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

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Introduction

Currently, the United States faces a shortage of STEM graduates while the amount of STEM occupations are expected to grow [1, 2]. 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 [3, 4]. To fill these roles in the future, today's high school students need more exposure to STEM [5]. Although high school programs explore the sciences, students still struggle to make the connection between the classroom environment and real world STEM applications [2, 6, 7]. 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 [8]. 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 [8].

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 [6-8]. Many programs devote one day a week to cover one engineering branch, thus covering all branches in a full week [2, 6, 9]. While these programs include biomedical engineering, many topics are omitted due to time constraints [6, 9]. 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 [8, 10]. While these programs provide access to biomedical engineering outside of high school curricula, many lack tissue engineering components [8]. Furthermore, tissue engineering specific programs are scarce and expensive due to the significant amount of personnel time to design and run them [9, 10]. 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 1 and Figure 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.

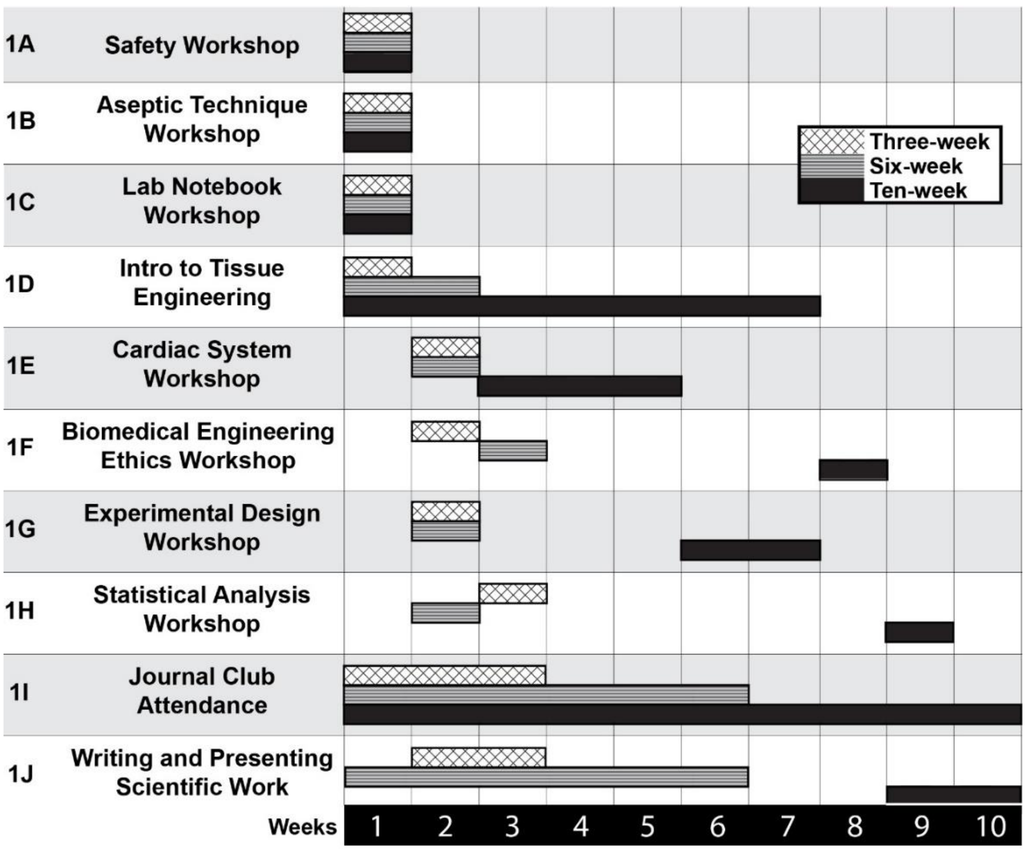

Figure 1: CardioStart Module-Based Learning Activities

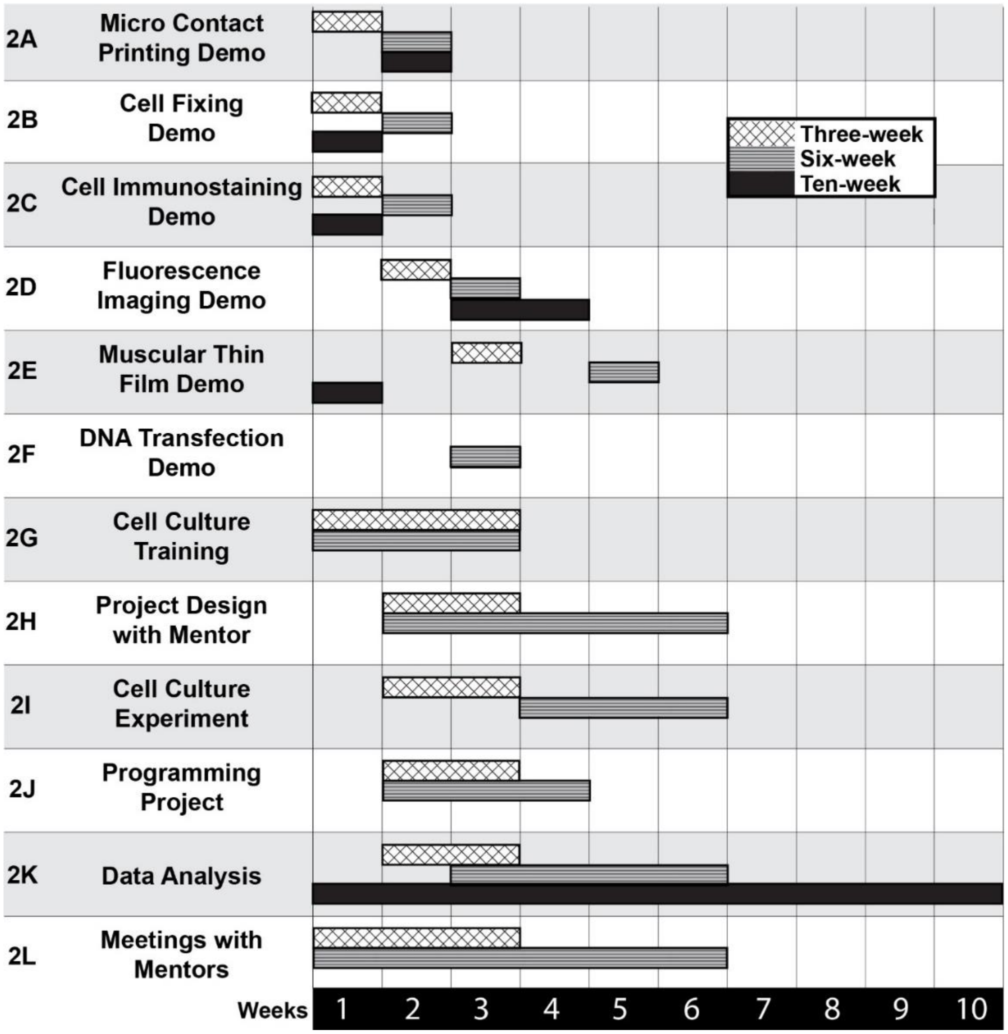

Figure 2: Demonstrations and Experimental Training

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 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 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 1C Lab Notebook Workshop, 1I Journal Club Attendance and 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 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 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 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 1D Intro to Tissue Engineering and 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 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 1G Experimental Design Workshop and 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 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 [11].

Cell Fixing and Immunostaining Demonstration

Once students understood microcontact printing, they learned about cell fixing and immunostaining (Figure 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 [11]. 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 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 [11].

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 [11]. Students were able to see how the assay works as well as cardiomyocytes beating and pacing (Figure 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 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 [12].

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 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 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 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 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 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 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.

