3-1E, Figure 3-6). Indeed, the gradual angle parquet tissues actin OOP and z-line OOP follow the predicted OOP values, respectively, unlike the sharp angle parquet tissues (Figure 3-5, Figure 3-1D, Figure 3-1E). This could be explained because cells sense force through gap junctions and mechanotransduction; if gap junctions aren't formed properly, cells may undergo apoptosis. In the larger parquet tiles, a large group of cells are oriented in the same direction with very few touching cells in a different orientation where gap junctions may not form. In the smaller parquet, every cell is touching a neighboring cell organized in a different orientation. Therefore, the confluency may suffer due to lack of continuous orientation (Figure 3-6). However, in the gradual angle parquet tiles, the smaller change in the neighboring cell is less likely to cause gap junction misalignment and cells can remain attached and follow the fibronectin pattern (Figure 3-1D, 3-1E).

The systolic, diastolic, and active stress mean values of the gradual angle parquets, for the most part fall within the 95% confidence limit of the model generated using the parameter calculated based on the data of the isotropic tissues. This implies that unlike the larger parquet (250 μm) which were shown to be very close to anisotropic tissues in terms of force generation capacity, the small parquets (100 μm) mimic the force generation capacity of the isotropic tissues. The current experiments would need to be repeated as there was significant variability in the harvest quality during these experiments. However, preliminary data alludes to the fact that local area of organization does dictate the force generated by the tissue.

To fully understand whether local area of organization does indeed affect force generation, more replicates of the gradual angle parquet tissues must be analyzed. Along with this data, new patterns can be generated in different sizes of local organization and tested. This would make the empirical model more robust and give researchers a greater understanding of the consequences of cardiac remodeling.

Acknowledgements

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Supplemental Figures

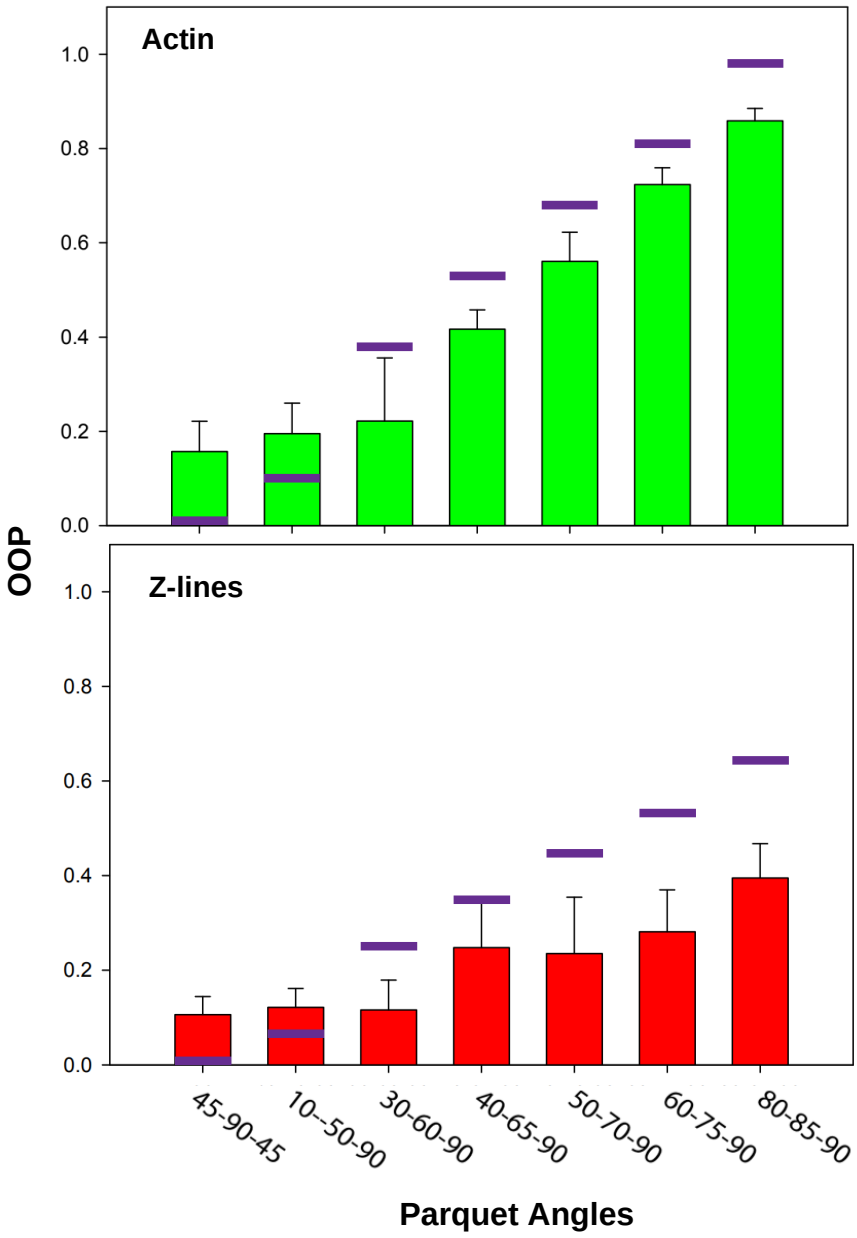

Figure 3-5: Sharp angle small parquet OOP for Actin and Z-lines

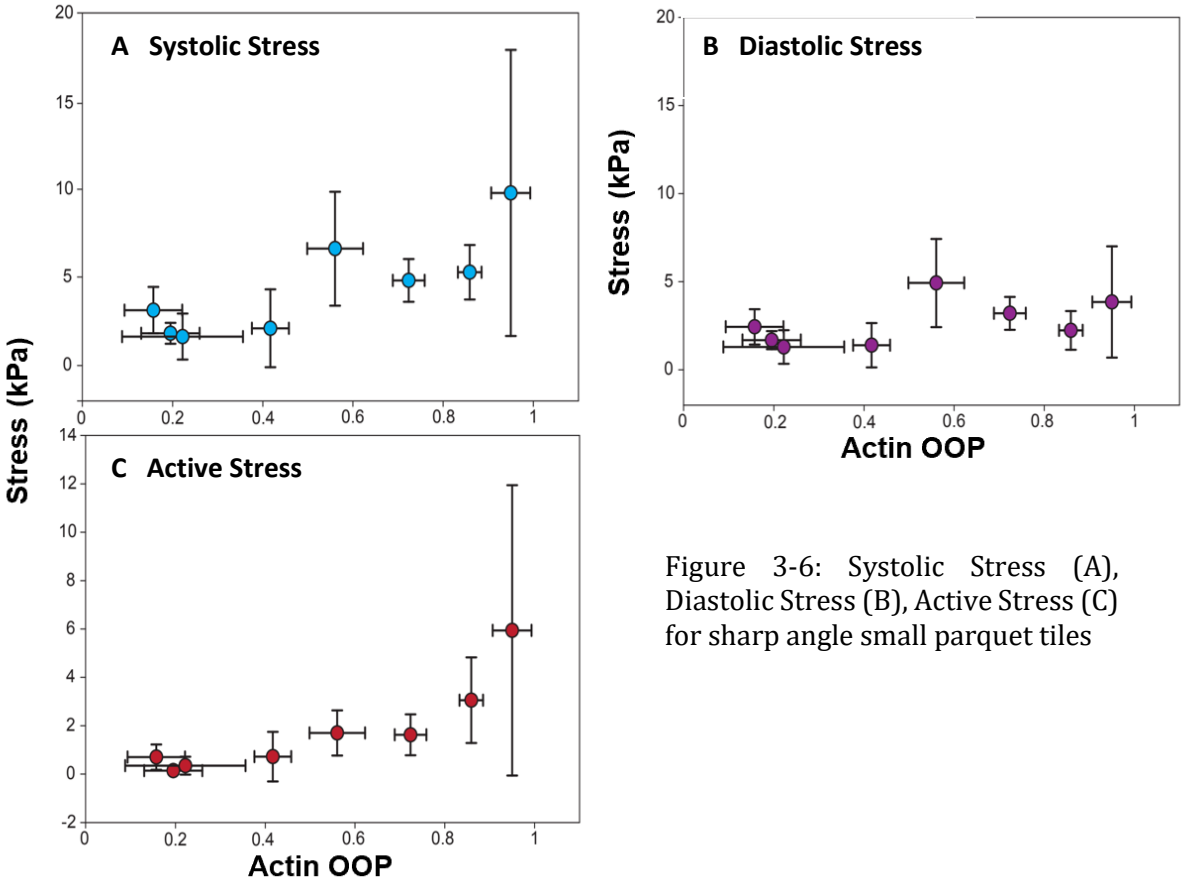

Figure 3-6: Systolic Stress (A), Diastolic Stress (B), Active Stress (C) for sharp angle small parquet tiles

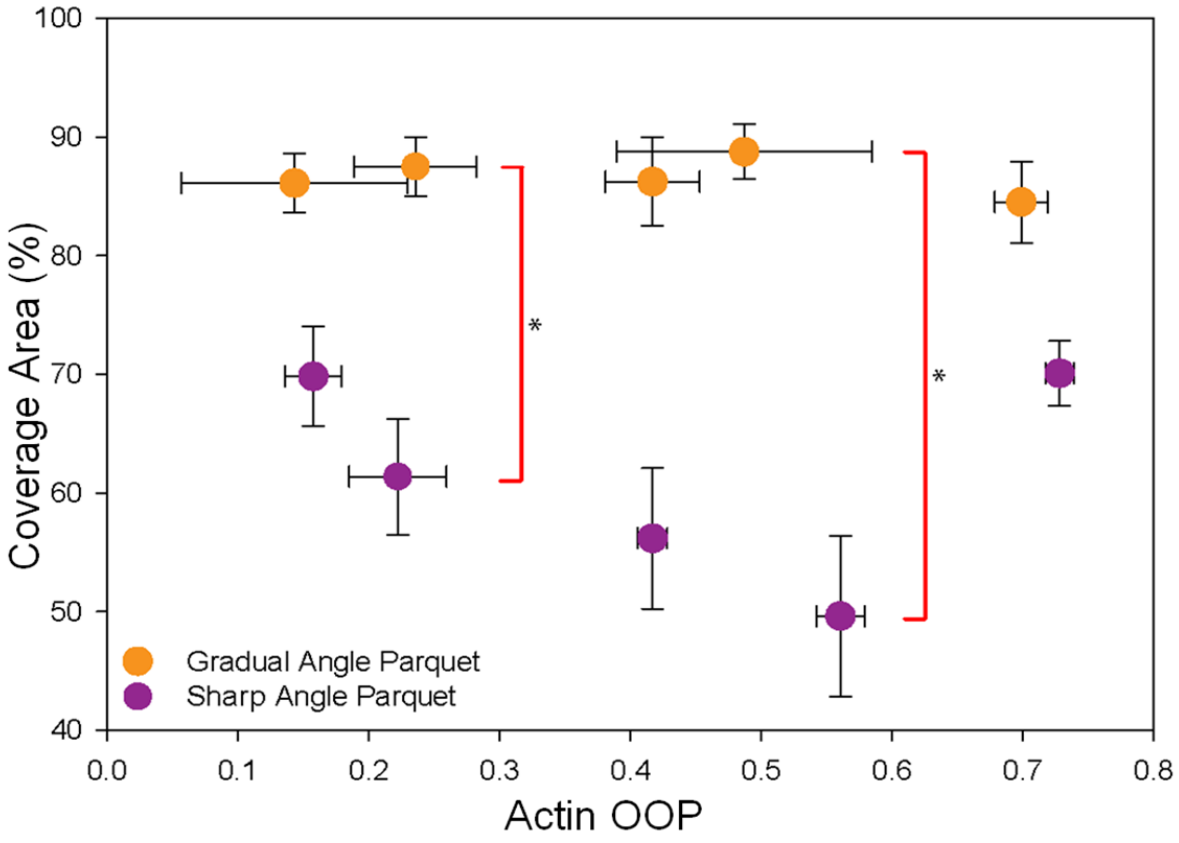

Figure 3-7: Actin OOP vs coverage area from sharp angle small parquet and gradual angle parquet

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Chapter 4 : Perturbations and Their Effect on Cardiac Force Production

Abstract

Evaluating cardiac function has become extremely important in biomedical and pharmacological research. However, cardiac cells are heterogenous in nature, making drawing conclusions difficult as deviations can be attributed to the experimental condition but also biological variability. Large sample sizes and high throughput assays have been used previously but are limiting in resources and type of data collected. Here, we propose a new platform to monitor stress changes over time to better predict biological variability within each sample. Through the creation of a simple force model, we are able to predict biological variability changes using muscular thin films allowing researchers to parse stress changes from experiments and natural changes due to cellular differences. This platform can then be used to evaluate how different perturbations affect cardiac stress generation.

Introduction

In vitro platforms for evaluating cardiac function have become essential in biomedical and pharmacological research [1-3]. Current platforms for determining cardiac contractility of tissues include, but are not limited to, cardiac fibers [4], micro electrode assays [5, 6], cantilever devices [7-9], and impedance multi-well plate assays [10]. These platforms can utilize many different cardiomyocyte sources including primary, induced pluripotent stem cell derived, and embryonic stem cell derived [11-13]. While combining these platforms with cardiomyocytes from different origins provides a tantalizing promise of eliminating animal testing, avoiding drug effect species mismatch, and introducing patient-disease-specific

cardiotoxicity assays, the heterogenous nature of cardiac cell cultures becomes a barrier in utilizing these *in vitro* systems [1, 14, 15]. Indeed, this heterogeneity causes a large variability in response; therefore, when comparing conditions, for example a variety of cell patterns, a large sample size is required to observe statistically significant differences [16, 17]. For many studies, where the effect of stimuli is subtle, the sample size is therefore prohibitively large [16, 17].

To minimize effects of a large sample size, high throughput plate readers can be used but limit the type of data collected as generated force (stress) cannot be measured [10, 18]. Platforms capable of stress measurements have used the same tissue sample to collect control and experimental data to alleviate large sample sizes [19-22]. However, cardiac function characteristics, such as beating rate or stress generation, change as a function of time after 24 hours in culture [19-22]. Therefore, experiments that require significant incubation periods such as those in pharmacological and toxicological studies may have significant alterations in cardiac function response making using the same sample nearly impossible [23, 24]. To resolve this problem, it is important to determine if there is consistency in stress development over time in culture.

To determine if stress varies consistently during the cell culture period, a platform to monitor stress generation over time needs to be utilized. In this paper, we describe a method for collecting control data to monitor stress changes over a period of 24 hours. This was achieved using muscular thin films to measure stress on consecutive days to better determine if stress changes can be predicted. This method can then be utilized to test a

variety of conditions as variations of stress over time can be accounted for in the experimental group.

Methods

Fabrication of the Substrate

Coverslips were prepared as stated in Knight, et al. [25]. Briefly, a large cover glass was shielded with protective film (static cling; Grafix Plastics, Cleveland, OH) which was cut and removed using custom templates to provide 1 cm strips of exposed glass. Next, 1 g of PIPPAm (poly(n-isopropylacrylamide)); Polysciences, Warrington, PA) was dissolved in 10 mL of 1-butanol (Macron Fine Chemicals, Center Valley, PA) and was then spin coated onto the glass and dried in a 60°C oven overnight. Then, PDMS was made in a 10:1 base/curing agent ratio and incubated at room temperature for 5 hours. After the protective cling film was removed from the surface of the glass, it was spin coated with the thickened PDMS and left to cure overnight in a 60°C oven. Lastly, the glass was cut with a diamond scribe (Musco Sports Lighting, Oskaloosa, IA) into individual coverslips using a custom template for "heart chips" suitable for 6 well plates.

Extracellular Matrix Patterning

Stamp patterns were designed using Adobe Illustrator software (Adobe Systems, San Jose, CA). Stamps were made to be 1.5 cm x 1.5 cm to produce fibronectin lines of 20 μm with 5μm gaps between lines for a globally anisotropic tissue. Designs were etched into a 5" by 5" chrome with soda-lime glass masks by a third-party vendor (FrontRange Photo Mask, Lake Havasu City, AZ) based on Adobe Illustrator files. The glass masks were used to create silicon

wafers via SU-8 deposition in the Bio-Organic Nanofabrication Facility (University of California, Irvine) and used to create PDMS stamps consisting of a 10:1 base/curing agent.

The stamps were sonicated in 95% ethanol for 15 min and were then dried using compressed nitrogen to prepare the stamps for microcontact printing. The stamps were coated with a 0.1 mg/mL concentration of fibronectin (Fisher Scientific Company, Hanover Park, IL) and incubated at room temperature for 1 hour. After 1-hour, compressed nitrogen was used to dry the stamp and the remaining fibronectin was printed onto PDMS coated coverslips that had been exposed to UV light (Jelight Company, Irvine, CA) for 8 min. The stamped coverslips were submerged in a 1 mg/mL Pluronics solution (Sigma Aldrich, St. Louis, MO) for 5 min and washed 3 times with PBS (phosphate buffered saline; Life Technologies, Carlsbad, CA). All the steps of microcontact printed were performed in a biosafety cabinet under sterile conditions.

Well Reducers

Well reducers were created to contain the media on the films. To create the reducers, a 1x1 LEGO™ brick was surrounded by PDMS in a 10:1 ratio. The PDMS was cured for 4 hours at 65°C and the LEGO™ was then removed. A razor blade was then used to cut a square around the LEGO™ cutout to create a square 1.5 cm x 1.5 cm in length with the LEGO™ hole in the center.

Cardiomyocyte Harvest and Culture

Neonatal rat ventricular myocytes were isolated from 2-day old neonatal rats (Charles River Laboratories, Wilmington, MA). Briefly, ventricular myocardium was extracted in a biosafety cabinet under sterile conditions. Heart tissue was rinsed in Hanks balanced salt solution

buffer (HBSS; Life Technologies) and then incubated in 1 mg/mL solution of trypsin (Sigma-Aldrich, St. Louis, MO) dissolved in HBSS and was placed on a rotating shaker in a 4°C refrigerator overnight for 12 hours. Next, the trypsin solution was removed and neutralized with warmed M199 medium (Invitrogen, Carlsbad, CA) which was supplemented with 10% heat inactivated fetal bovine serum, 10mM HEPES, 0.1 mM MEM non-essential amino acids, 3.5 g/L glucose, 2mM L-glutamine (Life Technologies), 2 mg/L vitamin B12 and 50 U/mL penicillin (Sigma-Aldrich) (10% M199). Media was carefully aspirated to avoid the loss of tissue. Tissue was then dissociated using 1mg/mL solution of collagenase type 2 dissolved in HBSS. Collagenased tissues were centrifuged at 1200 rpm for 10 min and the supernatant was aspirated. After, cells were resuspended in warm 10% M199 media.

The cell solution was then purified with 3 consecutive preplates to isolate cardiomyocytes from fibroblasts using cell culture flasks (BD Biosciences, San Diego, CA). After the final preplate, cells were counted using a disposable hemocytometer (Fisher Scientific, Waltham, MA) and were seeded at $4.5x10^5$ cells/mL.

After 24 hours, warmed PBS was used to rinse the cultures three times after gently banging the plates to remove dead cells. Warmed 10% M199 was added after the last rinse and cells were placed back in the incubator and after 24 hours, 10% M199 was replaced with warmed 2% M199 media.

Contractility Experiments

Heart on a chip experiments were performed day 3 after seeding and time studies began day 3 and readings were taken after 10 min, 1 hour, 3 hours, 6 hours, and 24 hours as specified. These experiments were performed in warmed Tyrode's solution of 5 mM HEPES (Acros

Organics, Thermo Fisher Scientific, Bridgewater, NJ); 1 mM magnesium chloride (Santa Cruz Biotechnology, Dallas, TX); 5 mM glucose, 1.8 mM calcium chloride, 5.4 mM potassium chloride, 135 mM sodium chloride, and 0.33 mM sodium phosphate (Sigma-Aldrich). First, cell seeded on patterned coverslips were placed in a 60 mm petri dish with warmed Tyrode's solution. Then, the coverslips were cut into thin films as previously described by Grosberg et al. [7]. The Tyrode's solution was then cooled to below 37°C to allow the PIPPAm to dissolve thus releasing the thin films from the coverslip surface. Coverslips were moved into a 35 mm petri dish filled with warm Tyrode's solution. The petri dish was then placed inside an INUL-MS2 Stage Top Incubator (Tokai Hit, Fujinomiya-shi, Shizuoka-ken, Japan) to allow for temperature control. Customized electrodes were placed on the 35 mm petri dish and films were paced with 15 volts at 1-3 Hz using a MyoPacer Field Stimulator (IonOptix, Milton, MA).

Contractility images were acquired on a model no. SZX-ILLB2 Stereoscope (Olympus America, Center Valley, PA) mounted with a model no. A601f/A602f camera (Basler, Exton, PA). Short videos clips were recorded for each coverslip and then analyzed using ImageJ and MATLAB software as previously described in Grosberg et al. [7]. Briefly, film bending was tracked for each film and diastole and systole were detected using custom software.

To calculate the stress generated from film bending, cell and substrate thickness are also important parameters as the films are modeled as a two-layer elastic beam. Cell thicknesses for cardiomyocytes were estimated to be 5 μm. PDMS thickness was measured for each chip preparation using a DektakXT profilometer (Bruker, Tucson, AZ).

Statistics

To determine significance between systolic, diastolic and isotropic tissues across multiple days, a One-way ANOVA with the Tukey Test were used. Significance was considered for an unadjusted p-value of less than the critical level.

Results

Control Experiments

Muscular thin film assays were performed to model controls without monitoring stress changes over time in order to compare a new method (Figure 4-1).

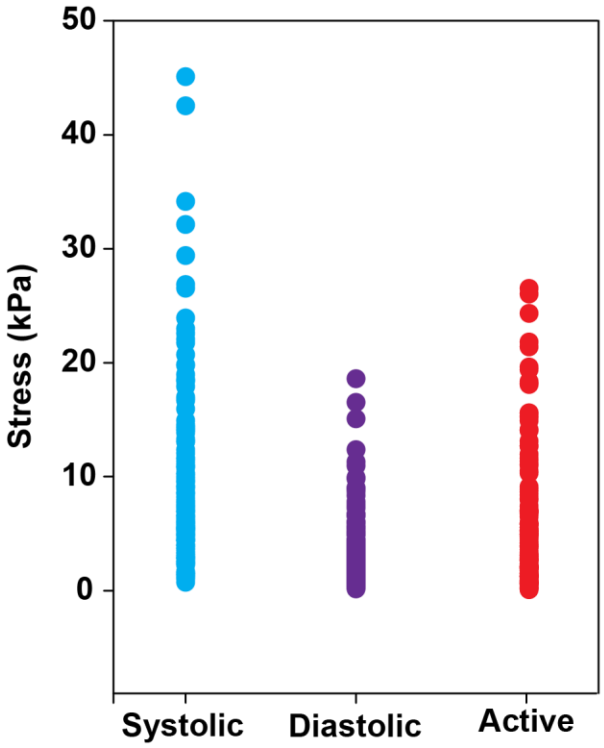

Figure 4-1: Biological Variability seen in measured stress values. Data includes 131 films from 26 assays across 6 cell harvests

With such a high biological variability, a new control method was created to account for the natural deviations and determine if the stress changes remained constant. Muscular thin films were completed a day prior to the experiment and data was collected twice (Figure 4-2).

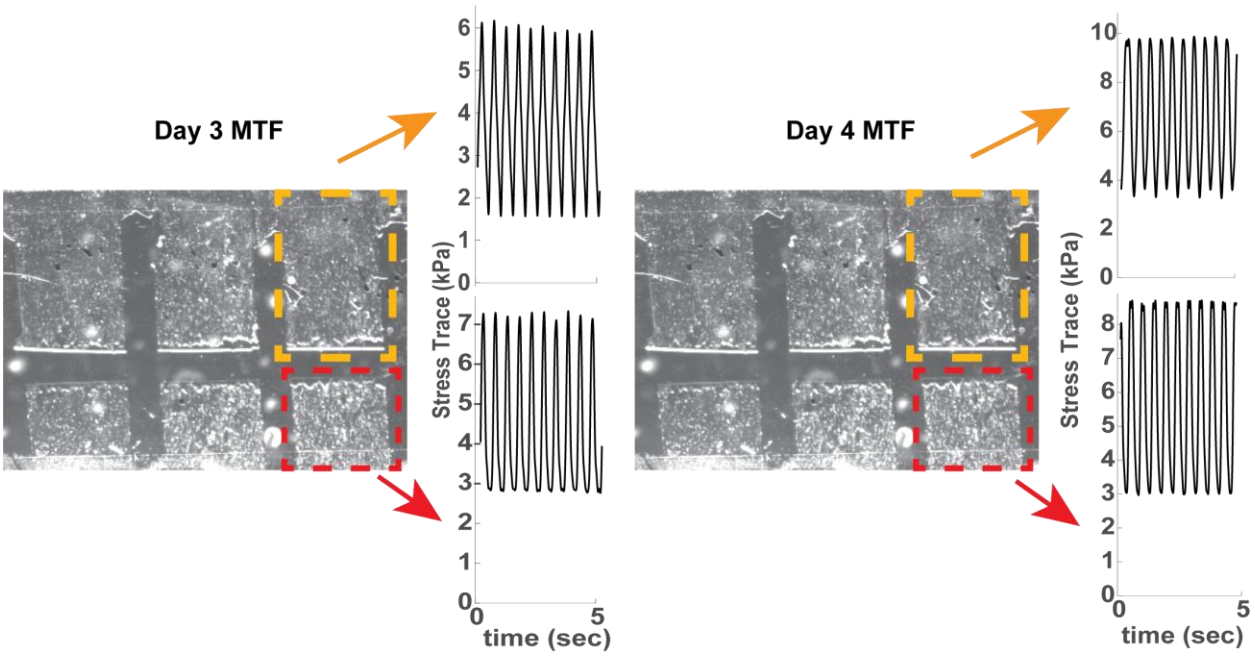

Figure 4-2: Stress was measured for each film on Day 3 and remeasured on Day 4. Here, two representative films (orange and red) are shown as well as their respective stress outputs for both days of measurement

To decrease the number of cells needed for each experiment, a well reducer made of PDMS was utilized (Figure 4-3). The reducer was attached to the coverslip with vacuum grease to create a tight seal. MTF measurements were then taken with the reducer on the coverslip to reduce contaminations.