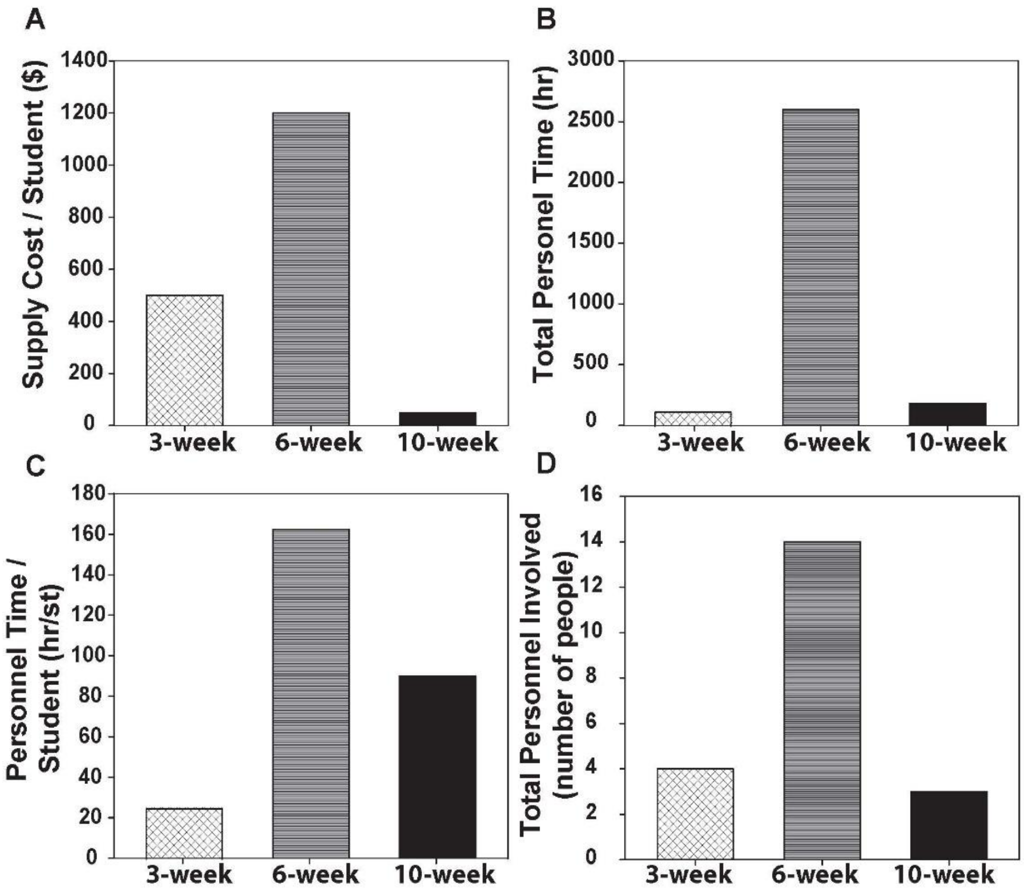

Figure 3: Comparison of program costs across the three program iterations (A) Supply cost normalized by the number of students in each program (B) Total personnel time required per program (C) Personnel time normalized by the number of students in each program (D) Total personnel involved in each program

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 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 3A). Personnel time cost was further broken into time cost per student (Figure 3C) as well as the total number of people involved in mentoring the students in each program (Figure 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 3D). The breakdowns in Figure 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 4). This could be explained by a 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.