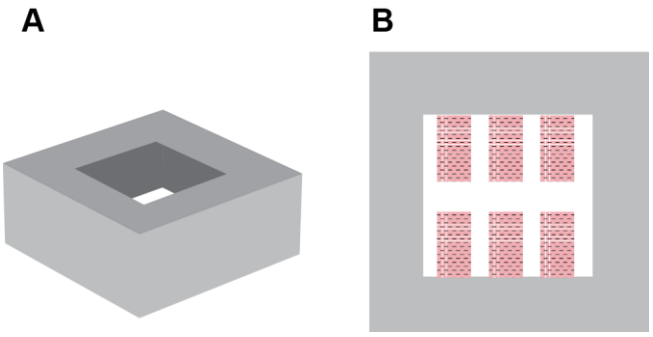

Figure 4-3: Well reducer schematic (A) PDMS well reducer (B) top view of well reducer with thin films cut for measurement

Once stresses were measured across day 3 and day 4, the differences between the 2-day measurements were quantified (Figure 4-4).

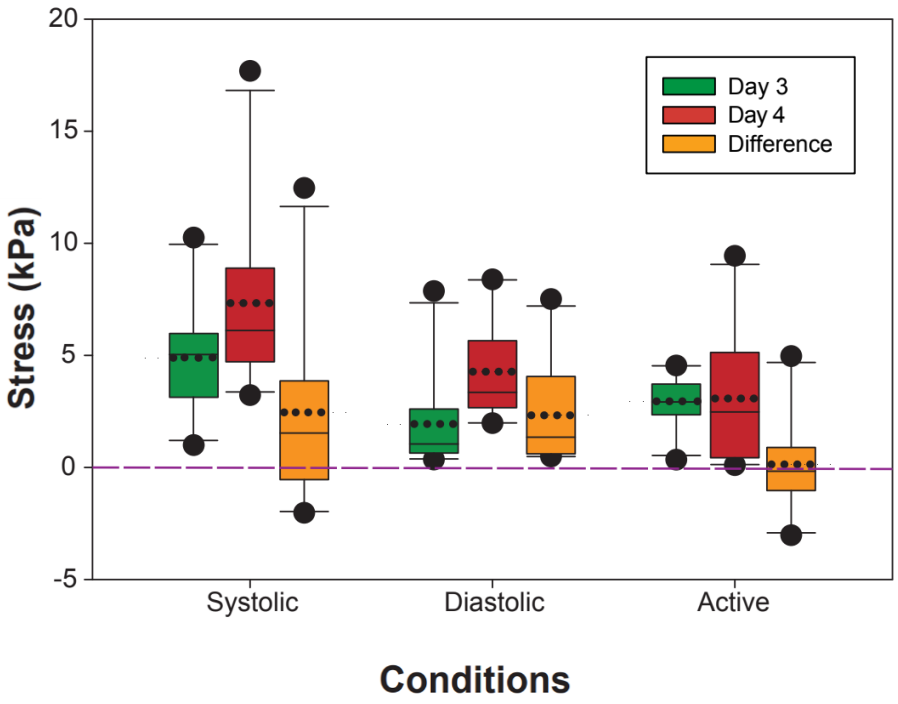

Figure 4-4: Stress Differences across MTF days

To predict the variability seen between day 3 and day 4, a simple model was generated (Table 4-1). One data set was used to calculate a prediction factor, f_0, by dividing the stress at day 4 by the stress at day 3. To validate the model, a unique data set was used. Stress measured on day 3 was multiplied by the generated prediction factor, and root mean squared error (RMSE) and average percentage error were calculated using equation 1 and equation 2.

$$RMSE = \frac{1}{N}\sqrt{\sum_{i}^{N}(D4_{pi} - D4_i)^2} \quad (1)$$

$$Ave\ \%\ error = \frac{\overline{D4_p} - \overline{D4}}{\overline{D4}} * 100 \quad (2)$$

In these equations, N is the number of films, $D4_p$ is the predicted stress value on day 4, $D4$ is the stress measured on day 4, $\overline{D4_p}$ is the average predicted stress value on day 4 and $\overline{D4}$ is the average stress measured on day 4.

Table 4-1: Predictive Model Data

Generate Model			Validate Model		
D3 (kPa)	D4 (kPa)	Predictive Factor f_0 (D4/D3)	D3 (kPa)	D4 (kPa)	$D4_p$ (kPa) ($D4*f_0$)
3.7	0.46	0.124	2.35	2.03	2.99
3.4	4.05	1.18	4.44	6.34	5.65
2.5	4.84	1.91	5.46	10.74	6.94
3.14	8.52	2.71	2.19	0.62	2.79
Ave 4.59	Ave 5.80	f_{0ave} 1.27	Ave 3.14	Ave 4.19	Ave 4.00

Calculating the RMSE yields a value of 0.57 while the average percentage error is 4.6%. If day 4 stress values from both sets were compared without predicting the values, the average percentage error is 28%.

Future Work and Applications

Future work will test cardiac tissue stress generation in the presence of pro-inflammatory cytokines. Cytokines were chosen for validation as patients who were hospitalized with COVID-19 and showed signs of heart ailments had elevated levels of pro-inflammatory cytokines [26-28]. Through the addition of pro-inflammatory cytokines, it will be possible to understand the direct effect of each cytokine on cardiac tissue stress generation.

Discussion

As cardiac cell sources are heterogenous in nature, it is important to have a large sample size to determine differences between conditions. However, this is not always possible as experimental groups can become prohibitively large. To compensate, high throughput plate readers are used but are limited to certain data types. For stress measurements, a single sample is used for both controls and experiments, but stress has been shown to change over time as demonstrated in Figure 1. To properly account for this change in stress over time, a new platform for analyzing controls was developed.

This method measured a films stress one day prior to experimental measurement. For controls, a films stress was measured day 3 and then again on day 4 (Figure 4-2). This inherently made each film its own control. When comparing the stress values between day 3 and day 4, we noticed that the difference between active stress values was relatively small

and created a simple model to predict the difference in stress (Figure 4). The force model (Table 4-1) was able to predict day 4 values with a 4.6% error. This demonstrates that collecting longitudinal data can greatly increase the predictive power of these assays.

Conclusion

Elucidating the relationship between stress fluctuations and time in culture is important as many scientists rely on changes in stress to determine toxicity of experimental conditions. If changes to stress due to biological variability are not accounted for, experimental conclusions may be drawn incorrectly. Here, we aimed to determine how cardiac stress changes after 2 days in culture to create a prediction model. This model can then be used to elucidate the direct effects of different perturbations as the model will account for natural biological variability. Such perturbations can include pro-inflammatory cytokines and other biological markers or drugs. In the future, this platform will allow researchers to better predict how perturbations affect cardiac tissue as biological variability is already accounted for.

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Chapter 5 : CardioStart: Development and Implementation of a Tissue Engineering Summer High School Program

Science, technology, engineering, and math (STEM) high school summer programs have been shown to be effective at introducing students to research experiences; however, insufficient program capacities in implementing and designing these programs are still major limitations. Among STEM programs, tissue engineering remains an especially difficult challenge as cell culture requires many resources that are found only in specific laboratory settings. We aimed to develop an adaptable and scalable summer tissue engineering program, CardioStart, in order to increase tissue engineering knowledge and maximize program efficiency. Students were enrolled in either a three-week, six-week, or ten-week iteration of CardioStart that took place at the University of California Irvine every summer from 2014-2019. The three-week and six-week programs consisted of seminars, hands-on lab work, tissue engineering modules, and communication modules. The ten-week program excluded hands-on activities but included data analysis. To evaluate the effectiveness of CardioStart, pre- and post-assessments were given to the students and comments were collected from the staff on the overall program effectiveness. Students' assessments showed improved tissue engineering and communication skills across all three programs as well as an interest in pursuing tissue engineering as an undergraduate degree. In addition, students that participated in the ten-week program also stated they wanted hands-on experiences to supplement the modules. Staff commented that the six-week and ten-week program was time-consuming while the three-week iteration was more easily managed and could be adapted for use by other engineering laboratories. To further improve CardioStart, tissue engineering workshops could be converted to online modules that would supplement hands-

on experiences, thus streamlining the program and increasing the accessibility of tissue engineering programs.

Introduction

Currently, the United States faces a shortage of STEM graduates while the amount of STEM occupations are expected to grow [28, 29]. One such occupation is biomedical engineering with the number of jobs expected to increase by 23% over the next ten years—with a notable fraction of these jobs in tissue engineering [39, 40]. To fill these roles in the future, today's high school students need more exposure to STEM [41]. Although high school programs explore the sciences, students still struggle to make the connection between the classroom environment and real world STEM applications [29, 32, 38]. Often times these programs exclude biomedical engineering or, more specifically, tissue engineering from their curriculum leading to students not being fully informed when choosing an undergraduate major [33]. Therefore, there is a strong need to motivate students to pursue degrees in STEM fields through summer programs, which expose students to engineering topics they would not experience otherwise such as tissue engineering research [33].

To encourage high school students to explore all branches of engineering as an undergraduate major, summer programs have been run that cover a multitude of engineering topics across a few weeks [32, 33, 38]. Many programs devote one day a week to cover one engineering branch, thus covering all branches in a full week [29, 34, 38]. While these programs include biomedical engineering, many topics are omitted due to time constraints [34, 38]. Biomedical engineering specific programs are often structured to encompass the multidisciplinary nature of the field by presenting students with a variety of

projects to complete [33, 42]. While these programs provide access to biomedical engineering outside of high school curricula, many lack tissue engineering components [33]. Furthermore, tissue engineering specific programs are scarce and expensive due to the significant amount of personnel time to design and run them [34, 42]. To expose more students to the tissue engineering field, a summer program that is scalable and less time consuming is essential.

Thus, we aimed to create a high school program that provides the students with a depth of exposure to tissue engineering, which is normally not possible in broad overview biomedical engineering programs. In this paper, we report on three variations of a high school tissue engineering program, CardioStart, that can be adapted for use at other research universities. The original six-week program was created to allow students ample time to understand cell culture and complete a tissue engineering project alongside a graduate student. Other iterations of this program include a ten-week program without hands on experiments, and a three-week program in which students cultured cells and learned proper tissue engineering techniques but remained in a group with one instructor. Through these iterations of CardioStart, we aimed to demonstrate that high school tissue engineering programs are not only beneficial for students but are also feasible for other university labs to adopt using material created for Cardiostart.

CardioStart Program

CardioStart has undergone three iterations, a six-week program, a ten-week program, and a three-week program. Both the three and six-week programs consisted of cell culture training, tissue engineering modules and the completion of a cell-culture technique based

project. The ten-week program consisted of live demos and an introduction to tissue engineering through presentations. A brief summary of all experimental and module-based learning completed in all CardioStart iterations can be seen in Figure 5-1 and Figure 5-2. The six-week program required student participation for 35 hours per week. The ten-week program involved students' participation for 16 hours a week. The three-week program was 25 hours a week in person with around 5 hours of self-guided learning throughout the week.

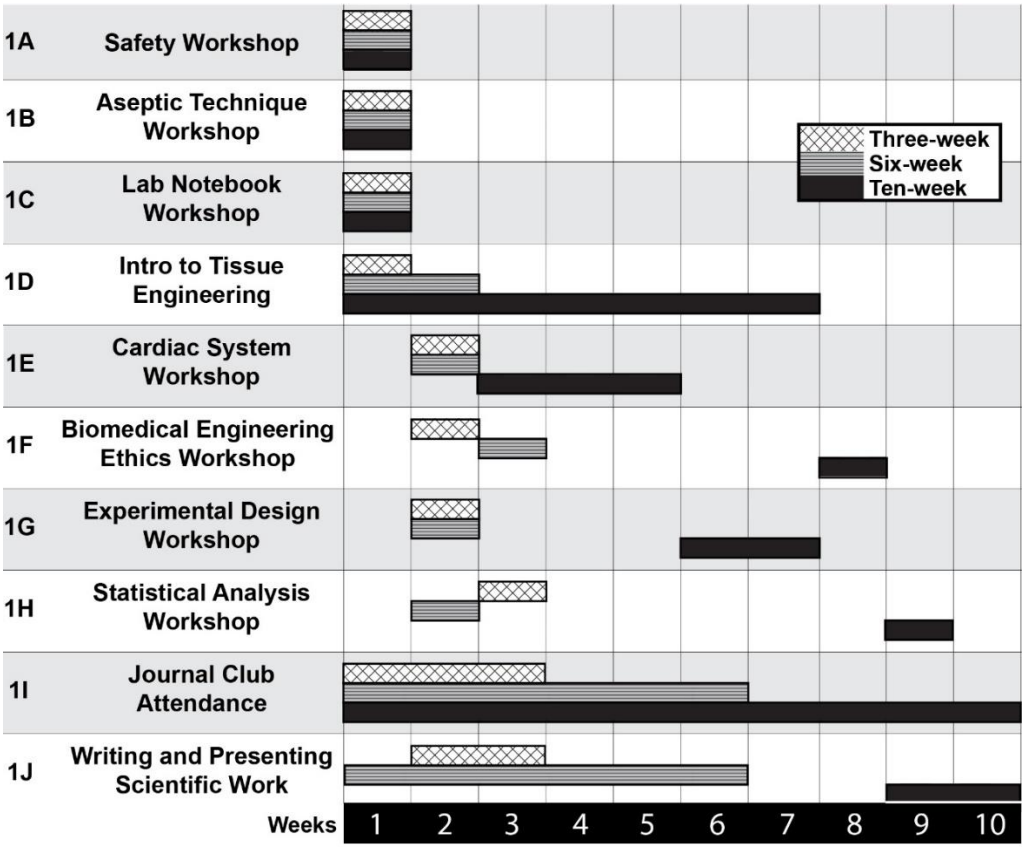

Figure 5-1: CardioStart Module-Based Learning Activities

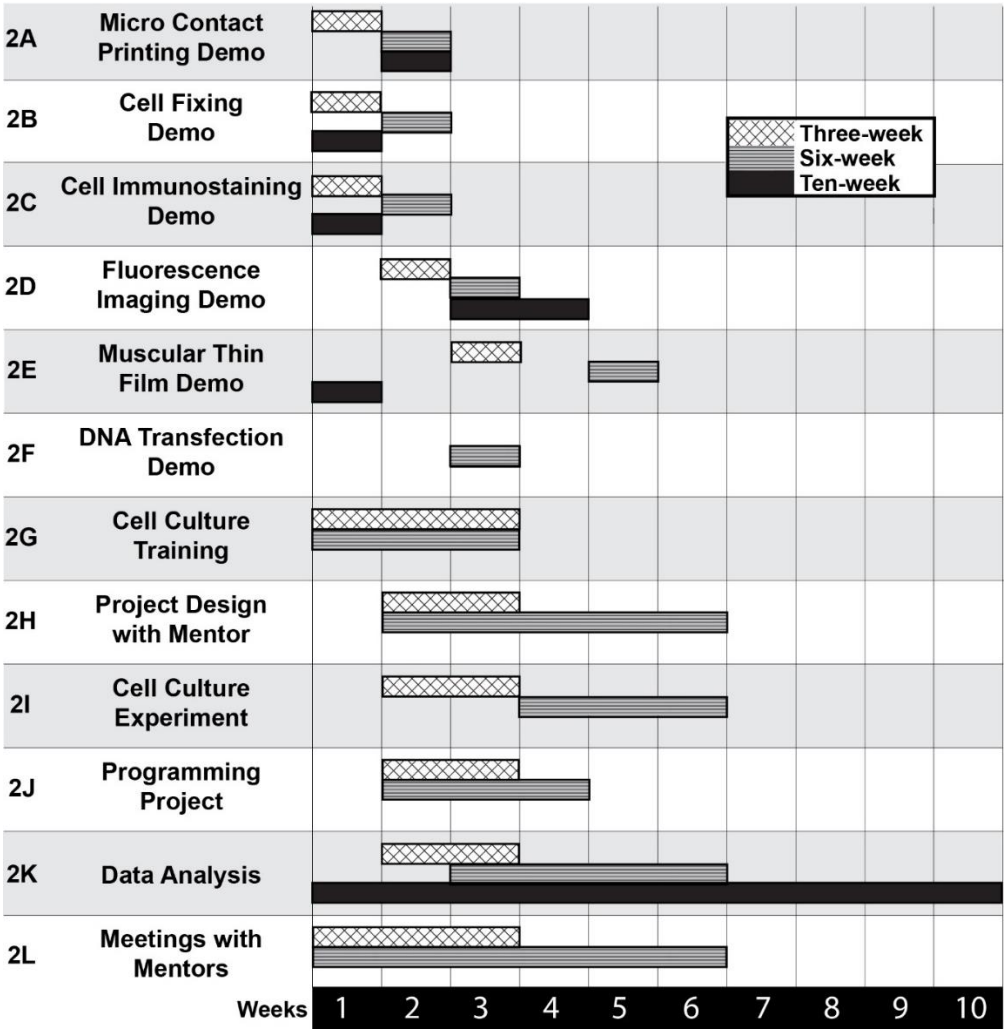

Figure 5-2: Demonstrations and Experimental Training

Program Experiences

Safety and Aseptic Technique Workshop

Before entering the laboratory space, online environmental health and safety training courses administered by the University of California Irvine were completed (Figure 5-1A Safety Workshop). Students were then taken on a tour of the tissue engineering lab and given the basic rules for working safely. This included wearing proper personal protective

equipment (PPE), where to find and store chemicals properly, and what to do if an injury does occur. Students were also taught the importance of aseptic technique in cell culture (Figure 5-1B). This presentation included proper ways to sterilize laminar flow hoods, materials needed for cell culture, correct ways to place items into a laminar flow hood, and what contamination looks like in both culture plates and flasks. In these workshops, students learned the importance of safety as well as how to properly culture cells for use in experiments. This training is standard for anyone who desires to culture cells.

Research and Dissemination

In a group of modules focused on research and dissemination (Figure 5-1C Lab Notebook Workshop, 5-1I Journal Club Attendance and 5-1J Writing and Presenting Scientific Work), students learned how to properly keep a laboratory notebook, comprehend journal articles, and articulate scientific findings through writing and presenting. The first module consisted of what to document when doing experiments and how to keep a notebook organized (Figure 5-1C Lab Notebook Workshop). Students were also able to attend journal clubs hosted by lab groups in the Edwards Lifesciences Center for Advanced Cardiovascular Technology throughout the program (Figure 5-1I Journal Club Attendance). To prepare for journal clubs, journal articles were given to students two days prior to the meeting to discuss the paper and ask an instructor any questions they may have. As the last module in this group, students were taught how to present scientific work as well as write abstracts (Figure 5-1J Writing and Presenting Scientific Work). To practice scientific writing, students wrote an abstract for a published journal article. Students were also able to update their resumes

during writing workshops and have them edited by graduate mentors. At the end of the program, students were able to use their laboratory notebook, journal articles, and writing skills to compose a cohesive abstract based on their experiences in the program.

Introduction to Tissue Engineering and Cardiac System

The next group of modules introduced students to the basics of tissue engineering and the cardiovascular system (Figure 5-1D Intro to Tissue Engineering and 5-1E Cardiac System Workshop). The first lecture covered the basics of cells, what types are commonly used in tissue engineering, and where we collect them from. The next portion focused on scaffolds: materials used to make them, importance of extracellular matrix, and their purpose in tissue engineering. The last portion consisted of device implantation and current products available on the market that utilize cells and scaffolds. Some of the challenges of tissue engineering were also listed and students were asked to read journal articles on technologies currently in development. Students then continued to learn about cardiovascular tissue engineering challenges by first learning how blood flows through the body. The lesson then shifted to action potentials within the heart and how the heart contracts. Blood vessel formation and valves were then covered and how tissue engineering can positively affect the cardiac field. The lecture ended with the difficulties in tissue engineering heart muscle to repair damage.

Biomedical Engineering Ethics Workshop

In the biomedical engineering ethics workshop (Figure 5-1F Biomedical Engineering Ethics Workshop), students focused on understanding ethical dilemmas faced by biomedical and tissue engineers. Current questions in the field were presented, and students were asked to argue both sides of problems such as whether to use embryonic stem cells. After the presentation, case studies were completed on ethics and plagiarism.

Experimental Design and Statistical Analysis Workshops

Before performing individual experiments, students were taught how to properly design experiments, perform analysis, and interpret results (Figure 5-1G Experimental Design Workshop and 5-1H Statistical Analysis Workshop). Examples of experimental design were given with positive controls, negative controls, experiments, and unnecessary experiments performed. Students were asked to complete a worksheet where situations were to be matched with type of control and the hypothesis they expected. Once students completed the worksheet on experimental design, they were taught how to analyze collected results. Basic statistics such as averages, standard deviation, z-score and t-test were explained to enable students to analyze cell project results. After students completed the exercises on statistics and when to use which test, they were given a tutorial in Microsoft Excel on how to easily calculate these values. Students were then able to determine p-values and learned whether to accept or reject their hypothesis. To reinforce the workshop, students were given a worksheet to complete.

Micro Contact Printing Demonstration

In the microcontact printing demonstration (Figure 5-2A Micro Contact Printing Demo), students observed the patterning of extracellular matrix which anchors cells and allows them to grow and communicate which results in tissues. In tissue engineering, micro-contact printing is a critical step to forming tissues used in various applications. Graduate students walked participants through the micro contact printing protocol in which the end result was creating a layer of extracellular matrix. For demonstration purposes, students were then able to watch cardiomyocyte placement on the matrix and a resulting cardiac tissue formation [18].

Cell Fixing and Immunostaining Demonstration

Once students understood microcontact printing, they learned about cell fixing and immunostaining (Figure 5-2B Cell Fixing Demo and 2C Cell Immunostaining Demo). Students were able to watch graduate students fix cardiac tissues with 4% paraformaldehyde and Triton-X. The result of fixing yielding a preserved cardiac tissue, which was then immunostained for four internal cell constructs. Before watching this process, students read and understood the protocol and were able to correctly select the immunostains used to image four internal cell constructs simultaneously. This is a powerful tool in tissue engineering to determine tissue quality [18]. A worksheet to reinforce immunostain selection was also completed.

Fluorescence Imaging Demonstration

Using coverslips previously fixed and immunostained, students observed imaging on an IX-83 inverted motorized microscope mounted with a digital CCD camera (Figure 5-2D Fluorescence Imaging Demo). A 40X oil immersion objective was used to acquire images later used to complete the programming projects. The images acquired were of the cardiac cell's nuclei, actin filaments, and sarcomeres, which are unique to cardiac cells. These images are then used to quantify the overall architecture of the cells [18].

Muscular Thin Film Demonstration

Muscular thin films are used to determine the stress cardiac tissues can generate. This is a useful tool when testing drugs for cardiotoxic effects [18]. Students were able to see how the assay works as well as cardiomyocytes beating and pacing (Figure 5-2E Muscular Thin Film Demo). They were then asked to determine how muscular thin film devices are useful in research and industrial settings.

DNA Transfection Demonstration

Students observed the process of DNA transfection (Figure 5-2F DNA Transfection Demo). A simple DNA mini-prep was completed and cells were transfected with green fluorescent protein which could be imaged by students to determine if the transfection was successful [43].

Cell Culture and Experiment

Students were taught proper technique when culturing cells in a laminar flow cabinet. This included completed modules in aseptic technique and demos on the tools required to passage correctly and efficiently. Students practiced these skills by seeding, feeding, passaging, and cryopreserving a commercial lung cancer cell line multiple times throughout the program (Figure 5-2G Cell Culture Training). A commercial lung cancer cell line was used for practice as these cells are more cost effective than cardiomyocytes. Once students completed the required training, they were given a cell project based on techniques and lessons learned previously in the program. Before starting the project, students used their knowledge of experimental design to propose an experiment and create a protocol with the help of a graduate student (Figure 5-2H Project Design with Mentor). The students were then able to collect the appropriate data required and perform statistical analysis to determine if they should accept or reject their initial hypothesis (Figure 5-2I Cell Culture Experiment). For example, one project conducted by a student sought to discover how changing the freezing cell protocol affected the viability of cells by comparing the amount of live vs dead cells in culture.

Programming Project

Students were given a tutorial on ImageJ and MATLAB for use in a research setting. The ImageJ tutorial focused on image analysis while the MATLAB tutorial focused on understanding how to write and run scripts. Students were then given a series of tissue images and tasked with creating a video within ImageJ using Macros (Figure 5-2J

Programming Project). They were then asked to create a simple calculator in MATLAB using basic scripts taught in the tutorial.

Data Analysis

Using MATLAB scripts, students were asked to analyze previously acquired images of actin and nuclei (Figure 5-2K Data Analysis). Custom scripts were given to students to determine orientation within these tissues, and students were taught how to interpret these codes and results.