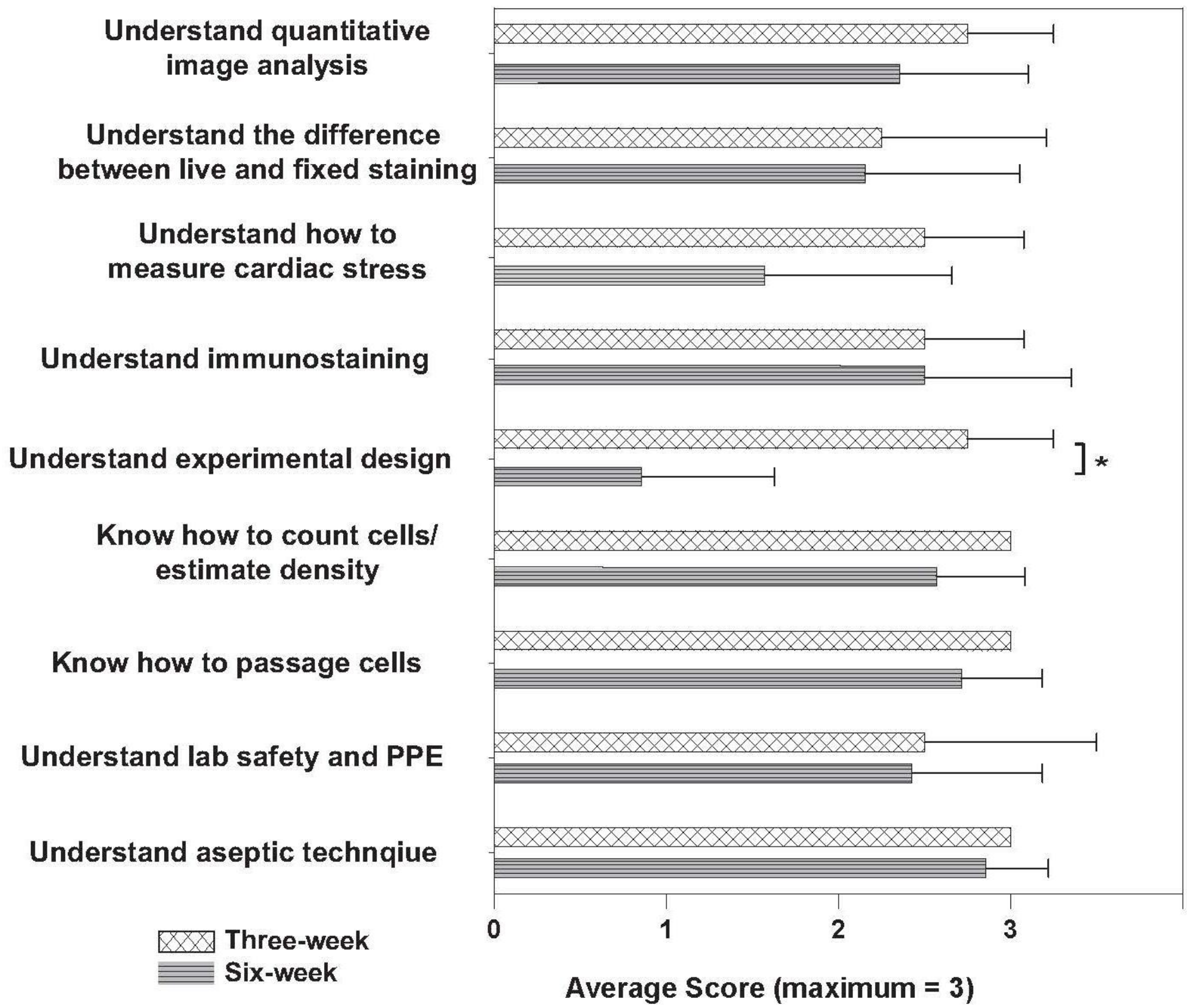

Figure 4: Self improvement survey results comparing 3-week and 6-week programs. 3-week program N=4, 6-week program N=14; *indicated statistical significance with $p < 0.001$. Error bars represent the standard deviation of the data