Meetings with Mentors

Students met with 2-5 professors and graduate students throughout the program to gain insight into cardiac biomedical engineering (Figure 5-2L Meetings with Mentors). Students were able to ask professors about their research and the future goals of the laboratories. Many asked for advice on which major to pursue in the future. Similar questions were asked of graduate students with the most notable being the path taken to become a graduate student. The Edwards Lifesciences Center for Advance Cardiovascular Technology helped make these experiences possible as the professors were members of the center.

Results

Comparison of Program Costs

CardioStart had two major costs associated with running the program: personnel time and supply costs. Personnel time included experimental setup as well as time spent with students in the program. Supply costs included experimental supplies in addition to welcome and reception meals.

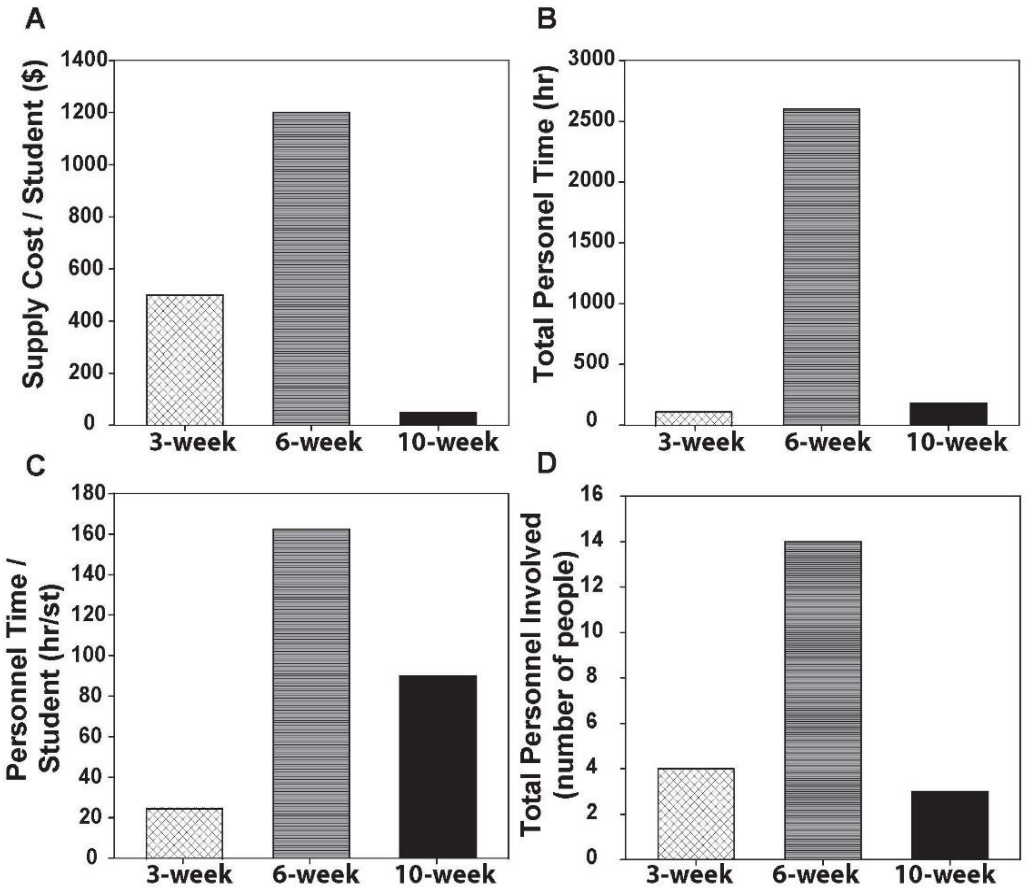

Figure 5-3: Comparison of program costs across the three program iterations (A) Supply cost normalized by the number of students in each program (B) Total personnel time required per program (C) Personnel time normalized by the number of students in each program

The six-week program had the highest supply cost and personnel time, while the ten-week program had the lowest supply cost, and the three-week program had the lowest personnel time cost (Figure 5-3A and 3B). The supply cost for the 10-week program was lower than the other programs due to lack of experiments performed by students (Figure 5-3A). Personnel time cost was further broken into time cost per student (Figure 5-3C) as well as the total

number of people involved in mentoring the students in each program (Figure 5-3D). This revealed that the three-week program had the least individual time spent with students while the 6-week had the most mentor-student interaction time. This is due to each student pair having a graduate student mentor for three of the six weeks, which is reflected in the greater number of personnel involved in the six-week program (Figure 5-3D). The breakdowns in Figure 5-3 showcase the overall cost reduction of the three-week program in comparison to the six- and ten-week programs.

Assessment of Program

While the cost assessment determined the 3-week program optimized overall program costs, student learning outcome results were needed to compare the effectiveness of each program to judge whether cost cutting measures were detrimental to the overall objectives. To determine whether students learned the concepts taught throughout each iteration, pre- and post-surveys (included in the supplemental appendix) were given on the first and last day. This assessment was approved by the Institutional Review Board at the University of California Irvine IRB No: 2018-4211. The surveys consisted of qualitative questions from each workshop, demonstrations and experiments presented during the program. The quantitative questions were then analyzed using a two-tailed, paired t-test with a significance level of 0.05 since the programs were paired. Results comparing the three-week and six-week CardioStart program exhibit students achieve the same level of understanding across both programs with the exception of a better understanding of experimental design in the three-week program (Figure 5-4). This could be explained by a

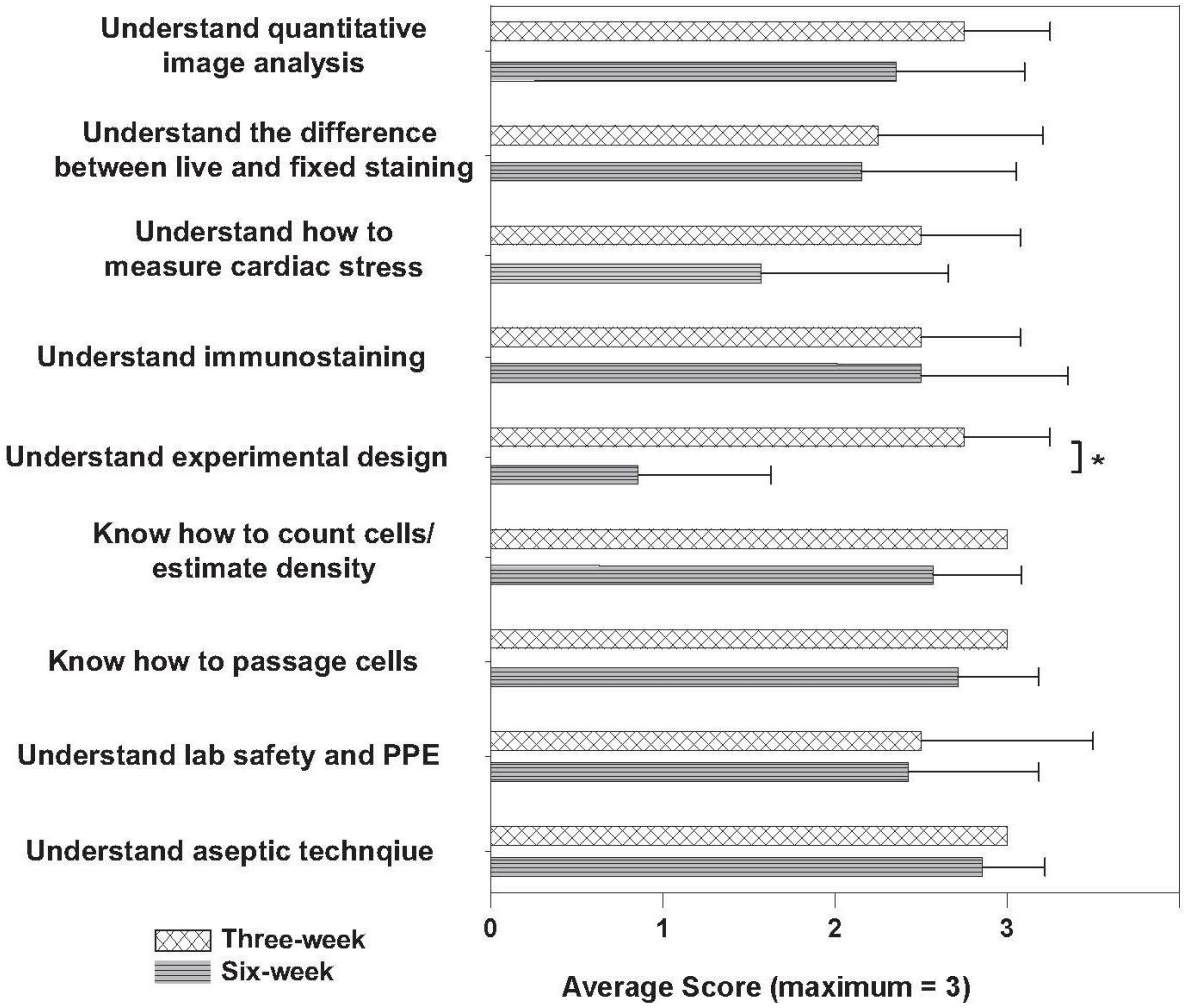

Figure 5-4: Self-improvement survey results comparing 3-week and 6-week programs. 3-week program N=4, 6-week program N=14; *indicated statistical significance with $p < 0.001$. Error bars represent the standard deviation of the data

more structured workshop due to centralized instruction and simpler projects to complete due to the time constraint. The ten-week program was not included in the comparison as the students did not participate in hands on experiments.

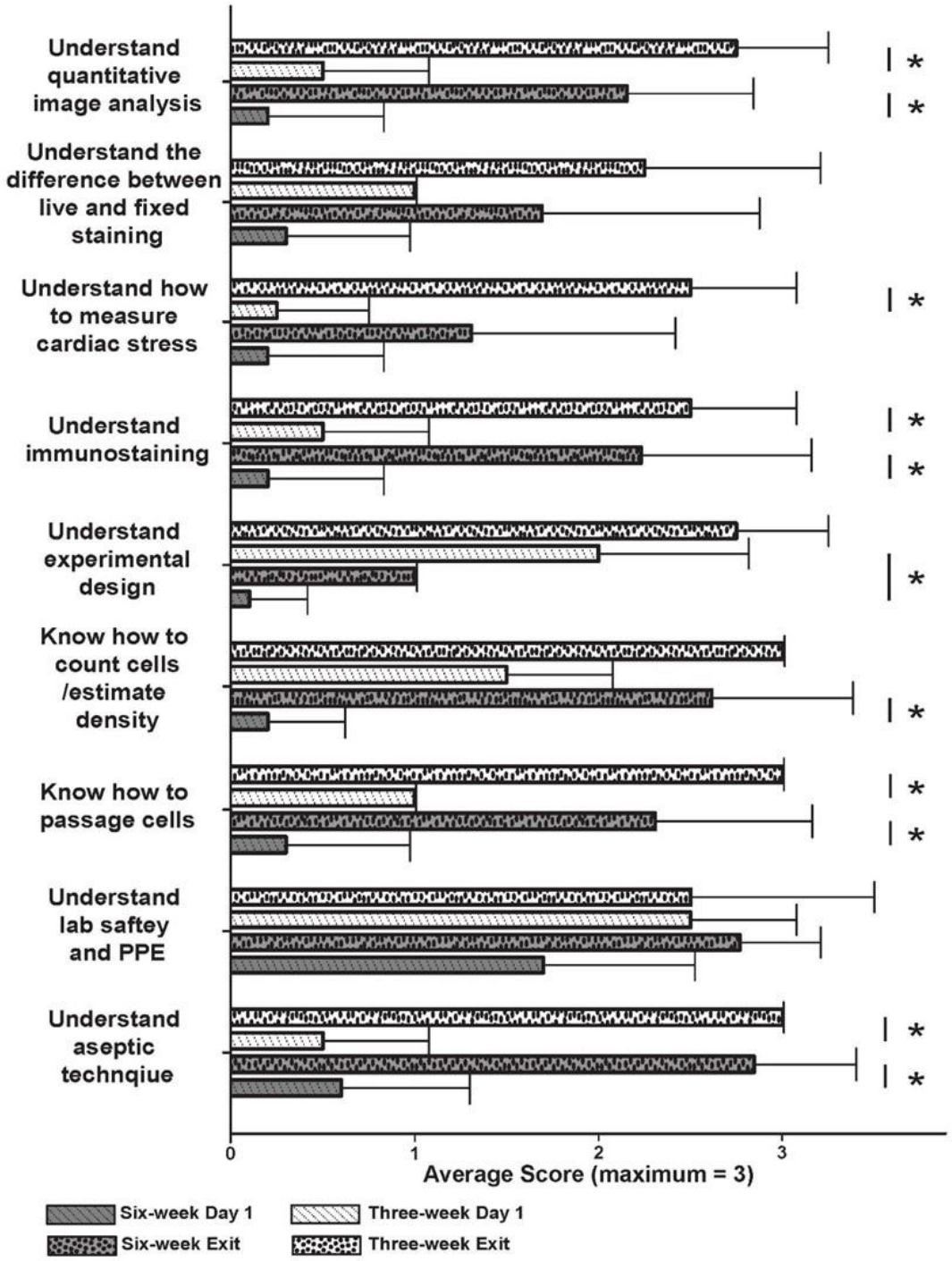

Figure 5-5: Pre- and post-survey results. The following pairs were compared: (1) three- and six-week pre-survey, (2) three- and six-week post-survey, (3) three-week pre- and post-survey, and (4) six-week pre- and post-survey. The *indicated statistical significance

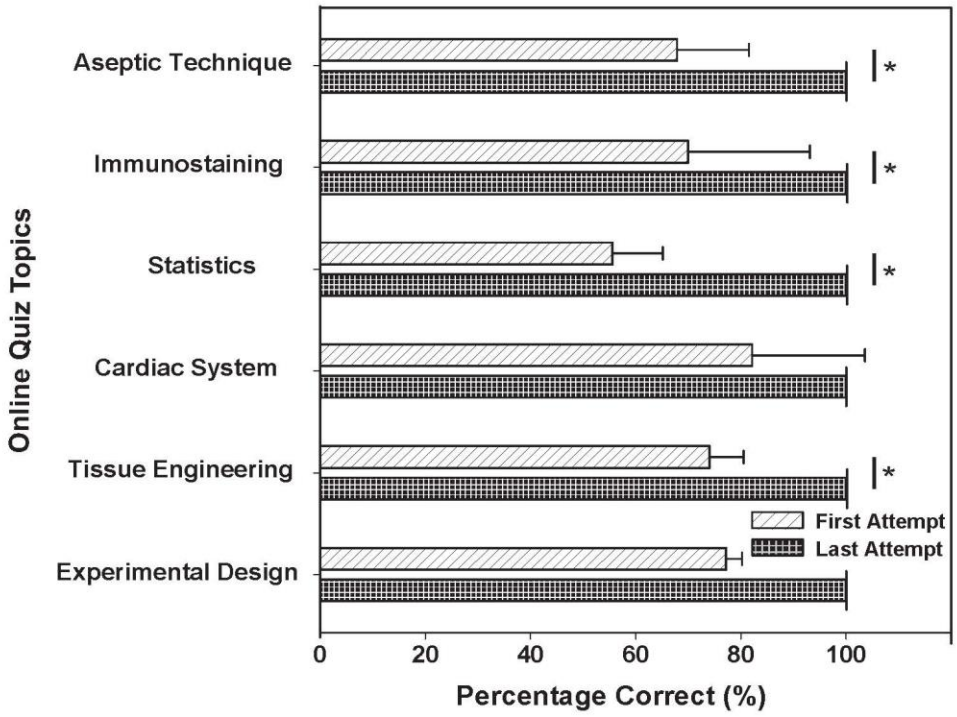

Figure 5-6: Online quiz first and last attempts in three-week program. The first attempt was after completing the corresponding module while the remaining attempts were completed after further discussion and teamwork. Three-week program N=4, *indicated statistical significance

:

Figure 5-5 compared pre- and post- survey results based on understanding of the topics when the assessment was given. To better assess students learning throughout the 3-week program, online quizzes were given on each topic. The first attempt and last attempt of these quizzes can be seen in Figure 5-6. Quizzes were taken after the module given on each topic. Students were allowed to take the quizzes multiple times to achieve as high a score as they were satisfied with. This comparison serves as a tool to monitor real time learning.

The overall success of the program can be determined by the open-ended questions related to what students enjoyed about the program and what can be improved. The following comments on the post-survey from the three-week program relate to the following questions: “What was your favorite part of CardioStart?”

"Being able to have hands on experience in the lab and getting comfortable with the lab equipment and techniques."

"Cell culturing and learning new techniques"

"The ability to do my own cell project"

When the students in the three-week program were asked "What was your least favorite part of CardioStart?", the majority of responses were geared toward program length as students felt they did not get to finish their projects. Comments included

"Getting contaminations in flasks so I couldn't complete my project"

"Length of lectures were long"

"Too much data analysis"

Students were also able to comment on how to improve the program. Comments for the following question are below: "Do you have any comments/suggestions that can help make CardioStart better for students in the future?"

"Adding in a lecture on Microsoft Excel to further help with data analysis"

"Having more cell culture projects to choose from"

"Changing the program to be more online based to allow more students to access the program"

Overall, students that participated in CardioStart seemed pleased with the overall program planning and curriculum. Indeed, while the structuring of the program is important, the excitement generated by the topic is crucial as more students are needed in the STEM field.

Discussion

We have designed and tested multiple iterations of a high school summer tissue engineering program, CardioStart, that gives students an understanding of a field they might not experience otherwise. Most engineering summer camps briefly cover biomedical engineering without tissue engineering components [29], while biomedical engineering specific camps aim to cover the breadth of the field and may not devote enough time to tissue engineering [33]. Moreover, tissue engineering camps are hindered due to the scarcity and expenses needed to design and run them [34, 42]. CardioStart allows students to fully immerse themselves in the tissue engineering field through hands on cell culturing experiences, tissue and cardiac engineering modules as well as data analysis and scientific communication practice, all while aiming to reduce costs and improve adaptability for use at other universities.

To achieve the first goal of reducing costs, each iteration was examined to compare overall personnel time costs, personnel time cost per student, and supply cost per student. The six-week program had the highest supply cost per student as well as personnel time per student. The supply costs were greater as they were spending more time on experimental work as opposed to the three-week and ten-week programs. The six-week program also required more one-on-one time with graduate student mentors to complete their cell culture projects. The ten-week program was created to reduce supply costs and personnel time. However, the ten-week program required more personnel time than the three-week program due to more graduate student guidance during workshops and modules. Thus, the three-week program effectively reduced program costs for both supplies and personnel

time. This was a major goal as many limits on student achievement are financial, but costs are still high.

To assess students learning between the three- and six- and ten-week programs, a pre- and post- survey including both qualitative and quantitative questions were given. Qualitative results collected revealed students enjoyed both the three- and six-week programs, while students in the ten-week program requested hands on lab experiences. Due to this finding, the ten-week program was not included in the quantitative analysis due to the lack of hands on experiments. Students in all programs showed enthusiasm towards the topic and the majority continued on to achieve a higher education degree in a STEM field. Based on quantitative results collected, students achieved the same level of understanding across both the three- and six-week programs with exception to experimental design which could be due to students confidence of the topic upon entering the three-week program. While the number of students remains low, the program is still being tested and optimized for best results. These preliminary results in combination with the reduction of program costs demonstrate that the three-week program is the most effective of the three and will continue to be optimized to further reduce costs and improve student learning.

Toward achieving the second goal of improving adaptability and further reducing costs, a fourth online hybrid program will be created. Students will complete the modules from the 3-week course online with short assessments and include hands on workshops at a local university. This course would reduce personnel time significantly as they would only be required to run demos and cell culture training. Another benefit is higher throughput since the program would be able to accommodate more students by staggering students in

cell culture since space is a limiting factor. This hybrid course would improve adaptability as well since most universities will be able to use the fully developed online course and would only require spaces for cell culturing. The hybrid course is in development and will be tested in the summer of 2020.

Conclusion

In this paper, three iterations of a tissue engineering high school summer camp, CardioStart, were evaluated to determine adaptability at other universities as well as encourage students to pursue tissue engineering. A detailed comparison of modules presented to students as well as cost analysis were completed for each program. Student learning outcomes were also assessed and taken into account. Overall, the three-week CardioStart program yielded the best outcome in reducing overall program and personnel costs as well as student learning goals. Future work consists of creating a hybrid course based on the three-week program to improve adaptability to other universities.

Acknowledgments

The authors would like to thank the Edwards Lifesciences Center for Advanced Cardiovascular Technology for the use of its facilities and Assistant Director Ann Fain for making this program possible.

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Supplemental Appendix

Pre-Assessment Survey

General Questions	0 - Never heard of it	1 – Sort of	2 – OK	3 – Very well
I understand the importance of aseptic techniques to successful cell culture	0	1	2	3
I understand how the choice of fluorophores for immunostaining depends on the filter cubes in the microscope	0	1	2	3
I understand the importance of safety in the lab	0	1	2	3
I understand the importance of experimental design	0	1	2	3
I know how to passage cells	0	1	2	3
I know how to count cells and how to estimate seeding density	0	1	2	3
I understand the importance of control experiments	0	1	2	3
I understand the importance of measuring the stress produced by cardiac tissues	0	1	2	3
I understand the difference between staining cells live and after they are fixed	0	1	2	3
I understand the importance of ethical behavior in science and engineering	0	1	2	3
I understand the importance of a well composed presentation	0	1	2	3
I understand the importance of putting effort into scientific writing	0	1	2	3
I am comfortable with quantitative image analysis using ImageJ	0	1	2	3
I can use micropipette	0	1	2	3
I can use a brightfield microscope	0	1	2	3
I can record and keeping laboratory data successfully	0	1	2	3
I can read and understand journal articles	0	1	2	3
I understand how engineers and doctors work together	0	1	2	3
I feel comfortable explaining the cardiovascular system	0	1	2	3

I can perform statistical analysis on data	0	1	2	3
I feel comfortable writing scientific reports	0	1	2	3
I can recognize plagiarism	0	1	2	3

Future Goals Questions	1 - strongly disagree	2 - disagree	3 - neutral	4 - agree	5 - strongly agree
I am interested in pursuing a degree in STEM	1	2	3	4	5
How likely are you to attend college?	1	2	3	4	5
I am interested in pursuing a career in biomedical engineering	1	2	3	4	5
I have participated in similar programs in the past	1	2	3	4	5

What are you most excited to learn about?

What topics are you already familiar with?

Post Assessment Survey

General Questions At the present time	0 - Never heard of it	1 – Sort of	2 – OK	3 – Very well
I understand the importance of aseptic techniques to successful cell culture	0	1	2	3
I understand how the choice of fluorophores for immunostaining depends on the filter cubes in the microscope	0	1	2	3
I understand the importance of safety in the lab	0	1	2	3
I understand the importance of experimental design	0	1	2	3
I know how to passage cells	0	1	2	3
I know how to count cells and how to estimate seeding density	0	1	2	3
I understand the importance of control experiments	0	1	2	3
I understand the importance of measuring the stress produced by cardiac tissues	0	1	2	3
I understand the difference between staining cells live and after they are fixed	0	1	2	3
I understand the importance of ethical behavior in science and engineering	0	1	2	3
I understand the importance of a well composed presentation	0	1	2	3
I understand the importance of putting effort into scientific writing	0	1	2	3
I am comfortable with quantitative image analysis using ImageJ	0	1	2	3
I can use micropipette	0	1	2	3
I can use a brightfield microscope	0	1	2	3
I can record and keeping laboratory data successfully	0	1	2	3
I can read and understand journal articles	0	1	2	3
I understand how engineers and doctors work together	0	1	2	3
I feel comfortable explaining the cardiovascular system	0	1	2	3
I can perform statistical analysis on data	0	1	2	3

CardioStart Questions	1 - strongly disagree	2 - disagree	3 - neutral	4 - agree	5 - strongly agree
CardioStart helped my understanding of scientific research	1	2	3	4	5
CardioStart helped the development of knowledge about the cardiac system	1	2	3	4	5
CardioStart helped me understand the difficulties of entering a scientific research career	1	2	3	4	5
CardioStart strengthened my skills as a scientist	1	2	3	4	5
My expectations of CardioStart were met	1	2	3	4	5
I am interested in pursuing a degree in STEM	1	2	3	4	5
How likely are you to attend college?	1	2	3	4	5
I am interested in pursuing a career in biomedical engineering	1	2	3	4	5
How likely are you to recommend CardioStart to a friend	1	2	3	4	5

What was your favorite part of CardioStart?

What was your least favorite part of CardioStart?

Do you have any comments/suggestions that can help make CardioStart better for students in the future?

Chapter 6 : CardioStart Online: A Virtual High School Tissue Engineering Course

CardioStart Online: A Virtual High School Tissue Engineering Course

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Abstract

In this paper, we altered an in-person high school tissue engineering program to create a virtual course. Through this alteration, we aimed to show that online programs can still be engaging to students but also provide greater accessibility and flexibility to students. This was achieved through utilizing google classroom as a virtual platform for students to engage with course modules and assessments. Students' responses to assessments were positive and showed improvement in all topics covered during the course. When understanding of topics was compared between the in-person and online courses, results showed insignificant differences between students understanding with the exception being a greater understanding in the topic of statistics. We were also able to engage five times the number of students online as compared to the in-person program, which was conducted yearly for six summers. However, many students suggested including hands-on activities to supplement their knowledge of cell culture techniques after completing the course. Overall, the online program improved accessibility and scalability of the in-person program. Future

work will consist of bridging this virtual course and the hands-on experiments performed in during the in-person program to provide interested students access to laboratory experiences.