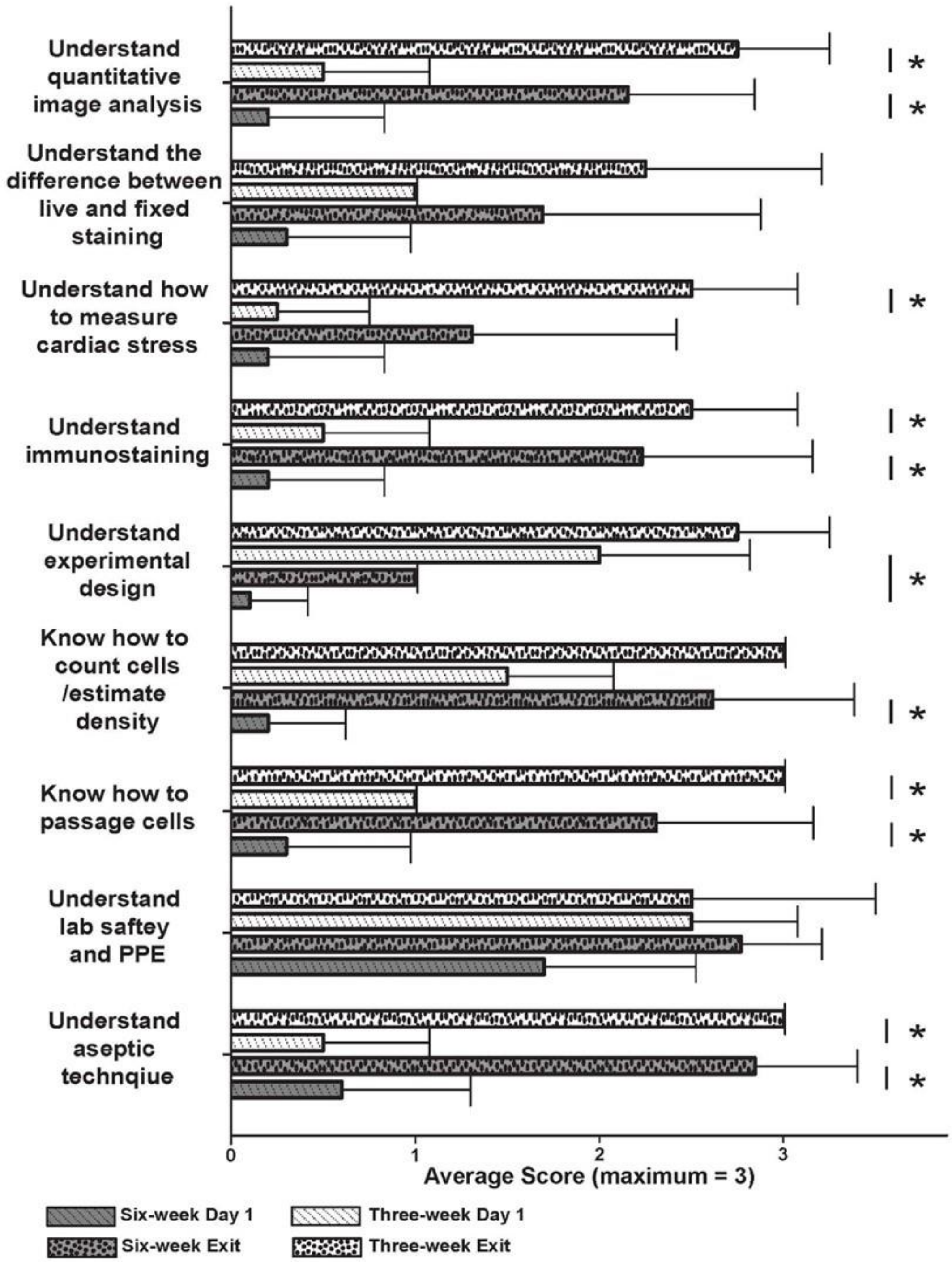

Figure *5*: Pre- and post-survey results. The following pairs were compared: (1) three- and six-week pre-survey, (2) three- and six-week post-survey, (3) three-week pre- and post-survey, and (4) six-week pre- and post-survey. The *indicated statistical significance with $p < 0.001$. Error bars represent the standard deviation of the data

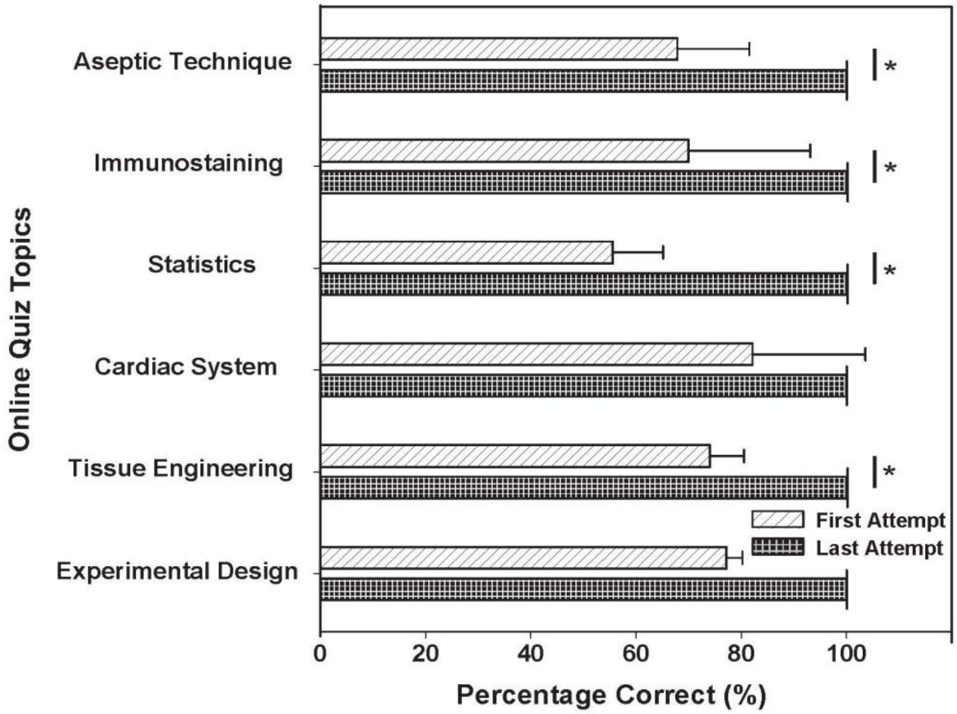

Figure 6: Online quiz first and last attempts in three-week program. The first attempt was after completing the corresponding module while the remaining attempts were completed after further discussion and teamwork. Three-week program N=4, *indicated statistical significance with $p < 0.001$. Error bars represent the standard deviation of the data

Figure 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 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 [2], while biomedical engineering specific camps aim to cover the breadth of the field and may not devote enough time to tissue engineering [8]. Moreover, tissue engineering camps are hindered due to the scarcity and expenses needed to design and run them [9, 10]. 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.

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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?