Keywords

Tissue Engineering, High School STEM, Online Learning

Declarations:

Funding: This study was funded by the Edwards Lifesciences Center for Advanced Cardiovascular Technology, and the Stacey Nicholas Office of Access and Inclusion at UCI.

Conflicts of interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval: Work presented is provided from program surveys and was approved by the Institutional Review Board at the University of California Irvine IRB No: 2020-5964.

Consent to participate: Informed consent was obtained from all individual participants and parent/guardian of participants included in the study.

Consent for publication: Students were informed of our intent to publish the survey results via email and voluntarily consented to share their responses.

Availability of data and material: Not applicable

Code availability: Not applicable

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jasmine Naik. The first

draft of the manuscript was written by Jasmine Naik and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Introduction

As biotechnology and pharmaceutical companies push to supply vaccines and COVID-19 supplies, Science, Technology, Engineering, and Math (STEM) related jobs, such as biomedical engineering, have been projected to exhibit more stable growth than other jobs [30, 44, 45]. To fill these roles, the country will require a supply of STEM trained graduates to enter the workforce; therefore, youth STEM education is now more critical than ever [46-48]. In the current job market, even as these careers surge, many people remain unemployed because they do not have a degree in STEM [44]. The relatively small amount of STEM degrees can be attributed to a lack of encouragement, role models, and access to quality education [28, 29, 45, 49]. Education curricula content across the US varies significantly depending on the resources available, leaving some students lacking STEM exposure [29, 38, 47, 50]. Further, to bridge the gaps between fundamental knowledge and the ability to apply knowledge to real-world problems, students need access to engaging extracurricular programs and role models to emulate them [49, 51].

To fill this need, many schools and communities provide after school programs, of which 50% offer two or more days of STEM instruction in the hopes of inspiring the next generation of STEM graduates [31]. Indeed, these programs have improved students' attitude towards STEM fields and careers, increased STEM knowledge and skills, and increased the likelihood of student graduation and pursuing a STEM career; however, there is still an unmet need for afterschool programs as only 8.4 million students are enrolled,

leaving more than 40 million students without programs to attend. In fact, as many as 19.4 million students would sign up if a program were available [31, 35, 52-55]. The main reasons for students not enrolling are program costs and a lack of program availability in safe locations, both of which were cited to be higher in rural areas [31, 56, 57]. Along with funding challenges, some programs are also not aligned with students interests, with only half of them offering stimulating STEM activities [31, 56]. In order to allow for both more rigorous STEM involvement and to reach even more students, universities are looked to for additional programs.

Many universities have research and laboratory spaces where they can offer summer programs for students to explore their interests with more in-depth STEM integration than after school programs [56]. Despite a seeming abundance of resources, the summer programs offered by many universities are limited to local students or are expensive due to a variety of program costs such as housing, personnel and supplies needed [35]. The costs associated with running these programs also limit the number of students that can attend each program, which increases competition to attend and lowers accessibility [35-37]. Further, many engineering summer programs are not able to devote significant time to each engineering discipline, therefore omitting many topics that students may find engaging [32-34, 38, 42]. To expose more students to biomedical engineering and more specifically tissue engineering, a summer program that is easily accessible and scalable is essential for inspiring the next generation of scientists and engineers.

In this paper, we describe a scalable online program centered on tissue engineering that was altered from our in-person program, CardioStart [58]. Through the creation of a

modular online platform, students from across the country were able to engage with PowerPoints and virtual projects, even while the COVID-19 pandemic limited extracurricular activities. In the virtual format, we aimed to access whether online tissue engineering programs and in-person programs are equally engaging, while being mindful of the fact that online programs have greater accessibility to students.

Methods

After researching multiple online platforms, we chose to use Google Classroom as it is widely accessible for all students and free of charge. Through this platform, modules were adapted from the previous version of CardioStart [58], thus keeping the content the same while adding training videos where in-person laboratory procedures would normally be performed. The Google Classroom consisted of modules covering the following topics: introduction to tissue engineering, cardiovascular system, ethics, experimental design, statistics, writing and presenting scientific work, laboratory techniques, and image processing. Within each module, students could view recorded PowerPoint presentations, collections of engaging YouTube videos to provide real world references, journal articles which aided in the completion of small projects, and discussion boards. To further engage with students, office hours held by graduate students were offered twice a week on zoom for enrolled students to ask questions they may have about the material covered.

To gauge student knowledge before, during, and after the program, multiple surveys and assessments were built into the course. Before beginning the course and after course completion, students were instructed to take a pre- and post-survey. Throughout the course, each module also contained a short assessment, which could be taken multiple times until

the students were satisfied with their score. These surveys and assessments let the students evaluate the knowledge they had at the beginning of the course, what they learned after the completion of each module, and what they mastered by completing CardioStart. The analysis and publication of the cumulative results of these assessments were approved by the Institutional Review Board at the University of California Irvine IRB No: 2020-5964.

Students were recruited by sending flyers to schools' science departments. Interested students then emailed the CardioStart program director, who then asked students to submit IRB consent and assent forms to participate in the CardioStart research study. Students who did not submit these forms still participated, however survey and assessment responses were not downloaded or analyzed.

Results

Student Recruitment

During this study, 85 students enrolled in the online CardioStart program and 65 consented to have their data used for research purposes. Of these students who consented to participate, 18 have completed the course when this article was prepared. The recruitment effort resulted in a more diverse student population than would be possible with the small cohorts admitted to an in-person program (Figure 6-1). While the 18 students were interested in all modules available, the remaining students were able to just complete the modules they found interesting. It is likely that by starting the program during the summer months, the students were able to have adequate time to complete the program (Figure 6-2). Those students who joined during the school year may have had difficulty delegating time to extra activity as many have intense course loads.

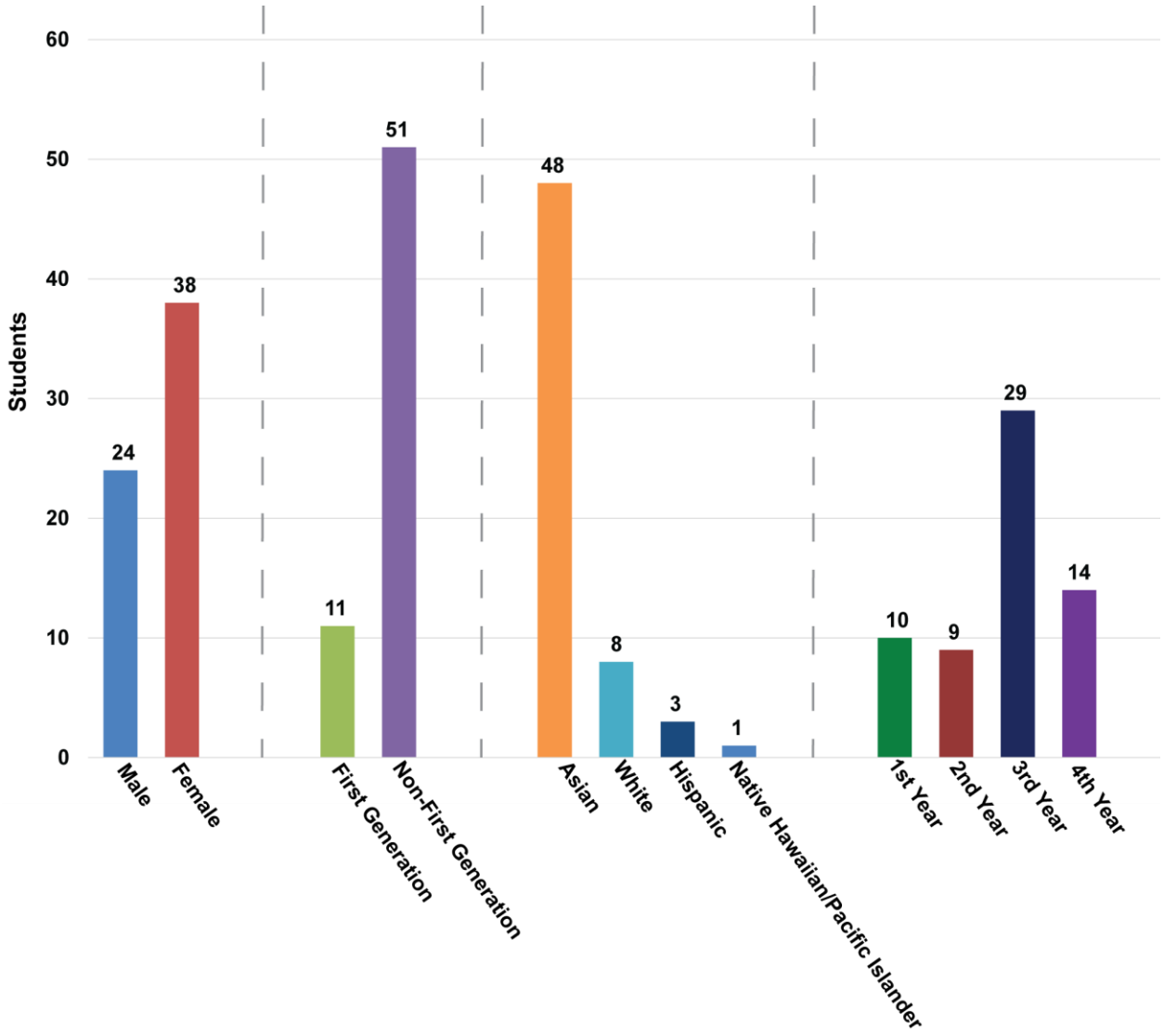

Figure 6-1: Demographics of Enrolled Students : Male vs Female students enrolled, First-Generation vs Non-First Generation students, Ethnicity of Students, Year in High School *data collected from July 13th 2020 through June 4th 2021

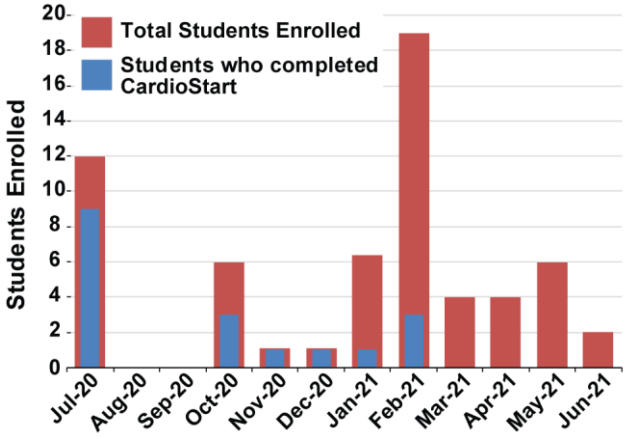

Figure 6-2: Total students enrolled per month and students' completion of program *data collected from July 13th 2020 through June 4th 2021

Costs of Running Program

As one of the main barriers to having students participate in these programs are costs to the universities and students, we analyzed the expenses of online CardioStart and compared it to the three-week in person program (Figure 6-3) [58].

The online program supply cost per student was $0 as the online Google Classroom was free to access and no experimental supplies were needed unlike the three-week programs, which cost $500 per student (Figure 6-3a). Additionally, the total number of required personnel (Figure 6-3b), was equivalent as both the online program and the three-week in-person program required two instructors and the support of two academic advisors. In contrast, the overall time spent by personnel in program setup is greater in the online program (Figure 6-3c). The initial startup time for the online program was 70 hours for content creation while the three-week in-person start up time was 20 hours. However, the online program had a greatly reduced run-time per week as 2 hours of course maintenance

done per week, while the three-week in-person program required meeting with students for 25 hours each week. Moreover, the online program significantly reduced the total personnel time spent per student as there was no cap on student enrollment and thus made the online program more efficient (Figure 6-3d).

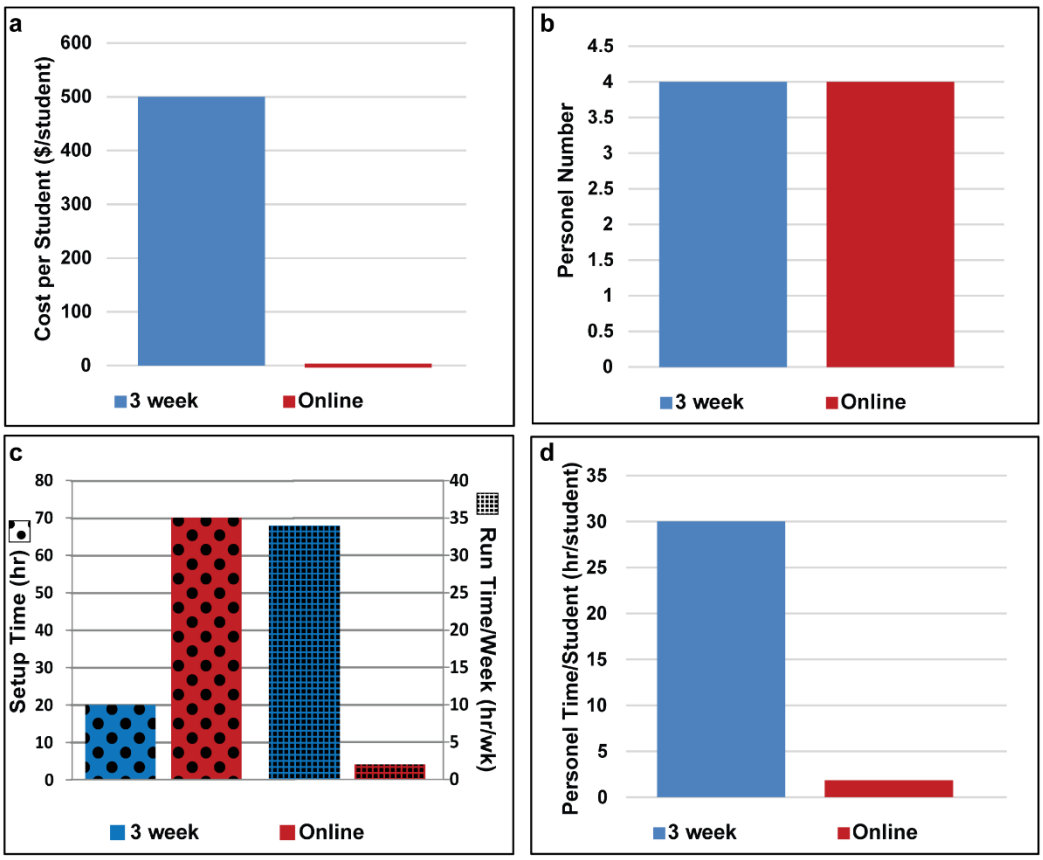

Figure 6-3: Overall Program Costs. (a) Cost per student in 3-week program vs online program (b) Personnel number required for the 3-week program and online program (c) Personnel Time required for Setup and Hours/week (d) Personnel Time per student for the 3-week program and online program *data collected from July 13th 2020 through June 4th 2021

Pre- and Post-Survey Comparison

For those students who consented, we were able to analyze their participation in the program. To determine how effective an online version of CardioStart was, the pre- and post-survey responses from the online program and the three-week in-person version were compared (Figure 6-4). Students self-evaluated survey questions on a scale from 0 – students never heard of the topic to 3- students understand the topic well. Score results illustrate a shift from blue (score of 0-1) in the pre-survey to red (score of 2-3) in the post-survey in every topic after completing CardioStart (Figure 6-4). Overall, the survey results indicate that this trend exists regardless of whether they participated in the online or three-week in-person version of CardioStart.

Figure 6-4: Pre- and Post-Survey Response comparisons in the 3-week and online programs *data collected from July 13th 2020 through June 4th 2021

Assessment scores

To determine how well each module translated to the online platform, we performed a comparison between the average assessments scores from the online version of CardioStart and the three-week in-person version. These assessments were short quizzes given after the completion of each module and scored out of 100. When comparing the two programs, there is no significant difference with the exception of the topic of statistics in students' outcomes between the three-week in person and online programs (Figure 6-5). A Tukey Test was used to determine significance between the programs.

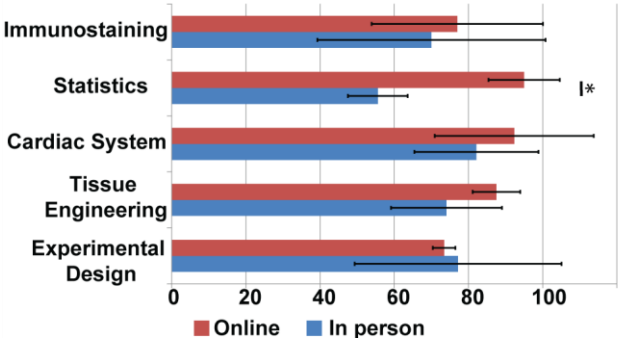

Figure 6-5: Assessment Scores of the 3-week vs online program *data collected from July 13th 2020 through June 4th 2021

Students' thoughts on program

To more comprehensively evaluate the program, quantitative questions were also asked on what topics they though CardioStart covered and responses were collected. Students were asked to give survey questions a score of 0 – CardioStart did not address this topic to 5 – CardioStart greatly addressed this topic. Student responses were overall positive and can be seen in Figure 6-6.

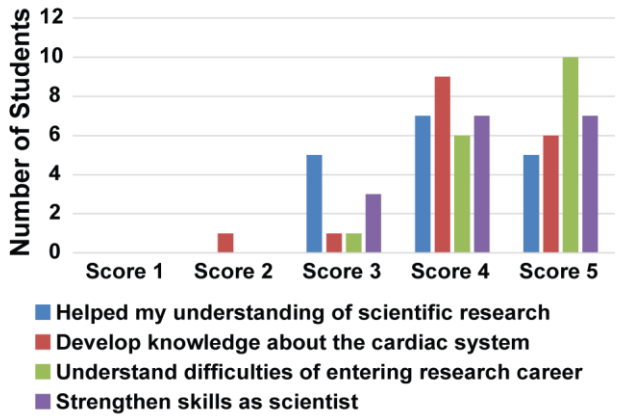

Figure 6-6: Qualitative student responses to skills learned through CardioStart *data collected from July 13th 2020 through June 4th 2021

Along with these questions, students were also asked open ended qualitative questions where they expanded on what they enjoyed most about the program and what could be improved:

> "I found learning about tissue engineering and the cardiac system something that interests me, and I therefore went about researching these topics in more detail for fun."
>
> "The course was very informative and the assignments were helpful in reviewing the content."
>
> "This program has allowed me to expand my knowledge especially in tissue engineering, the cardiovascular system, and statistics."
>
> "All of the information, presentations, and assignments really helped me learn about various aspects of cardiac and tissue engineering."

Students were also invited to provide feedback on areas they would like improvement on:

> "Although I felt that this program was great online, the only improvement I would suggest is to incorporate more interactive elements. Some aspects, like the Fiji project, were engaging, but it would be more fun if there were hands-on experiences and live exposure."
>
> "I may have been better prepared for CardioStart if I had some background in advanced cell biology. A background video or vocabulary list introducing commonly used terms relating to tissue engineering would have been helpful.

Discussion

In this work, we described the implementation of a scalable, online program for high school students, online CardioStart, which allows for greater scalability, accessibility, and cost effectiveness than similarly run in-person programs. Through the use of online surveys and quizzes, we were able to compare the online program to the previous held in-person programs (Figure 6-4). Generally, programs like these introduce students to topics not covered in high school curricula; however, one deficiency is that they are not widely available to many students [35-37]. This lack of accessibility has become more evident due to COVID-19 closures, as students' education has been significantly impacted [48]. Through the creation of an online program, many students are now able to participate in a tissue engineering course that would not have been available to them due to limited accessibility or high program costs.

One of the main factors that contributes to the ease of online scalability is the amortization of the main cost, program set-up. Oftentimes, universities are willing to support

an effort to set up a program rather than the program itself, thus allowing many students to access the program free of charge. As a result, online CardioStart is not only scalable, but also accessible to students who may be unable to pay program costs otherwise.

Another benefit of increased scalability is the number of students able to participate in online programs. Utilizing the google classroom, we experienced a 12-fold increase in student participation as we were not limited by lab capacity or conflicts with student schedules. The google classroom can hold 1,000 students and those who have completed the course can be removed to make space for new participants. Additionally, google classrooms can be duplicated to accommodate more students. To make the program even more scalable, more research groups can develop similar programs with different modules, allowing all student interests to be addressed.

The online program also allows for greater student flexibility, thus allowing greater accessibility. As seen in Fig II, many more students were able to engage with the content than previous in-person programs. Due to the modular format of online CardioStart, students had the option of choosing the modules that interested them. This is highly beneficial as students had the freedom to learn what they enjoyed, instead of being locked in a program for an entire summer while they were only interested in parts of the program. Still, the 18 students who completed the program is an approximate three-fold increase to what our lab could host for the past six years in any given summer.

As programs are converted to be more scalable and accessible through online education, there is a danger of sacrificing student engagement and learning. For the CardioStart online conversion, based on pre- and post-survey and assessments comparisons

seen in Figure 6-4, students do master the same concepts regardless of the virtual format. However, when asked to leave comments about the program students wanted more hands-on activities. Post-COVID, re-introducing hands on experiments would be a great complement to the online program, and students that expressed an interest could be invited to the university to complete their training.

In the future, we will continue to improve the online CardioStart program by adding modules as topics become relevant. In the fall of 2020, we included a module on COVID-19 and the heart as research became available. This demonstrates that the modular platform described in this paper allows for continuous improvements to be made and for content to remain relevant to students. During the next program offering, we aim to merge the in-person and online CardioStart programs, which will address students' comments about their desire to work in a tissue engineering lab. Students will first complete the online CardioStart course and learn the basics of the tissue engineering field. Interested students can then join a lab for cell culture training, experimental design practice, and learn more about day-to-day life as a researcher. With this pipeline in place, we can then partner with other universities to leverage lab spaces across the country in order for students to complete the hands-on training. With the creation of online CardioStart, we hope that more universities will adopt similar programs to close the 19 million student gap of those who do not have access to STEM programs [31, 55].

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Chapter 7 : Conclusions and Future Work

As the population continues to age, heart disease will become more prevalent across the world and will require more research to understand disease progression, possible treatments and healing the native tissue [1-3]. Although much research is devoted to these topics, there are still many mysteries left unsolved as the heart is a complex organ. Through the work in this dissertation, we aimed to discover how various perturbations to cardiac tissue affect force production as well as inspire the next generation of students to pursue degrees in STEM fields, in particular, cardiovascular tissue engineering.

A common perturbation seen in many patients suffering from heart disease is cardiac remodeling which leads to arrythmia or loss of cardiac output [19, 59-61]. To explore the effects of cellular remodeling on force generation, cardiac tissues were engineered to contain area of local organization (~100 micron) while keeping the overall tissue organization (1 mm) varied. The size of local organization was chosen based on two results: (1) isotropic tissues self-assemble these small areas of organization and (2) a simplified force model relating size of local organization (~250 micron) to force production was not able to predict isotropic tissue force. Therefore, we sought to determine whether isotropic tissues are inherently different because there is no guidance leading to a difference in tissue formation since organization is spontaneous or if the size of organization determines the force generated. By measuring the force produced by tissues locally organized at a length scale of 100 microns while keeping the mm length scale varied, we gained knowledge of how organization size affects tissue force generation. To continue this work, further patterns can be tested with different size local areas of organization to produce a more robust model. This will in turn lead to a better understanding of cardiac tissue self-assembly.

Though remodeling is a common perturbation leading to heart disease, COVID-19 mortality has overtaken that of heart disease [62, 63]. As COVID-19 cases increased over the past year, more clinical data has shown cardiac distress related mortalities leading to the belief that other mechanisms other than virus load are responsible for severe cases [24, 64-66]. Upon further examination of patients suffering with a cardiac comorbidity, many had increased levels of pro-inflammatory cytokines leading to the belief cytokine storm was responsible for cardiac involvement [67, 68]. Through the addition of pro-inflammatory cytokines TNF-α, CCL2, and IL-6, to heart tissue, we aimed to discover how force generation was directly impacted by cytokines. In the future, we will test varying concentrations of cytokines found within the physiological range. To begin to add complexity back into the system, cytokines will then be added in pairs to determine cytokine interaction with one another. As this research continues, future work will aim to continue to add complexity into the system through coculture of cardiomyocytes and macrophages in both inflammatory and healing stages to elucidate the mechanisms behind cytokine storm and tissue force production.

While contributing to the field of cardiac tissue engineering is important, researchers also share a role in recruiting the next generation of students to continue upon their work. This has become especially important as the population continues to age and the prevalence of cardiovascular disease will continue to grow [28-30]. To encourage students to purse research in the area of cardiac tissue engineering, we developed a high school tissue engineering program, CardioStart. Through the iteration of the in-person CardioStart program, we discovered that the short 3-week program was most cost effective while promoting student learning as compared to the 6-week program. The transition of the 3-

week in-person CardioStart program to an online platformed allowed five-fold the number of students to enroll and engage with the material. Students' learning was also unhindered using the online platform but many missed the hands-on experiments which the in-person program provided. The next step in providing enrichment to these students would be combining the two programs together. This would entail students learning about the field through the online course before entering a tissue engineering lab to practice what they learnt. To continue to expand engineering education, the lab hopes to partner with other universities to create similar programs to encompass many topics as students' interests are vast. By creating a network of programs, students will have access to topics they may not have thought to explore before in hopes to inspire creative thinking to problems that do not yet have solutions.

This dissertation focuses on understanding the fundamentals of cardiac architecture, the affects perturbations have on tissue function, and how to involve the next generation of scientists to continue this work.

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